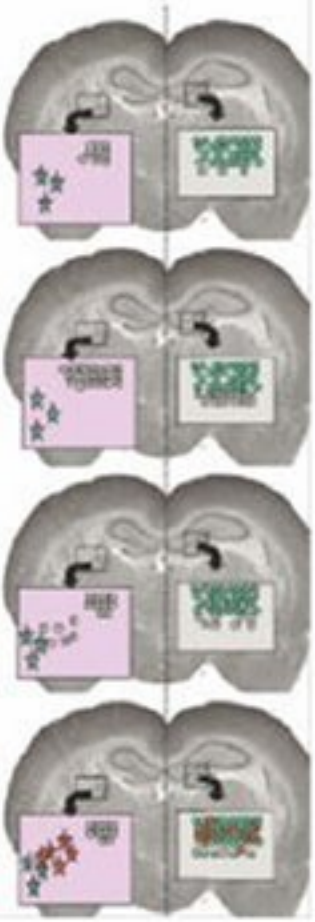
print
21 52 06
online
www.karger.com/ced

Cerebrovascular Diseases

www.cerebrovascular-diseases.com



Neuroprotection in Brain Ischemia: An Update

Editors
Markku Kaste, Helsinki
José Castillo, Santiago de Compostela

KARGER

Cerebrovascular Diseases

Neuroprotection in Brain Ischemia: An Update

Editors
Markku Kaste, Helsinki
José Castillo, Santiago de Compostela

22 figures, 3 in color, and 5 tables, 2006

KARGER

Basel · Freiburg · Paris · London · New York ·
Bangalore · Bangkok · Singapore · Tokyo · Sydney

S. Karger
Medical and Scientific Publishers
Basel • Freiburg • Paris • London
New York • Bangalore • Bangkok
Singapore • Tokyo • Sydney

Printed in Switzerland on acid-free paper by
Reinhardt Druck, Basel
ISBN 3–8055–8122–X

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Cerebrovascular Diseases

Vol. 21, Suppl. 2, 2006

Contents

V **Preface**

1 **Targets of Cytoprotection in Acute Ischemic Stroke: Present and Future**
Lizasoain, I.; Cárdenas, A.; Hurtado, O.; Romera, C. (Madrid); Mallolas, J. (Girona); Lorenzo, P. (Madrid); Castillo, J. (Santiago de Compostela); Moro, M.A. (Madrid)

9 **Apoptosis: Future Targets for Neuroprotective Strategies**
Ferrer, I. (Barcelona)

21 **Vascular Protection in Brain Ischemia**
Rodríguez-Yáñez, M. (Santiago de Compostela); Castellanos, M. (Girona); Blanco, M.; Mosquera, E.; Castillo, J. (Santiago de Compostela)

30 **Gene Expression in Cerebral Ischemia: A New Approach for Neuroprotection**
Millán, M.; Arenillas, J. (Badalona)

38 **Ischemic Preconditioning: A Novel Target for Neuroprotective Therapy**
Blanco, M. (Santiago de Compostela); Lizasoain, I. (Madrid); Sobrino, T. (Santiago de Compostela); Vivancos, J. (Madrid); Castillo, J. (Santiago de Compostela)

48 **Estrogens as Neuroprotectants against Ischemic Stroke**
Alonso de Leciñana, M.; Egido, J.A. (Madrid)

54 **Neurorepair versus Neuroprotection in Stroke**
Hurtado, O.; Pradillo, J.M.; Alonso-Escolano, D.; Lorenzo, P. (Madrid); Sobrino, T.; Castillo, J. (Santiago de Compostela); Lizasoain, I.; Moro, M.A. (Madrid)

64 **The Ischemic Penumbra: A New Opportunity for Neuroprotection**
Fisher, M. (Worcester, Mass.)

71 **Evolving Paradigms for Neuroprotection: Molecular Identification of Ischemic Penumbra**
Castellanos, M. (Girona); Sobrino, T.; Castillo, J. (Santiago de Compostela)

80 **Deterioration in Acute Ischemic Stroke as the Target for Neuroprotection**
Serena, J. (Girona); Rodríguez-Yáñez, M. (Santiago de Compostela); Castellanos, M. (Girona)

89 **Non-Pharmacological Neuroprotection: Role of Emergency Stroke Management**
Leira, R.; Blanco, M.; Rodríguez-Yáñez, M.; Flores, J.; García-García, J. (Santiago de Compostela)

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Access to full text and tables of contents, including tentative ones for forthcoming issues: www.karger.com/ced_issues

99 Neuroprotection in Malignant MCA Infarction
Delgado, P.; Sahuquillo, J.; Poca, M.A.; Álvarez-Sabin, J. (Barcelona)

106 Neuroprotection in Vascular Dementia
Martínez-Vila, E.; Murie-Fernández, M.; Gállego Pérez-Larraya, J.; Irimia, P. (Pamplona)

118 Thrombolysis and Neuroprotection in Cerebral Ischemia
Gutiérrez, M.; Díez Tejedor, E.; Alonso de Leciñana, M.; Fuentes, B.; Carceller, F.; Roda, J.M. (Madrid)

127 Other Neuroprotective Therapies on Trial in Acute Stroke
Ferro, J.M. (Lisboa); Dávalos, A. (Badalona)

131 Author Index
132 Subject Index

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):V–VI
DOI: 10.1159/000091697

Published online: May 2, 2006

Preface

Protecting the Ischemic Brain

Despite many years of research, treatment and prevention of ischemic stroke continue to be one of the major challenges in current medicine.

Progressive understanding of the complex mechanisms of cerebral ischemia has influenced the development of a large number of molecules to block the ischemic cascade at different levels. Many of these drugs have demonstrated considerable efficacy in a range of animal models of cerebral ischemia. The transfer of these results to human clinical practice has not, however, proved to be sufficiently satisfactory. More than 140 clinical trials have been conducted in stroke and some 250 drugs, either alone or in combination, have been studied both in ischemic stroke and in cerebral hemorrhages. From this research, only revascularization of the occluded cerebral arteries has proven to be effective, but only when it has been utilized at a very early stage, and in selected patients.

Much recent neurovascular research has been designed to identify molecular changes deriving from cerebral ischemia that are capable of becoming therapeutic targets: mechanisms associated with energy failure, excitotoxicity, lesions associated with free radicals, inflammation and apoptosis. The hope of having a drug available for universal application that by itself cures ischemic stroke is, without a doubt, an unattainable utopia. However, analyses and reviews of successes as well as of various failures that have occurred in clinical trials developed for studying neuroprotection in ischemic stroke, permit us to be reasonably certain that in the near future we will have available neuroprotectors that are effective in particular situations and for particular patients with stroke.

The therapeutic margin for neuroprotectors, which at present is very narrow, may be even more challenged by the progressively more common use of thrombolytic treatment in regular clinical practice. However, the knowledge that recanalization may permit the arrival of a neuroprotector to the ischemic zone with greater ease and effectiveness does not allow us to be despondent.

Faced with this situation, it seems to be appropriate to ask what the therapeutic margin for pharmacological neuroprotection will be. Considering that the therapeutic objective in acute cerebral infarction is to boost reperfusion and to reduce damage by ischemia-reperfusion, the most reasonable expectations for neuroprotection suggest that this will have its place in a variety of clinical situations: maintaining viability of the ischemic tissue longer and lengthening the therapeutic window for thrombolysis, inhibiting or controlling adverse reactions arising from reperfusion and reducing the risk of brain hemorrhage in those patients who receive thrombolytic treatment. This gives us the opportunity for a combined therapy for thrombolysis with neuroprotection, for which there already are satisfactory experiences in animal models.

Pressure from industry to develop a neuroprotective drug for universal application that is highly effective and has maximum tolerance, has probably led to two major methodological errors: (1) simplifying the complexity of ischemic damage that affects the brain as a functional unit by blocking one single molecular mechanism, and (2) overlooking the value of neuroprotection in enhancing neurorecovery and plasticity, which is essential for the recovery of a stroke patient.

Consequently, in order to achieve better results in acute stroke trials in the future, many changes are needed

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ced

in study designs. Future therapeutic options will probably include, in the following order and in accordance with the various time windows, neuroprotection, reperfusion, neuroprotection once again and treatments to enhance neurorepair.

We think that only optimism is acceptable, because never before have so many positive factors existed simultaneously: extensive understanding of the mechanisms involved in cerebral ischemia, overwhelming biotechnological development, investment by the pharmaceutical industry that would have been unimaginable in the past times of therapeutic nihilism, and a growing awareness among health authorities and the public that stroke is a treatable neurological emergency.

In this context, the present supplement of *Cerebrovascular Diseases* is well justified. The supplement addresses widely neuroprotection in cerebral ischemia. It covers not only molecular, cellular, cerebral and vascular aspects, with particular mention of neurorepair, but also clinical entities that are most susceptible. We hope that the timely information offered here will be of use to the readers.

Markku Kaste, Helsinki
José Castillo, Santiago de Compostela

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):1–8
DOI: 10.1159/000091698

Published online: May 2, 2006

Targets of Cytoprotection in Acute Ischemic Stroke: Present and Future

I. Lizasoain[a] A. Cárdenas[a] O. Hurtado[a] C. Romera[a] J. Mallolas[b]
P. Lorenzo[a] J. Castillo[c] M.A. Moro[a]

[a]Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid;
[b]Departamento de Neurología, Hospital Universitario Doctor Josep Trueta, Girona, and
[c]Departamento de Neurología-Neurología Vascular, Laboratorio de Investigación Neurovascular,
Hospital Clínico Universitario, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Key Words
Antioxidants · Glutamate · Inflammation · Neuroprotection

Abstract
Although the management of stroke has improved remarkably over the last decade due mainly to the advent of thrombolysis, most neuroprotective agents, although successful in animal studies, have failed in humans. Our increasing knowledge concerning the ischemic cascade is leading to a considerable development of pharmacological tools suggesting that each step of this cascade might be a target for cytoprotection. Glutamate has long been recognized to play key roles in the pathophysiology of ischemia. However, although some trials are still ongoing, the results from several completed trials with drugs interfering with the glutamatergic pathway have been disappointing. Regarding the inhibition of glutamate release as a possible target for cytoprotection, it might be afforded either by decreasing glutamate efflux or by increasing glutamate uptake. In this context, it has been shown that glutamate transport is the primary and only mechanism for maintaining extracellular glutamate concentrations below excitotoxic levels. This transport is executed by the five high-affinity, sodium-dependent plasma membrane glutamate transporters. Among them, the transporter EAAT2 is responsible for up to 90% of all glutamate transport. We will discuss the effect of different neuroprotective tools (membrane stabilizers or endogenous neuroprotection) affecting glutamate efflux and/or expression of EAAT2. We will also describe the finding of a novel polymorphism in the EAAT2 promoter region which could be responsible for differences in both gene function and regulation under pathological conditions such as cerebral ischemia, and which might well account for the failure of glutamate antagonists in the clinical practice. These results may possess important therapeutic implications in the management of patients at risk of ischemic events, since it has been demonstrated that those patients with progressing stroke have higher plasma concentrations of glutamate which remain elevated up to 24 h when compared to the levels in patients without neurological deterioration.

Cytoprotection and Ischemic Cascade

In the last years, much has been written about neuroprotection. Although we normally use the word neuroprotection, the term cytoprotection is likely more accurate since our objective is to recover the entire brain tissue including not only neurons but also non-neuronal cells.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0001$23.50/0

Accessible online at:
www.karger.com/ced

Dr. Ignacio Lizasoain
Departamento de Farmacología, Facultad de Medicina
Universidad Complutense de Madrid
ES-28040 Madrid (Spain)
Tel. +34 91 3941 478, Fax +34 91 3941 463, E-Mail ignacio.lizasoain@med.ucm.es

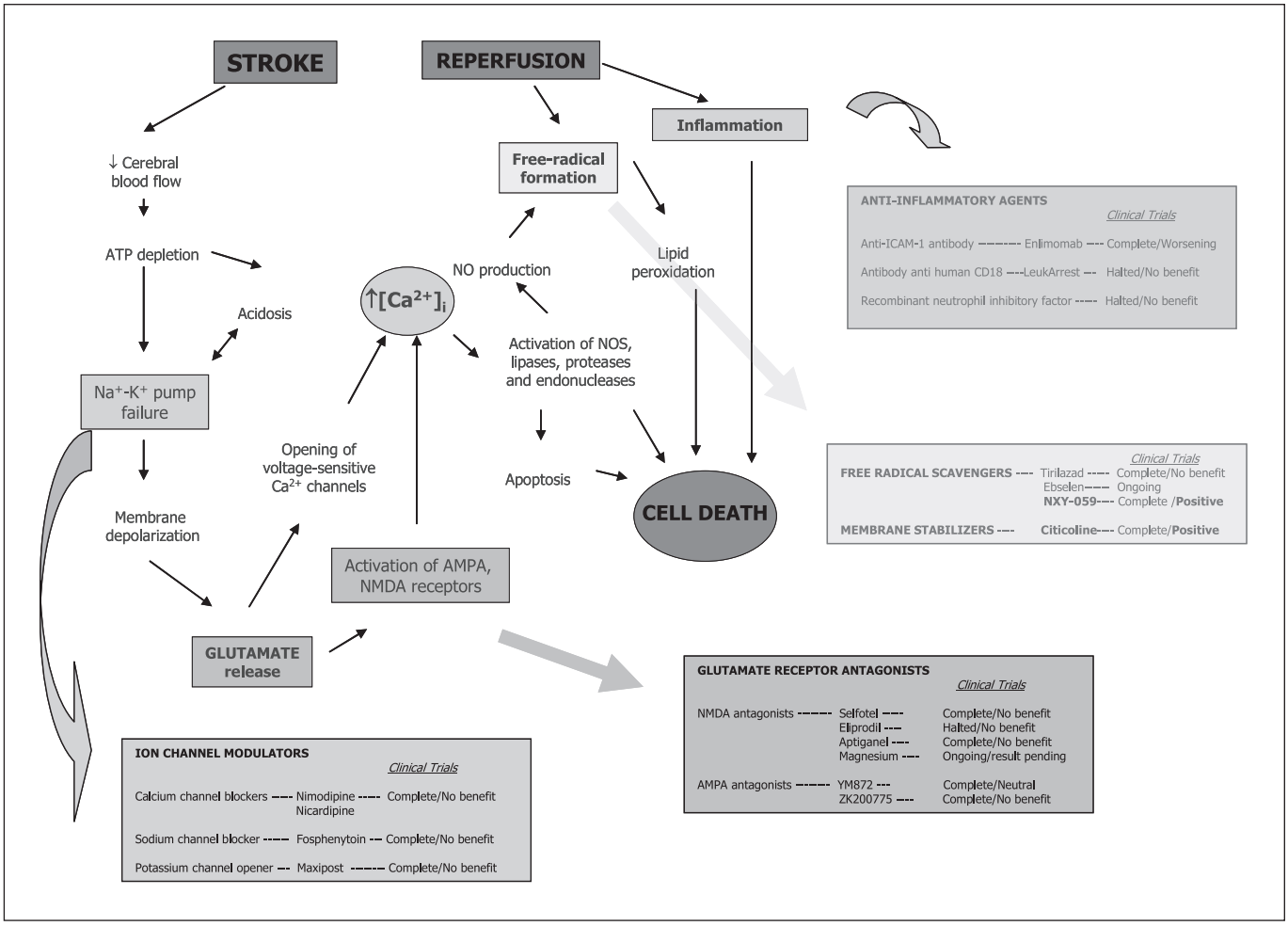


Fig. 1. Neuroprotective drugs acting on the ischemic cascade.

In order to define the targets of cytoprotection, we will first illustrate briefly the ischemic cascade (see fig. 1) in which, at least theoretically, each step of this cascade might be a target for therapeutic intervention.

In most cases, stroke results from the obstruction of blood flow in a major cerebral vessel. The brain requires a continuous supply of oxygen and glucose to maintain normal function and viability. When this supply is interrupted, a cascade of events takes place: energy source depletion mainly due to reduction of ATP leads to an altered cell function by interruption of ATP-dependent processes, such as the disruption of ionic gradients across membranes due to the failure of the Na^+,K^+-ATPase. This causes an increase in extracellular K^+ as well as an influx of Na^+, Cl^- and Ca^{2+} into the cells. The initial increase in extracellular K^+ concentration may spread, triggering depolarizations and reversal of the amino acid transporters. In these conditions, both voltage-operated and receptor-operated calcium channels are recruited, thus provoking an elevation of free cytosolic Ca^{2+}. A massive release of excitatory amino acids, mainly glutamate, may derive from both reversal of glutamate transporters and Ca^{2+}-dependent exocytosis. Glutamate, an excitatory amino acid which has been implicated in the pathogenesis of brain injury, results in excitotoxicity in which excessive extracellular glutamate kills neurons through Ca^{2+}-dependent and -independent mechanisms. Glutamate-mediated excitotoxicity is thought to occur mainly because of the overactivation of AMPA and NMDA synaptic glutamate receptors [for review, see 1]. Such overactivation, mainly of the NMDA subtype, further leads to Na^+, Ca^{2+}, Cl^- and H_2O accumulation, cell swelling and cytotoxic

edema. In addition, elevated intracellular Ca^{2+} causes mitochondrial calcium overload, termination of ATP production and vast breakdown of phospholipids, proteins and nucleic acids by activation of calcium-dependent phospholipases, proteases, and endonucleases. In addition, the augmented intracellular Ca^{2+} enhances the increase in extracellular glutamate, thus propagating excitotoxicity.

A main event during ischemia is the generation of free radicals; due to their high reactivity, they provoke damage to lipids, DNA and proteins and produce neuronal death. They also contribute to the breakdown of the blood-brain barrier and brain edema. One of these radicals which is elevated after the ischemic insult is nitric oxide (NO). NO generated primarily by neuronal and inducible NO synthases (NOS) promotes neuronal damage following ischemia. In addition, the conversion of xanthine dehydrogenase to xanthine oxidase promotes the cellular formation of toxic oxygen free radicals such as the superoxide anion which further breaks down membrane, cytoskeletal, and nuclear structures. An important source of oxidative stress-mediated brain damage is the oxidant reactions due to the formation of peroxynitrite, a powerful oxidant that results from the interaction between NO and superoxide. This anion has been shown to cause cell damage by several mechanisms that include lipid peroxidation, tyrosine nitration, sulfhydryl oxidation and nitrosylation, and DNA breakage.

Ischemic injury is associated to an inflammatory response in which several mediators are released or activated, such as inflammatory cytokines and adhesion molecules. The result is the infiltration of leukocytes to the brain parenchyma, and the activation of resident microglial and astroglial cells [for review, see 2]. The inflammatory response is a consequence of an active gene expression which is triggered by ischemia. In this context, important transcription factors are activated and/or synthesized, including NF-κB, hypoxia-inducible factor 1, interferon regulatory factor 1 and STAT3 [for review, see 3]. Apart from the expression of adhesion molecules and the initiation of the inflammatory reaction, cytokines might activate the expression of inflammation-related genes, such as iNOS and cyclooxygenase-2 [for review, see 4]. Other important inflammatory mediators being expressed and/or activated in this setting are several proteolytic enzymes belonging to the family of the metalloproteases, including matrix metalloproteases, implicated in damage to extracellular matrix [2], as well as their endogenous protease inhibitors.

During the last 20 years, many neuroprotective drugs have been studied in experimental stroke models [for review, see 5], however very few have shown efficacy in clinical trials (see fig. 1 and for further details see Stroke Trials Directory in The Internet Stroke Center at http://www.strokecenter.org/trials and see Ferro and Dávalos, pp. 127–130, this supplement). Several families of compounds have been studied according to different targets of cytoprotection, which include calcium channel blockers, sodium channel blockers, potassium channel openers, calcium chelators, antioxidants or free radical scavengers, GABA agonists, glutamate antagonists, growth factors, leukocyte adhesion inhibitors, NO inhibitors, opioid antagonists, phosphatidylcholine precursors, and serotonin agonists.

Among them, the major group corresponds to drugs interfering with the glutamatergic transmission. Again, preclinical studies demonstrating its utility are contradictory when compared with the clinical trials. As regards drugs that act on the NMDA receptor, NMDA antagonists were tested in well-controlled trials with negative results: Selfotel, a potent competitive antagonist, Eliprodil, a polyamine site antagonist, Aptiganel, an NMDA channel blocker, and Gavestinel (GV150526), an NMDA antagonist at the glycine site [6–9]. Phase III trials are still ongoing with Troxoprodil (CP-101,606, an antagonist of the NR2B subunit of the NMDA receptor). Magnesium is an ideal neuroprotectant because of its different mechanisms of action (NMDA channel blocker, calcium channel blocker, promotes vasodilation, inhibits inflammatory response), low cost, easy administration and good safety profile. It is being studied in two ongoing phase III trials [for review, see 10, and FAST-MAG website]. The competitive AMPA antagonist, YM872 (Zonampanel, a high-affinity competitive antagonist) is still under study in two ongoing clinical trials; however, the studies with the AMPA antagonist ZK200775 (MPQX) were halted due to adverse effects [11, 12]. Metabotropic glutamate receptors are quite different from ionotropic receptors because its activation or inhibition would modulate glutamate release. Therefore, these drugs are expected to have a better safety profile, free of psychotropic side effects, than those acting on ionotropic receptors [for review, see 13].

Regarding glutamate release, there have been some attempts with negative results, such as the sodium channel blocker and anticonvulsant fosphenytoin, the sodium and calcium channel blocker Sipatrigine (619C89), or the potassium channel activator BMS-204352 (MaxiPost) [14], although a second multicenter trial of the latter is

being considered. A trial is also ongoing with ONO-2506, a compound that modulates uptake capacity of glutamate transporters.

The phosphatidylcholine precursor citicoline has demonstrated a neuroprotective effect in animal models of stroke. In this case, a meta-analysis of seven controlled clinical stroke trials showed that treatment with oral citicoline within the first 24 h after onset in patients with moderate to severe stroke increases the probability of complete recovery at 3 months [15]. We will discuss how this drug may be targeting glutamate release as a major mechanism of action.

In other chapters of this supplement issue new approaches for neuroprotection are discussed, such as caspase inhibitors to reduce apoptosis (see Ferrer, pp. 9–20), maneuvers to confer vascular protection (see Rodríguez-Yáñez et al., pp. 21–29) or to modify gene expression (see Blanco et al., pp. 38–47) and estrogen or its derivatives phytoestrogens as future neuroprotective treatment (see Alonso de Leciñana and Egido, pp. 48–53).

In summary, preclinical studies have demonstrated that numerous drugs are effective for treating stroke in animals; however, most of them have failed in clinical trials. Therefore, the study of the discrepancies between preclinical studies and clinical trials to form a basis for future trials together with the search of new targets for neuroprotection will help us to find a better neuroprotective drug.

Strategies to Search New Targets: Ischemic Preconditioning

Since 1990, when Kitagawa et al. [16] described the phenomenon called *ischemic tolerance,* we know that a short or mild ischemic event called ischemic preconditioning (IPC) can result in subsequent resistance to severe ischemic injury. Thus, elucidation of mechanisms that regulate the acquisition of brain tolerance could guide efforts to develop effective and safe pharmacological agents to protect the brain or reduce ischemic injury (see Alonso de Leciñana and Egido, pp. 48–53).

The mechanisms by which IPC can produce tolerance have been reviewed [17]. It has been described that antiexcitotoxicity, anti-inflammatory, antiapoptotic and regeneration mechanisms can be involved at different times. It is well known that mechanisms, which are important during the phase of ischemic cell death, are also targets of ischemic tolerance. In this sense, glutamate has long been recognized to play key roles in the pathophysiology of ischemia, due to its excessive accumulation in the extracellular space and the subsequent activation of its receptors, mainly the N-methyl-*D*-aspartate (NMDA) type of glutamate receptor [18, 19]. In humans, the concentrations of glutamate in plasma have been found to be significantly higher in patients with large cerebral infarcts, and in those with a higher risk of early neurological deterioration, supporting the excitotoxic activity of glutamate in patients with cerebral infarction [19, 20]. Furthermore, although glutamate release is very rapid after the insult in stable infarcts, its release may last longer as it has been demonstrated in progressing infarcts [21].

There are few references in the literature associating glutamate and ischemic tolerance. In this context, it has been demonstrated that glutamate receptor subtypes are differentially expressed after ischemic tolerance with suppression of mGluR1b and 5 [22] or GluR2 genes expression [23]. Glutamate release is also affected as it is inhibited in rat brain cortical slices preconditioned by exposure to hypoxia/hypoglycemia [24], as well as in murine cortical neurons preconditioned with KCl [25].

We have recently studied antiexcitotoxicity mechanisms in an experimental model of ischemic tolerance by using a primary culture of rat neurons or astrocytes exposed to an experimental ischemia (oxygen-glucose deprivation (OGD)) [26]. Our data have confirmed that IPC produced by sublethal OGD causes ischemic tolerance to subsequent lethal OGD exposures, as previously reported [27–29]. We have also shown that OGD-induced increase in extracellular glutamate is lower in IPC-exposed cultures, in agreement with previous reports in other settings [24, 25]. And, more interestingly, we have demonstrated that glutamate uptake is increased in cells exposed to IPC [26], suggesting the involvement of glutamate transporters in the phenomenon of tolerance.

Glutamate transport is the primary and only mechanism for maintaining extracellular glutamate concentrations below excitotoxic levels [30, 31]. To date, five high-affinity, sodium-dependent glutamate transporters have been cloned from mammalian tissue [32]: EAAT1/GLAST and EAAT2/GLT-1 are localized primarily in astrocytes (it has been shown recently that a splicing variant of EAAT2, EAAT2b, is found also in neurons), EAAT3/EAAC1 and EAAT4 are distributed in neuronal membranes, and EAAT5 is retinal. When we evaluated the involvement of transporter regulation in ischemic tolerance, our data showed that IPC increases the expression of the glutamate transporters EAAT2 and EAAT3, an effect that mediates ischemic tolerance by decreasing excitotoxicity due to OGD-induced increase in extracellular

glutamate in rat cortical cultures [26]. These new data point to glutamate transporters as a new target for cytoprotection.

Clinical Relevance of Glutamate Transporters (EAAT2) as New Targets of Cytoprotection

As mentioned before, the inhibition of glutamate actions has demonstrated to be a very powerful strategy to decrease brain damage after experimental ischemia and, indeed, the larger part of efforts to reduce ischemia-induced brain injury has primarily focused on attenuating excitotoxicity with several neuroprotective drugs which block glutamate receptors or inhibit glutamate release induced by brain ischemia [33]. However, the notion of the glutamate transporters as a new target of neuroprotection has not been explored. Among the five high-affinity, sodium-dependent glutamate transporters, the astroglial transporter EAAT2 (GLT-1) [34, 35], also found in some neurons, is responsible for up to 90% of all glutamate transport in adult tissue [reviewed in 36]. Reductions of EAAT2 protein expression have been suggested to take place in ischemia [37]. Furthermore, several studies have shown that EAAT2 deletion using either antisense or gene deletion strategies is related to larger increases in extracellular glutamate, neuronal damage and brain edema after experimental brain ischemia [38–41]. Therefore, an increased function and/or expression of this transporter would be expected to play a protective role in pathologies in which extracellular glutamate levels lead to neuronal damage, such as stroke. As it will be discussed below, some neuroprotective drugs may be acting via this mechanism.

Interestingly, we have recently described a novel and highly prevalent polymorphism in the promoter of the EAAT2 glutamate transporter [42, 43]. This polymorphism is not associated with increased risk for stroke because its prevalence is comparable in stroke patients and in healthy subjects. However, this polymorphism is associated with higher and maintained plasma glutamate concentrations as well as with higher frequency of neurological deterioration in patients with acute hemispheric stroke. Transfection experiments in rat astrocytes show that the mutant EAAT2 promoter has a 30% reduction in activity when compared with the wild-type, in agreement with greater glutamate concentrations found in patients with the mutant genotype. Moreover, our data show that the polymorphism abolishes an AP2 consensus sequence and creates a new sequence that corresponds to the consensus binding site for the transcription factor GCF2. Whereas AP2 is a transcriptional activator [44], GCF2 is a transcriptional repressor that decreases activity of several genes [45]. This finding further explains the differences in the glutamate concentrations and clinical evolution in patients with the polymorphism. We have also shown that GCF2 is remarkably induced after an ischemic insult, suggesting that newly created consensus binding site for GCF2 is active, thus leading to an active repression of EAAT2 expression [46].

This new polymorphism supports the hypothesis that, in patients with acute ischemic stroke, the magnitude of the excitotoxic damage could be genetically determined due to a decrease in the glutamate uptake. Plasma and CSF glutamate levels are threefold higher in patients with subsequent early neurological deterioration than in those with stable or improving stroke [19, 20]. In addition, CSF glutamate levels on admission have been found to remain elevated up to 24 h in patients who develop progressing stroke in the following 48 h, whereas they drop to the normal range within the first 6 h after onset of the ischemia in patients without later neurological worsening [21]. Thus, it remains to be explored whether progressing stroke is due to the presence of this polymorphism.

In conclusion, this study has revealed a novel functional polymorphism in the EAAT2 promoter region and a pattern of regulation that decreases promoter activity. This polymorphism is associated with higher plasma glutamate levels and a clinically relevant trend towards neurologic worsening after stroke. These findings may explain the reported failure of glutamate antagonists in human stroke, and prompt the use of pharmacogenetics in future clinical trials with drugs blocking or modifying the excitotoxic pathway.

A New Neuroprotective Drug Acting on EAAT2 Expression or Functionality: Citicoline

Cytidine-5′-diphosphocholine (citicoline or CDP-choline) is a compound normally present in all cells throughout the body and an intermediate in the biosynthesis of phosphatidylcholine (PtdCho). It has been shown that citicoline produces neuroprotective effects in a variety of CNS injury models including cerebral ischemia. At the experimental level, it has been reported to decrease infarct volume and edema, and/or to improve neurological deficits, either alone or in combination with other agents [47–55]. In humans, citicoline is the only neuroprotectant that has shown positive trends in all randomized, double-

blind trials and has demonstrated efficacy in a meta-analysis with an overall safety similar to placebo [15].

The effects proposed to explain the neuroprotective actions of citicoline have been thoroughly reviewed [56–60], but its precise mechanism is not well defined.

We have demonstrated a neuroprotective effect of citicoline in experimental brain ischemia, as indicated by a reduction in infarct volume and/or neurological deficits after middle cerebral artery occlusion (MCAO) [61]. Citicoline, in our study, decreases extracellular glutamate accumulation after ischemia by a dual mechanism involving both a decreased neuronal glutamate efflux and an increased astrocytic glutamate uptake.

Using in vivo and in vitro models of ischemia, we have shown that citicoline is able to recover the ischemic-induced fall on neuronal ATP levels and this effect is responsible, at least in part, for the decrease in extracellular glutamate and the subsequent neuroprotection after ischemia, very likely due to a reduced reversal of the neuronal transporters. Several mechanisms might explain the effect of citicoline on ATP levels both in control and ischemia, either in vivo or in vitro. In this context, it has been demonstrated that citicoline prevents the loss of cardiolipin which is an exclusive inner mitochondrial phospholipid and it is essential for mitochondrial electron transport [62]. Furthermore, citicoline restores Na^+,K^+-ATPase activity in vivo [63] and has a direct stimulatory effect in vitro [64]. Although the inhibition of phospholipase A_2 has been recently shown [59], the precise mechanism by which citicoline produces these effects is not known, and further studies are required to clarify this point.

Interestingly, our results also show that citicoline causes an increase in glutamate uptake in astrocytes, which was not observed in neurons. Furthermore, citicoline increased remarkably EAAT2 plasma membrane expression. Citicoline induces the translocation of this transporter from the cytosol to the membrane, where it is functional and helps to decrease extracellular glutamate concentrations. Previously reported actions of citicoline on cell membrane [56–60] might play a role in its effect on astrocytic membrane.

In summary, our results show that citicoline exhibits a remarkable and specific protection which occurs concomitantly with an inhibition of ischemia-induced neuronal glutamate release and an increase in astrocytic glutamate uptake by increasing EAAT2 translocation to the membrane.

Conclusion

Preclinical studies have demonstrated that numerous drugs are effective for treating stroke in animals; however, most of them have failed in clinical trials. Therefore, the study of the discrepancies between preclinical studies and clinical trials to form a basis for future trials together with the search of new targets for neuroprotection will help us to find a better neuroprotective drug.

Elucidation of mechanisms that regulate the acquisition of brain tolerance could guide efforts to develop effective and safe pharmacological agents to protect the brain or reduce ischemic injury. In this sense, we have demonstrated that IPC increases the expression of the glutamate transporters EAAT2 and EAAT3, an effect that mediates ischemic tolerance by decreasing excitotoxicity.

We have also revealed a novel functional polymorphism in the EAAT2 promoter region, which is associated with higher plasma glutamate levels and a clinically relevant trend towards neurologic worsening after stroke. These findings reinforce the clinical relevance of EAAT2 as a new target for neuroprotection and may re-open the research on glutamate antagonists for acute stroke treatment by designing new trials in those patients affected by the polymorphism.

Finally, we have demonstrated that citicoline exhibits a remarkable and specific protection which occurs concomitantly with an inhibition of ischemia-induced neuronal glutamate release and an increase in astrocytic glutamate uptake by increasing EAAT2 translocation to the membrane. These results may possess important therapeutic implications in the management of patients at risk of ischemic events and open a new line of investigation to search for compounds able to increase either the function or the expression of glutamate transporters.

References

1 Arundine M, Tymianski M: Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. Cell Calcium 2003;34:325–337.
2 Feuerstein GZ, Wang X, Barone FC: Inflammatory gene expression in cerebral ischemia and trauma. Potential new therapeutic targets. Ann NY Acad Sci 1997;825:179–193.
3 Dirnagl U, Iadecola C, Moskowitz MA: Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391–397.
4 Del Zoppo G, Ginis I, Hallenbeck JM, Iadecola C, Wang X, Feuerstein GZ: Inflammation and stroke: putative role for cytokines, adhesion molecules and iNOS in brain response to ischemia. Brain Pathol 2000;10:95–112.
5 Labiche LA, Grotta JC: Pharmacologic modification of acute cerebral ischemia, in Mohr JP, Choi DW, Grotta JC, Weir B, Wolf PA (eds): Stroke Pathophysiology, Diagnosis and Management, ed 4. Philadelphia, Churchill Livingstone, 2004, pp 1025–1058.
6 Davis S, Albers GW, Diener HC, Lees KR, Norris J: Termination of acute stroke studies involving selfotel treatment: ASSIST Steering Committee. Lancet 1997;349:32.
7 Lees KR, Asplund K, Carolei A, Davis SM, Diener HC, Kaste M, Orgogozo JM, Whitehead J: Glycine antagonist (gavestinel) in neuroprotection (GAIN International) in patients with acute stroke: a randomized trial. Lancet 2000;355:1949–1954.
8 Albers GW, Goldstein LB, Hall D, Lesko LM, Aptiganel Acute Stroke Investigators: Aptiganel hydrochloride in acute ischemic stroke: a randomized controlled trial. JAMA 2001;286:2673–2682.
9 Sacco RL, DeRosa JT, Haley EC Jr, Levin B, Ordronneau P, Phillips SJ, Rundek T, Snipes RG, Thompson JL, Glycine Antagonist in Neuroprotection Americas Investigators: Glycine antagonist in neuroprotection for patients with acute stroke. GAIN Americas: a randomized controlled trial. JAMA 2001;285:1719–1728.
10 IMAGES Study Investigators: Magnesium for acute stroke (intravenous magnesium efficacy in stroke trial): randomised controlled trial. Lancet 2004;363:439–445.
11 Van Hoogdalem E, de Vos R, Hefting NR, Napoliello MJ: Clinical experience with YM872, a novel AMPA receptor antagonist for acute ischemic stroke. Stroke 1999;30:264.
12 Elting JW, Sulter GA, Kaste M, Lees KR, Diener HC, Hommel M, Versavel M, Teelken AW, De Keyser J: AMPA antagonist ZK200775 in patients with acute ischemic stroke: possible glial cell toxicity detected by monitoring of S-100B serum levels. Stroke 2002;33:2813–2818.
13 Kew JNC, Kemp JA: Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology 2005;179:4–29.
14 Bozic ME, Hommel M, Grotta J, Fisher M, Fayad P, Bogousslavsky J: POST-011: Efficacy and safety of MaxiPost in patients with acute stroke. Cerebrovasc Dis 2001;11:127.
15 Dávalos A, Castillo J, Alvarez-Sabín J, Secades JJ, Mercadal J, Lopez S, Cobo E, Warach S, Sherman D, Clark WM, Lozano R: Oral citicoline in acute ischemic stroke. An individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.
16 Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K: Ischemic tolerance phenomenon found in the brain. Brain Res 1990;528:21–24.
17 Dirnagl U, Simon RP, Hallenbeck JM: Ischemic tolerance and endogenous neuroprotection. Trends Neurosci 2003;26:248–254.
18 Choi DW, Rothman SM: The role of glutamate neurotoxicity in hypoxic-ischaemic neuronal death. Annu Rev Neurosci 1990;13:171–182.
19 Castillo J, Dávalos A, Naveiro J, Noya M: Neuroexcitatory amino acids and their relationship to infarct size and neurological deficit in ischemic stroke. Stroke 1996;27:1060–1065.
20 Castillo J, Dávalos A, Noya M: Progression of ischaemic stroke and excitotoxic amino acids. Lancet 1997;349:79–83.
21 Dávalos A, Castillo J, Serena J, Noya M: Duration of glutamate release after acute ischemic stroke. Stroke 1997;28:708–710.
22 Sommer C, Roth SU, Kuhn R, Kiessling M: Metabotropic glutamate receptor subtypes are differentially expressed after transient cerebral ischemia without, during and after tolerance induction in the gerbil hippocampus. Brain Res 2000;872:172–180.
23 Tanaka H, Calderone A, Jover T, Grooms SY, Yokota H, Zukin RS, Bennett MV: Ischemic preconditioning acts upstream of GluR2 downregulation to afford neuroprotection in the hippocampal CA1. Proc Natl Acad Sci USA 2002;99:2362–2367.
24 Johns L, Sinclair AJ, Davies JA: Hypoxia/hypoglycemia-induced amino acid release is decreased in vitro by preconditioning. Biochem Biophys Res Commun 2000;276:134–136.
25 Grabb MC, Lobner D, Turetsky M, Choi DW: Preconditioned resistance to oxygen-glucose deprivation-induced cortical neuronal death: alterations in vesicular GABA and glutamate release. Neuroscience 2002;115:173–183.
26 Romera C, Hurtado O, Botella SH, Lizasoain I, Cárdenas A, Fernández-Tomé P, Leza JC, Lorenzo P, Moro MA: In vitro ischaemic tolerance involves up-regulation of glutamate transport partly mediated by the TACE/ADAM17-TNF-α pathway. J Neurosci 2004;24:1350–1357.
27 Bruer U, Weih MK, Isaev NK, Meisel A, Ruscher K, Bergk A, Trendelenburg G, Wiegand F, Victorov IV, Dirnagl U: Induction of tolerance in rat cortical neurones: hypoxic preconditioning. FEBS Lett 1997;414:117–121.
28 Grabb MC, Choi DW: Ischemic tolerance in murine cortical cell culture: critical role for NMDA receptors. J Neurosci 1999;19:1657–1662.
29 Liu J, Ginis I, Spatz M, Hallenbeck JM: Hypoxic preconditioning protects cultured neurons against hypoxic stress via TNF-α and ceramide. Am J Physiol 2000;278:C144–C153.
30 Takahashi M, Billups B, Rossi D, Sarantis M, Hamann M, Attwell D: The role of glutamate transporters in glutamate homeostasis in the brain. J Exp Biol 1997;200:401–409.
31 Maragakis NJ, Rothstein JD: Glutamate transporters in neurologic diseases. Arch Neurol 2001;58:365–370.
32 Seal RP, Amara SG: Excitatory amino acid transporters: a family in flux. Annu Rev Pharmacol Toxicol 1999;39:431–456.
33 Lees KR: Neuroprotection. Br Med Bull 2000;56:401–412.
34 Pines G, Danbolt NC, Bjoras M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E, Kanner BI: Cloning and expression of a rat brain *L*-glutamate transporter. Nature 1992;360:464–467.
35 Shadshidharan P, Wittenberg I, Plaitakis A: Molecular cloning of human brain glutamate/aspartate transporter II. Biochim Biophys Acta 1994;1191:393–396.
36 Robinson MB: The family of sodium-dependent glutamate transporters: a focus on the GLT-1/EAAT2 subtype. Neurochem Int 1999;33:479–491.
37 Torp R, Lekieffre D, Levy LM, Haug FM, Danbolt NC, Meldrum BS, Ottersen OP: Reduced postischemic expression of a glial glutamate transporter, GLT1, in the rat hippocampus. Exp Brain Res 1995;103:51–58.
38 Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF: Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron 1996;16:675–686.
39 Rao VL, Dogan A, Todd KG, Bowen KK, Kim BT, Rothstein JD, Dempsey RJ: Antisense knockdown of the glial glutamate transporter GLT-1, but not the neuronal glutamate transporter EAAC1, exacerbates focal cerebral ischemia-induced neuronal damage in rat brain. J Neurosci 2001;21:1876–1883.
40 Hamann M, Rossi DJ, Marie H, Attwell D: Knocking out the glial glutamate transporter GLT-1 reduces glutamate uptake but does not affect hippocampal glutamate dynamics in early simulated ischemia. Eur J Neurosci 2002;15:308–314.
41 Namura S, Maeno H, Takami S, Jiang XF, Kamichi S, Wada K, Nagata I: Inhibition of glial glutamate transporter GLT-1 augments brain edema after transient focal cerebral ischemia in mice. Neurosci Lett 2002;324:117–120.

42 Mallolas J, Hurtado O, Castellanos M, Blanco M, Sobrino T, Serena J, Vivancos J, Castillo J, Lizasoain I, Moro MA, Dávalos A: A polymorphism in the *EAAT2* promoter is associated with higher glutamate concentrations and higher frequency of progressing stroke. J Exp Med 2006; doi:10.1084/jem.20051979.

43 Mallolas J, Hurtado O, Moro MA, Castellanos M, Lizasoain I, Serena J, Silva Y, Leza JC, Castillo J, Dávalos A: Different pattern of regulation of mutant EAAT2 promoter explains higher glutamate concentrations and brain injury in patients with mutant genotype after acute stroke. Cerebrovasc Dis 2005;19:24.

44 Imagawa M, Chiu R, Karin M: Transcription factor AP-2 mediates induction by two different signal-transduction pathways: protein kinase C and cAMP. Cell 1987;51:251–260.

45 Reed AL, Yamazaki H, Kaufman JD, Rubinstein Y, Murphy B, Johnson AC: Molecular cloning and characterization of a transcription regulator with homology to GC-binding factor. J Biol Chem 1998;273:21594–21602.

46 Mallolas J, Hurtado O, Castellanos M, Serena J, Castillo J, Silva Y, Lorenzo P, Lizasoain I, Dávalos A, Moro MA: GCF2 transcription factor is up-regulated in brain during ischemia. Cerebrovasc Dis 2005;19:11.

47 Kakihana M, Fukuda N, Suno M, Nagaoka A: Effects of CDP-choline on neurologic deficits and cerebral glucose metabolism in a rat model of cerebral ischemia. Stroke 1988;19:217–222.

48 Schabitz WR, Weber J, Takano K, Sandage BW, Locke KW, Fisher M: The effects of prolonged treatment with citicoline in temporary experimental focal ischemia. J Neurol Sci 1996;138:21–25.

49 Schabitz WR, Li F, Irie K, Sandage BW Jr, Locke KW, Fisher M: Synergistic effects of a combination of low-dose basic fibroblast growth factor and citicoline after temporary experimental focal ischemia. Stroke 1999;30:427–431.

50 Aronowski J, Strong R, Grotta JC: Citicoline for treatment of experimental focal ischemia: histologic and behavioral outcome. Neurol Res 1996;18:570–574.

51 Onal MZ, Li F, Tatlisumak T, Locke KW, Sandage BW Jr, Fisher M: Synergistic effects of citicoline and MK-801 in temporary experimental focal ischemia in rats. Stroke 1997;28:1060–1065.

52 Andersen M, Overgaard K, Meden P, Boysen G, Choi SC: Effects of citicoline with thrombolytic therapy in a rat embolic stroke model. Stroke 1999;30:1464–1471.

53 Shuaib A, Yang Y, Li Q: Evaluating the efficacy of citicoline in embolic ischemic stroke in rats: neuroprotective effects when used alone or in combination with urokinase. Exp Neurol 2000;161:733–739.

54 Grieb P, Gadamski R, Wojda R, Janisz M: CDP-choline, but not cytidine, protects hippocampal CA1 neurones in the gerbil following transient forebrain ischemia. Folia Neuropathol 2001;39:141–145.

55 Alkan T, Kahveci N, Goren B, Korfali E, Ozluk K: Ischemic brain injury caused by interrupted versus uninterrupted occlusion in hypotensive rats with subarachnoid hemorrhage: neuroprotective effects of citicoline. Arch Physiol Biochem 2001;109:161–167.

56 D'Orlando KJ, Sandage BW Jr: Citicoline (CDP-choline): mechanisms of action and effects in ischemic brain injury. Neurol Res 1995;17:281–284.

57 Adibhatla RM, Hatcher JF, Dempsey RJ: Effects of citicoline on phospholipid and glutathione levels in transient cerebral ischemia. Stroke 2001;32:2376–2381.

58 Adibhatla RM, Hatcher JF, Dempsey RJ: Citicoline: neuroprotective mechanisms in cerebral ischemia. J Neurochem 2002;80:12–23.

59 Adibhatla RM, Hatcher JF: Citicoline decreases phospholipase A_2 stimulation and hydroxyl radical generation in transient cerebral ischemia. J Neurosci Res 2003;73:308–315.

60 Secades JJ: CDP-choline: update and review of its pharmacology and clinical use. Methods Find Exp Clin Pharmacol 2002;24:1–53.

61 Hurtado O, Moro MA, Cárdenas A, Sánchez V, Fernández-Tomé P, Leza JC, Lorenzo P, Secades JJ, Lozano R, Dávalos A, Castillo J, Lizasoain I: Neuroprotection afforded by prior citicoline administration in experimental brain ischemia: effects on glutamate transport. Neurobiol Dis 2005;18:336–345.

62 Hoch FL: Cardiolipins and biomembrane function. Biochim Biophys Acta 1992;1113:71–133.

63 Rigoulet M, Guerin B, Cohadon F, Vandendreissche M: Unilateral brain injury in the rabbit: reversible and irreversible damage of the membranal ATPases. J Neurochem 1979;32:535–541.

64 Plataras C, Tsakiris S, Angelogianni P: Effect of CDP-choline on brain acetylcholinesterase and Na^+,K^+-ATPase in adult rats. Clin Biochem 2000;33:351–357.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):9–20
DOI: 10.1159/000091699

Published online: May 2, 2006

Apoptosis: Future Targets for Neuroprotective Strategies

Isidro Ferrer

Institut de Neuropatologia, Servei Anatomia Patològica, IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona, Hospitalet de Llobregat, Barcelona, Spain

Key Words
Apoptosis · Necrosis · Stroke · Focal ischemia · Neuroprotection

Abstract
Focal permanent or transient cerebral artery occlusion produces massive cell death in the central core of the infarction, whereas in the peripheral zone (penumbra) nerve cells are subjected to various determining survival and death signals. Cell death in the core of the infarction and in the adult brain is usually considered a passive phenomenon, although events largely depend on the partial or complete disruption of crucial metabolic pathways. Cell death in the penumbra is currently considered an active process largely dependent on the activation of cell death programs leading to apoptosis. Yet cell death in the penumbra includes apoptosis, necrosis, intermediate and other forms of cell death. A rather simplistic view implies poor prospects regarding cell survival in the core of the infarction and therapeutic expectations in the control of cell death and cell survival in the penumbra. However, the capacity for neuroprotection depends on multiple factors, primarily the use of the appropriate agent, at the appropriate time and during the appropriate interval. Understanding the mechanisms commanding cell death and survival area is as important as delimiting the therapeutic time window and the facility of a drug to effectively impact on specific targets. Moreover, the detrimental effects of homeostasis and the activation of multiple pathways with opposing signals following ischemic stroke indicate that better outcome probably does not depend on a single compound but on several drugs acting in combination at the optimal time in a particular patient.

Introduction

Neuroprotection in ischemic stroke has been a topic of increasing concern and massive research [1–9]. More than one hundred putative targets and compounds have been assayed in vitro and in vivo with variable results. A large number of agents have proved to be neuroprotective in animal models subjected to transient or focal cerebral ischemia, usually produced by middle cerebral artery occlusion, or subjected to global cerebral ischemia. Yet most of the same compounds have failed in human stroke trials [8]. Several reasons may account for these discrepancies. One of them is that experimental animal models are designed so that the same insult is applied to the cohort which is maintained under the same basal conditions to minimize individual variations and to optimize reproducibility. Certainly, this is not the real scenario in human stroke, in which individual variations and differences in the characteristics of the insult are the rule. Another important fact is that unique observations in one

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0009$23.50/0

Accessible online at:
www.karger.com/ced

Prof. I. Ferrer
Institut de Neuropatologia, Servei Anatomia Patològica
IDIBELL-Hospital Universitari de Bellvitge, Universitat de Barcelona
carrer Feixa Llarga sn, ES–08907 Barcelona (Spain)
Tel. +34 93 403 5808, E-Mail 8082ifa@comb.es

species are not necessarily valid in other species, including humans, and that the beneficial effects when the agent is administered alone are not applicable when the agent is given in combination with other products [10]. Finally, it is worth stressing that the administration of certain elements is limited to very reduced therapeutic windows in which the agent is active; no benefit can be expected when the subject affected by a stroke is treated with even a very powerful agent at the wrong time. Together, these comments will serve to remind us that neuroprotection in human stroke has to be based on the combination of precise knowledge of the chronology of pathogenic and pathological events with the application of fuzzy logic related with individual variations.

Guidelines for the early management of patients with ischemic stroke have been published by the Stroke Council of the American Stroke Association and the European Stroke Initiative [11, 12]. Early diagnosis and treatment is a cardinal factor in patient outcome. Stroke units have been proved to reduce stroke mortality, clinical complications and neurological deficits. Rapid re-canalization is the primary focus during the first hours of ischemic stroke. Other measures include monitoring of the neurological status, adequate hydration and treatment of low blood pressure, prevention of venous thrombosis, treatment of hyperthermia, maintenance of normoglycemia, monitoring heart rhythm, removal of Foley catheter as soon as possible, treatment of pulmonary and urinary infections, and skin care to avoid decubiti [13]. At present, thrombolysis with tissue plasminogen activator or related compounds within 3 h of the onset is the most specific therapy effective in reducing mortality and disabilities associated with stroke [14, 15]. Immediate antithrombotic therapy is no longer recommended for most patients because of possible hemorrhagic complications [16, 17].

Since metabolic function is lowered with hypothermia, reducing body temperature has been suggested as a putative treatment in cerebral ischemia [18]. This has indeed been proven to be useful, although prevention of side effects such as hypotension, cardiac arrhythmia and pneumonia, in combination with precise control of timing, degree and duration of mild to moderate hypothermia, are needed to optimize the efficacy of this procedure [19, 20].

This chapter is focused on key events during early stages of ischemic stroke, and on pathogenic roles of distinctive molecules and metabolic pathways that compromise cell survival and affect infarct progression. This information is needed to procure a rationale for neuroprotective measures in stroke patients. In this context, the present review is not a compendium but rather an approach to some salient aspects of neuroprotection in ischemic stroke. Major information is focused on focal cerebral ischemia, although comments on global ischemia are also introduced in some relevant aspects.

Focal Cerebral Ischemia

Focal permanent or transient cerebral artery occlusion produces massive cell death in the central core of the infarction. These effects depend on several factors including duration of the occlusion, extent of the reperfusion and basal metabolic status, among others. Necrosis is characterized by rapid energy and metabolic failure, consisting of reduced ATP, impairment of membrane ionic pumps with massive Ca^{2+}, Na^{+} and water influx, undermining mitochondrial ATP-sensitive potassium channels, followed by membrane disruption and cell death. Proteases, calpains, endonucleases and other enzymes disrupt protein and nucleic acid assemblies. Enhanced poly(ADP-ribose)polymerase-1 (PARP-1) and poly(ADP-ribosyl)ation after focal ischemia promote cell death by NAD^{+} and ATP depletion, whereas PARP gene disruption and drug-dependent PARP inhibition generate resistance to cerebral ischemia [21, 22].

Necrosis is accompanied by glutamate release and additional excitotoxic cell damage, and it is followed by activation of various responses in neighboring cells, such as activation of phospholipases, cyclooxygenase-2 (COX-2), STATs, lipolysis, oxidative stress and increased nitric oxide (NO) production. Glial cells, and leukocytes and monocytes, are sources of inflammatory cytokines and chemokines which, in turn, activate complex metabolic pathways with variable, often opposing effects depending on the basal metabolic state, resulting in cell death or in favoring scavenging and reparative responses. These findings point to the likelihood that necrosis is a more complex phenomenon than conventionally sanctioned.

Although the infarct core has produced little therapeutic expectation, several studies have been focused on very early events following ischemia. Targeting mitochondrial ATP-sensitive potassium channels is being considered as a novel approach to neuroprotection [23]. Drugs developed to selectively inhibit PARP-1 while preventing the function of PARP-2 look promising as putative neuroprotectors following ischemia [24]. Last, but not least, the study of inflammatory mediators has permitted the delineation of possible therapies in central nervous system ischemia [25, 26].

The Area around the Core of the Infarction: The Penumbra

The penumbra is defined as the rim of tissue that is hypoperfused around the ischemic core in which the blood flow is too low to maintain electrical activity but is sufficient to preserve ion channels. In addition to low perfusion levels, cells in the penumbra are subject to deleterious factors produced by neighboring cells, including excitotoxicity, spreading depression propagating through the penumbral tissue that can induce additional energy demand contributing to cellular energy failure, oxidative stress, NO overproduction, inflammatory cytokines, and adhesion molecule and metalloproteinase production, all of which facilitate the penetration of leukocytes. These chemical environmental signals act on membrane receptors and then activate intracellular signaling pathways inducing cellular responses. For instance, it is well recognized that many cytokines trigger a cascade of events that can drive cells to death. Furthermore, direct cell-matrix and cell-cell interactions, which are nowadays also recognized as remarkable communicating networks in brain tissue, might also contribute to the propagation of signals from the core to the neighboring penumbra.

Early responses to cellular ischemia in the vicinity of the ischemic core are reduced protein synthesis, and hsp70 mRNA induction and HSP70 heat shock protein expression [27]. This represents a protective response, as mice overexpressing HSP-70 are protected against cerebral infarction [28]. Together, these observations have permitted consideration of heat shock proteins in neuroprotection [29].

Other early responses include c-fos mRNA induction and c-Fos protein expression, the effects of which in the damaged tissue are not clearly understood.

It is generally believed that cells in the penumbra die by an active process named programmed cell death or apoptosis. The term programmed cell death is here applied because it refers to the dependence on protein synthesis and activation of cell death programs. The term apoptosis is used to indicate that some cells have early chromatin condensation and endonuclease activation, manifested as a ladder pattern on agarose gels, which corresponds to internucleosomal nuclear DNA break multiples of 180–200 base pairs. A large number of studies have been focused on apoptosis following focal ischemia [30–34]. In some instances, apoptosis has been considered the prototypical form of cell death in the penumbra, in contrast with necrosis as the paradigm of cell death in the infarct core. Yet the penumbra is a very imprecise compartment in which cells may suffer necrosis or apoptosis, as well as intermediate and other forms of cell death [30, 33]. In a different setting, apoptosis is the predominant form of cell death in most regions in the hypoxic developing nervous system, including those common lesions described as ischemic pontosubicular necrosis.

More importantly, the penumbra is a compartment in which cell outcome will depend on multiple factors that take place during a relatively dilated period of time after the ischemic insult. This aspect is crucial to operating with a selected battery of therapeutic agents to reduce infarct progression.

Programmed cell death may be initiated by external signals, by the activation of the mitochondrial pathway or by endoplasmic reticulum stress. The external pathway is mediated by the Fas receptor, a surface receptor that belongs to the tumor necrosis factor family, which binds to the Fas ligand (Fas-L). The Fas/Fas-L signaling system activates Fas-associated death domain (FADD) and caspase 8 (cleavage of the inactive zymogen pro-caspase-8 to active caspase-8), which in turn activates caspase-3 (cleavage of pro-caspase-3 to the active 17 kDa product). The mitochondrial pathway is initiated by Bax translocation to the mitochondria membrane and competition with other members of the Bcl-2 family. This is followed by cytochrome c leakage to the cytosol, the binding of cytochrome c to Apaf-1, dATP and pro-caspase-9, comprising the apoptosome, and subsequent activation of caspase-9. Active caspase-9 cleaves caspase-3 and other active caspases.

External stimuli also activate the mitochondrial pathway, as caspase-8 may impact on cytochrome c release through cleavage of pro-apoptotic Bid, another member of the Bcl-2 family.

The inhibitor of apoptosis (IAP) family includes X-linked IAP (XIAP), which blocks the apoptosome machinery and inhibits apoptosis. However, XIAP is regulated by Smac/DIABLO (second mitochondrial activator of caspases/direct IAP-binding protein with low pI) released from mitochondria during apoptosis, thus preventing IAP inhibition of caspases. Interestingly, Smac/DIABLO is inhibited by Bcl-2 and Bcl-xL, thus preventing inactivation of XIAP.

All these pathways are activated in the penumbra following cerebral ischemia [33, 35–39]. Although most studies have been carried out in animal models, a similar scenario seems to occur in human stroke [40] (fig. 1).

Caspase inhibitors may block apoptosis in experimental models. However, this process is transient and cell

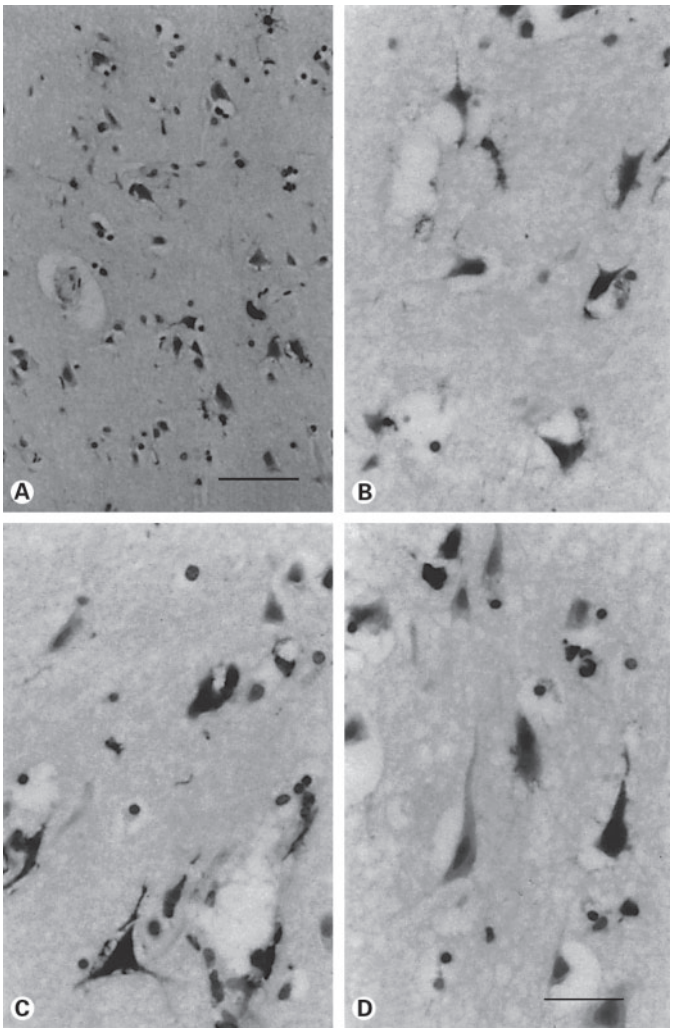


Fig. 1. Border region of the infarct at 48 h after human ischemic stroke. **A**, **B** Method of in situ end-labeling of nuclear DNA fragmentation (TUNEL) showing positive cells without apoptotic morphology. **C**, **D** Active, cleaved caspase-3 (17 kDa) immunohistochemistry showing positive cells mingled with negative neurons. Paraffin sections slightly counterstained with hematoxylin. Bars: **A** = 50 μm, **B–D** (bar in **D**) = 25 μm.

death progresses later. Therefore, direct inhibition of caspases is not a primary target for neuroprotection, as cells at this stage are committed to die. More exciting prospects appear following manipulation of several upstream signals. Overproduction of Bcl-2 in transgenic mice is associated with reduction of the infarct size following focal cerebral ischemia [41]. Human Bcl-2 overexpression with herpes simplex virus vectors limits neuronal death in focal cerebral ischemia [42]. Further evidence in vitro and in vivo has extended the properties of members of the Bcl-2 family as very powerful anti-apoptotic agents and feasible protectors of mitochondria and apoptosis [43–45].

Finally, Bad is a pro-apoptotic member of the Bcl-2 family which is normally phosphorylated and bound to the protein 14-3-3. Phosphorylation, and then inhibition of Bad, depends on several signals, including Ras/ERK, PI3K/Akt and PKA. Following apoptotic stimuli, Bad is dephosphorylated, and it translocates to the outer mitochondrial membrane, dimerizes with Bcl-xL and promotes cytochrome c release [46–48]. In contrast, Akt activity is associated with cell survival following ischemia, thus appearing as a potential target for neuroprotective drugs [49].

In addition to cytochrome c and Smac/DIABLO, which are key factors in the caspase-dependent pathway of apoptosis, apoptosis inducing factor (AIF) is also extruded from the mitochondria to the cytosol after apoptotic stimuli. AIF translocates to the nucleus where it determines peripheral chromatin condensation and large DNA strands. The mitochondrial caspase-independent pathway of apoptosis is also activated following cerebral ischemia [33, 36, 50].

Glutamate Release and Cellular Damage in Ischemia. Calcium Influx

Neuronal ischemia is followed by rapid calcium-dependent and calcium-independent release of glutamate, extracellular glutamate accumulation and excitotoxic cell damage. The efflux of glutamate induced by ischemia is reduced by tetrodotoxin and non-NMDA antagonists, whereas excitotoxic cell damage is mainly mediated by NMDA receptors [51]. Several studies in experimental stroke have demonstrated the benefits of the glutamate blockade in the resolution of brain infarcts following middle cerebral artery occlusion. However, no evidence of significant advantage or harm has been found in several clinical trials using drugs modulating excitatory amino acid action [52]. This is not surprising as results from experimental focal ischemia in rats have demonstrated a therapeutic window of 3–4 h at most [53]. Treatment in humans with drugs geared to reducing glutamate release or to blocking glutamate receptors administered within the first 24 h is far from the ideal therapeutic window.

However, there is no reason for dismay in the face of these negative results. Rather, these observations further support the necessity to optimize the use of specific drugs at the appropriate time. Moreover, studies have shown metabotropic glutamate receptors as possible targets for

neuroprotective drugs. For example, mGlu2/3 receptor agonists inhibit glutamate release and promote the synthesis and release of neurotrophic factors by astrocytes [54]. Moreover, adenosine receptors modulate several neurotransmitter receptors, and adenosine 2A receptors have proved beneficial in experimental stroke, probably counteracting the effects of excitatory amino acids [55].

Excessive elevation of intracellular calcium levels is deleterious for cells. However, calcium/calmodulin (CaM)-dependent protein kinase kinase (CaM-KK), an upstream activator for CaM kinase which is increased in the vicinity of the infarct area, has the capacity to phosphorylate cyclic AMP-responsive element-binding protein (CREB) and Akt, thus preventing apoptosis [56, 57]. Consequently, activation of CaM-KK may be useful in preventing ischemic cell death [57].

Mitogen-Activated Protein Kinases and Phosphorylated Substrates following Ischemia

The family of mitogen-activated protein kinases (MAPKs) is composed of several members, including extracellular signal-regulated kinases (ERKs), stress-activated protein kinases (SAPKs), c-Jun N-terminal kinases (JNKs), and p38 kinases. All these members are activated by phosphorylation by specific upstream kinases which are regulated by trophic factors, membrane signals and stress. MAPKs, in turn, regulate, by phosphorylation at specific sites, several transcription factors including CREB, c-Myc, ATF-2, c-Jun and Elk-1 (fig. 2). In addition to cellular growth and differentiation, MAPKs are involved in cell death and cell survival. Some studies have shown decreased phosphorylation of MAPK/ERK, SAPK/JNK and p38 in the core of the infarction, but increased phosphorylation in the penumbra at 4 h after ischemia. Phosphorylated CREB is markedly reduced in the infarct area as early as 1 h after middle cerebral artery occlusion, whereas c-Myc-P, CREB-P, Elk-1-P, ATF-2-P and c-Jun-P are increased in the penumbra at 4 h after ischemia. Interestingly, SAPK/JNK-P and c-Jun-P (which is phosphorylated at Ser^{63} by SAPK/JNK-P) are markedly increased in the infarct area 1 h after the occlusion. These data suggest that early activation of SAPK/JNK/c-Jun together with decreased CREB-P is associated with cell death, whereas delayed activation of MAPKs and their substrates in the penumbra might be associated with cell death or with cell survival [58].

Previous studies have shown that CREB is associated with survival following ischemia [59]. On the other hand, c-Jun has been associated with cell death, but also with neuronal survival and differentiation, as well as with neuronal regeneration [60]. Recently, a cell-permeable peptide designed to inhibit SAPK/JNK signaling has proven successful following cerebral ischemia [61].

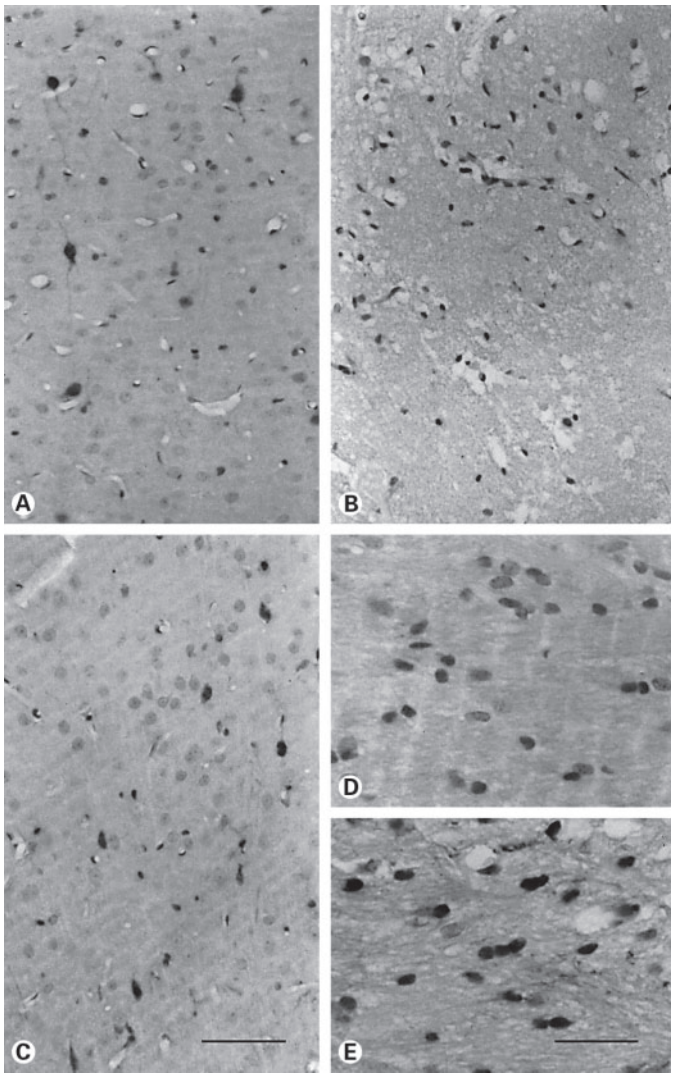


Fig. 2. Phosphorylated mitogen-activated protein kinase (MAPK/ERK-P) immunohistochemistry in human ischemic stroke at 24 h. **A** Cerebral cortex of the contralateral hemisphere; **B** core of the infarction; **C** penumbra area; **D** subcortical white matter of the contralateral hemisphere; **E** subcortical white matter in the vicinity of the infarction. Increased numbers of MAPK/ERK-P-immunoreactive cells are found in the cerebral cortex and subcortical white matter in the vicinity of the infarction. Paraffin sections, slightly counterstained with hematoxylin. Bars: **A–C** (bar in **C**) = 50 μm; **D**, **E** (bar in **E**) = 25 μm.

Oxidative Stress, Nitric Oxide, and Molecular Damage and Cell Death following Ischemia

Mitochondria produce hydrogen peroxide and superoxide anion radicals under normal conditions. These reactive oxygen species (ROS) are eliminated by superoxide dismutases, catalase and glutathione peroxidase. In addition, other antioxidants such as endogenous glutathione and enzymatic antioxidants, and the dietary free-radical scavengers ascorbate and α-tocopherol, are involved in this process. Increased intracellular ROS have deleterious effects on lipids, proteins and nucleic acids that lead to oxidative molecular damage, loss of function and cell death. Cerebral ischemia produces a dramatic increase in mitochondrial ROS which may bring about oxidative damage of lipids, proteins, RNA and DNA, and promote cell death [62, 63].

Several studies with transgenic and knockout mice have shown that overexpression of SOD1 (cytosolic, copper/zinc superoxide dismutase) is associated with a marked reduction in infarct volume following permanent focal ischemia [64, 65]. SOD1 overexpression is also neuroprotective following global ischemia. In contrast, SOD1-deficient mice are more vulnerable to focal and global cerebral ischemia. In the same line, transgenic mice overexpressing SOD2 (mitochondrial, manganese superoxide dismutase) are relatively protected following transient focal ischemia, whereas SOD2-null mice show increased infarct volume after focal cerebral ischemia [66–69]. Finally, ECSOD (extracellular SOD isoform: SOD3) overexpression is associated with increased cell survival, whereas ECSOD ablation is associated with increased cell death following global and focal cerebral ischemia, respectively [70–72].

Genetic manipulation of glutathione peroxidases (GSHPx) has provided stimulating results. Transgenic mice overexpressing human GSHPx-1 have a marked reduction in the volume of the infarct after transient focal ischemia, whereas GSHPx-$1^{-/-}$ mice suffer from increased infarct volume following ischemia and reperfusion [73, 74]. Interestingly, a basal GSHPx threshold is necessary for cell survival following ischemia, as crossed mice with SOD1 over-overproduction and GSPHPx-1 ablation have larger infarcts when compared with wild mice.

Among mitochondrial proteins damaged by ischemia that affect respiratory function and redox homeostasis are pyruvate dehydrogenase (PDH) and NADH-CoQ oxidoreductase (complex I of the electron transport chain). It is feasible that PDH is a target of oxidative damage which results in increased oxidative stress and lactic acid production [75, 76]. Depression of NADH-CoQ oxidoreductase blocks the rate-limiting step of the electron transport chain. In addition, NADH and NADPH are released from the mitochondrial matrix to the cytosol following activation of the mitochondrial membrane permeability transition pore [77]. However, P53 is also susceptible to redox disturbances and may activate the mitochondrial apoptotic pathway [78].

In addition to oxidative damage, cerebral ischemia induces the expression and activation of nitric oxide synthases (NOS) in neurons and glial cells. Generated NO and reactive peroxynitrites may promote cell death via apoptosis by decreasing mitochondria membrane potential, releasing cytochrome c from the mitochondria, activating caspase, and degrading caspase inhibitors [79–83]. Nitration of proteins may result in further cellular damage. Ischemia-induced NO overproduction is, in part, related with a glutamatergic-mediated increase in intracellular calcium resulting in calmodulin-dependent up-regulation of NOS [84].

During cerebral ischemia, the concentrations of free fatty acids, mainly arachidonic and docosahexaenoic acid (DHA), are markedly increased. Oxidation of arachidonic acid may increase thromboxane and prostaglandin levels via the cyclooxygenase pathway. Reaction of NO with superoxide causes the formation of peroxynitrite, lipid peroxidation and toxic lipid peroxidation products as hydroxynonenal that may stimulate apoptosis [85].

Since membrane lipids may be damaged by ischemia citicoline (CDP-choline) has been used as a stimulator of phosphatidylcholine synthesis in several paradigms. Citicoline has been effective in reducing infarct size and cell death following middle cerebral occlusion in the rat [86]. In addition, many other properties of CDP-choline appear to be contributory to the outcome of experimental stroke [87]. Results of clinical trials are unconvincing, and further studies are needed in the selection of patients.

Together, these observations have prompted the development of drugs to reduce oxidative stress and nitration molecular damage, as well as to increase antioxidant activity. These are compounds with SOD-like properties, catalase-like activity, and the capacity to oxidize NO and oxidized nitroxides, or drugs with the ability to eliminate peroxynitrite and peroxynitrite-derived products [84]. An alternative approach has also been proposed, using spin traps to capture ROS. Modern spin trap NXY-059 is particularly promising as it improves ischemic outcome in primates subjected to permanent focal cerebral ischemia [88, 89].

Recent experimental studies have been directed to modulate the mitochondrial membrane permeability transition (MPT) pore. Thus, inhibition of MPT with cyclosporine A, which interacts with cyclophilin D, and antibodies to elements of the MPT pore, act as neuroprotectors against ischemia [90, 91].

In addition to these oxidative target-directed compounds, other substances also have antioxidant properties, including CDP-choline (citicoline), statins and docosahexaenoic acid-derived mediators. Citicoline increases GSH levels and GSSG reductase activity, and attenuates glutathione oxidation ratio in transient forebrain ischemia and other experimental models [92, 93]. Moreover, citicoline reduces oxidative stress in rat cerebral postischemic reperfusion [94]. HMG-CoA reductase inhibitors (statins) up-regulate eNOS, inhibit iNOS and reduce inflammatory cytokine responses. Statins ameliorate ischemic oxidative stress and reduce infarct size in experimental models [95]. Statins offer promising results for the treatment of acute ischemic stroke [96].

Neuroprotectin D1 is a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress [97]. In addition, NPD1 up-regulates BCl-2 and BClxL, down-regulates Bax, and inhibits caspase-3 activation and COX-2 expression.

Early Vascular Factors following Ischemia

Ischemia/reperfusion is accompanied by key events in microcirculation, including disruption of the blood-brain barrier, edema and swelling of perivascular astrocytes, abnormal potassium channel function, abnormal expression of water channel aquaporins, altered expression of proteases and metalloproteinases, and increased inflammatory mediators and inflammation [98, 99]. Statins, angiotensin modulators, erythropoietin, minocycline and thiazolidinediones are putative agents acting on key targets of vascular protection [100].

Endothelium-derived NO is a potent vasodilator and vascular protector produced by eNOS through oxidative conversion of *L*-arginine to *L*-citrulline. Inhibition of eNOS by *L*-NAME decreases cerebral blood flow and increases brain infarct size in animals lacking nNOS. Activation of eNOS by statins, corticosteroids, estrogen and *L*-arginine further supports the use of these compounds as protectors against stroke [101].

However, blood vessels are also vulnerable to brain ischemia. Endothelial cells die by apoptosis, and the mechanisms commanding cell death in endothelial cells are probably similar to those found in neurons. SAPK/JNK and p38 kinase, and apoptosis signal-regulating kinase-1 (ASK-1), are activated in endothelial cells, and activation of these metabolic pathways is associated with activation of caspase-9 and caspase-3 [102–104].

Astrocytes in Brain Ischemia

Neurons and astrocytes may respond differently to ischemia because astrocytes are the principal containers of glycogen in the brain, whereas neurons largely depend on oxygen and have little glycogen [105]. However, there is increasing evidence that astrocytes are also vulnerable to cerebral ischemia [105]. Several factors cause cell death in astrocytes in vitro, including ischemia, acidosis, oxidative stress and cytokines. Experimental models of ischemia in vivo have also shown Bax up-regulation, caspase activation and nuclear condensation. Ischemia-induced astrocytic death is blocked by caspase inhibitors [106]. Further details of this process are obtained from distinct paradigms demonstrating abnormal mitochondrial function of astrocytes in a cell culture model of stroke [107].

In addition, astrocytic swelling is a major event in cerebral ischemia. This circumstance leads to the release of excitatory amino acids via volume-activated anion channels (VRACs). Tamoxifen is a potent inhibitor of these channels, and it is neuroprotective in focal ischemia with a window of 3 h. Although it is not known whether the action of tamoxifen is due solely to VRAC inhibition, these results validate protection of astrocytes as an additional goal in ischemia neuroprotection [108].

Finally, glial cells in the vicinity of the infarct are a source of multiple factors including cytokines of the interleukin family, chemokines, tumor necrosis factor-α, transforming growth factor-β, matrix metalloproteinases and plasminogen activator system, iNOS, heat shock proteins, erythropoietin, antioxidants, and a variety of trophic factors: glial cell line-derived neurotrophic factor, nerve growth factor, brain-derived neurotrophic factor, neurotrophins-3, -4, -5, ciliary neurotrophic factor and fibroblast growth factor. Some of these factors have deleterious effects, but many of them are also beneficial and may contribute to tolerance and neuroprotection [109, 110].

Trophic Factors and Neuroprotection in Stroke

Trophic factors and their receptors are dramatically regulated following cerebral ischemic insults. Increased mRNA and protein expression levels are rapidly observed following stroke and persist for a long period [111]. It seems clear that trophic factors are involved in cellular remodeling and regeneration following brain damage. In addition, there is strong evidence that many trophic factors may play crucial roles at early stages following brain ischemia, and thus may act as putative neuroprotective agents.

Pioneering studies with trophic factors in brain ischemia utilized intraventricular, intracerebral and intravascular routes, or were based on grafts of transfected cell lines. These approaches are difficult when applied in the current clinical practice. Moreover, most trophic factors do not cross the blood-brain barrier, and large doses are accompanied by harmful peripheral side effects. Yet modern methods using targeted trophic factors bound to specific ligands or viral vectors carrying specific trophic factors are promising tools, at least in experimental models, when applied at the appropriate time windows.

As an example, vasoactive intestinal peptide (VIP) is a potent vasodilator in peripheral tissues and when applied directly to brain arteries. However, VIP cannot cross the blood-brain barrier. For this reason, a VIP chimeric analog bound to a murine monoclonal antibody against rat transferrin receptors (OX26-SA) has been infused through the carotid artery in anesthetized rats. Radiolabeled assays have shown good pharmacodynamics and a marked increase in the cerebral blood flow, thus suggesting that targeted VIP may be a useful tool in the treatment of stroke [112].

A similar approximation has been used with human recombinant basic fibroblast growth factor (bFGF) bound to OX26-SA. Intracarotid infusion reduces infarct size following middle cerebral occlusion in the rat. This effect is dose- and time-dependent, as no effects are found if the treatment is delayed beyond 2 h [112]. These observations have renewed engagement on bFGF. It is worth stressing that bFGF had no longer been considered a suitable treatment in human ischemic stroke, in spite of its neuroprotective effects, because of the peripheral side effects related with the high doses required to cross the blood-brain barrier [113, 114].

Brain-derived neurotrophic factor (BDNF) has been proposed as a putative neuroprotective agent because of the increased expression of BDNF and TrkB, its specific receptor, in the rat brain following ischemia. BDNF administration in the cerebrospinal fluid reduced cell death following global ischemia in rats [115], whereas grafting of BDNF-producing fibroblasts diminished ischemic cell death in the hippocampus following transient forebrain ischemia in gerbils [116]. A similar approach with grafted BDNF-transfected fibroblasts protected nerve cells from dying in the area of penumbra in a model of focal cerebral ischemia in rats [117]. In order to obviate the blood-brain barrier, BDNF was conjugated to a blood-brain barrier drug targeting system. This procedure has been demonstrated to be effective in reducing cell death following cerebral ischemia [118, 119]. Interestingly, a recent study has demonstrated that the combination of hypothermia at 33°C and intravenous BDNF infusion reduces striatal glutamate and cell death more than in animals treated with hypothermia or BDNF alone [120].

Transforming growth factor (TGF)-α and epidermal growth factor receptor (EGF-R) co-localize in the majority of neurons and in maturing astrocytes. Since TGF-α is a membrane-anchored protein which may be cleaved, leading to the formation of a soluble form, in addition to distant effects due to the soluble form, local effects of TGF-α may be produced by juxtacrine as well as by autocrine stimulation [121]. TGF-α also has neuroprotective effects following intraventricular administration in ischemic rats [122], and this property is associated with the prevention of ERK phosphorylation [123].

Numerous studies have shown that TGF-β expression is increased in the brain following cerebral ischemia. Moreover, TGF-β is neuroprotective following middle cerebral artery occlusion and after ischemic insults in vitro [124]. This capacity is mediated by MAPK/ERK activation, and it is associated with phosphorylation of Bad at Ser^{112} thus inhibiting Bad and caspase-3 activation [125–127]. In addition, TGF-β induces translocation of nuclear factor-κB (NFκB) transcriptional activity in the presence of apoptotic stimuli, as well as increased phosphorylation of Iκβ kinase with a subsequent degradation of Iκβα. This action through NFκB is necessary for TGF-β neuroprotection [127].

Glial cell line-derived neurotrophic factor (GDNF), a member of the TGF-β superfamily, has a proven capacity to reduce brain damage following ischemia [128–130]. This effect is time-dependent, as no neuroprotective effect is observed if GDNF is given 3 h after transient middle cerebral occlusion [131]. The neuroprotective effect is mediated by GDNF binding to the GFRα-1 receptor [132]. Several mechanisms of neuroprotection have been proposed for GDNF, including reduction of NMDA-in-

duced calcium influx, reduction of iNOS activity and NO production and release, and down-regulation of caspase-dependent apoptotic pathways [132–135].

Bone morphogenic proteins (BMPs) are also members of the TGF-β superfamily, most of them expressed in the nervous system. They bind to specific type I and type II serine-threonine kinase receptors. BMPs are important factors during the development of the nervous system, but the presence of the ligands and receptors in adults gives support to additional functions in the adult brain. In fact, several BMP mRNAs and proteins are up-regulated following cerebral insults. BMP6 and BMP7 administration 24 h prior to middle cerebral artery occlusion results in reduced brain infarcts [136, 137]. However, much more limited benefits are obtained when BMP is administered after ischemia [138].

Vascular endothelial growth factor (VEGF) binds to the receptor kinases VEGFR-1 (Flt-1) and VEGFR-2 (KDR/Flk-1), and promotes angiogenesis, vascular permeability and endothelial proliferation. Several VEGF isoforms are produced as a result of alternative splicing of the VEGF gene. Neuropilin 1 and 2 bind semaphorins and VEGF, suggesting that, in addition to vascular effects, VEGF is implicated in neurogenesis [139]. VEGF is up-regulated in the penumbra of the infarct between 6 and 24 h, and precedes angiogenesis [140, 141]. Intravenous infusion of VEGF within 48 h after the onset of focal ischemia enhances angiogenesis in the penumbra and ameliorates neural recovery [142, 143]. In addition, exogenous administration of VEGF directly administered or overexpressed by gene delivery reduces ischemic brain infarct and decreases cell death [144–148]. Several mechanisms are involved, including modulation of the PI3K/Akt/NFκB signaling pathway, inhibition of caspase-3 activity, and reduction of apoptosis, as well as modulation of potassium channels [149].

Together, these observations concerning neurotrophins and other trophic factors support the concept that they are, with proper decoding of the restraints concomitant to route of administration, dose, timing and schedule, combination of factors and reduction of peripheral side effects, potent neuroprotective agents.

References

1 Hickenbottom SL, Grotta J: Neuroprotective therapy. Semin Neurol 1998;18:485–492.
2 Sun A, Cheng J: Novel targets for therapeutic intervention against ischemic brain injury. Clin Neuropharmacol 1999;2:164–171.
3 Jonas S, Avigari V, Viera D, Waterman P: Neuroprotection against cerebral ischemia. A review of animal studies and correlation with human trial results. Ann NY Acad Sci 1999; 890:2–3.
4 Dávalos A: Neuroprotective agents. Neurologia 1999;14(suppl 4):49–53.
5 Jain KK: Neuroprotection in cerebrovascular disease. Expert Opin Invest Drugs 2000;9: 695–711.
6 Martínez-Vila E, Sierira PI: Current status and perspectives of neuroprotection in ischemic stroke treatment. Cerebrovasc Dis 2001; 11(suppl 1):60–70.
7 Ovbiagele B, Kidwell CS, Starkman S, Saver JL: Neuroprotective agents for the treatment of acute ischemic stroke. Curr Neurol Neurosci Rep 2003;3:9–20.
8 Wahlgren NG, Ahmed N: Neuroprotection in cerebral ischaemia: facts and fancies – the need for new approaches. Cerebrovasc Dis 2004; 17(suppl 1):153–166.
9 Slevin M, Krupinski J, Kumar P, Gaffney J, Kumar S: Gene activation and protein expression following ischemic stroke: strategies towards neuroprotection. J Cell Mol Med 2005; 9:85–102.
10 Hoyte L, Kaur J, Buchan AM: Lost in translation: taking neuroprotection from animal models to clinical trials. Exp Neurol 2004;188: 200–204.
11 Adams HP, Adams RJ, Brott T, et al: Guidelines for the early management of patients with ischaemic stroke. A scientific statement from the Stroke Council of the American Stroke Association. Stroke 2003;34:1056–1083.
12 European Stroke Initiative Executive Committee and Writing Committee: The European Stroke Initiative recommendations for stroke management: update 2003. Cerebrovasc Dis 2003;16:311–318.
13 Broderick JP, Hacke W: Treatment of acute ischemic stroke. II. Neuroprotection and medical management. Circulation 2002;106:1736–1740.
14 Klijn CMJ, Hankey GJ: Management of acute ischaemic stroke: new guidelines from the American Stroke Association and European Stroke Initiative. Lancet Neurol 2003;2:698–701.
15 Toni D, Lorenzano S, Sacchetti ML, Fiorelli M, De Michelle M, Principe M: Specific therapies for ischemic stroke: rTPA and others. Neurol Sci 2005;26:S26–S28.
16 Chamorro A: Heparin in acute ischemic stroke: the case for a new clinical trial. Cerebrovasc Dis 1999;9(suppl 3):16–23.
17 Gubitz G, Counsell C, Sandercock P, Signorini D: Anticoagulants for acute ischemic stroke. Cochrane Database Syst Rev, CD00024.
18 Gunn AJ, Gunn TR: Te pharmacology of neuronal rescue with cerebral hypothermia. Early Hum Dev 1998;53:19–35.
19 Hammer MD, Krieger DW: Hypothermia for acute ischemic stroke: not just another neuroprotectant. Neurologist 2003;9:280–289.
20 Olsen TS, Weber UJ, Kammersgaard LP: Therapeutic hypothermia for acute stroke. Lancet Neurol 2003;2:410–416.
21 Endres M, Wang ZQ, Namura S, Waeber C, Moskowitz MA: Ischemic brain injury is mediated by the activation of poly(ADP-ribose) polymerase. J Cereb Blood Flow Metab 1997; 17:1143–1151.
22 Eliasson MJ, Sampei K, Mandir AS, Hum PD, Traystman RJ, Bao J, et al: Poly(ADP-ribose)polymerase gene disruption renders mice resistant to cerebral ischemia. Nat Med 1997; 3:1089–1095.
23 Busija DW, Lacza Z, Rajapakse N, Shimizu K, Kis B, Bari F, Domoki F, Horiguchi T: Targeting mitochondrial AT-sensitive potassium channels – a novel approach to neuroprotection. Brain Res Rev 2004;46:282–294.
24 Chiarugi A: Poly(ADP-ribosyl)ation and stroke. Pharmacol Res 2005;52:15–24.
25 Barone F, Feuerstein GZ: Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab 1999;19:819–834.

26 Clark WM, Lutsep HL: Potential of anticytokine therapies in central nervous system ischaemia. Expert Opin Biol Ther 2001;1:227–237.
27 Planas AM, Soriano MA, Estrada A, Sanz O, Martin E, Ferrer I: The heat shock stress response after brain lesions: induction of 72 kDa heat shock protein (cell types involved, axonal transport, transcriptional regulation) and protein synthesis inhibition. Prog Neurobiol 1997; 51:607–636.
28 Rajdev S, Hara K, Kokubo Y, Mestrill R, Dillmann W, Weinstein PR, Sharp FR: Mice overexpressing rat heat shock protein 70 are protected against cerebral infarction. Ann Neurol 2000;47:782–791.
29 Yenari MA: Heat shock proteins and neuroprotection. Adv Exp Med Biol 2002;513:281–299.
30 MacManus JP, Buchan AM: Apoptosis after experimental stroke: fact or fashion. J Neurotrauma 2000;17:899–914.
31 Zheng Z, Zhao H, Steinberg GK, Yenari MA: Cellular and molecular events underlying ischemia-induced neuronal apoptosis. Drug News Perspect 2003;16:497–503.
32 Love S: Apoptosis and brain ischemia. Prog Neuropsychopharmacol Biol Psychiatr 2003; 27:267–282.
33 Ferrer I, Planas AM: Signaling of cell death and cell survival following focal cerebral ischemia: life and death struggle in the penumbra. J Neuropathol Exp Neurol 2003;62:329–339.
34 Zhang F, Yin W, Chen J: Apoptosis in cerebral ischemia: executional and regulatory signaling mechanisms. Neurol Res 2004;26:835–845.
35 KrupinskiJ, Lopez E, Martí E, Ferrer I: Expression of caspases and their substrates in the rat model of cerebral ischemia. Neurobiol Dis 2000;7:332–342.
36 Ferrer I, Friguls B, Dalfó E, Justicia C, Planas AM: Caspase-dependent and caspase-independent signaling of apoptosis in the penumbra following middle cerebral artery occlusion in the adult rat. Neuropathol Appl Neurobiol 2003;29:472–481.
37 Chan PH: Mitochondria and neuronal death/survival signaling pathways in cerebral ischemia. Neurochem Res 2004;29:1943–1949.
38 Siegelin MD, Kossatz LS, Winckler J, Rami A: Regulation of XIAP and Smac/DIABLO in the rat hippocampus following transient forebrain ischemia. Neurochem Int 2005;46:41–51.
39 Prunell GF, Arboleda VA, Troy CM: Caspase function in neuronal death: delineation of caspases in ischemia. Curr Drug Targets CNS Neurol Disord 2005;4:51–61.
40 Love S, Barber R, Wilcock GK: Neuronal death in brain infarcts in man. Neuropathol Appl Neurobiol 2000;26:55–66.
41 Martinou JC, Dubois-Dauphin M, Staple JK, Rodriguez F, Francowski H, Missotten M, et al: Over-expression of Bcl-2 in transgenic mice protects neurons from naturally-occurring cell death and experimental ischemia. Neuron 1994;13:1017–1030.
42 Linnik MD, Zahos P, Geschwind MD, Fedoroff HJ: Expression of bcl-2 from a defective herpes simplex virus-1 vector limits neuronal death in focal cerebral ischemia. Stroke 1995; 26:1670–1674.
43 Kowaltowski AJ, Fenton RG, Fiskum G: Bcl-2 family proteins regulate mitochondrial reactive oxygen production and protect against oxidative stress. Free Radic Biol Med 2004;37: 1845–1853.
44 Ouyang YB, Giffard RG: Cellular neuroprotective mechanisms in cerebral ischemia: Bcl-2 family proteins and protection of mitochondrial function. Cell Calcium 2004;36:303–311.
45 Soane L, Fiskum G: Inhibition of mitochondrial neural cell death pathways by protein transduction of bcl-2 family proteins. J Bioenerg Biomembr 2005;37:179–190.
46 Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ: Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not Bcl-xL. Cell 1996;87: 619–628.
47 White BC, Sullivan JM, deGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, et al: Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. J Neurol Sci 2000; 179:1–39.
48 Saito A, Hayashi T, Okuno S, Ferrand-Drake M, Chan PH: Overexpression of copper/zinc superoxide dismutase in transgenic mice protects against neuronal cell death after transient focal ischemia by blocking activation of the bad cell death signaling pathway. J Neurosci 2003;23:1710–1718.
49 Fukunaga K, Kawano T: Akt is a molecular target for signal transduction therapy in brain ischemic insult. J Pharmacol Sci 2003;92:317–327.
50 Zhan RZ, Wu C, Fujihara H, Taga K, Qi S, Naito M, Shimoji K: Both caspase-dependent and caspase-independent pathways may be involved in hippocampal CA1 neuronal death because of loss of cytochrome c from mitochondria in a rat forebrain ischemia model. J Cereb Blood Flow Metab 2001;21:529–540.
51 Nishizawa Y: Glutamate release and neonatal damage in ischemia. Life Sci 2001;69:369–381.
52 Muir KW, Lees KR: Excitatory amino acid antagonists for acute stroke. Cochrane Database Syst Rev 2003; CD001244
53 Kaplan B, Brint S, Tanabe J, Jacewicz M, Wang XJ, Pulsinelli W: Temporal thresholds for neocortical infarction in rats subjected to reversible focal cerebral ischemia. Stroke 1991; 22:1032–1039.
54 Bruno V, Battaglia G, Copani A, D'Onofrio M, Di Iorio P, De Blasi A, Melchiorri D, Flor PJ, Nicoletti F: Metabotropic glutamate receptor subtypes as targets for neuroprotective drugs. J Cereb Blood Flow Metab 2001;21:1013–1033.
55 Ongini E, Adami M, Ferri C, Bertorelli R: Adenosine A2A receptors and neuroprotection. Ann NY Acad Sci 1997;825:30–48.
56 Nagahiro S, Uno M, Sato K, Goto S, Morioka M, Ushio Y: Pathophysiology and treatment of cerebral ischemia. J Med Invest 1998;45:57–70.
57 Yano S, Morioka M, Kuratsu J, Fukunaga K: Functional proteins involved in regulation of intracellular Ca^{2+} for drug development: role of calcium/calmodulin-dependent protein kinases in ischemic neuronal death. J Pharmacol Sci 2005;97:351–354.
58 Ferrer I, Friguls B, Dalfó E, Planas AM: Early modifications in the expression of mitogen-activated protein kinase (MAPK/ERK), stress-activated kinases SAPK/JNK and p38, and their phosphorylated substrates following focal cerebral ischemia. Acta Neuropathol 2003; 105:425–437.
59 Tanaka K: Alteration of second messengers during acute cerebral ischemia: adenylate cyclase, cyclic AMP-dependent protein kinase, and cyclic AMP response element binding protein. Prog Neurobiol 2001;65:173–207.
60 Herdegen T, Skene P, Bähr M: The c-Jun transcription factor bipotential mediator of neuronal death, survival and regeneration. Trends Neurosci 1997;20:227–231.
61 Bonny C, Borsello T, Zine A: Targeting the JNK pathways as a therapeutic protective strategy for nervous system diseases. Rev Neurosci 2005;16:57–67.
62 Love S: Oxidative stress in brain ischemia. Brain Pathol 1999;9:119–131.
63 Fiskum G, Rosenthal RE, Verecki V, Martin E, Hoffman GE, Chinopoulos C, Kowaltowski A: Protection against ischemic brain injury by inhibition of mitochondrial oxidative stress. J Bioenerg Biomembr 2004;36:347–352.
64 Lewen A, Matz P, Chan PH: Free radical pathways in CNS injury. J Neurotrauma 2000;17: 871–890.
65 Sugawara T, Fujimura M, Noshita N, Whan Kim G, Saito A, Hayashi T, Narasimhan P, Maier CM, Chan PH: Neuronal death/survival signaling pathways in cerebral ischemia. NeuroRx 2004;1:17–25.
66 Murakami K, Kondo T, Kawase M, Li Y, Sato S, Chen SF, et al: Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. J Neurosci 1998;18: 205–213.
67 Fujimura M, Morita-Fujimura Y, Kawase M, Copin JC, Calagui B, Epstein CJ, et al: Manganase superoxide dismutase mediates the early release of mitochondrial cytochrome c and subsequent DNA fragmentation after permanent focal cerebral ischemia in mice. J Neurosci 1999;19:3414–3422.
68 Noshita N, Sugawara T, Fujimura M, Morita-Fujimura Y, Chan PH: Manganese superoxide dismutase affects cytochrome c release and caspase-9 activation after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 2001;21:557–567.

69 Kim GW, Kondo T, Noshita N, Chan PH: Manganese superoxide dismutase deficiency exacerbates cerebral infarction after focal cerebral ischemia/reperfusion in mice: implications for the production and role of superoxide radicals. Stroke 2002;33:809–815.
70 Sheng H, Bart RD, Oury TD, Pearlstein RD, Crapo JD, Warner DS: Mice overexpressing extracellular superoxide dismutase have increased resistance to focal cerebral ischemia. Neuroscience 1999;88:185–191.
71 Sheng H, Brady TC, Pearltein RD, Crapo JD, Warner DS: Extracellular superoxide dismutase deficiency worsens outcome from focal cerebral ischemia in the mouse. Neurosci Lett 1999;267:13–16.
72 Sheng H, Kudo M, Mackensen GB, Pearlstein RD, Crapo JD, Warner DS: Mice overexpressing extracellular superoxide dismutase have increased resistance to global cerebral ischemia. Exp Neurol 2000;163:392–398.
73 Weisbrot-Lefkowitz M, Reuhl K, Perry B, Chan PH, Inoyue M, Mirochnitchenko O: Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. Mol Brain Res 1998;53:333–338.
74 Crack PJ, Taylor JM, Flentjar NJ, de Haan J, Hertzog P, Iannello RC: Glutathione peroxidase-1 contributes to the neuroprotection seen in the superoxide dismutase-1 transgenic mouse in response to ischemia/reperfusion injury. J Cereb Blood Flow Metab 2003;23:19–22.
75 Fiskum G, Murphy AN, Beal MF: Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases. J Cereb Blood Flow Metab 1999;19:351–369.
76 Bogaert YE, Sheu KF, Hof PR, Brown AM, Blass JP, Rosenthal RE, Fiskum G: Neuronal subclass-selective loss of pyruvate dehydrogenase immunoreactivity following canine cardiac arrest and resuscitation. Exp Neurol 2000;161:115–126.
77 Chinopuolos C, Starkov AA, Fiskum G: Cyclosporine A-insensitive permeability transition in brain mitochondria: inhibition by 2-aminoethoxydiphenyl borate. J Biol Chem 2003;278:27382–27389.
78 Miller FD, Pozniak CD, Walsh GS: Neuronal life and death: an essential role for the p53 family. Cell Death Differ 2000;7:880–888.
79 Brown GC, Borutaite V: Nitric oxide, cytochrome c and mitochondria. Biochem Soc Symp 1999;66:17–25.
80 Brown GC, Borutaite V: Inhibition of mitochondrial respiratory complex I by nitric oxide, peroxynitrite and S-nitrosothiols. Biochim Biophys Acta 2004;1658:44–49.
81 Kowaltowski AJ, Vercesi AE, Fiskum G: Bcl-2 prevents mitochondrial permeability transition and cytochrome C release via maintenance of reduced pyridine nucleotides. Cell Death Differ 2000;7:903–910.
82 Kikugawa K, Hiramoto K, Ohkawa T: Effects of oxygen on the reactivity of nitrogen oxide species including peroxynitrite. Biol Pharmacol Bull 2004;27:17–23.
83 Nomura Y: Neuronal apoptosis and protection: effects of nitric oxide and endoplasmic reticulum-related proteins. Biol Pharm Bull 2004;27:961–963.
84 Warner DS, Sheng H, Batinié-Haberle I: Oxidants, antioxidants and the ischemic brain. J Exp Biol 2004;207:3221–3231.
85 Mattson MP, Culmsee C, Yu ZF: Apoptotic and anti-apoptotic pathways mechanisms in stroke. Cell Tissue Res 2000;301:173–187.
86 Krupinski J, Ferrer I, Barrachina M, Secades JJ, Mercadal J, Lozano R: CDP-choline reduces pro-caspase and cleaved caspase-3 expression, nuclear DNA fragmentation, and specific PARP-cleaved products of caspase activation following middle cerebral artery occlusion in the rat. Neuropharmacology 2002;42:846–854.
87 Adibhatla RM, Hatchler JF, Dempsey RJ: Citicoline: neuroprotective mechanisms in cerebral ischemia. J Neurochem 2002;80:12–23.
88 Marshall JWB, Duffin KJ, Green R, Ridley RM: NXY-059, a free radical-trapping agent, substantially lessens the functional disability resulting from cerebral ischemia in a primate species. Stroke 2003;32:190–198.
89 Green AR, Ashwood T: Free radical trapping as a therapeutic approach to neuroprotection in stroke: experimental and clinical studies with NXY-059 and free radical scavengers. Curr Drug Targets CNS Neurol Disord 2005;4:109–118.
90 Waldmeier PC, Zimmermann K, Qian T, Tintelnot-Blomley M, Lemasters JJ: Cyclophilin D as a drug target. Curr Med Chem 2003;10:1485–1506.
91 Perez-Velazquez JL, Kokarovtseva L, Weiss-papir M, Frantseva MV: Anti-porin antibodies prevent excitotoxic and ischemic damage to brain tissue. J Neurotrauma 2003;20:633–647.
92 Adibhatla RM, Hatcher JF, Dempsey RJ: Effects of citicoline on phospholipid and glutathione levels in transient cerebral ischemia. Stroke 2001;32:2376–2381.
93 Barrachina M, Dominguez I, Ambrosio S, Secades J, Lozano R, Ferrer I: Neuroprotective effect of citicoline in 6-hydroxydopamine-lesioned rats and in 6-hydroxydopamine-treated SH-SY5Y human neuroblastoma cells. J Neurol Sci 2003;215:105–110.
94 Fresta M, Puglisi G: Biological effects of CDP-choline loaded along circulating liposomes on rat post-ischemic reperfusion. Int J Pharmaceut 1996;134:89–97.
95 Vaughan CJ, Delanty N: Neuroprotective properties of statins in cerebral ischemia and stroke. Stroke 1999;30:1969–1973.
96 Montaner J: Treatment with statins in the acute phase of ischemic stroke. Expert Rev Neurother 2005;5:211–221.
97 Bazan NG: Neuroprotectin D1 (NPD1): a DHA-derived mediator that protects brain and retina against cell injury-induced oxidative stress. Brain Pathol 2005;15:159–166.
98 Takahashi M, Macdonald RL: Vascular aspects of neuroprotection. Neurol Res 2004;26:862–869.
99 Ferrer I: Pathology of brain edema and cerebral swelling; in Kalimo H (ed): Pathology and Genetics. Cerebrovascular Diseases. Basel, ISN Neuropath Press, 2005, pp 32–38.
100 Fagan SC, Hess DC, Machado LS, Hohnandel EJ, Pollock DM, Ergul A: Tactics for vascular protection after acute ischemic stroke. Pharmacotherapy 2005;25:387–395.
101 Endres M, Laufs U, Liao JK, Moskowitz MA: Targeting eNOS for stroke protection. Trends Neurosci 2004;27:283–289.
102 Scarabelli TM, Stephanon A, Pasini E, et al: Different signaling pathways induce apoptosis in endothelial cells and cardiac myocytes during ischemia/reperfusion injury. Circ Res 2002;90:745–748.
103 Machino T, Hashimoto S, Maruoka S, Gon Y, Hayashi S, Mizumura K, Nishitoh H, Ichijo H, Horie T: Apoptosis signal-regulating kinase-1-mediated signaling pathway regulates hydrogen peroxide-induced apoptosis in human pulmonary vascular endothelial cells. Crit Care Med 2003;31:2776–2778.
104 Lee SR, Lo EH: Interactions between p38 mitogen-activated protein kinase and caspase-3 in cerebral endothelial cells after hypoxia-reoxygenation. Stroke 2003;34:2704–2709.
105 Bambrick L, Kristian T, Fiskum G: Astrocyte mitochondrial mechanisms of ischemic brain injury and neuroprotection. Neurochem Res 2004;29:601–608.
106 Giffard RG, Swanson RA: Ischemia-induced programmed cell death in astrocytes. Glia 2005;50:299–306.
107 Dugan LL, Kim-Ham JS: Astrocyte mitochondria in in vitro models of ischemia. J Bioenerg Biomembr 2004;36:317–321.
108 Kimelberg HK: Astrocytic swelling in cerebral ischemia as a possible cause of injury and target for therapy. Glia 2005;50:389–397.
109 Dirnagl U, Simon RP, Hallenbeck JM: Ischemic tolerance and endogenous neuroprotection. Trends Neuroci 2003;22:391–397.
110 Trendelenburg G, Dirnagl U: Neuroprotective role of astrocytes in cerebral ischemia: focus on ischemic preconditioning. Glia 2005;51:307–320.
111 Ferrer I, Lopez E, Pozas E, Ballabriga J, Marti E: Multiple neurotrophic signals converge in surviving CA1 neurons of the gerbil hippocampus following transient forebrain ischemia. J Comp Neurol 1998;394:416–430.
112 Wu D: Neuroprotection in experimental stroke with targeted neurotrophins. NeuroRx 2005;2:120–128.
113 FIBLAST Safety Study Group: Clinical safety of intravenous bFGF in acute stroke. Stroke 1998;29:287.
114 Bogousslavsky J, Victor SJ, Salinas EO, Pallay A, Donnan GA, Fieschi C, et al: Fiblast (trafermin) in acute stroke: results of the European-Australian phase II/III safety and efficacy trial. Cerebrovasc Dis 2002;14:239–251.

115 Kiprianova I, Freiman TM, Desiderato S, Schwab S, Galmbacher R, Guillardon E, Spranger M: Brain-derived neurotrophic factor prevents neuronal death and glial activation after global ischemia in the rat. J Neurosci Res 1999;56:21–27.

116 Ferrer I, Ballabriga J, Martí E, Perez E, Alberch J, Arenas E: BDNF up-regulates TrkB protein and prevents the death of CA1 neurons following transient forebrain ischemia. Brain Pathol 1998;8:253–261.

117 Ferrer I, Krupinski J, Goutan E, Martí E, Ambrosio S, Arenas E: Brain-derived neurotrophic factor reduces cortical cell death by ischemia after middle cerebral artery occlusion in the rat. Acta Neuropathol 2001;101:229–238.

118 Zhang Y, Pardridge WM: Conjugation of brain-derived neurotrophic factor (BDNF) to a blood-brain barrier targeting system enables neuroprotection in regional brain ischemia following intravenous injection of the neurotrophin. Brain Res 2001;889:49–56.

119 Zhang Y, Pardridge WM: Neuroprotection in transient focal brain ischemia after delayed intravenous administration of brain-derived neurotrophic factor conjugated to a blood-brain barrier drug targeting system. Stroke 2001;32:1378–1384.

120 Berger C, Schabitz WR, Wolf M, Mueller H, Sommer C, Schwab S: Hypothermia and brain-derived neurotrophic factor reduce glutamate synergistically in acute stroke. Exp Neurol 2004;185:305–312.

121 Ferrer I, Alcántara S, Ballabriga J, Olivé M, Blanco R, Rivera R, Carmona M, Berruezo M, Pitarch S, Planas AM: Transforming growth factor-α and epidermal growth factor-receptor immunoreactivity in normal and pathologic brain. Prog Neurobiol 1996;49:99–123.

122 Justicia C, Perez-Asensio FJ, Burguete MC, Salom JB, Planas AM: Administration of transforming growth factor-α reduces infarct volume after transient focal cerebral ischemia in the rat. J Cereb Blood Flow Metab 2001;21:1097–1104.

123 Friguls B, Petegnief V, Justicia C, Pallas M, Planas AM: Activation of ERK and Akt signaling in focal cerebral ischemia: modulation by TGF-α and involvement of NMDA receptors. Neurobiol Dis 2002;11:443–456.

124 Dhandapani KM, Brann DW: Transforming growth factor-β: a neuroprotective factor in cerebral ischemia. Cell Biochem Biophys 2003;39:13–22.

125 Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, Klumpp S, Kriegstein J: Transforming growth factor-β_1 increases bad phosphorylation and protects neurons against damage. J Neurosci 2002;22:3898–3909.

126 Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET, Vivien D: Transforming growth factor-β and ischemic brain injury. Cell Mol Neurobiol 2003;23:539–550.

127 Zhu Y, Culmsee C, Klumpp S, Krieglstein J: Neuroprotection by transforming growth factor-β_1 involves activation of the nuclear factor-κB through phosphatidylinositol-3-OH kinase/Akt and mitogen-activated protein kinase-extracellular-signal regulated kinase-1,2 signaling pathways. Neuroscience 2004;123:897–906.

128 Wang Y, Lin SZ, Chiou AL, Williams LR, Hoffer BJ: GDNF protects against ischemia-induced injury in the cerebral cortex. J Neurosci 1997;17:4341–4348.

129 Miyazaki H, Okuma Y, Fujii Y, Nagashima K, Nomura Y: Glial cell line-derived neurotrophic factor protects against delayed neuronal death after transient forebrain ischemia in rats. Neuroscience 1999;89:643–647.

130 Kilic U, Kilic E, Dietz GP, Bahr M: Intravenous TAT-GDNF is protective after focal cerebral ischemia in mice. Stroke 1998;29:1417–1422.

131 Zhang WR, Sato K, Iwai M, Nagano I, Manabe Y, Abe K: Therapeutic time window of adenovirus-mediated GDNF gene transfer after transient middle cerebral artery occlusion in rat. Brain Res 2002;947:140–145.

132 Wang Y, Chang CF, Morales M, Chaing YH, Hoffer J: Protective effects of glial cell line-derived neurotrophic factor in ischemic brain injury. Ann NY Acad Sci 2002;962:423–437.

133 Kitagawa H, Sasaki C, Sakai K, Mori A, Mitsumoto Y, Mori T, et al: Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents ischemic brain injury after transient middle cerebral artery occlusion in rats. J Cereb Blood Flow Metab 1999;19:1336–1344.

134 Tsai TH, Chen SL, Chiang YH, Lin SZ, Ma HI, Kuo SW, et al: Recombinant adeno-associated virus vector expressing glial cell line-derived neurotrophic factor reduces ischemia-induced damage. Exp Neurol 2000;166:266–275.

135 Harvey BK, Hoffer BJ, Wang Y: Stroke and TGF-β proteins: glial cell line-derived neurotrophic factor and bone morphogenic protein. Pharmacol Ther 2005;105:113–125.

136 Lin SZ, Hoffer BJ, Kaplan P, Wang Y: Osteogenic protein 1 protects against cerebral infarction induced by MCA ligation in adult rats. Stroke 1999;30:126–133.

137 Wang Y, Chang CF, Morales M, Chou J, Chen HL, Chaing YH, et al: Bone morphogenic protein-6 reduces ischemia-induced brain damage in rats. Stroke 2001;32:2170–2178.

138 Chang CF, Lin SZ, Chiang YH, Morales M, Chou J, Lein P, et al: Intravenous administration of bone morphogeneic protein-7 after ischemia improves motor function in stroke rats. Stroke 2003;34:558–564.

139 Storkebaum E, Lambrechts D, Carmeliet P: VEGF: once regarded as aspecific angiogenic factor, now implicated in neuroprotection. Bioessays 2004;26:943–954.

140 Plate KH, Beck H, Danner S, Allegrini PR, Wiessner C: Cell type-specific upregulation of vascular endothelial growth factor in an MCA-occlusion model of cerebral infarct. J Neuropathol Exp Neurol 1999;58:654–666.

141 Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, et al: Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol 2000;156:965–976.

142 Zhang ZG, Zhang L, Jiang Q, Zhang R, Davies K, et al: VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J Clin Invest 2000;106:829–838.

143 Zhang ZG, Zhang L, Tsang W, Soltanian-Zadeh H, Morris D, et al: Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after focal cerebral ischemia. J Cereb Blood Flow Metab 2002;22:379–392.

144 Hayashi T, Abe K, Itoyama Y: Reduction of ischemic damage by application of vascular endothelial growth factor in rat brain after transient ischemia. J Cereb Blood Flow Metab 1998;18:887–895.

145 Jin K, Mao XO, Batteur SP, McEachron E, Leahy A, Greenberg DA: Caspase-3 and the regulation of hypoxic neuronal death by vascular endothelial growth factor. Neuroscience 2001;108:351–358.

146 Yang ZJ, Bao WL, Qiu MH, Zhang LM, Lu SD, Huang YL, Sun FY: Role of vascular endothelial growth factor in neuronal DNA damage and repair in rat brain following a transient cerebral ischemia. J Neurosci Res 2002;70:140–149.

147 Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA: VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest 2003;111:1843–1851.

148 Manoonkitiwongsa PS, Schultz RL, McCreery DB, Whitter EF, Lyden PD: Neuroprotection of ischemic brain by vascular endothelial growth factor is critically dependent on proper dosage and may be compromised by angiogenesis. J Cereb Blood Flow Metab 2004;24:693–702.

149 Sun FY, Guo X: Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. J Neurosci Res 2005;79:180–184.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):21–29
DOI: 10.1159/000091700

Published online: May 2, 2006

Vascular Protection in Brain Ischemia

Manuel Rodríguez-Yáñez[a] Mar Castellanos[b] Miguel Blanco[a]
Esther Mosquera[c] José Castillo[a]

[a]Department of Neurology, Division of Vascular Neurology, Hospital Clínico Universitario, University of Santiago de Compostela, Santiago de Compostela; [b]Stroke Unit, Hospital Universitario Dr. Josep Trueta, Girona, and [c]Laboratory of Neurovascular Research, Hospital Clínico Universitario, University of Santiago de Compostela, Santiago de Compostela, Spain

Key Words
Vascular protection · Ischemic stroke · Stroke, treatment · Statin treatment

Abstract
Vascular damage occurring after cerebral ischemia may lead to a worse outcome in patients with ischemic stroke, as it facilitates edema formation and hemorrhagic transformation. There are several phases in the development of vascular injury (acute, subacute and chronic) and different mediators act in each one. Therapeutic options to avoid vascular injury must be focused on acting in each phase. However, even though experimental studies have demonstrated the benefit of therapeutic interventions both in the acute and chronic phases of cerebral ischemia, only the chronic phase offers a therapeutic window sufficiently wide enough to provide vascular protection in clinical practice. Several drugs including erythropoietin and HMG-CoA reductase inhibitors (statins), antihypertensive (angiotensin modulators), antibiotics (minocycline) and antihyperglycemic drugs (thiazolidinediones) have been proved to provide vascular protection in patients with ischemic stroke. Anti-inflammatory, antioxidant, and antiapoptotic actions are responsible for the vascular protective effect related to these drugs.

After ischemic stroke, damage to cerebral blood vessels occurs early and is progressive over time. The result of vascular damage is an increase in the endothelial cell permeability leading to edema formation and hemorrhagic transformation of the ischemic lesion which in turn is associated with a worse functional outcome.

Vascular endothelium is an indispensable organ in the regulation of the tone and vascular homeostasis. Antioxidant, anti-inflammatory, vasodilatory, antiaggregant, anticoagulant and profibrinolytic effects have all been described among the functions of the endothelium. These effects disappear as a result of endothelial dysfunction secondary to vascular damage [1]. Vascular protection has traditionally been approached through the enhancement of endothelial function, the prolongation of endothelial cell survival, and the suppression of the thrombotic and anti-inflammatory effects within the vasculature [2].

Although any strategy reducing the incidence of the vascular event responsible for endothelial dysfunction could be considered to be 'vascular protective' (including antithrombotic and antihypertensive therapies), this term is only used for therapeutic agents with direct beneficial effects on the vascular endothelium. Vascular protection is defined as an increase in the endothelial function with the aim of preventing vascular smooth muscle cell proliferation, inflammation, thrombosis, and apoptosis.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0021$23.50/0

Accessible online at:
www.karger.com/ced

Prof. José Castillo
Department of Neurology, Hospital Clínico Universitario
Travesa da Choupana, s/n
ES–15706 Santiago de Compostela (Spain)
Tel. +34 981 951 348, Fax +34 981 951 098, E-Mail mecasti@usc.es

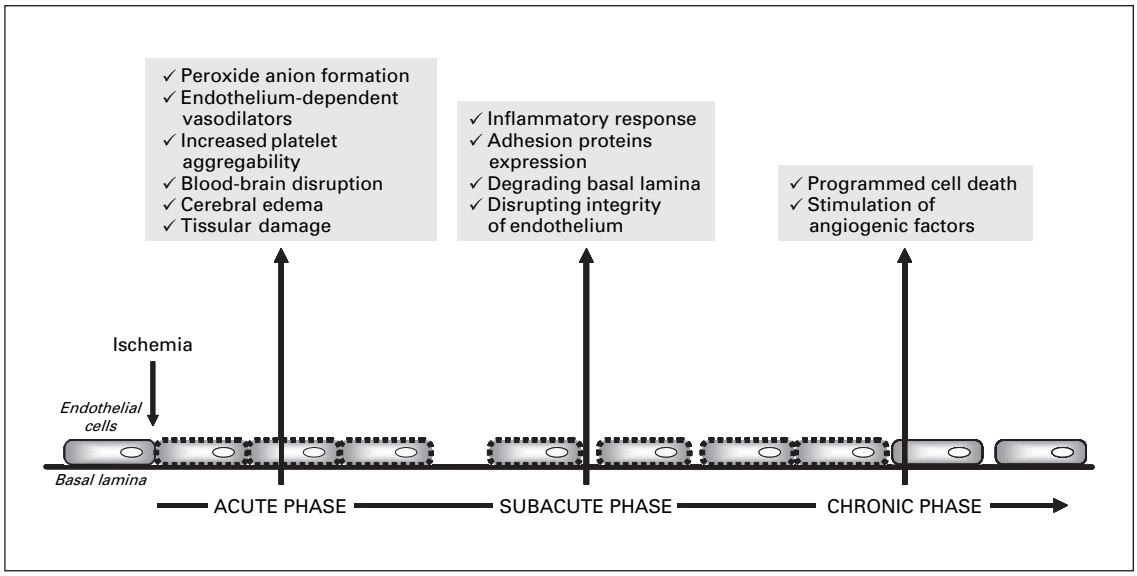


Fig. 1. Pathophysiology of vascular injury after ischemic stroke. Mechanisms of vascular damage depend on the temporal evolution of cerebral ischemia.

Cerebral ischemia results in a cascade of events that takes place at different times, a fact that allows us to distinguish between an acute phase of stroke (occurring within hours), a subacute phase (occurring within hours to days) and a chronic phase of evolution (occurring within days to months). Targets of vascular protection after ischemic stroke differ depending on the time of evolution and must be identified within each one of these phases [3].

Pathophysiology of Vascular Injury after Ischemic Stroke (fig. 1)

Cerebral blood flow reduction is followed by a series of processes that affect microvasculature provoking blood-brain barrier (BBB) disruption and vascular tone disturbance [4]. Several factors including free oxygen radicals, oxygen, nitric oxide (NO), endothelin-1 (ET-1), vascular endothelial growth factor (VEGF) and angiopoietin-1 play an important role in the maintenance of vascular tone and structure regulation during the acute phase of stroke [4, 5]. As a result of the reperfusion, a large amount of free oxygen radicals including hydrogen peroxide, hydroxyl radicals, and especially the superoxide anion are released [6, 7]. Free oxygen radicals alter the vascular response to CO_2, stimulate the release of endothelium-dependent vasodilators such as acetylcholine, and increase platelet aggregability as well as endothelial and BBB permeability [7]. BBB disruption results in albumin and other high-molecular-weight protein extravasation, which produces edema and an increase in the intracranial pressure. In addition to its effects on BBB integrity and vascular tone, the superoxide anion reacts with NO forming the highly toxic peroxynitrite radical, which is responsible for delayed tissular damage, and is an important signal mechanism triggering inflammation and apoptosis in the acute and chronic phases of ischemic stroke. Other molecules released during the acute phase of ischemia are also responsible for the increase in BBB permeability, such as ET-1 [8, 9], VEGF, which also promotes angiogenesis [10], and angiopoietin-1, which also mediates angiogenesis and participates in BBB stability, and whose levels decrease immediately after ischemia coinciding with the increase in BBB permeability [11].

The generation of free radicals and the increase in intracellular calcium occurring in the acute phase of ischemia activates different inflammatory genes in the subacute phase of stroke. The result is the release of several inflammatory mediators including interleukin (IL)-1β, tumor necrosis factor α (TNF-α), and transcription factors such as hypoxia-inducible factor-1, nuclear factor-κB (NF-κB), and interferon regulatory factor-1 [12–15], which in turn stimulates the expression of adhesion proteins including the intercellular adhesion molecule 1 (ICAM-1), P-selectins and E-selectins, which are impor-

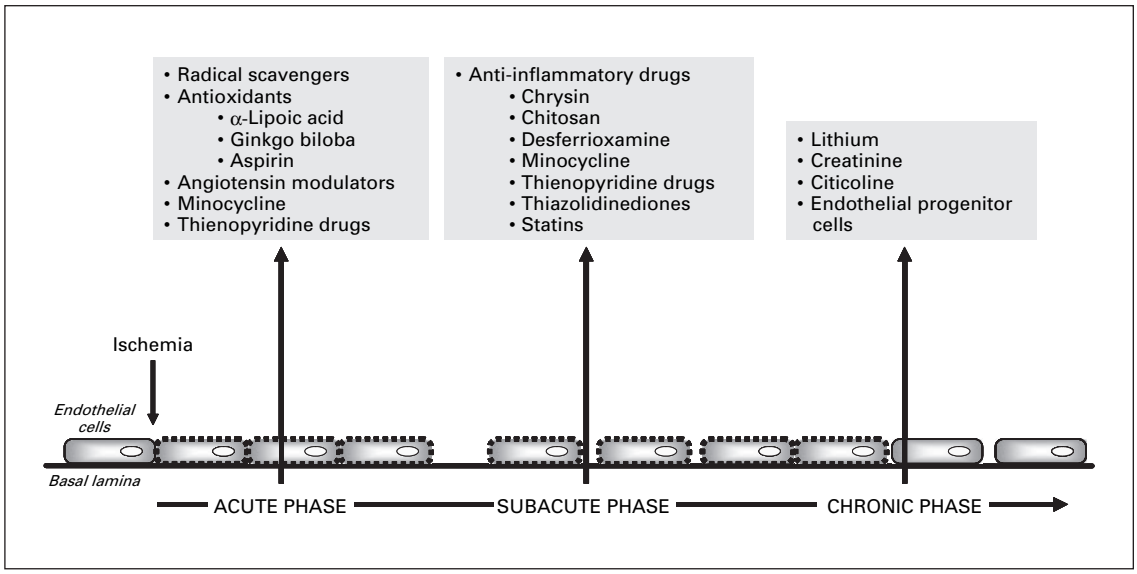


Fig. 2. Therapeutic targets for vascular protection.

tant mediators of endothelium vascular integrity. Adhesion proteins interact with neutrophils and facilitate their migration through the vascular wall to the cerebral tissue. Inflammatory molecules also induce the release of matrix metalloproteinases (MMP), a group of proteolytic enzymes related to the remodeling of the basal lamina and the disruption of the endothelium [16, 17].

In the chronic phase of stroke, the expression of genes participating in apoptosis and in the stimulation of angiogenic factors is induced in endothelial cells. Programmed cell death is triggered by the activation of different factors in cellular surface receptors such as TNF-α, superoxide and IL-1β. In response to this stimulus, proteolytic enzymes, caspases and other proteins such as B-cell lymphoma-leukemia 2 (Bcl2)-associated C protein (Bax) and transformation-related protein 53 (Trp53), as well as antiapoptotic enzymes such as Bcl2 and inhibitor of apoptosis protein (Iap) are activated [18–21].

Therapeutic Targets for Vascular Protection in Brain Ischemia (fig. 2)

As we have seen, vascular protection therapeutic options must be considered in the context of the physiopathology of vascular injury after ischemic stroke. In the acute phase, the superoxide anion is the predominant mediator. In the subacute phase, the most important factors are inflammatory mediators and proteases. Finally, proapoptotic gene expression is the most important mediator of vascular injury in the chronic phase.

Acute Phase of Stroke

Free oxygen radicals play an important role during the acute phase of stroke. Several studies have demonstrated that neutralizing free oxygen radicals by spin traps or scavenger enzymes such as superoxide dismutase or catalase prevents abnormal vasoreactivity and the increase in the permeability of the BBB [22, 23] providing evidence that limiting oxidant stress in the acute phase of stroke is critical for improving outcome.

The administration of antioxidants such as α-lipoic acid and *Ginkgo biloba* extract before the induction of ischemia also provides neuroprotection and reduces infarct volume [24]. A recent study reported that in addition to its antithrombotic effect, aspirin also provides neuroprotection due to its antioxidant effect in cerebral tissue subjected to hypoxia [25]. However, the effect of antioxidants on cerebrovascular function and vascular integrity and the appropriate therapeutic window in the acute phase of stroke still remain unclear.

Subacute Phase of Stroke

Inflammatory mediators and proteases play an important role within hours to days after stroke. The inflammatory reaction triggered as a consequence of cerebral isch-

emia makes it an attractive pharmacological option considering its rapid initiation and progression over many hours after stroke as well as its contribution to the evolution of tissue injury. The inflammatory reaction secondary to brain ischemia provides fertile ground for the investigation into novel therapeutic agents for the treatment of stroke. The inhibition of the mitogen-activated protein kinase (MAPK) cascade through the administration of cytokine suppressive anti-inflammatory drugs, which block p38 MAPK and hence the production of IL-1 and TNF-α, would seem to be the most promising future therapeutic option [26]. One of these drugs is chrysin, a natural and biologically active compound extracted from many plants, honey, and propolis, which has a powerful anti-inflammatory effect that induces p38-MAPK activation [27] although its clinical utility has not yet been demonstrated.

Chitosan, another anti-inflammatory drug prepared by the chemical N-deacetylation of chitin, has been observed to accelerate wound healing and the production of cytokines [28]. The iron chelator desferrioxamine (DFX) is capable of inducing NF-κB activation and hypoxia-dependent gene expression, probably by replacing or removing the central iron of the putative heme oxygen sensor [29]. Chitosan inhibited the DFX-induced proinflammatory cytokine production (IL-6, IL-8, and TNF-α) by the blockage of NF-κB activation in human mast line (HMC)-1 cells [30].

Different agonists of peroxisome proliferator-activated receptor (PPAR)-γ such as 15-dPGJ_2 may have a neuroprotective effect. This prostaglandin is related to good neurological outcome and smaller infarct volume in ischemic stroke [31] and can also act through PPAR-γ-independent mechanisms including the inhibition of NF-κB signaling [32] and some kinases involved in the MAPK cascade [33].

Chronic Phase of Stroke

Ongoing delayed neuronal death, in part mediated by apoptosis, contributes to the progression of cerebral infarction during the recovery period. The inhibition of apoptotic mechanisms may provide sustained neuroprotection [34]. Different treatments have been tested to stop apoptosis in animal models of ischemic stroke. It has been proved that hyperbaric oxygen therapy limits infarct volume preserving more brain tissue and promoting neurological functional recovery [35]. It has also been demonstrated that chronic treatment with low doses of lithium provides neuroprotection in a transient model of focal cerebral ischemia by blocking apoptotic mechanisms [36]. The administration of oral creatine results in a remarkable reduction in ischemic brain infarction volume and neuroprotection by the reduction of postischemic caspase-3 activation and cytochrome c release [37].

Endothelial progenitor cells derived from bone marrow circulate in the peripheral blood and have been related to in neoangiogenesis after tissue ischemia [38]. These cells are capable of proliferating and differentiating into endothelial cells and so are ideal candidates for vascular regeneration [39]. Neoangiogenesis has been observed in perilesional tissue from endothelial progenitor cells in animal models of cerebral ischemia [40] and so the use of these cells should be considered as a potential therapeutic target in the future.

Drugs Providing Vascular Protection

There are several currently marketed safe therapeutic agents including statins, angiotensin modulators, erythropoietin, minocycline and thiazolidinediones which have been found to act against some key targets of vascular protection [41].

Statins

Statins, or 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, have been associated with a significant decrease in the incidence of ischemic stroke in patients with a previous history of coronary artery disease, both with and without high serum levels of cholesterol. Several studies have found that statins may reduce the incidence of stroke by around 30% [42–44]. They have also been found to slow down the development of atherosclerosis in both coronary and carotid arteries and to reduce the progression of carotid intima-media thickening [45]. Over and above the undoubtedly important effects of statins in stroke prevention, they may have further beneficial effects including endothelial, anti-inflammatory, antioxidant-protective properties [46].

Statins improve the endothelial vascular function. Vascular endothelial injury is a major initiating factor of atherosclerosis. The endothelium has important antiatherogenic properties as endothelial cells synthesize several mediators that protect the vessel from atherosclerosis [47]. NO is particularly important in this protective process. Different nitric oxide synthase (NOS) forms play important but opposing roles in cerebral ischemia. The inducible form of NOS (iNOS) has been found to be an important mediator in the ischemia and reperfusion inflammatory response [48]. Astrocytes elaborate iNOS in

response to of several mediators such as IL-1β, TNF-α and IL-6. The expression of iNOS has also been demonstrated to occur in neutrophils infiltrating the ischemic brain and blood vessels within the ischemic territory in human ischemic stroke [49]. Additionally, neuronal NOS (nNOS) may contribute to neuronal damage by promoting glutamate-mediated neurotoxicity. In contrast, NO produced by endothelial NOS (eNOS) has a protective effect and orchestrates the paracrine homeostatic functions of the endothelium, with leukocyte and platelet adhesion inhibition, control of vascular tone, and the maintenance of the thromboresistant interface between the bloodstream and the vessel wall. NO produced by eNOS stimulates guanylyl cyclase activity, which results in several potentially antiatherogenic and antithrombotic actions including vascular smooth muscle proliferation inhibition [50], and platelet adhesion [51] activation and aggregation inhibition [52]. Statin treatment produces a beneficial effect in cerebral ischemia due to brain eNOS modulation. In a murine model of ischemic stroke prophylactic statin therapy has been found to increase cerebral blood flow, to reduce infarct volume by approximately 30% and improve neurological outcome in normocholesterolemic animals [53]. In this intriguing investigation, statin therapy directly upregulated eNOS activity in the brain without altering the expression of nNOS independently of the change in cholesterol levels. Even though these effects have not been found in humans, these observations suggest that statins may protect the cerebral endothelium and attenuate the ischemic burden.

In addition to biochemically remodeling the endothelium, statins have been shown to inhibit a number of inflammatory processes known to be important during cerebral ischemia and reperfusion. They have anti-inflammatory properties as they reduce the inflammatory cell accumulation in atherosclerotic plaques [54] and so contribute to plaque stability. The statins also help plaque stability by inhibiting MMP and tissue factor expression in plaques [55]. Statin treatment reduces enhanced leukocyte-endothelium interactions in hypercholesterolemic animals [56] and inhibits neutrophil adhesion to coronary endothelium [57]. In in vivo studies, both simvastatin and lovastatin reduce monocyte CD11b expression and ex vivo CD11b-dependent monocyte adhesion to the endothelium in subjects with hypercholesterolemia [58]. In addition to these potentially salutary effects, statin therapy may modulate central nervous system cytokine production. Statin treatment may represent a novel means of suppressing cytokine response that occurs during ischemia and reperfusion by directly reducing the in vivo induction of inflammatory mediators such as iNOS, IL-1β and TNF-α in astrocytes and macrophages.

Finally, statins may be neuroprotective due to their potentially antioxidant effects. The generation of free radical generation results in neuronal damage by lipid peroxidation induction, protein oxidation and direct damage to nucleic acids. Several studies have found that statin therapy may reduce lipoprotein oxidation and ameliorate free radical injury. They also have favorable antioxidant effects including an increase in the lag time of copper-induced low density lipoprotein (LDL) oxidation [59] and a reduction in leukocyte-induced LDL oxidation [60]. Hydroxyl metabolites of atorvastatin have been shown to inhibit oxidation in a concentration-dependent manner in an in vitro model of cerebral ischemia [61], and in a study of hypercholesterolemic patients, treatment with simvastatin increased the α-tocopherol/total cholesterol ratio [62], thus possibly boosting membrane-specific antioxidant defenses.

Angiotensin Modulators

Angiotensin II is the main product of the renin-angiotensin system and tends to be elevated in most of hypertensive patients. Besides its vasoconstrictive action, it can contribute to atherogenesis by stimulating smooth muscular cell growth [63]. Angiotensin II binds to specific receptors of smooth muscular cells, inducing phospholipase C activation, which increases calcium concentrations and provokes muscular contraction. It also increases protein synthesis and provokes smooth muscular cell hypertrophy [64]. Angiotensin II has also been found to increase lipoxygenase activity, which results in increased inflammation and LDL oxidation. Furthermore, angiotensin II increases the expression of proinflammatory cytokines such as IL-6 and ICAM-1 by arterial wall smooth muscular cells [65–67].

Increased production of reactive oxygen species, especially the superoxide anion, contributes to functional and structural alterations. By stimulating the angiotensin II type 1 (AT1) receptor, angiotensin II contributes to the overexpression of cytosolic proteins involved in the activation of NAD(P)H oxidase, which is a major source of superoxide production [68, 69]. The overexpression of these cytosolic proteins might lead to vascular hypertrophy and remodeling in hypertension [68, 69].

Besides its effect on blood pressure, angiotensin modulators have other effects. Recent animal model studies show that previous treatment with losartan, an angiotensin inhibitor, reduces infarct size after cerebral focal ischemia by the stimulation of cerebral angiogenesis

[70]. Angiotensin inhibitors may offer benefit with respect to endothelial dysfunction and vascular remodeling [71]. It has been observed that the AT1 receptor antagonists reduce overall oxidative stress in hypertensive patients independently of its effects on blood pressure [72].

Angiotensin modulators reduce mortality after cerebral ischemia, and a long-term benefit in decreasing the risk of stroke. Angiotensin-converting enzyme inhibitor treatment decreases short-term mortality in older patients with acute ischemic stroke [73]. It has been proved that antihypertensive treatment with perindopril reduces and prevents cardiovascular disease in a large range of patients with vascular diseases, whether or not they are hypertensive [74]. Perindopril is a long-acting, once-daily lipophilic angiotensin-converting enzyme inhibitor with a high tissue angiotensin-converting enzyme affinity, which decreases angiotensin II and potentiates bradykinin.

For this reason, angiotensin modulators are first-line agents for the treatment of hypertension and cardiovascular diseases [75, 76]. The block of the renin-angiotensin system by using these agents has special advantages due to specific vascular and antiatherosclerotic effects that contribute to better vascular protection.

Erythropoietin

Erythropoietin is a glycoprotein that is produced mainly by interstitial fibroblasts in the kidneys of adults and in hepatocytes in the fetus. Released into the circulation, it makes its way to the bone marrow, where it regulates red cell production by preventing apoptosis of erythroid progenitor cells. Recently, erythropoietin has emerged as a multifunctional growth factor that plays a significant role in the nervous system. Both erythropoietin and its receptor are expressed throughout the brain in glial cells [77], neurons [78] and endothelial cells. Hypoxia and ischemia have been recognized as important driving forces of erythropoietin expression in the brain. Erythropoietin has potent neuroprotective properties [79] and appears to act in a dual way by directly protecting neurons from ischemic damage and by stimulating endothelial cells and thus supporting the angiogenic effect of VEGF in the nervous system.

Erythropoietin modulates a broad array of cellular processes that include progenitor stem cell development, cellular integrity, and angiogenesis, and inhibits the apoptotic mechanisms of injury, including the preservation of cellular membrane asymmetry to prevent inflammation [80].

Minocycline

Minocycline is a semisynthetic second-generation tetracycline with demonstrated anti-inflammatory [81], glutamate antagonist [82], and antiapoptotic effects [83]. Apart from its antimicrobial properties, minocycline has been found to have beneficial effects on microglial activation, MMP, NO production, and apoptotic cell death [84].

Minocycline has neuroprotective effects in vivo against permanent focal cerebral ischemia and in vitro against glutamate-induced cell death. The inhibition of oxidative stress by minocycline may be partly responsible for these effects [85]. Minocycline may also provide protection by interfering with MMP [86]. In animal models of focal cerebral ischemia, minocycline has been shown to reduce infarct size by more than 50% [81]. However, the minocycline doses used in these studies were almost 30 times the weight-based dose routinely administered to humans for anti-infective and anti-inflammatory purposes.

Minocycline may represent a prototype of an anti-inflammatory compound that provides protection against ischemic stroke and has a clinically relevant therapeutic window, but these results must be confirmed in clinical trials.

Thiazolidinediones

Thiazolidinediones are a new kind of antihyperglycemic that improve insulin-mediated glucose uptake into skeletal muscle without increasing endogenous insulin secretion [87] and have been demonstrated to be effective in the treatment of non-insulin-dependent diabetes mellitus with insulin resistance. They exert their primary effects by activating specific receptors, called peroxisome proliferator-activated receptor (PPAR). There are three types of such receptors: PPAR-α, PPAR-δ and PPAR-γ. Thiazolidinediones activate PPAR-γ receptor, which is a ligand-dependent nuclear transcription factor that has been implicated in a broad range of cellular functions, including anti-inflammatory effects [88, 89].

Besides their insulin and glucose metabolism effect, thiazolidinediones have another non-glucemic effects. As we have seen, thiazolidinediones are PPAR-γ nuclear receptor agonists, which are present in several tissues, including adipose tissue, endothelial cells, macrophages, and smooth muscular cells, therefore thiazolidinediones administration improves most of metabolic syndrome compounds [90]. Treatment with thiazolidinediones decreases free serum fatty acid, increases HDL cholesterol levels by 10–20%, convert small and heavy LDL cholesterol particles into big and light, and modestly reduces

serum triglyceride levels if they are >200 mg/dl. These PPAR-γ receptor agonists improve endothelial dysfunction. They reduce adhesion molecule and growth factor production and limit smooth muscular cell and fibroblast proliferation. Besides these effects it has been demonstrated that thiazolidinediones have an anti-inflammatory effect since they reduce some molecular inflammatory markers such as TNF-α and C-reactive protein. It has also been demonstrated that thiazolidinediones have vasculoprotective effects in both acute and chronic vascular injury through the inhibition of vascular smooth muscle cell proliferation [91].

Other Therapeutic Agents

Another drug that can be effective in vascular protection is clopidogrel. The exact mechanism of the benefit of clopidogrel is still being elucidated but it is related to the inhibition of vascular inflammation, endothelial dysfunction, and localized angiogenesis, and not just aggregation [92]. It has been proved that this drug exerts anti-inflammatory effects in animal models. Clopidogrel administration reduces P-selectin expression and CD40 ligand and tissue factor expression and also protects endothelial NOS protein expression [93]. Clopidogrel has also been shown to inhibit CD40 ligand expression in the platelets of healthy volunteers [94].

Citicoline can also exert a vascular neuroprotective effect since it has an antioxidant action by stimulating phospholipase A_2 and decreasing OH^- generation [95]. Citicoline can also cooperate in reducing brain glutamate release after ischemia [96].

References

1 Blanco M, Rodríguez-Yañez M, Sobrino T, Leira R, Castillo J: Platelets, inflammation and atherothrombotic neurovascular disease: the role of endothelial dysfunction. Cerebrovasc Dis 2005;20(suppl 2):32–39.

2 Zachary I, Mathur A, Yla-Herttuala S, Martin J: Vascular protection: a novel nonangiogenic cardiovascular role for vascular endothelial growth factor. Arterioscler Thromb Vasc Biol 2000;34:327–329.

3 Fagan SC, Hess DC, Hohnadel EJ, Pollock DM, Ergul A: Targets for vascular protection after acute ischemic stroke. Stroke 2004;35:2220–2225.

4 Dirnagl U, Iadecola C, Moskowitz MA: Pathobiology of ischemic stroke: an integrated view. Trends Neurosci 1999;22:391–397.

5 Lipton P: Ischemic cell death in brain neurons. Physiol Rev 1999;79:1431–1568.

6 Kontos CD, Wei EP, Williams JI, Kontos HA, Povlishock JT: Cytochemical detection of superoxide in cerebral inflammation and ischemia in vivo. Am J Physiol 1992;263:H1234–H1242.

7 Kontos HA: Oxygen radicals in cerebral ischemia: the 2001 Willis Lecture. Stroke 2001;32:2712–2716.

8 Matsuo Y, Mihara S, Ninomiya M, Fujimoto M: Protective effect of endothelin type A receptor antagonist on brain edema and injury after transient middle cerebral artery occlusion in rats. Stroke 2001;32:2143–2148.

9 Macrae IM, Robinson MJ, Graham DI, Reid JL, McCulloch J: Endothelin-1-induced reductions in cerebral blood flow: dose dependency, time course, and neuropathological consequences. J Cereb Blood Flow Metab 1993;13:276–284.

10 Zhang Z, Chopp M: Vascular endothelial growth factor and angiopoietins in focal cerebral ischemia. Trends Cardiovasc Med 2002;12:62–66.

11 Zhang ZG, Zhang L, Croll SD, Chopp M: Angiopoietin-1 reduces cerebral blood vessel leakage and ischemic lesion volume after focal cerebral embolic ischemia in mice. Neuroscience 2002;113:683–687.

12 Rothwell NJ, Hopkins SJ: Cytokines and the nervous system. II. Actions and mechanisms of action. Trends Neurosci 1995;18:130–136.

13 Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM: TNF-α pre-treatment induces protective effects against focal cerebral ischemia in mice. J Cereb Blood Flow Metab 1997;17:483–490.

14 Sairanen T, Carpen O, Karjalainen-Lindsberg ML, Paetau A, Turpeinen U, Kaste M, Lindsberg PJ: Evolution of cerebral tumor necrosis factor-α production during human ischemic stroke. Stroke 2001;32:1750–1758.

15 Vila N, Castillo J, Dávalos A, Chamorro A: Proinflammatory cytokines and early neurological worsening in ischemic stroke. Stroke 2000;31:2325–2329.

16 Rosenberg GA, Estrada EY, Dencoff JE: Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke 1998;29:2189–2195.

17 Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ: Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab 1999;19:624–633.

18 Chen J, Zhu RL, Nakayama M, Kawaguchi K, Jin K, Stetler RA, Simon RP, Graham SH: Expression of the apoptosis-effector gene, Bax, is upregulated in vulnerable hippocampal CA1 neurons following global ischemia. J Neurochem 1996;67:64–71.

19 Hara A, Iwai T, Niwa M, Uematsu T, Yoshimi N, Tanaka T, Mori H: Immunohistochemical detection of Bax and Bcl-2 proteins in gerbil hippocampus following transient forebrain ischemia. Brain Res 1996;711:249–253.

20 Sairanen TR, Lindsberg PJ, Brenner M, Siren AL: Global forebrain ischemia results in differential cellular expression of interleukin-1β and its receptor at mRNA and protein level. J Cereb Blood Flow Metab 1997;17:1107–1120.

21 Saito T, Nagao T, Okabe M, Saito K: Neurochemical and histochemical evidence for an abnormal catecholamine metabolism in the cerebral cortex of the Long-Evans cinnamon rat before excessive copper accumulation in the brain. Neurosci Lett 1996;216:195–198.

22 Nelson CW, Wei EP, Povlishock JT, Kontos HA, Moskowitz MA: Oxygen radicals in cerebral ischemia. Am J Physiol 1992;263:H1356–H1362.

23 Weisbrot-Lefkowitz M, Reuhl K, Perry B, Chan PH, Inouye M, Mirochnitchenko O: Overexpression of human glutathione peroxidase protects transgenic mice against focal cerebral ischemia/reperfusion damage. Brain Res Mol Brain Res 1998;53:333–338.

24 Clark WM, Rinker LG, Lessov NS, Lowery SL, Cipolla MJ: Efficacy of antioxidant therapies in transient focal ischemia in mice. Stroke 2001;32:1000–1004.

25 De La Cruz JP, Guerrero A, Gonzalez-Correa JA, Arrebola MM, de la Cuesta SF: Antioxidant effect of acetylsalicylic and salicylic acid in rat brain slices subjected to hypoxia. J Neurosci Res 2004;75:280–290.

26 Barone FC, Feuerstein GZ: Inflammatory mediators and stroke: new opportunities for novel therapeutics. J Cereb Blood Flow Metab 1999;19:819–834.

27 Weng MS, Ho YS, Lin JK: Chrysin induces G1 phase cell cycle arrest in C6 glioma cells through inducing p21Waf1/Cip1 expression: involvement of p38 mitogen-activated protein kinase. Biochem Pharmacol 2005;69:1815–1827.

28 Mori T, Okumura M, Matsuura M, Ueno K, Tokura S, Okamoto Y, Minami S, Fujinaga T: Effects of chitin and its derivatives on the proliferation and cytokine production of fibroblasts in vitro. Biomaterials 1997;18:947–951.

29 Jimenez LA, Thompson J, Brown DA, Rahman I, Antonicelli F, Duffin R, Drost EM, Hay RT, Donaldson K, MacNee W: Activation of NF-κB by PM(10) occurs via an iron-mediated mechanism in the absence of IκB degradation. Toxicol Appl Pharmacol 2000;166:101–110.

30 Seo SB, Jeong HJ, Chung HS, Lee JD, You YO, Kajiuchi T, Kim HM: Inhibitory effect of high molecular weight water-soluble chitosan on hypoxia-induced inflammatory cytokine production. Biol Pharm Bull 2003;26:717–721.

31 Blanco M, Moro MA, Davalos A, Leira R, Castellanos M, Serena J, Vivancos J, Rodriguez-Yanez M, Lizasoain I, Castillo J: Increased plasma levels of 15-deoxy-Δ-prostaglandin J2 are associated with good outcome in acute atherothrombotic ischemic stroke. Stroke 2005;36:1189–1194.

32 Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK: 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2 inhibits multiple steps in the NF-κB signaling pathway. Proc Natl Acad Sci USA 2000;97:4844–4849.

33 Caivano M, Rodriguez C, Cohen P, Alemany S: 15-Deoxy-$\Delta^{12,14}$-prostaglandin J2 regulates endogenous cot MAPK kinase kinase 1 activity induced by lipopolysaccharide. J Biol Chem 2003;278:52124–52130.

34 Inoue S, Drummond JC, Davis DP, Cole DJ, Patel PM: Combination of isoflurane and caspase inhibition reduces cerebral injury in rats subjected to focal cerebral ischemia. Anesthesiology 2004;101:75–81.

35 Yin D, Zhou C, Kusaka I, Calvert JW, Parent AD, Nanda A, Zhang JH: Inhibition of apoptosis by hyperbaric oxygen in a rat focal cerebral ischemic model. J Cereb Blood Flow Metab 2003;23:855–864.

36 Xu J, Culman J, Blume A, Brecht S, Gohlke P: Chronic treatment with a low dose of lithium protects the brain against ischemic injury by reducing apoptotic death. Stroke 2003;34:1287–1292.

37 Zhu S, Li M, Figueroa BE, Liu A, Stavrovskaya IG, Pasinelli P, Beal MF, Brown RH Jr, Kristal BS, Ferrante RJ, Friedlander RM: Prophylactic creatine administration mediates neuroprotection in cerebral ischemia in mice. J Neurosci 2004;24:5909–5912.

38 Kalka C, Masuda H, Takahashi T, et al: Transplantation of ex vivo expanded endothelial progenitor cells for therapeutic neovascularization. Proc Natl Acad Sci USA 2000;97:3422–3427.

39 Gehling UM, Ergun S, Schumacher U, et al: In vitro differentiation of endothelial cells from AC133-positive progenitor cells. Blood 2000;95:3106–3112.

40 Zhang ZG, Zhang L, Jiang Q, Chopp M: Bone marrow-derived endothelial progenitor cells participate in cerebral neovascularization after focal cerebral ischemia in the adult mouse. Circ Res 2002;90:284–288.

41 Fagan SC, Hess DC, Machado LS, Hohnadel EJ, Pollock DM, Ergul A: Tactics for vascular protection after acute ischemic stroke. Pharmacotherapy 2005;25:387–395.

42 Scandinavian Simvastatin Survival Study Group: Randomised trial of cholesterol lowering in 4,444 patients with coronary heart disease: the Scandinavian Simvastatin Survival Study (4S). Lancet 1994;344:1383–1389.

43 Blauw GJ, Lagaay AM, Smelt AHM, Westendorp RGJ: Stroke, statins, and cholesterol: a meta-analysis of randomized, placebo-controlled, double-blind trials with HMG-CoA reductase inhibitors. Stroke 1997;28:946–950.

44 Crouse JR III, Byington RP, Hoen HM, Furberg CD: Reductase inhibitor monotherapy and stroke prevention. Arch Intern Med 1997;157:1305–1310.

45 Crouse JR III, Byington RP, Bond MG, Espeland MA, Craven TE, Sprinkle JW, McGovern ME, Furberg CD: Pravastatin, lipids, and atherosclerosis in the carotid arteries (PLAC-II). Am J Cardiol 1995;75:455–459.

46 Vaughan CJ, Delanty N: Neuroprotective properties of statins in cerebral ischemia and stroke. Stroke 1999;30:1969–1973.

47 Ross R: Cell biology of atherosclerosis. Annu Rev Physiol 1995;57:791–804.

48 Samdani AF, Dawson TM, Dawson VL: Nitric oxide synthase in models of focal ischemia. Stroke 1997;28:1283–1288.

49 Forster C, Clark HB, Ross ME, Iadecola C: Inducible nitric oxide synthase expression in human cerebral infarcts. Acta Neuropathol 1999;97:215–220.

50 Garg UC, Hassid A: Nitric oxide-generating vasodilators and 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. J Clin Invest 1989;83:1774–1777.

51 Radomski MW, Palmer RM, Moncada S: Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 1987;ii:1057–1058.

52 Azuma H, Ishikawa M, Sekizaki S: Endothelium-dependent inhibition of platelet aggregation. Br J Pharmacol 1986;88:411–415.

53 Endres M, Laufs U, Huang Z, Nakamura T, Huang P, Moskowitz MA, Liao JK: Stroke protection by 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors mediated by endothelial nitric oxide synthase. Proc Natl Acad Sci USA 1998;95:8880–8885.

54 Vaughan CJ, Gotto AM Jr, Basson CT: The evolving role of statins in the management of atherosclerosis. J Am Coll Cardiol 2000;35:1–10.

55 Aikawa M, Rabkin E, Sugiyama S, Voglic SJ, Fukumoto Y, Furukawa Y, et al: An HMG-CoA reductase inhibitor, cerivastatin, suppresses growth of macrophages expressing matrix metalloproteinases, and tissue factor in vivo, and in vitro. Circulation 2001;103:276–283.

56 Kimura M, Kurose I, Russell J, Granger DN: Effects of fluvastatin on leukocyte-endothelial cell adhesion in hypercholesterolemic rats. Arterioscler Thromb Vasc Biol 1997;17:1521–1526.

57 Lehr HA, Seemuller J, Hubner C, Menger MD, Messmer K: Oxidized LDL-induced leukocyte/endothelium interaction in vivo involves the receptor for platelet activating factor. Arterioscler Thromb 1993;13:1013–1018.

58 Weber C, Erl W, Weber KSC, Weber PC: HMG-CoA reductase inhibitors decrease CD11b expression and CD11b-dependent adhesion of monocytes to endothelium and reduce increased adhesiveness of monocytes isolated from patients with hypercholesterolemia. J Am Coll Cardiol 1997;30:1212–1217.

59 Hussein O, Schlezinger SA, Rosenblat M, Keidar S, Aviram M: Reduced susceptibility of low density lipoprotein (LDL) to lipid peroxidation after fluvastatin therapy is associated with the hypocholesterolemic effect of the drug and its binding to LDL. Atherosclerosis 1997;128:11–18.

60 Chen L, Haught WH, Yang B, Saldeen TGP, Parthasarathy S, Mehta JL: Preservation of endogenous antioxidant activity and inhibition of lipid peroxidation as common mechanisms of antiatherosclerotic effects of vitamin E, lovastatin and amlodipine. J Am Coll Cardiol 1997;30:569–575.

61 Aviram M, Rosenblat M, Bisgaier CL, Newton RS: Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. Atherosclerosis 1998;138:271–280.

62 Human JA, Ubbink JB, Jerling JJ, Delport R, Vermaak WJH, Vorster HH, Lagendijk J, Potgieter HC: The effect of simvastatin on the plasma antioxidant concentrations in patients with hypercholesterolaemia. Clin Chem Acta 1997;263:67–77.

63 Chobanian AV, Dzau VJ: Renin angiotensin system and atherosclerosis vascular disease; in Fuster V, Ross R, Topol EJ (eds): Atherosclerosis and coronary artery disease, vol 1. Philadelphia, Lippincott-Raven, 1996, pp 237–242.

64 Gibbons GH, Pratt RE, Dzau VJ: Vascular smooth muscle cell hypertrophy vs. hyperplasia: autocrine transforming growth factor-β_1 expression determinates growth response to angiotensin II. J Clin Invest 1992;90:456–461.

65 Kranzhöfer R, Schmidt J, Pfeiffer CAH, Hagl S, Libby P, Kübler W: Angiotensin induces inflammatory activation of human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1999;19:1623–1629.

66 Hernandez-Presa M, Bustos C, Ortego M, Tuñon J, Renedo G, Ruiz-Ortega M, Egido J: Angiotensin-converting enzyme inhibition prevents arterial nuclear factor-κB activation, monocyte chemoattractant protein-1 expression, and macrophage infiltration in a rabbit model of early accelerated atherosclerosis. Circulation 1997;95:1532–1541.

67 Tummala PE, Chen XL, Sundell CL, Laursen JB, Hammes CP, Alexander RW, Harrison DG, Medford RM: Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: a potential link between the renin-angiotensin system and atherosclerosis. Circulation 1999;100:1223–1229.

68 Griendling KK, Sorescu D, Lassegue B, Ushio-Fukai M: Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arterioscler Thromb Vasc Biol 2000;20:2175–2183.

69 Cai H, Griendling KK, Harrison DG: The vascular NAD(P)H oxidases as therapeutic targets in cardiovascular diseases. Trends Pharmacol Sci 2003;24:471–478.

70 Forder JP, Munzenmaier DH, Greene AS: Angiogenic protection from focal ischemia with angiotensin II type 1 receptor blockade in the rat. Am J Physiol 2005;288:H1989–H1996.

71 Schmieder RE: Mechanisms for the clinical benefits of angiotensin II receptor blockers. Am J Hypertens 2005;18:720–730.

72 Dohi Y, Ohashi M, Sugiyama M, Takase H, Sato K, Ueda R: Candesartan reduces oxidative stress and inflammation in patients with essential hypertension. Hypertens Res 2003;26:691–697.

73 Zuliani G, Cherubini A, Volpato S, Atti AR, Ble A, Vavalle C, Di Todaro F, Benedetti C, Ruggiero C, Senin U, Fellin R: Treatment with angiotensin-converting enzyme inhibitors is associated with a reduction in short-term mortality in older patients with acute ischemic stroke. J Gerontol A Biol Sci Med Sci 2005;60:463–465.

74 PROGRESS Collaborative Group: Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. Lancet 2001;358:1033–1041.

75 Ferrari R, Pasanisi G, Notarstefano P, Campo G, Gardini E, Ceconi C: Specific properties and effect of perindopril in controlling the renin-angiotensin system. Am J Hypertens 2005;18(suppl):142–154.

76 Sierra C, de la Sierra A: Antihypertensive, cardiovascular, and pleiotropic effects of angiotensin-receptor blockers. Curr Opin Nephrol Hypertens 2005;14:435–441.

77 Masuda S, Okano M, Yamagishi K, Nagao M, Ueda M, Sasaki R: A novel site of erythropoietin production: oxygen-dependent production in cultured rat astrocytes. J Biol Chem 1994;269:19488–19493.

78 Bernaudin M, Marti HH, Roussel S, Divoux D, Nouvelot A, MacKenzie ET, Petit E: A potential role for erythropoietin in focal permanent cerebral ischemia in mice. J Cereb Blood Flow Metab 1999;19:643–651.

79 Sakanaka M, Wen TC, Matsuda S, Masuda S, Morishita E, Nagao M, Sasaki R: In vivo evidence that erythropoietin protects neurons from ischemic damage. Proc Natl Acad Sc USA 1998;95:4635–4640.

80 Maiese K, Li F, Chong ZZ: New avenues of exploration for erythropoietin. JAMA 2005;293:90–95.

81 Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J: A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. Proc Natl Acad Sci USA 1999;96:13496–13500.

82 Tikka TM, Koistinaho JE: Minocycline provides neuroprotection against N-methyl-*D*-aspartate neurotoxicity by inhibiting microglia. J Immunol 2001;166:7527–7533.

83 Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, Ferrante RJ, Kristal BS, Friedlander RM: Minocycline inhibits caspase-independent and -dependent mitochondrial cell death pathways in models of Huntington's disease. Proc Natl Acad Sci USA 2003;100:10483–10487.

84 Zemke D, Majid A: The potential of minocycline for neuroprotection in human neurologic disease. Clin Neuropharmacol 2004;27:293–298.

85 Morimoto N, Shimazawa M, Yamashima T, Nagai H, Hara H: Minocycline inhibits oxidative stress and decreases in vitro and in vivo ischemic neuronal damage. Brain Res 2005;1044:8–15.

86 Koistinaho M, Malm TM, Kettunen MI, Goldsteins G, Starckx S, Kauppinen RA, Opdenakker G, Koistinaho J: Minocycline protects against permanent cerebral ischemia in wild type but not in matrix metalloprotease-9-deficient mice. J Cereb Blood Flow Metab 2005;25:460–467.

87 Nolan JJ, Ludvik B, Beerdsen P, Joyce M, Olefsky J: Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med 1994;331:1188–1193.

88 Jiang C, Ting AT, Seed B: PPAR-γ agonist inhibit production of inflammatory cytokines. Nature 1998;391:82–86.

89 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK: The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. Nature 1998;391:79–82.

90 Parulkar AA, Pendergrass ML, Granda-Ayala R, Lee TR, Fonseca VA: Non-hypoglycemic effects of thiazolidinediones. Ann Intern Med 2001;134:61–71.

91 Yoshimoto T, Naruse M, Shizume H, Naruse K, Tanabe A, Tanaka M, Tago K, Iries K, Muraki T, Demura H, Zardi L: Vasculo-protective effects of insulin sensitizing agent pioglitazone in neointimal thickening and hypertensive vascular hypertrophy. Atherosclerosis 1999;145:333–340.

92 Santa-Cruz RA, Steinhubl SR: Clopidogrel: how good is it and how does it work? Curr Cardiol Rep 2004;6:264–268.

93 Molero L, Lopez-Farre A, Mateos-Caceres PJ, Fernandez-Sanchez R, Luisa Maestro M, Silva J, Rodriguez E, Macaya C: Effect of clopidogrel on the expression of inflammatory markers in rabbit ischemic coronary artery. Br J Pharmacol 2005;146:419–424.

94 Chamorro A: Role of inflammation in stroke and atherothrombosis. Cerebrovasc Dis 2004;17(suppl 3):1–5.

95 Adibhatla RM, Hatcher JF: Citicoline decreases phospholipase A_2 stimulation and hydroxyl radical generation in transient cerebral ischemia. J Neurosci Res 2003;73:308–315.

96 Hurtado O, Moro MA, Cardenas A, Sanchez V, Fernandez-Tome P, Leza JC, Lorenzo P, Secades JJ, Lozano R, Davalos A, Castillo J, Lizasoain I: Neuroprotection afforded by prior citicoline administration in experimental brain ischemia: effects on glutamate transport. Neurobiol Dis 2005;18:336–345.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):30–37
DOI: 10.1159/000091701

Published online: May 2, 2006

Gene Expression in Cerebral Ischemia: A New Approach for Neuroprotection

Mónica Millán Juan Arenillas

Neurology Service, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

Key Words
Gene expression · Cerebral ischemia · Apoptosis · Neuroprotection · Angiogenesis

Abstract
Cerebral ischemia is one of the strongest stimuli for gene induction in the brain. Hundreds of genes have been found to be induced by brain ischemia. Many genes are involved in neurodestructive functions such as excitotoxicity, inflammatory response and neuronal apoptosis. However, cerebral ischemia is also a powerful reformatting and reprogramming stimulus for the brain through neuroprotective gene expression. Several genes may participate in both cellular responses. Thus, isolation of candidate genes for neuroprotection strategies and interpretation of expression changes have been proven difficult. Nevertheless, many studies are being carried out to improve the knowledge of the gene activation and protein expression following ischemic stroke, as well as in the development of new therapies that modify biochemical, molecular and genetic changes underlying cerebral ischemia. Owing to the complexity of the process involving numerous critical genes expressed differentially in time, space and concentration, ongoing therapeutic efforts should be based on multiple interventions at different levels. By modification of the acute gene expression induced by ischemia or the apoptotic gene program, gene therapy is a promising treatment but is still in a very experimental phase. Some hurdles will have to be overcome before these therapies can be introduced into human clinical stroke trials.

Introduction

In the last decade, important investigative work has been undertaken with notable repercussions of the knowledge of pathophysiology, diagnosis and treatment of cerebrovascular disease [1]. One of the most complex investigation areas but with more significant advances in recent years is the study and identification of genes, their protein products, their functions and their implications in the pathophysiology of cerebral ischemia [2, 3]. Molecular biology techniques and more recently DNA microarray analyses have allowed the development of many experimental studies in vitro or in animal models of cerebral ischemia. However, there are limited works after stroke in humans. These studies have contributed interesting data in the understanding of genetic regulation and protein expression following ischemic stroke [2, 3].

Cerebral ischemia is a powerful stimulus for gene regulation [4–8]. It is responsible for activation of genes involved as much in cell death as in cell recovery (fig. 1). Some genes are involved in excitotoxicity, cell depolarization, post-ischemic inflammatory alterations and

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0030$23.50/0

Accessible online at:
www.karger.com/ced

Mónica Millán
Neurology Service, Hospital Universitari Germans Trias i Pujol
Carretera del Canyet, s/n
ES–08916 Badalona (Spain)
Tel. +34 93 497 8911, Fax +34 93 497 8742, E-Mail mmillan.germanstrias@gencat.net

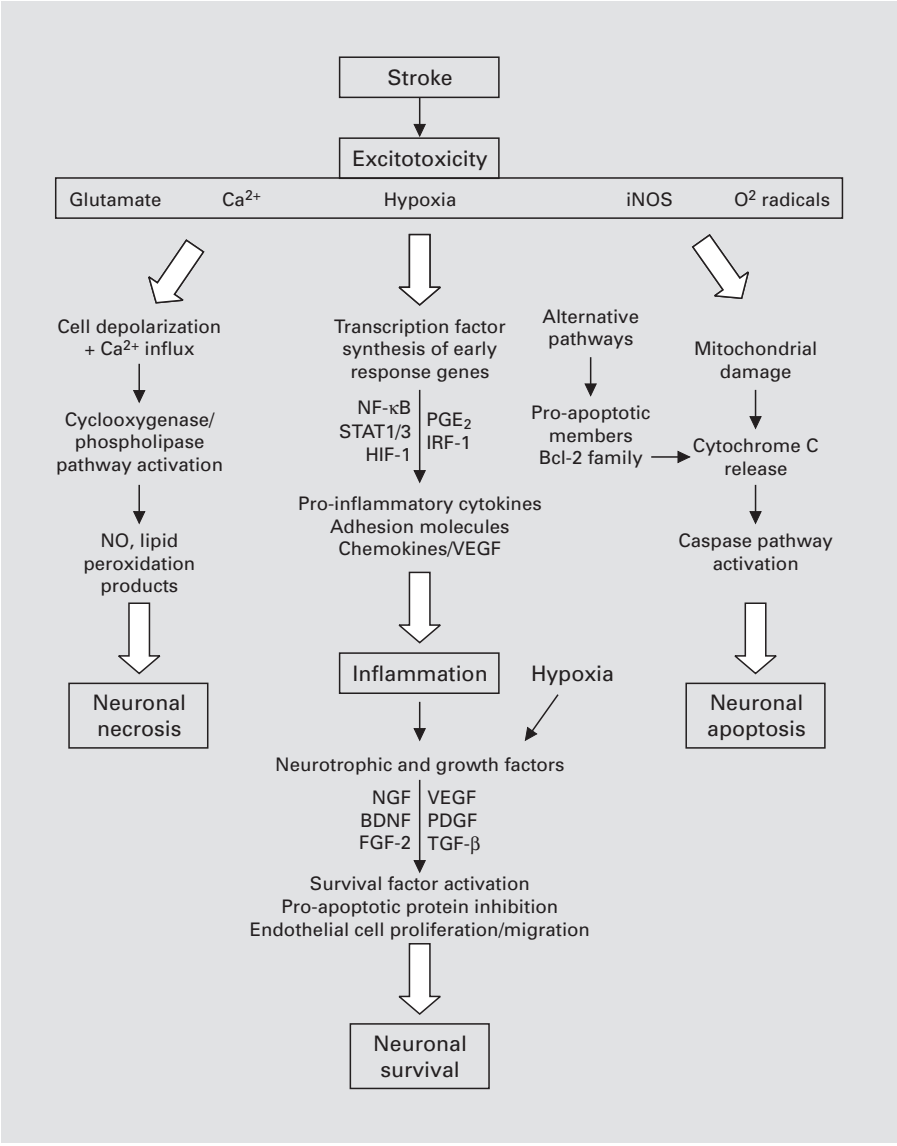


Fig. 1. Gene activation and protein expression following ischemic stroke. Cerebral ischemia is a potent modulator of gene expression. Some genes are involved in cell death, but others are neuroprotective and important for recovery. iNOS = Inducible nitric oxide synthase; NO = nitric oxide; NF-κB = nuclear factor-κB; STAT1/3 = signal transducers and activators of transcription; HIF-1 = hypoxia-inducible factor; PGE_2 = prostaglandin E_2; IRF-1 = insulin-responsive factor-1; VEGF = vascular endothelial cell growth factor; NGF = nerve growth factor; BDNF = brain-derived neurotrophic factor; FGF-2 = fibroblast growth factor; PDGF = platelet-derived growth factor; TGF-β = transforming growth factor-β.

apoptosis resulting in final tissue damage, while other genes exercise neuroprotective functions through neurotrophic and angiogenic factor expression [4–8]. A time course of gene response with many molecular interactions during acute and delayed phases of ischemic brain injury has been demonstrated [7, 8] (table 1). Therefore, it is a complex process to isolate target genes for a neuroprotection strategy which requires important efforts, especially when several genes may participate in both cellular responses.

Simultaneously, new treatments that modify biochemical, molecular and genetic changes that take place during cerebral ischemia to minimize brain injury and to improve thrombolytic treatment effectiveness have been investigated [9]. These therapies have their application especially in the area of ischemic penumbra, in which inactive but even viable neurons exist if cerebral blood flow is recovered [10, 11]. Although there seem to exist significant differences among the gene expression of animals and humans [7], and despite the fact that many drugs have been demonstrated to interfere with the biochemical changes associated to cerebral ischemia in experimental models but not clinical efficacy in humans [12], the future denounces good perspectives in ischemic stroke treatment through a better understanding of underlying pathogenesis, identification of therapeutic genes, development

Table 1. Gene category expression and regulation induced by cerebral ischemia

Gene category	Regulation	Time
Immediate early genes	Upregulated	30 min
Transcription factors	Upregulated	30 min
Heat shock proteins	Upregulated	30 min–24 h
Inflammation	Upregulated	4–24 h
Cytoskeletal structure	Upregulated	4–24 h
Metabolism	Upregulated	4–24 h
Apoptosis	Upregulated	8–24 h
Growth factors	Upregulated	24 h–7 days
Protein kinases	Up and down	
Ion channel genes	Downregulated	8–24 h
Neurotransmitter receptor genes	Downregulated	8–24 h
Synaptic protein genes	Downregulated	3–7 days

Time = Peak increase of overexpression or downexpression.

of appropriate experimental models to improve therapies and in the application of gene therapy beyond an experimental phase.

Excitotoxicity

Immediately after an arterial occlusion, the affected cerebral area is hypoxic and hypoglycemic. Within a few minutes, ATP deficiency originates from a failure of the Na^+ and K^+ pumps with a rapid decrease of intracellular K^+ followed by a neuronal depolarization. This causes the opening of the Ca^{2+} channels increasing intracellular calcium concentration and leading to a large cellular membrane depolarization [4, 7]. Next, a rapid release of glutamate takes place from the presynaptic nerve terminals and astrocytes [13]. Glutamate stimulates N-methyl-*D*-aspartate (NMDA) receptors and metabotropic receptors. Excitotoxicity for stimulation of the NMDA receptors causes an intense cellular depolarization with an influx of Ca^{2+} and Na^+, and an efflux of Cl^- and water resulting in edema and membrane failure that ends in the Ca^{2+}-dependent ischemic cascade that will lead to neuronal necrosis [4, 7, 13]. Three gene products of the sodium-calcium exchanger NCX family (NCX1, NCX2, NCX3) couple the movement of these ions across the cell membrane. In an experimental study it has been demonstrated that NCX gene expression is regulated after ischemia in a differential manner, depending on the exchanger isoform and region involved in the insult. In the ischemic core all three NCX were downregulated, whereas in the peri-infarct area NCX2 was downregulated but NCX3 was significantly upregulated and in non-ischemic brain regions, both NCX1 and NCX3 were upregulated [14]. These data may provide a better understanding of each NCX role to design appropriate pharmacological strategies to reduce excitotoxicity. Besides glutamate, other neurotransmitters like glycine and γ-aminobutyric acid (GABA) also appear in the extracellular space during brain ischemia [15]. Glycine acts as a coactivator of NMDA receptor and causes more neuronal damage. Synthesis of GABA (inhibitor neurotransmitter) is increased during cerebral ischemia due to glutamate increment, increased activity of the glutamate descarboxylase (GAD) that synthesizes GABA and inhibition of GABA-transaminase (GABA-T) that removes GABA [15, 16]. Increment of GABA activity during brain ischemia has been shown to be neuroprotective in experimental studies [15].

An increase of intracellular Ca^{2+} mediates immediate early gene expression such as c-fos or c-jun and heat shock proteins (HSP) and induces enzymatic activation of several intracellular metabolic pathways as cyclooxygenase-2 (COX-2), phospholipase C/A_2, nitric oxide synthase (NOS), among others [7, 16] (fig. 1). Cyclooxygenase activation causes nitric oxide release, while phospholipase activates mitogen-activated protein (MAP) kinase and lipolysis, increasing both the expression of lipid peroxidation products [7, 16]. During cerebral ischemia, formation of reactive oxygen species (ROS) can exceed the antioxidative capacity of neurons and generate an oxidative stress. The main ROS in neurons are oxygen free radicals, such as superoxide anion (O_2^-), nitric oxide (NO) and hydroxyl radical (^-OH), among others. However, O_2^- is the one that generates oxidative stress in ischemic brain [7, 16]. Recently, the presence of ROS produced by an increased expression of NOX4 (NADPH oxidase isoform) has been demonstrated in cortical neurons. NOX4 could exert a role in the angiogenesis induced by ischemia/hypoxia since it is overexpressed in newly formed capillaries [17]. Ischemic brain also originates from a high production of NO which it is synthesized from *L*-arginine through NOS [18]. The effect of NO increase can be neurotoxic or neuroprotective according to the NOS isoform starting from which is synthesized. Stimulation of the NMDA receptors is largely mediated by NO formation [18].

Multiple pharmacological interventions to reduce excitotoxicity have been evaluated in clinical trials. Glutamate release inhibitors, NMDA receptor antagonists, calcium channel blockers or oxygen free radical antagonists

have been assessed. Most of them have not obtained positive results [12]. Potential reasons are delayed drug administration (excitotoxicity begins within minutes) and a harmful effect on neuronal survival due to the blockade of normal synaptic transmission [19]. In spite of this, new therapies are being investigated that act especially by blocking ion channels, stimulating HSP expression that has been shown to be neuroprotective, or modulating the expression of glutamate transporters. Recently, it has been demonstrated that treatment with the sodium channel blocker RS100642 during the first 6 h of the middle cerebral artery occlusion (MCAO) was able to selectively reverse downregulation of the sodium channel gene Na_v 1.1 to normalize electric brain activity and reduce infarct size [20]. Also an inducer of HSP-70 expression, geranylgeranylacetone showed in an animal model of MCAO that it significantly reduces infarct volume when administered very early [21]. Finally, β-lactam antibiotics seem to offer neuroprotection by increasing glutamate transporter GLT1 (or EAAT2) expression. In short, ceftriaxone has been demonstrated to be a potent stimulator of GLT1 expression in brain of animals, as well as its biochemical and functional activity [22].

Inflammatory Response

Intracellular Ca^{2+}, oxygen free radicals, inducible NOS (iNOS), hypoxia and glutamate act by activating the inflammatory process in damaged neurons, astrocytes, microglia, endothelial cells, leukocytes and other immune cells in ischemic tissue. A rapid induction of transcription factors takes place a few hours after ischemic insult, resulting in an increased expression of inflammatory cytokines and chemokines [6–8].

Nuclear factor-κB (NF-κB) activates tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β starting the inflammatory response. Subsequently, these interleukins induce other cytokines such as IL-1α, IL-6 and IL-8 [23–25]. Interleukins play an important role in the development of acute phase reactants and in the release of a group of molecules that maintain a more persistent inflammatory response. Hypoxia inducible factor 1 (HIF-1) induces vascular endothelial cell-derived growth factor (VEGF) that increases blood-brain barrier (BBB) leakage and secondary brain edema. Interferon regulatory factor-1 (IRF-1) stimulates production of γ-interferon which stimulates macrophages [7, 8]. Transcription factors STAT1 and STAT3 cause an overexpression of platelet-activating factor (PAF), monocyte chemoattractant protein-1 (MPC-1) and intercellular adhesion molecule (ICAM-1) [25]. PGE_2 produced by the cyclooxygenase pathway and lipolysis induces inflammation through upregulation of TNF-α and IL-6 [7, 8] (fig. 1).

On the other hand, inflammatory cytokines induce expression of adhesion molecules such as ICAM-1, platelet endothelial cell adhesion molecule (PECAM-1) and endothelial cell leukocyte adhesion molecule (ELAM-1) on endothelial cell surface [23–25]. There is a peak increase in mRNA expression of inflammation mediators 4–24 h post-injury, which is the critical time window of the maturation of ischemic injury [8]. These genes are involved in peripheral inflammatory cell recruitment into the brain through migration and agglutination of neutrophils, macrophages and monocytes to brain tissue. Later on, these cells are added and adhered to the arterial wall and contribute to brain injury by microvascular obstruction and by producing neurotoxic mediators like ROS and NO [6–8]. Matrix metalloproteinases (MMP), in charge of extracellular matrix remodeling, also intervene in tissue damage following cerebral ischemia. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are proteolytic enzymes that produce BBB leakage and secondary brain edema, and facilitate hemorrhagic transformation of cerebral infarct [26–28]. Cytokines IL-6 and TNF-α stimulate MMP-9 production [7].

Therapeutic strategies used to reduce the inflammatory response can have a longer time window before they can be effective. However, inflammation decrease can exert deleterious effects in terms of tissue repair, since it diminishes neurotrophic factor expression [7, 8] (fig. 1). NF-κB plays a key role in starting inflammatory changes. A potential neuroprotection strategy is to inhibit the upregulation of a family of delayed cell death genes, for example by inhibition of NF-κB activation with the proteasome inhibitor MLN519. This has shown to effectively attenuate upregulation of several inflammatory genes (IL-1, IL-6, TNF-α, ICAM-1), reduce neutrophil and macrophage infiltration, and consequently decrease infarction with delayed administration up to 6 h after transient MCAO in rats [29]. Another therapeutic alternative is based on the specific inhibition of the expression of adhesion molecules or cytokines. Inhibition of ICAM-1 expression by antisense oligonucleotides and inhibition of TNF-α-converting enzyme by the selective antagonist DPH-067517 have demonstrated a neuroprotective effect by reduction of infarct size and neurological deficits [30, 31].

Apoptosis Induction

Mechanisms of the cellular death by apoptosis are not fully elucidated. Excitotoxicity and excessive production of ROS cause disruption of the internal mitochondrial membrane and activation of transcription factors including NF-κB and the activating transcription factor-2 (ATF-2) [32–34]. ATF-2 induces expression of pro-apoptotic members of the Bcl-2 family (Bax, Bad and Bid) and their translocation towards the external mitochondrial membrane forming channels that allow the cytochrome c release from the mitochondrial intermembrane space. Release of cytochrome c is the main reason for apoptosis associated to mitochondria [32–34] (fig. 1). Cytochrome c induces oligomerization of an activator factor of apoptosis, the APAF-1 (apoptosis-activating factor-1) that binds later with pro-caspase-9. These cause activation of caspase-9 and caspase-3 that finally binds to ADP-ribose polymerase (PARP) and subsequently neuronal apoptosis begins [32–34]. Increased expression of pro-caspases 1, 2, 3, 6 and 8, and adhesion of caspase-3 were demonstrated in ischemic penumbra neurons between 12 and 24 h after MCAO in an experimental rat model [34].

Other components of the excitotoxic-inflammatory cascade, such as hypoxia, intracellular Ca^{2+}, overexpression of pro-inflammatory cytokines and glutamate increase, can promote expression of several transcription factors that finally lead to mitochondrial cytochrome c release through activation of pro-apoptotic members of the Bcl-2 family [7]. Hypoxia activates HIF-1 and p53; increment of Ca^{2+} activates calpain and p53 [35]; the release of pro-inflammatory cytokines such as TNF-α and IL-1 stimulates the caspase pathway through TNF-R, FAS and FADD expression, or the MAP kinase pathway through c-Jun N-terminal kinase (JNK) and p38 MAP kinase expression [36], and finally, glutamate induces p38 activation by means of the transcription factor ATF-2, growth arrest and DNA damage-inducible gene 153 and CHOP-19 [7]. Although not very well known, other pathways seem to exist where non-caspase components could be involved in regulation of neuronal apoptosis induced by ischemic stroke. However, the MAP kinase pathway operating through JNK and p38 seems to be a factor strongly associated to apoptosis [7, 36]. Thus, JNK inhibition has been demonstrated in an animal model that protects it from neuronal death. A new JNK inhibitor, SP600125, significantly diminished activation of a nuclear substrate (c-jun) and inactivated a non-nuclear substrate (Bcl-2) induced by ischemic insult, resulting in a potential new and effective strategy to treat ischemic stroke [37]. On the other hand, the protein transfer of Bcl-xl derived from HIV-1 was effectively delivered across the BBB and significantly reduced infarct size and caspase activation in a mouse model of stroke [38]. Caspase-3 expression and associated neuronal apoptosis were also notably reduced in the penumbra region in the presence of nucleoside citicoline [39].

Neuroprotective Mechanisms: Cellular Survival, Revascularization and Tissue Reperfusion

Neurons, leukocytes and microglia synthesize and release several neurotrophic factors after acute ischemic stroke in an attempt to neutralize the detrimental effects of excitotoxicity and inflammation (fig. 1). Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) activate a signal transduction pathway including phosphorylinositol 3-kinase (PI3K) and Akt, inhibitors of pro-apoptotic p53 and Bad [40]. Neurotrophic factors NGF and BDNF also stimulate phospholipase C (PLC) and protein kinase C (PKC) pathways, which activate survival pathways that involve NF-κB and anti-apoptotic members of the Bcl-2 family [40]. Other growth factors such as basic fibroblast growth factor (FGF-2) and vascular endothelial cell growth factor (VEGF) activate the MAP kinase pathway (ERK1/2) through PLC inhibiting pro-apoptotic effects of JNK and p38 and stimulating anti-apoptotic proteins production such as Bcl-2, AMPc-binding protein (CREB) and NF-κB [41, 42].

In the ischemic penumbra tissue there exists an increased expression of growth factors as VEGF, FGF-2, platelet-derived growth factor (PDGF-β) and transforming growth factor-β (TGF-β) associated with an increment of angiogenesis [43]. The revascularization process after MCAO has been described in an experimental rat model [44]. Data suggest that newly formed vessels (microvessels) form regular connections in the first week of cerebral ischemia, with a similar pattern to those of the normal brain [44]. Macrophages, leukocytes and damaged platelets secrete large quantities of angiogenic growth factors [43, 45]. Overexpression of angiogenic factors such as VEGF or FGF-2 in endothelial cells takes place through activation of ERK1/2, p38 and JNK MAP kinases in response to hypoxia. Cytokines TNF-α and IL-1 also induce mRNA transcription of these growth factors and stimulate angiopoietin-1 expression that can intervene in cell survival and cell migration [43]. Therefore, most angiogenic factors will regulate proliferation and

migration of endothelial cells and proliferation of smooth muscle cells, which also have an important role in the revascularization process [43, 45].

VEGF is the angiogenic factor with more influence on new blood vessel growth after cerebral ischemia. Endogenous neuronal VEGF increases within hours in the ischemic brain and plays a neuroprotective role in the pathophysiologic processes that follow stroke. Exogenous VEGF, directly administered or overexpressed by gene delivery into rat brains, reduces ischemic brain infarct and decreases hypoxic neuronal death. The main neuroprotective mechanisms of VEGF include modulation of the PI3K-Akt-NF-κB signaling pathway and inhibition of caspase-3 activity resulting in reduction of ischemic neuronal apoptosis; inhibition of potassium channel currents by an increase of tyrosine phosphorylation via activation of the PI3K pathway, and finally, enhancement of proliferation and migration of neural progenitors in the subventricular zone and improvement of striatal neurogenesis and maturation of newborn neurons in striatum [46, 47].

Therefore, selective upregulation of p38, MAP kinase ERK1/2 and JNK MAP kinases proteins, and VEGF, FGF-2, PDGF-B and TGF-B growth factors could have a critical role in the neuronal survival and revascularization process after cerebral ischemia. Preclinical studies have demonstrated that gene transfection with viral vectors of FGF-2 or hepatocyte growth factor reduces infarct size and improves neurological deficit [48–50]. Administration of neurotrophic factors as neuregulins have also shown a neuroprotective effect in rat. Treatment with neuregulin-1 (NRG-1) diminished expression of most of genes (HSP-70, IL-1β and MCP-1) in at least 50% in comparison with a control group with reduction of neuronal death. In in vitro studies, NRG-1 suppressed expression of inflammatory genes in activated macrophages [51].

Genetic Therapy

Gene transfer is a potential therapy of ischemic vascular disease. Viral (retro-, adeno- and herpesvirus) and non-viral delivery vehicles are currently being used in animal models and in gene therapy clinical trials [52]. Although purification of viral vectors may reduce the pathogenic properties or the host immune response, more progress is needed to ensure greater confidence before their use in clinical practice. However, liposomes containing the therapeutic DNA are non-pathogenic but the transfection efficiencies are quite low [52]. Another interesting aspect is the correct way to transfer the therapeutic genes and vectors into the appropriate tissue (penumbra, blood vessel, endothelial cell, etc.). Gene therapy is possible in acute stroke by modulating the excitotoxic and apoptotic elements of neuron death and stimulating angiogenesis [53, 54]. In animal models it has achieved a neuroprotective effect with viral vectors by modulating excitatory amino acids, reducing cytosolic calcium, reducing inflammation and increasing HSP, anti-apoptotic genes or angiogenic factors. Most animal studies have demonstrated efficacy of gene transfer in reducing infarct size, but in these studies vectors were introduced prior to experimental ischemia [53, 54]. Expression of genes in ischemic or reperfused tissue is blunted and delayed since transcriptional and translational processes are inhibited by ischemia. Thus, injection of a gene transfer vector must be in the penumbra area where transcriptional and translational processes are diminished but not inhibited [55].

Although gene therapy continues to progress, many obstacles still exist. It will be necessary to develop safer and more effective vectors, improve vector delivery, achieve very early transfection in acute ischemia and, finally, a much better understanding of which genes should be delivered in stroke gene therapy.

In summary, different physiopathological processes following acute ischemic stroke suggest that their treatment could improve through modulation of gene transcription and protein activation, especially in the ischemic penumbra around to the brain infarct. Owing to the complexity of the process and the fact that several genes encoding transcription factors may participate in both cellular responses (beneficial and harmful), a therapeutic effort should be based on multiple therapies with action taken at different levels and times. An immediate reduction of excitotoxicity within a few minutes, and an inflammatory response within a few hours, a better control of tissue reperfusion around the ischemic area with concomitant administration of neurotrophic and angiogenic factors to maintain neuronal viability and stimulate angiogenesis, and a bigger blockade of apoptotic cellular death, for example with viral transfection of anti-apoptotic genes or JNK or caspase inhibition, would allow to improve neuronal survival and the patient's outcome.

References

1 Hachinski V: Stroke: the next 30 years. Stroke 2002;33:1–4.
2 King HC, Sinha AA: Gene expression profile analysis by DNA microarrays. JAMA 2001; 286:2280–2288.
3 Read SJ, Parsons AA, Harrison DC, Philpott K, Kabnick K, O'Brien S, et al: Stroke genomics: approaches to identify, validate, and understand ischemic stroke gene expression. J Cereb Blood Flow Metab 2001;21:755–778.
4 Pulsinelli W: Pathophysiology of acute ischemic stroke. Lancet 1992;339:533–536.
5 Akins PT, Liu PK, Hsu CY: Immediate early gene expression in response to cerebral ischemia: friend or foe? Stroke 1996;27:1682–1687.
6 Ginsberg MD: Adventures in the pathophysiology of brain ischemia: penumbra, gene expression, neuroprotection. The 2002 Thomas Willis Lecture. Stroke 2003;34:214–223.
7 Slevin M, Krupinski J, Kumar P, Gaffney J, Kumar S: Gene activation and protein expression following ischaemic stroke: strategies towards neuroprotection. J Cell Mol Med 2005; 9:85–102.
8 May Lu XC, Williams AJ, Yao C, Berti R, Hartings JA, Whipple R, et al: Microarray analysis of acute and delayed gene expression profile in rats after focal ischemic brain injury and reperfusion. J Neurosci Res 2004;77:843–857.
9 Hong H, Liu GC: Current status and perspectives on the development of neuroprotectants for ischemic vascular disease. Drugs Today (Barc) 2003;39:213–222.
10 Heiss WD, Graf R: The ischemic penumbra. Curr Opin Neurol 1994;7:11–19.
11 Ginsberg MD: Injury mechanisms in the ischemic penumbra. Approaches to neuroprotection in acute ischemic stroke. Cerebrovasc Dis 1997;7(suppl 2):7–12.
12 Legos JJ, Tuma RF, Barone FC: Pharmacological interventions for stroke: failures and future. Expert Opin Invest Drugs 2002;11:603–614.
13 Rossi DJ, Oshima T, Atwell D: Glutamate release in severe brain ischemia is mainly by reserved uptake. Nature 2000;403:316–321.
14 Boscia F, Gala R, Pignataro G, de Bartolomeis A, Cicale M, Ambesi-Impiombato A, et al: Permanent focal brain ischemia induces isoform-dependent changes in the pattern of Na^+/Ca^{2+} exchanger gene expression in the ischemic core, periinfarct area, an intact brain regions. J Cereb Blood Flow Metab 2005 (Epub ahead of print).
15 Schwartz-Bloom RD, Sah R: Gamma-aminobutyric acid A neurotransmission and cerebral ischemia. J Neurochem 2001;77:353–371.
16 Castillo J, Rodríguez I: Biochemical changes and inflammatory response as markers of brain ischaemia: molecular markers of diagnostic utility and prognosis in human practice. Cerebrovasc Dis 2004;17:7–18.
17 Vallet P, Charnay Y, Steger K, Ogier-Denis E, Kovari E, Herrmann F, et al: Neuronal expression of the NADPH oxidase NOX4, and its regulation in mouse experimental brain ischemia. Neuroscience 2005;132:233–238.
18 Castillo J, Rama R, Dávalos A: Nitric oxide-related brain damage in acute ischemic stroke. Stroke 2000;31:852–857.
19 Ikonomidou C, Turski L: Why did NMDA receptor antagonists fail clinical trials for stroke and traumatic brain injury? Lancet Neurol 2002;i:383–386.
20 Yao C, Williams AJ, Lu XC, Price RA, Cunningham BS, Berti R, et al: The sodium channel blocker RS100642 reverses down-regulation of the sodium channel α-subunit Na(v) 1.1 expression caused by transient ischemic brain injury in rats. Neurotox Res 2003;5:245–254.
21 Yasuda H, Shichinohe H, Kuroda S, Ishikawa T, Iwasaki Y: Neuroprotective effect of a heat shock protein inducer, geranylgeranylacetone, in permanent focal cerebral ischemia. Brain Res 2005;1032:176–182.
22 Rothstein JD, Pastel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, et al: Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 2005;433:73–77.
23 Vila N, Castillo J, Dávalos A, Chamorro A: Proinflammatory cytokines and early stroke progression. Stroke 2000;31:2325–2329.
24 Iadecola C, Alexander M: Cerebral ischemia and inflammation. Curr Opin Neurol 2001;14: 89–94.
25 Danton GH, Dietrich WD: Inflammatory mechanisms after ischemia and stroke. J Neuropath Exp Neurol 2003;62:127–136.
26 Takagi Y, Harada J, Chiarugi A, Moskowitz MA: STAT1 is activated in neurons after ischaemia and contributes to ischaemic brain injury. J Cereb Blood Flow Metab 2002;22: 1311–1318.
27 Castellanos M, Leira R, Serena J, Pumar JM, Lizasoain I, Castillo J, et al: Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. Stroke 2003;34:40–46.
28 Montaner J, Alvarez-Sabín J, Molina CA, Anglés A, Abilleira S, Arenillas JF, et al: Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 2001; 32:1759–1766.
29 Berti R, Williams AJ, Velarde LC, Moffett JR, Elliot PJ, Adams J, et al: Effect of the proteasome inhibitor MLN519 on the expression of inflammatory molecules following middle cerebral artery occlusion and reperfusion in rat. Neurotox Res 2002;5:505–514.
30 Vemuganti R, Dempsey RJ, Bowen KK: Inhibition of intercellular adhesion molecule-1 protein expression by antisense oligonucleotides is neuroprotective after transient middle cerebral artery occlusion in rat. Stroke 2004; 35:179–184.
31 Wang X, Feuerstein GZ, Xu L, Wang H, Schumacher WA, Ogletree ML: Inhibition of tumor necrosis factor-α-converting enzyme by a selective antagonist protects brain from focal ischemic injury in rats. Mol Pharmacol 2004; 65:890–896.
32 Choi DW: Excitotoxicity, apoptosis and ischemic stroke. J Biochem Mol Biol 2001;34:8–14.
33 MacManus JP, Linnik MD: Gene expression induced by cerebral ischemia: an apoptotic perspective. J Cereb Blood Flow Metab 1997; 17:815–852.
34 Ferrer I, Planas AM: Signaling of cell death and cell survival following focal cerebral ischaemia: life and death struggle in the penumbra. J Neuropath Exp Neurol 2003;62:329–339.
35 Sedarous M, Keramaris E, O'Hare M: Calpains mediate p53 activation and neuronal death evoked by DNA damage. J Biol Chem 2003; 278:26031–26038.
36 Harper SJ, LoGrasso P: Signalling for survival and death in neurones. The role of the stress-activated kinases, JNK and p38. Cell Signal 2001;13:299–310.
37 Guan QH, Pei DS, Zhang QG, Hao ZB, Xu TL, Zhang GY: The neuroprotective action of SP600125, a new inhibitor of JNK, on transient brain ischemia/reperfusion-induced neuronal death in rat hippocampal CA1 via nuclear and non-nuclear pathways. Brain Res 2005; 1035:51–59.
38 Cao G, Pei W, Ge H, Liang Q, Luo Y, Sharp FR, et al: In vivo delivery of a Bcl-xl fusion protein containing the TAT protein transduction domain protects against ischemic brain injury and neuronal apoptosis. J Neurosci 2002;22:5423–5431.
39 Krupinski J, Ferrer I, Barrachina M, Secades JJ, Mercadal J, Lozano R: CDP-choline reduces pro-caspase and cleaved caspase-3 expression, nuclear DNA fragmentation, and specific PARP-cleaved products of caspase artery occlusion in the rat. Neuropharmacology 2002; 42:846–854.
40 Culmsee C, Gerling N, Lehmnan M, Nikolova-Karakashian M, Prehn JHM, Mattson MP, et al: Nerve growth factor survival signalling in cultured hippocampal neurons is mediated through TRKA and requires the common neurotrophin receptor p75. Neuroscience 2002; 115:1089–1108.
41 Fujiwara K, Date I, Shingo T, Yoshida H, Kobayashi K, Takeuchi A, et al: Reduction of infarct volume and apoptosis by grafting of encapsulated basic fibroblast growth factor-secreting cells in a model of middle cerebral artery occlusion in rats. J Neurosurg 2003;99: 1053–1062.
42 Ijichi A, Sakuma S, Tofilon PJ. Hypoxia-induced vascular endothelial growth factor expression in normal rat astrocyte cultures. Glia 1995;14:87–93.

43 Krupinski J, Kaluza J, Kumar P: Role of angiogenesis in patients with cerebral ischaemic stroke. Stroke 1994;25:1794–1798.
44 Krupinski J, Stroemer P, Slevin M: Three-dimensional structure of newly-formed blood vessels after focal cerebral ischemia in rat. Neuroreport 2003;14:1171–1176.
45 Croll SD, Wiegand SJ: Vascular growth factors in cerebral ischemia. Mol Neurobiol 2001;23:121–135.
46 Sun Y, Jin K, Xie L, et al: VEGF-induced neuroprotection, neurogenesis and angiogenesis after focal cerebral ischaemia. J Clin Invest 2003;111:1843–1851.
47 Sun FY, Guo X: Molecular and cellular mechanisms of neuroprotection by vascular endothelial growth factor. J Neurosci Res 2005;79:180–184.
48 Ay H, Ay I, Koroshetz WJ, et al.: Potential usefulness of basic fibroblast growth factor as a treatment for stroke. Cerebrovasc Dis 2000;9:131–135.
49 Watanabe T, Okuda Y, Nonoguchi N, Zhao MZ, Kajimoto Y, Furutama D, et al: Postischemic intraventricular administration of FGF-2 expressing adenoviral vectors improves neurologic outcome and reduces infarct volume after transient focal cerebral ischemia in rats. J Cereb Blood Flow Metab 2004;24:1205–1213.
50 Shimamura M, Sato N, Oshima K, et al: Novel therapeutic strategy to treat brain ischaemia: overexpression of hepatocyte growth factor gene reduced ischaemic injury without cerebral edema in rat model. Circulation 2004;109:424–431.
51 Xu Z, Ford GD, Croslan DR, Jiang J, Gates A, Allen R, et al: Neuroprotection by neuroregulin-1 following focal stroke is associated with the attenuation of ischemia-induced pro-inflammatory and stress gene expression. Neurobiol Dis 2005;19:461–470.
52 Heistad DD, Faraci F: Gene therapy for cerebrovascular disease. Stroke 1996;27:1688–1693.
53 Sapolsky RM, Steinberg GK: Gene therapy using viral vectors for acute neurologic insults. Neurology 1999;53:1922–1931.
54 Vila N: A genetic approach to ischemic stroke therapy. Cerebrovasc Dis 2004;17(suppl 1):63–69.
55 Ooboshi H, Ibayashi S, Takada J, Yao H, Kitazono T, Fujishima M: Adenovirus-mediated gene transfer to ischemic brain. Stroke 2001;32:1043–1047.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):38–47
DOI: 10.1159/000091702

Published online: May 2, 2006

Ischemic Preconditioning: A Novel Target for Neuroprotective Therapy

Miguel Blanco[a] Ignacio Lizasoain[b] Tomás Sobrino[a] José Vivancos[c] José Castillo[a]

[a]Department of Neurology, Division of Vascular Neurology, Laboratory of Neurovascular Research, Hospital Clínico Universitario, University of Santiago de Compostela, Santiago de Compostela; [b]Departamento de Farmacología, Facultad de Medicina, Universidad Complutense, Madrid, and [c]Department of Neurology, Stroke Unit, Hospital Universitario La Princesa, Madrid, Spain

Key Words
Ischemic tolerance · Ischemic preconditioning · Stress response · Neuroprotection

Abstract
Ischemic preconditioning involves a brief exposure to ischemia in order to develop a tolerance to injurious effects of prolonged ischemia. The molecular mechanisms of neuroprotection that lead to ischemic tolerance are not yet completely understood. However, it seems that two distinct phases are involved. Firstly, a cellular defense function against ischemia may be developed by the mechanisms inherent to neurons such as posttranslational modification of proteins or expression of new proteins via a signal transduction system to the nucleus. Secondly, a stress response and synthesis of stress proteins (heat shock proteins) may be activated. These mechanisms are mediated by chaperones. The objective of ischemic preconditioning research is to identify the underlying endogenous protective cellular receptors and signaling cascades, with the long-term goal of allowing therapeutic augmentation of the endogenous protective mechanisms in cerebral ischemia and possibly development of new neuroprotective strategies for ischemic stroke treatment.

Similia similibus curentur (Hippocrates, 460–370 BC), *The dose makes the poison* (Paracelsus, 1493–1541 AD), and *Adaptation to perturbation is the basis for homeostasis* (Cannon) are expressions of the idea that when exposed to a sufficient but sublethal alteration in the environment, most living organisms acquire transient tolerance to otherwise lethal changes. This cellular response can be observed in a wide variety of species from bacteria to mammalian cells [1]. In 1964, Janoff [2] introduced the terms 'tolerance' and 'preconditioning' for this phenomenon, and the same year the phenomenon was observed in relation to ischemia, when it was shown that short periods of global hypoxia can protect the entire mammalian organism and preserve brain energy metabolism during longer periods of hypoxia [3], though most authors appear to assume that ischemic preconditioning or ischemic tolerance was discovered in the heart [4, 5].

The objective of ischemic preconditioning research is to identify the underlying endogenous protective cellular receptors and signaling cascades, with the long-term goal of allowing therapeutic augmentation of the endogenous protective mechanisms in cerebral ischemia and possibly inducing a protected state of the brain in conditions in which ischemia can be anticipated, such as during angioplasty procedures. Tolerance to ischemia in the brain can be induced by a number of different mechanisms. Many of these mechanisms are potentially damaging to the

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0038$23.50/0

Accessible online at:
www.karger.com/ced

Prof. José Castillo
Department of Neurology, Hospital Clínico Universitario
Santiago de Compostela, Travesa da Choupana s/n
ES–15706 Santiago de Compostela (Spain)
Tel. +34 981 951 348, Fax +34 981 951 098, E-Mail mecasti@usc.es

brain but, when administered at a low level that is insufficient to cause permanent damage, they can stimulate protective responses that reduce the damage caused by more severe ischemic events.

Models and Types of Ischemic Tolerance

Many animal models have been used to reproduce the various modes of ischemic tolerance [6]. Natural causes of ischemia such as thrombosis or embolism can be simulated by occlusion of one of the major blood vessels supplying the brain. The first animal model used for cerebral ischemic tolerance was described by Kitagawa et al. [7]. The preconditioning was produced in gerbils by occlusion of both common carotids for 2 min and 1 or 2 days after a 5-min ischemia, with exhibition of drastically complete protection against neuronal death. Later, other models of focal ischemia with occlusion of the common carotid artery [8] or with occlusion of the middle cerebral artery were described [9, 10]. A common method to precondition neural tissue is exposure to reduced atmospheric oxygen concentration [11]. In some cases, reduced atmospheric pressure has been used in combination with reduced oxygen concentration [12]. It has also been used in in vitro models of ischemic preconditioning, in which cultures were deprived of oxygen-glucose for a short time, an insult that did not induce neuronal death [13, 90].

Another noxious stress can confer cellular tolerance to a subsequent ischemia, a phenomenon known as 'cross-tolerance' [14], for example, high atmospheric pressure and oxygen concentration have also been found to have protective effects [15], pathologic events such as inflammation [16], epilepsy [17] and both hyperthermia and hypothermia [18] have shown ischemic tolerance. Many chemical agents have been used for preconditioning, often by interfering in the action of major proteins involved in neuronal damage. Examples include inhibitors of succinic dehydrogenase [19], glutamate receptor agonists [20], lipopolysaccharide [21], and hormone analogs [22]. Finally, tolerance to ischemic insults can also be produced by cortical spreading depression [23], sleep deprivation [24] cerebellar stimulation [25] and dietary restriction [26].

The tolerance phenomenon has also been studied in other organs. In the heart, it has been shown that brief ischemia induces a marked increase in myocardial resistance to a later ischemic insult that would be lethal otherwise [27]. Murry et al. [5] found that four periods of 4-min coronary occlusion, each separated by 5 min of reperfusion, led to a reduction in infarct size produced by a subsequent 40-min occlusion of the coronary artery.

Ischemic tolerance comes in at least two temporal profiles. The early, or rapid, phase is established within minutes and may last for several hours [18, 28, 29]. Protection has been observed when an ischemic insult is administered as little as 30 min following preconditioning [30]. It is thought that changes in cell metabolism (posttranslational modification) may account for the immediate response. The activation of existing proteins and upregulation of normal processes such as the oxygen extraction fraction are probably involved as well.

The late, or delayed, phase of protection requires hours and days to develop [7] and provides lasting protection, and usually involves de novo protein synthesis [31, 32] and gene expression [33]. The effect of ischemic preconditioning on genomic response to cerebral ischemia showed a pronounced downregulation of genes expressed during the ischemia, resulted in transcriptional changes involved in suppression of metabolic pathways and immune responses, reduction of ion-channel activity, and decreased blood coagulation [34] (fig. 1).

To induce tolerance by means of ischemic episodes, we need to take into account three parameters: the duration, the interval, and the number of episodes. First, the preconditioning treatment must be severe enough to initiate a response, but not so severe as to cause permanent damage. In a study of ischemia in gerbils, for example, it was found that at least 2 min of ischemia was required in order to induce protection against a more severe insult 2 days later [35]. Second, the subsequent insult must be planned to occur at a time point at which tolerance has been established. In the experiment of Chen et al. [36], protection was not observed until 2 days after preconditioning, and had disappeared by 7 days after preconditioning. Third, in the experiment of Kitagawa et al. [7], double 2-min periods of preconditioning ischemia, which were induced with a 1-day interval, produced much more protection of CA1 neurons on 5 min of forebrain ischemia than a single 2-min period of ischemia.

In the brain, the time course of ischemic tolerance apparently follows the delayed pattern. The latency period for ischemic tolerance is usually longer than 1 day, and the protection of brain tissue is induced only when the preconditioning ischemia is performed at least 1 day before the test ischemic challenge [32]. Once induced, the condition of ischemic tolerance is believed to last for a few days and to wane gradually until it disappears a few weeks after acquisition [37].

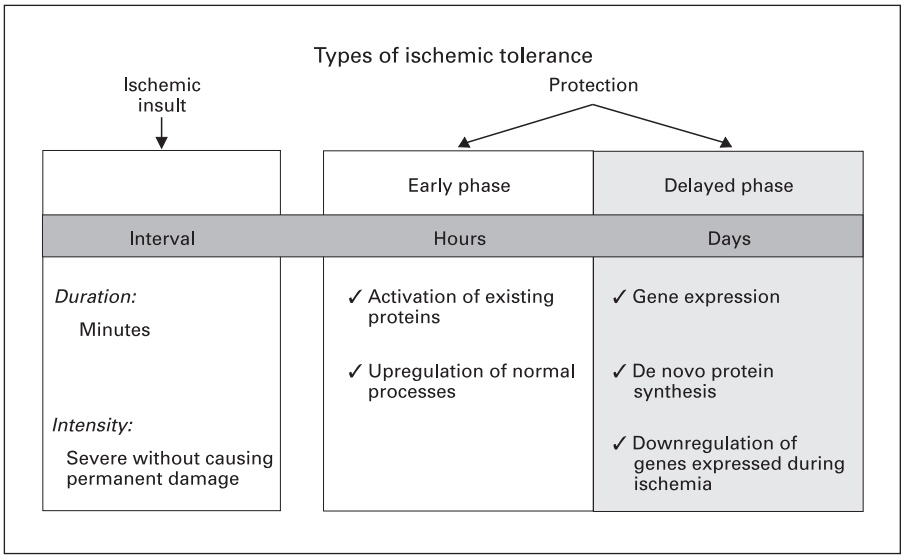


Fig. 1. Types of ischemic tolerance. The protection induced by ischemic preconditioning may be early/brief or delayed/permanent. Each involves different mechanisms.

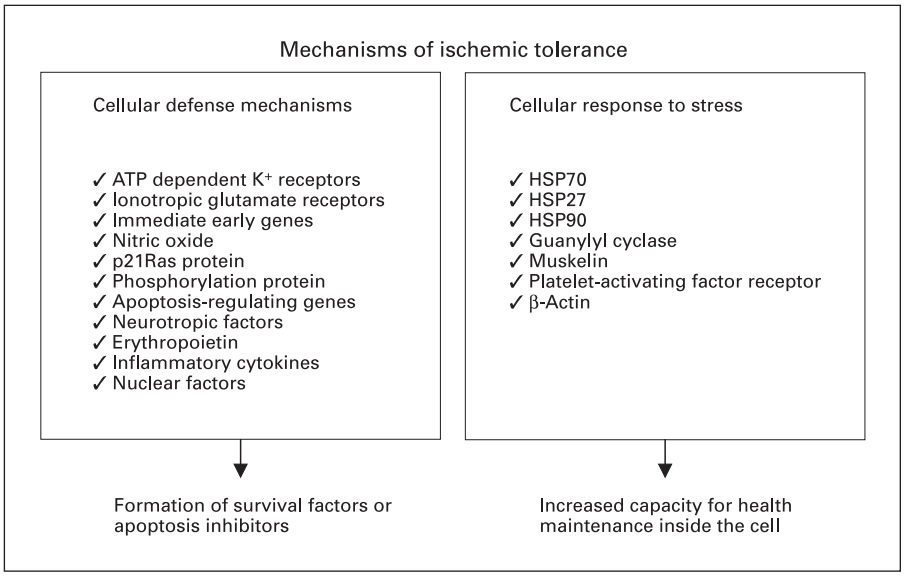


Fig. 2. Ischemic tolerance mechanisms. Cellular defense against ischemia can involve mechanisms of posttranslational modification of proteins or expression of new proteins. A cellular stress response may lead to greater health maintenance capacity inside the cell.

The Mechanism of Ischemic Tolerance

As Kirino [14] has shown in his review paper, acquisition of tolerance against various stresses requires at least two intracellular components: the stress sensor that can detect various stressful conditions and convert the information into intracellular signals, and the effector of tolerance induction. The author describes how various stress signals are captured by a small number of sensors, with the signals gradually converged to a stereotyped final common pathway, and he assumes that the mechanism of ischemic tolerance in the brain may be quite similar to that of other organs or other type of cells.

All potential mechanisms focused on ischemic tolerance may be divided in two categories: (1) A cellular defense function against ischemia which can involve mechanisms of posttranslational modification of proteins or expression of new proteins via a signal transduction system to the nucleus, which can activate cascades of events directed towards the formation of survival factors or apoptosis inhibitors. (2) A cellular stress response and synthesis of stress proteins may lead to an increased capacity for health maintenance inside the cell (chaperones), for example helping the cell to arrange unnecessary denatured proteins [14] (fig. 2).

Cellular Defense Mechanisms

ATP-Dependent K^+ Receptors

As in cardiac preconditioning, adenosine plays a role in neuronal preconditioning and in immediate ischemic tolerance [38, 39]. One of the initial events of ischemic tolerance is the opening of ATP-dependent K^+ channels via activation of adenosine A1 receptors [40]. This hypothesis is supported by the fact that impeding consumption of adenosine facilitates tolerance [41] whilst using antagonists to A1 receptors inhibits tolerance [42, 43]. Alteration of the K^+ channels sensitive to ATP hyperpolarizes the neuronal membrane, so protecting from a prejudicial depolarization [44].

Ionotropic Glutamate Receptors

Stimulation of NMDA receptors causes opening of ionic channels in the cellular membrane which mainly sets off entry of intracellular calcium and sodium and the exit of potassium to the extracellular medium, all of which originate from neuronal depolarization. There are five receptor subtypes (NR1, NR2A, NR2B, NR2C, NR2D). In experimental models it has been demonstrated that ischemic preconditioning is capable of inducing a fall in the expression of NR2A and NR2B receptors, and to a lesser extent of NR1 receptors, at the neocortex level, but not at the level of the hippocampus [45]. Consequently, following massive release of glutamate secondary to the lethal ischemic event, the neurons experience greater difficulty in allowing entry of intracellular Ca^{2+} which in turn sets off cellular damage.

AMPA glutamate receptors activate the opening of an ionotropic channel which mainly permits the entry of intracellular sodium and the exit of potassium to the extracellular medium. This channel is formed of four subunits that leave a pore between them through which both ions circulate. Depending on the electrical charge originating from the amino acids that form the pore, passage of the ion calcium is also permitted. One of the subunits, GluR2, has been well studied; the DNA that codifies this subunit always does so with the same sequence of amino acids, but in certain circumstances on being translated to RNAm, one of the codons changes a glutamine for arginine. When this new subunit is synthesized with the change of glutamine (GluR2 Q) for arginine (GluR2 R), this causes the receptor to be impermeable to the passage of calcium. Experimental studies have demonstrated how following preconditioning in neurons of the CA1 region of the hippocampus, the proportion of AMPA channels with their channel impermeable to calcium (GluR2 R) falls by up to 20%. This change in the intracellular current of calcium may be implicated in the neuroprotective effect, although the mechanism is as yet unknown [46].

Immediate Early Genes

Following an episode of global or focal ischemia, there is an almost immediate increase in the expressivity of certain genes that are known as immediate early genes (IEG). Although we do not yet completely understand the significance of the expressivity of these genes in response to cerebral ischemia, we do know that they can act both in benefit of and in detriment to cellular activity [47].

There are many stimuli that induce the expression of RNAm proceeding from IEG. First, IEGs were implicated in the regulation of the cellular cycle, but subsequently many IEGs were identified with multiple functions.

In normal conditions, IEGs display low expressivity, but with physiological stimuli IEG expressivity is raised modestly in regions that are functionally related to the stimulus. For example, dehydration results in a selective increase in the expressivity of the c-fos gene in the paraventricular nucleus (ADH producer) [48]. In contrast, in pathological conditions such as cerebral ischemia, there is an increase in the expressivity in extensive areas of the central nervous system. This rise in expressivity is seen both in zones directly affected by the ischemia and in zones outside of this [49].

A stimulus such as the release of glutamate induces an intracellular increase in calcium or activation of second messengers such as protein kinase, and in particular protein kinase A (AMPc-dependent) and C (calcium-dependent). These kinases phosphorylate a series of proteins that act directly on the DNA (DNA-binding proteins) such as SRE (serum response element) and Ca/CRE (calcium/cAMP response element). These DNA-binding proteins join to certain points of the genome regulating the transcription of the respective genes [47].

The prototype of IEG is the c-fos gene, which codifies a protein of 380 amino acids known as the Fos protein. The c-fos gene was one of the first to be discovered, sequenced from an oncogene found in a sarcoma of viral origin. The Fos protein has some structural characteristics of interest, having a region formed by basic amino acids that constitute a point of union to the DNA. Near to this region, there is a zone that has remains of leucine every seven amino acids, known as the leucine zipper. Owing to the spatial structure of the protein in an α-helix, the leucine remains stay aligned to one side. These leucine zippers act as points of union with other proteins, form-

ing dimers. These dimers join to specific regions of the DNA, known as AP-1 areas, which are implicated in the regulation of expression of other genes (the neurotrophin gene, proenkephalin, glial fibrillary acid protein, neuropeptide Y, vasoactive intestinal peptide, or tyrosine hydroxylase).

Cerebral ischemia is a powerful stimulant for the synthesis of IEG. In models of global ischemia in rats, by means of in situ hybridization, we can observe an increase in ARNm of the c-fos gene in the dentate cells of the cerebellum, in the CA1 and CA3 regions of the hippocampus, in the neocortex and in the Purkinje cells of the cerebellum [50]. By means of preconditioning, the rise in expressivity is limited to the ischemic zone.

Ischemic preconditioning induces a rise in the expression of the c-JUN protein but not other proteins regulated by IEGs [51]. The expression of c-Fos has been implicated in protection of the contralateral hippocampus following unilateral ischemic preconditioning [52].

Role of Nitric Oxide

In a newborn rat model, hypoxic preconditioning rendered the animals resistant to hypoxic ischemia. Acquisition of tolerance in this model depended on nitric oxide (NO) production by endothelial nitric oxide synthase (NOS) [53]. In the rat slice model, neuronal NOS (nNOS)-mediated NO was involved in anoxic preconditioning [54]. NO production depended on NMDA receptor activation [55], which activated nNOS. Increased NO can activate the Raf/Mek/Erk cascade and can induce new protein synthesis [56]. In cell cultivation models, a major loss of neuroprotection can be observed if an antagonist of NMDA receptors is added during preconditioning [55, 57]. Applying an NOS inhibitor such as nitro-*L*-arginine during preconditioning episodes results in blocking of up to 70% of the neuroprotector effect, and supplying an excess of substrate for formation of NOS, *L*-arginine, results in restored protection. Recently, prenatal hypoxia models show as the ischemic tolerance may be iNOS-dependent [58, 59].

p21Ras Protein

Lander et al. [60] have demonstrated in their work that p21ras is a target for NO. It has recently been demonstrated that NO could induce activation of p21ras following stimulation of cortical neuron cultures with NMDA [61]. After submitting a cellular culture to 5 min of OGD preconditioning, powerful activation has been observed of the Ras via NMDA receptors, via NO, but independently of CMPc. With inhibition of Ras during preconditioning, whether pharmacologically or by means of Ras mutants, the tolerance phenomenon is entirely lost. When an active form of Ras is added (by means of a viral vector) this is sufficient to induce tolerance [57]. The Ras protein induces cellular survival by activation of a cascade of phosphoinositide 3-kinase (PI3K)/Akt or Raf/Erk [62–64]. The (PI3K)/Akt route is related to anti-apoptotic signals [65, 66].

Phosphorylation Protein

Ischemia activates protein phosphorylation. This enhanced phosphorylation is blocked by preconditioning ischemia. Since the cascade of phosphorylation may operate as an amplifier of neuronal injury, preconditioning could cancel this detrimental cascade and normalize intracellular signal transduction [45].

Apoptosis-Regulating Genes

In gerbil models of focal ischemia, it has been demonstrated that the anti-apoptotic Bcl-2 gene is widely expressed by the neurons of the zone of ischemic penumbra at around the fourth day [67]. If in preconditioning cultured neurons in vitro we add cycloheximide (which is a powerful inductor of Bcl-2), we can see that tolerance is not produced. Other models of preconditioning have demonstrated diminution of expression of p53 (pro-apoptotic gene) and consequently their target genes p21 (WAF1/Cip1) and PAG608/Wig-1 [68], suggesting that the repression of pro-apoptotic genes may also contribute to neuroprotection.

Neurotrophic Factors

In models of ischemia in cellular cultures, it has been demonstrated that neurotrophic factors produce protective effects [69]. The effect on mature cells subject to ischemia is still not well understood. Brief episodes of ischemia induce production of NGF (nerve growth factor) in some interneurons of the hippocampus and in the granular cells. The relative resistance of these interneurons of the hippocampus to ischemia suggests that these cells can continuously produce NGF following ischemia [70]. BDNF (brain-derived neurotrophic factor) has been implicated in a neuroprotective effect in neonatal hypoxia [71] and in preconditioning for spreading depression [72, 73].

Role of Erythropoietin

Erythropoietin (EPO) is induced after cerebral ischemia as well as after hypoxic preconditioning in vivo [74]. Astrocytes are the main cellular source of the glycoprotein hormone EPO in the brain and low oxygen tension stimulates EPO-mRNA expression in astrocytes via hypoxia-

inducible factor-1 (HIF-1) [75]. In an in vitro model of ischemic preconditioning, EPO was shown to act as a paracrine mediator of neuroprotection [76]. Experimental data suggest a central role for JAK2, PI3K pathways, and nuclear factor-κB (NF-κB) signaling as mediators of paracrine EPO-induced neuroprotection [77].

Inflammatory Cytokines

Inflammatory cytokines, particularly tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, have been implicated in the mechanism of ischemic tolerance [78–80]. IL-1 receptor antagonist can block tolerance induced by brief priming ischemia [32]. A transitory occlusion of 10 min from MCA can induce the expression of ARNm of TNF-α [81]. The use of TNF-α as preconditioning has been demonstrated both in animal models [82] and in cultured neurons [83]. Both the intracellular route of ceramide [84, 85] and activation of nuclear factor NF-κB [86] have been related to the neuroprotective effect of TNF-α.

TNF-α is shed in its soluble form by a membrane-anchored zinc protease, identified as a disintegrin and metalloproteinase (ADAM) called TNF-α convertase (TACE/ADAM17). We have demonstrated, using in vitro models, that TACE is upregulated after ischemic brain damage and that the increase in TACE expression contributes to a rise in TNF-α and a subsequent neuroprotective effect after excitotoxic stimuli [87, 88]. Recently, we have shown that a TACE upregulation occurs after ischemia preconditioning and that this event plays a major role in TNF-α shedding. In turn the TNF-α released plays a neuroprotective role in ischemic tolerance [89]. Furthermore upregulation of glutamate uptake (glutamate transporter EAAT3) has been suggested as a mechanism involved in this neuroprotective effect [90].

Recently, the toll-like receptors (TLR) have been implicated in the inflammatory mechanism of ischemic tolerance. The rapid form of tolerance is achieved by direct interference with membrane fluidity, causing disruption of lipid rafts leading to inhibition of TLR/cytokine signaling pathways. In the delayed form of tolerance, the preconditioning stimulus first triggers the TLR/cytokine inflammatory pathways, leading not only to inflammation but also to simultaneous upregulation of feedback inhibitors of inflammation [91].

Nuclear Factors

The NF-κB is a final pathway for different signals, cytokines, neurotrophic factors, neurotransmitters, oxidative stress and intracellular elevation of Ca^{2+}. NF-κB induces the expression of neuroprotector genes such as MnSOD and Bcl-2. Expression of NF-κB can be activated by three types of preconditioning – ischemia, epilepsy and polyunsaturated fatty acids [92]. Pretreatment with NF-κB inhibitor blocked NF-κB activity eventually cancelled the neuroprotective effect of preconditioning [14].

Cellular Response to Stress; Stress Proteins

When the cell is exposed to a hostile environment via stress receptors, it sends signals to the cellular nucleus where transcription of the stress proteins (HSP or chaperones) will be induced. The expression of these proteins contributes to the induction of tolerance. These characteristics are common to a large number of cells. The response to stress is essential for cellular survival, as the cells are not able to survive with a sharp accumulation of denaturalized or aggregated proteins. These protein aggregates do not accumulate in healthy cells as they are continually degraded by intracellular 'cleaning' mechanisms. These mechanisms are mainly chaperones, which impede folding of the proteins on themselves, and proteolytic enzymes. Approximately one third of newly synthesized proteins in a cell are degraded a few minutes following their synthesis [93]. When these cellular cleaning systems fail, apoptosis ensues. This pathogenic mechanism has been implicated in the physiopathology of Alzheimer's disease, Parkinson's disease, Huntington's chorea [94] and cerebral ischemia [95].

When ischemic tolerance is induced by preceding ischemia, HSP70 increases in hippocampal CA1 pyramidal cells in gerbils [37, 96, 97] and rats [98, 99]. In animal models of ischemic tolerance, there was a very good correlation found between production of HSP70 and the degree of tolerance [100], and blocking the function of HSP70 leads to loss of tolerance [101]. Injecting MK801, an antagonist of NMDA channels, also resulted in interrupted production of HSP70 [102]. Other stress proteins have also been shown to rise following ischemic preconditioning, including for example HSP27 [103] and HSP110/105 [104]. Neither ubiquitin nor HSP70 appear until 5 min from ischemia in the gerbil model. However, where the gerbil has previously undergone ischemic preconditioning, they appear in 2 min [105, 106]. A recent proteomic analysis of adult ischemic preconditioning rat brain showed increased expression of HSP70, HSP27, HSP90, guanylyl cyclase, muskelin, platelet-activating factor receptor and β-actin at 24 h after preconditioning [107].

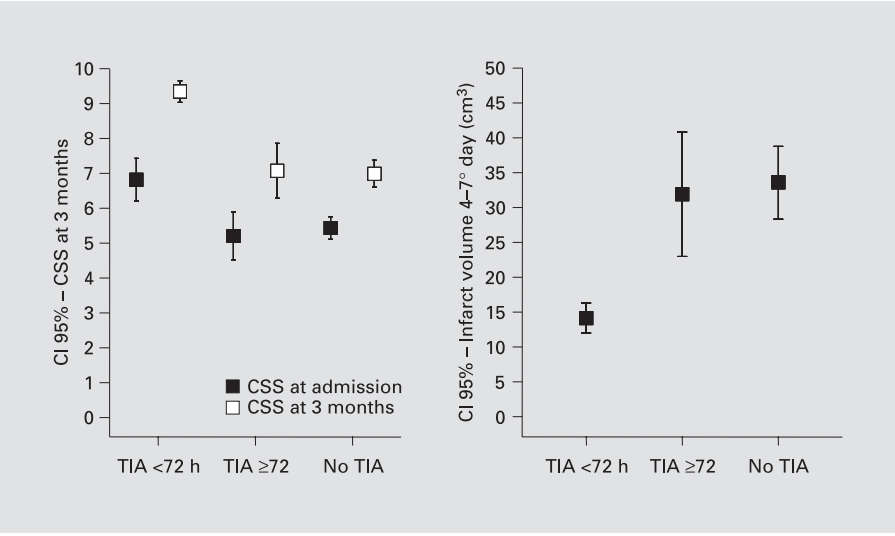


Fig. 3. Clinical human evidence of the benefit of ischemic tolerance. Taken from a prospective study including 339 patients who suffered a hemispheric ischemic stroke <24 h onset. 45 patients had a previous ipsilateral TIA ≤72 h before stroke, 54 patients a previous contralateral TIA or ≥72 h and 240 patients had no previous TIA.

Clinic Expression of Ischemic Tolerance

The phenomenon of cerebral ischemic tolerance has not only been described at the molecular level and in animal experimental models; there is also some clinical evidence of its presence. In 1999, our group presented a study that demonstrated how the presence of a transient ischemic attack prior to fatal stroke can induce ischemic tolerance [108]. In 26 patients with previous ipsilateral TIA 72 h to ischemic stroke, the Canadian Stroke Scale on admission, 48 h, 7 days and 3 months were significantly higher than that recorded in the group without TIA. Furthermore, more neurological deterioration and higher infarct volume was found in the group without previous TIA. Weih et al. [109] conducted a retrospective case-control study in 148 stroke patients (with and without antecedent TIA) and showed that TIA before stroke was an independent predictor of mild stroke (Canadian Neurological Scale score ≥6.5). Similar results were observed in more recent retrospective analyses [110–112]. Wegener et al. [113] demonstrated that initial diffusion lesions tended to be smaller and final infarct volumes were significantly reduced in patients with a previous TIA. Our group has recently published that ischemic tolerance is associated with increased levels of TNF-α in the presence of reduced concentrations of IL-6 in plasma [114]. These clinical findings are in tune with experimental models of ischemic tolerance (fig. 3). Nevertheless, further research is still necessary to determine the human mechanism of ischemic tolerance.

Ischemic Tolerance as a Therapeutical Target

Induction of ischemic tolerance has been suggested as a promising clinical strategy to prepare the brain for situations of possible ischemia, such as cardiac or brain surgery and in high-risk stroke patients. Dirnagl et al. [18] see even greater potential of the ischemic tolerance paradigm as an elegant experimental probe into the mechanisms of endogenous brain neuroprotection. Ischemic stroke outcome is conditioned by the balance of endogenous neuroprotective mechanisms and events that lead to cell death. The aim should be to stimulate the brain's own protective mechanism and this can be achieved by administering an exogenous tolerance effector. Blondeau et al. [17] induced ischemic tolerance injecting linolenic acid (neuroprotection induced by HSP70 expression) to rats and suggested the potential therapeutical value of polyunsaturated fatty acids in neuronal protection. Ischemic tolerance can be induced by clinically approved drugs such as desferroxamine, EPO [115], isoflurane [116] and K_{ATP} openers [117]. It is possible that these drugs could be effectively applied as potential neuroprotective agents.

Conclusion

Research on ischemic tolerance is potentially useful in identifying targets of acute therapy for cerebral ischemia. A clearer understanding of preconditioning could lead to better prevention and treatment of ischemic brain damage.

References

1 Feder ME, Hofmann GE: Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu Rev Physiol 1999;61:243–282.
2 Janoff A: Alterations in lysosomes (intracellular enzymes) during shock; effects of preconditioning (tolerance) and protective drugs. Int Anesthesiol Clin 1964;2:251–259.
3 Dahl NA: Prolonged anoxic survival due to anoxia preexposure: brain ATP, lactate, and pyruvate. Am J Physiol 1964;1:452–456.
4 Meldrum DR: Mechanisms of cardiac preconditioning: ten years after the discovery of ischemic preconditioning. J Surg Res 1997;73:1–13.
5 Murry CE, Jennings RB, Reimer KA: Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. Circulation 1986;74:1124–1136.
6 Baron FC: Endogenous brain protection: models, gene expression, and mechanisms. Methods Mol Med 2005;104:105–184.
7 Kitagawa K, Matsumoto M, Tagaya M, Hata R, Ueda H, Niinobe M, Handa N, Fukunaga R, Kimura K, Mikoshiba K, Kamada T: 'Ischemic tolerance' phenomenon found in the brain. Brain Res 1990;528:21–24.
8 Plaschke K, Weigand MA, Michel A, Martin E, Bardenheuer HJ: Permanent cerebral hypoperfusion: 'preconditioning-like' effects on rat energy metabolism towards acute systemic hypotension. Brain Res 2000;858:363–370.
9 Stagliano NE, Perez-Pinzon MA, Moskowitz MA, Huang PL: Focal ischemic preconditioning induces rapid tolerance to middle cerebral artery occlusion in mice. J Cereb Blood Flow Metab 1999;19:757–761.
10 Mackay KB, Stiefel TH, Foster AC: Ischemic preconditioning reduces infarct volume after subdural hematoma in the rat. Brain Res 2002;930:200–205.
11 Miller BA, Perez RS, Shah AR, Gonzales ER, Park TS, Gidday JM: Cerebral protection by hypoxic preconditioning in a murine model of focal ischemia-reperfusion. Neuroreport 2001;12:1663–1669.
12 Romanovskii DY, Tyulkova EI, Samoilov MO: Preconditioning hypobaric hypoxia prevents anoxia-induced inhibition of generation of focal potentials in slices of olfactory cortex from rat brain. Bull Exp Biol Med 2001;132:1154–1156.
13 Bruer U, Weih MK, Isaev NK, Meisel A, Ruscher K, Bergk A, Trendelenburg G, Wiegand F, Victorov IV, Dirnagl U: Induction of tolerance in rat cortical neurons: hypoxic preconditioning. FEBS Lett 1997;414:117–121.
14 Kirino T: Ischemic tolerance. J Cereb Blood Flow Metab 2002;22:1283–1296.
15 Prass K, Wiegand F, Schumann P, Ahrens M, Kapinya K, Harms C, Liao W, Trendelenburg G, Gertz K, Moskowitz MA, Knapp F, Victorov IV, Megow D, Dirnagl U: Hyperbaric oxygenation induced tolerance against focal cerebral ischemia in mice is strain dependent. Brain Res 2000;871:146–150.
16 Zimmermann C, Ginis I, Furuya K, Klimanis D, Ruetzler C, Spatz M, Hallenbeck JM: Lipopolysaccharide-induced ischemic tolerance is associated with increased levels of ceramide in brain and in plasma. Brain Res 2001;895:59–65.
17 Blondeau N, Widmann C, Lazdunski M, Heurteaux C: Polyunsaturated fatty acids induce ischemic and epileptic tolerance. Neuroscience 2002;109:231–241.
18 Dirnagl U, Simon RP, Hallenbeck JM: Ischemic tolerance and endogenous neuroprotection. Trends Neurosci 2003;26:248–254.
19 Kuroiwa T, Yamada I, Endo S, Hakamata Y, Ito U: 3-Nitropropionic acid preconditioning ameliorates delayed neurological deterioration and infarction after transient focal cerebral ischemia in gerbils. Neurosci Lett 2000;283:145–148.
20 Lam AG, Soriano MA, Monn JA, Schoepp DD, Lodge D, McCulloch J: Effects of the selective metabotropic glutamate agonist LY354740 in a rat model of permanent ischaemia. Neurosci Lett 1998;254:121–123.
21 Rosenzweig HL, Lessov NS, Henshall DC, Minami M, Simon RP, Stenzel-Poore MP: Endotoxin preconditioning prevents cellular inflammatory response during ischemic neuroprotection in mice. Stroke 2004;35:2576–2581.
22 Urayama A, Yamada S, Kimura R, Zhang J, Watanabe Y: Neuroprotective effect and brain receptor binding of taltirelin, a novel thyrotropin-releasing hormone analogue, in transient forebrain ischemia of C57BL/6J mice. Life Sci 2002;72:601–607.
23 Yanamoto H, Hashimoto N, Nagata I, Kikuchi H: Infarct tolerance against temporary focal ischemia following spreading depression in rat brain. Brain Res 1998;784:239–249.
24 Hsu JC, Lee YS, Chang CN, Ling EA, Lan CT: Sleep deprivation prior to transient global cerebral ischemia attenuates glial reaction in the rat hippocampal formation. Brain Res 2003;984:170–181.
25 Galea E, Golanov EV, Feinstein DL, Kobylarz KA, Glickstein SB, Reis DJ: Cerebellar stimulation reduces inducible nitric oxide synthase expression and protects brain from ischemia. Am J Physiol 1998;274:H2035–H2045.
26 Yu ZF, Mattson MP: Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. J Neurosci Res 1999;57:830–839.
27 Tomai F, Crea F, Chiariello L, Gioffre PA: Ischemic preconditioning in humans: models, mediators, and clinical relevance. Circulation 1999;100:559–563.
28 Schurr A, Reid KH, Tseng MT, West C, Rigor BM: Adaptation of adult brain tissue to anoxia and hypoxia in vitro. Brain Res 1986;374:244–248.
29 Schaller B, Graf R: Cerebral ischemic preconditioning. An experimental phenomenon or a clinical important entity of stroke prevention? J Neurol 2002;249:1503–1511.
30 Perez-Pinzon MA, Xu GP, Dietrich WD, Rosenthal M, Sick TJ: Rapid preconditioning protects rats against ischemic neuronal damage after 3 but not 7 days of reperfusion following global cerebral ischemia. J Cereb Blood Flow Metab 1997;17:175–182.
31 Kato H, Kogure K, Nakata N, Araki T, Itoyama Y: Facilitated recovery from postischemic suppression of protein synthesis in the gerbil brain with ischemic tolerance. Brain Res Bull 1995;36:205–208.
32 Baron FC, White RF, Spera PA, Ellison J, Currie RW, Wang X, Feuerstein GZ: Ischemic preconditioning and brain tolerance: temporal histological and functional outcomes, protein synthesis requirement, and interleukin-1 receptor antagonist and early gene expression. Stroke 1998;29:1937–1950.
33 Bernaudin M, Tang Y, Reilly M, Petit E, Sharp FR: Brain genomic response following hypoxia and re-oxygenation in the neonatal rat. Identification of genes that might contribute to hypoxia-induced ischemic tolerance. J Biol Chem 2002;277:39728–39738.
34 Stenzel-Poore MP, Stevens SL, Xiong Z, Lessov NS, Harrington CA, Mori M, Meller R, Rosenzweig HL, Tobar E, Shaw TE, Chu X, Simon RP: Effect of ischaemic preconditioning on genomic response to cerebral ischaemia: similarity to neuroprotective strategies in hibernation and hypoxia-tolerant states. Lancet 2003;362:1028–1037.
35 Kitagawa K, Matsumoto M, Kuwabara K, Tagaya M, Ohtsuki T, Hata R, et al: 'Ischemic tolerance' phenomenon detected in various brain regions. Brain Res 1991;561:203–211.
36 Chen J, Graham SH, Zhu RL, Simon RP: Stress proteins and tolerance to focal cerebral ischemia. J Cereb Blood Flow Metab 1996;16:566–577.
37 Kirino T, Tsujita Y, Tamura A: Induced tolerance to ischemia in gerbil hippocampal neurons. J Cereb Blood Flow Metab 1991;11:299–307.
38 Plamondon H, Blondeau N, Heurteaux C, Lazdunski M: Mutually protective actions of kainic acid epileptic preconditioning and sublethal global ischemia on hippocampal neuronal death: involvement of adenosine A1 receptors and K(ATP) channels. J Cereb Blood Flow Metab 1999;19:1296–1308.
39 Reshef A, Sperling O, Zoref-Shani E: Adenosine-induced preconditioning of rat neuronal cultures against ischemia-reperfusion injury. Adv Exp Med Biol 1998;431:365–368.
40 Heurteaux C, Lauritzen I, Widmann C, Lazdunski M: Essential role of adenosine, adenosine A1 receptors, and ATP-sensitive K^+ channels in cerebral ischemic preconditioning. Proc Natl Acad Sci USA 1995;92:4666–4670.

41 Kawahara N, Ide T, Saito N, Kawai K, Kirino T: Propentofylline potentiates induced ischemic tolerance in gerbil hippocampal neurons via adenosine receptor. J Cereb Blood Flow Metab 1998;18:472–475.
42 Hiraide T, Katsura K, Muramatsu H, Asano G, Katayama Y: Adenosine receptor antagonists cancelled the ischemic tolerance phenomenon in gerbil. Brain Res 2001;910:94–98.
43 Nakamura M, Nakakimura K, Matsumoto M, Sakabe T: Rapid tolerance to focal cerebral ischemia in rats is attenuated by adenosine A1 receptor antagonist. J Cereb Blood Flow Metab 2002;22:161–170.
44 Horiguchi T, Kis B, Rajapakse N, Shimizu K, Busija DW: Opening of mitochondrial ATP-sensitive potassium channels is a trigger of 3-nitropropionic acid-induced tolerance to transient focal cerebral ischemia in rats. Stroke 2003;34:1015–1020.
45 Shamloo M, Wieloch T: Changes in protein tyrosine phosphorylation in the rat brain after cerebral ischemia in a model of ischemic tolerance. J Cereb Blood Flow Metab 1999;19:173–183.
46 Yamaguchi K, Yamaguchi F, Miyamoto O, Hatase O, Tokuda M: The reversible change of GluR2 RNA editing in gerbil hippocampus in course of ischemic tolerance. J Cereb Blood Flow Metab 1999;19:370–375.
47 Akins PT, Liu PK, Hsu CY: Immediate early gene expression in response to cerebral ischemia. Friend or foe? Stroke 1996;27:1682–1687.
48 Sagar SM, Sharp FR, Curran T: Expression of c-fos protein in brain: metabolic mapping at the cellular level. Science 1988;240:1328–1331.
49 Sonnenberg JL, Macgregor-Leon PF, Curran T, Morgan JI: Dynamic alterations occur in the levels and composition of transcription factor AP-1 complexes after seizure. Neuron 1989;3:359–365.
50 Wessel TC, Joh TH, Volpe BT: In situ hybridization analysis of c-fos and c-jun expression in the rat brain following transient forebrain ischemia. Brain Res 1991;567:231–240.
51 Sommer C, Gass P, Kiessling M: Selective c-JUN expression in CA1 neurons of the gerbil hippocampus during and after acquisition of an ischemia-tolerant state. Brain Pathol 1995;5:135–144.
52 Belayev L, Ginsberg MD, Alonso OF, Singer JT, Zhao W, Busto R: Bilateral ischemic tolerance of rat hippocampus induced by prior unilateral transient focal ischemia: relationship to c-fos mRNA expression. Neuroreport 1996;8:55–59.
53 Gidday JM, Shah AR, Maceren RG, Wang Q, Pelligrino DA, Holtzman DM, Park TS: Nitric oxide mediates cerebral ischemic tolerance in a neonatal rat model of hypoxic preconditioning. J Cereb Blood Flow Metab 1999;19:331–340.
54 Centeno JM, Orti M, Salom JB, Sick TJ, Perez-Pinzon MA: Nitric oxide is involved in anoxic preconditioning neuroprotection in rat hippocampal slices. Brain Res 1999;836:62–69.
55 Grabb MC, Choi DW: Ischemic tolerance in murine cortical cell culture: critical role for NMDA receptors. J Neurosci 1999;19:1657–1662.
56 Nandagopal K, Dawson TM, Dawson VL: Critical role for nitric oxide signaling in cardiac and neuronal ischemic preconditioning and tolerance. J Pharmacol Exp Ther 2001;297:474–478.
57 Gonzalez-Zulueta M, Feldman AB, Klesse LJ, Kalb RG, Dillman JF, Parada LF, Dawson TM, Dawson VL: Requirement for nitric oxide activation of p21(ras)/extracellular regulated kinase in neuronal ischemic preconditioning. Proc Nat Acad Sci USA 2000;97:436–441.
58 Cho S, Park EM, Zhou P, Frys K, Ross ME, Iadecola C: Obligatory role of inducible nitric oxide synthase in ischemic preconditioning. J Cereb Blood Flow Metab 2005;25:493–501.
59 Zhao P, Zuo Z: Prenatal hypoxia-induced adaptation and neuroprotection that is inducible nitric oxide synthase-dependent. Neurobiol Dis 2005;20:871–880.
60 Lander HM, Ogiste JS, Pearce SF, Levi R, Novogrodsky A: Nitric oxide-stimulated guanine nucleotide exchange on p21ras. J Biol Chem 1995;270:7017–7020.
61 Yun HY, Gonzalez-Zulueta M, Dawson VL, Dawson TM: Nitric oxide mediates N-methyl-*D*-aspartate receptor-induced activation of p21ras. Proc Natl Acad Sci USA 1998;95:5773–5778.
62 Bading H, Greenberg ME: Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. Science 1991;253:912–914.
63 Downward J: Mechanisms and consequences of activation of protein kinase B/Akt. Curr Opin Cell Biol 1998;10:262–267.
64 Rosen LB, Ginty DD, Weber MJ, Greenberg ME: Membrane depolarization and calcium influx stimulate MEK and MAP kinase via activation of Ras. Neuron 1994;12:1207–1221.
65 Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME: Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and independent mechanisms. Science 1999;286:1358–1362.
66 Dudek H, Datta SR, Franke TF, Birnbaum MJ, Yao R, Cooper GM, Segal RA, Kaplan DR, Greenberg ME: Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 1997;275:661–665.
67 Shimazaki K, Ishida A, Kawai N: Increase in bcl-2 oncoprotein and the tolerance to ischemia-induced neuronal death in the gerbil hippocampus. Neurosci Res 1994;20:95–99.
68 Tomasevic G, Shamloo M, Israeli D, Wieloch T: Activation of p53 and its target genes p21(WAF1/Cip1) and PAG608/Wig-1 in ischemic preconditioning. Mol Brain Res 1999;70:304–313.
69 Mattson MP, Cheng B, Smith-Swintosky VL: Mechanisms of neurotrophic factors protection against calcium- and free radical-mediated excitotoxic injury: Implications for treating neurodegenerative disorders. Exp Neurol 1993;124:89–95.
70 Matsushima K, Schmidt-Kastner R, Hakim AM: Genes and cerebral ischemia: therapeutic perspectives. Cerebrovasc Dis 1996;6:119–127.
71 Han BH, Holtzman DM: BDNF protects the neonatal brain from hypoxic-ischemic injury in vivo via the ERK pathway. J Neurosci 2000;20:5775–5781.
72 Kawahara N, Croll SD, Wiegand SJ, Klatzo I: Cortical spreading depression induces long-term alterations of BDNF levels in cortex and hippocampus distinct from lesion effects: implications for ischemic tolerance. Neurosci Res 1997;29:37–47.
73 Yanamoto H, Xue JH, Miyamoto S, Nagata I, Nakano Y, Murao K, Kikuchi H: Spreading depression induces long-lasting brain protection against infracted lesion development via BDNF gene-dependent mechanism. Brain Res 2004;1019:178–188.
74 Prass K, Scharff A, Ruscher K, Lowl D, Muselmann C, Victorov I, Kapinya K, Dirnagl U, Meisel A: Hypoxia-induced stroke tolerance in the mouse is mediated by erythropoietin. Stroke 2003;34:1981–1986.
75 Semenza GL: HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J Appl Physiol 2000;88:1474–1480.
76 Ruscher K, Freyer D, Karsch M, Isaev N, Megow D, Sawitzki B, Priller J, Dirnagl U, Meisel A: Erythropoietin is a paracrine mediator of ischemic tolerance in the brain: evidence from an in vitro model. J Neurosci 2002;22:10291–10301.
77 Digicaylioglu M, Bichet S, Marti HH, Wenger RH, Rivas LA, Bauer C, Gassmann M: Localization of specific erythropoietin binding sites in defined areas of the mouse brain. Proc Natl Acad Sci USA 1995;92:3717–3720.
78 Ohtsuki T, Ruetzler CA, Tasaki K, Hallenbeck JM: Interleukin-1 mediates induction of tolerance to global ischemia in gerbil hippocampal CA1 neurons. J Cereb Blood Flow Metab 1996;16:1137–1142.
79 Wang X, Li X, Currie RW, Willette RN, Barone FC, Feuerstein GZ: Application of real-time polymerase chain reaction to quantitate induced expression of interleukin-1β mRNA in ischemic brain tolerance. J Neurosci Res 2000;59:238–246.
80 Pera J, Zawadzka M, Kaminska B, Szczudlik A: Influence of chemical and ischemic preconditioning on cytokine expression after focal brain ischemia. J Neurosci Res 2004;78:132–140.
81 Wang X, Li X, Erhardt JA, Barone FC, Feuerstein GZ: Detection of tumor necrosis factor-α mRNA induction in ischemic brain tolerance by means of real-time polymerase chain reaction. J Cereb Blood Flow Metab 2000;20:15–20.
82 Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM: TNF-α pretreatment induces protective effects against focal cerebral ischemia in mice. J Cereb Blood Flow Metab 1997;17:483–490.

83 Liu J, Ginis I, Spatz M, Hallenbeck JM: Hypoxic preconditioning protects cultured neurons against hypoxic stress via TNF-α and ceramide. Am J Physiol 2000;278:C144–C153.

84 Ginis I, Schweizer U, Brenner M, Liu J, Azzam N, Spatz M, Hallenbeck JM: TNF-α pretreatment prevents subsequent activation of cultured brain cells with TNF-α and hypoxia via ceramide. Am J Physiol 1999;276:C1171–C1183.

85 Takahashi K, Ginis I, Nishioka R, Klimanis D, Barone FC, White RF, Chen Y, Hallenbeck JM: Glucosylceramide synthase activity and ceramide levels are modulated during cerebral ischemia after ischemic preconditioning. J Cereb Blood Flow Metab 2004;24:623–627.

86 Ginis I, Jaiswal R, Klimanis D, Liu J, Greenspon J, Hallenbeck JM: TNF-α induced tolerance to ischemic injury involves differential control of NF-κB transactivation: the role of NF-κB association with p300 adaptor. J Cereb Blood Flow Metab 2002;22:142–152.

87 Hurtado O, Cárdenas A, Lizasoain I, Bosca L, Leza JC, Lorenzo P, Moro MA: Upregulation of TNF-α convertase (TACE/ADAM17) after oxygen-glucose deprivation in rat forebrain slices. Neuropharmacology 2001;40:1094–1102.

88 Hurtado O, Lizasoain I, Fernández-Tomé P, Álvarez-Barrientos A, Leza JC, Lorenzo P, Moro MA: TACE/ADAM17-TNF-α pathway in rat cortical cultures after exposure to oxygen-glucose deprivation or glutamate. J Cereb Blood Flow Metab 2002;22:576–585.

89 Cárdenas A, Moro MA, Leza JC, O'Shea E, Davalos A, Castillo J, Lorenzo P, Lizasoain I: Up-regulation of TACE/ADAM17 after ischemic preconditioning is involved in brain tolerance. J Cereb Blood Flow Metab 2002;22:1297–1302.

90 Romera C, Hurtado O, Botella SH, Lizasoain I, Cárdenas A, Fernández-Tomé P, Leza JC, Lorenzo P, Moro MA: In vitro ischemic tolerance involves upregulation of glutamate transport partly mediated by the TACE/ADAM17-tumor necrosis factor-α pathway. J Neurosci 2004;24:1350–1357.

91 Kariko K, Weissman D, Welsh FA: Inhibition of toll-like receptor and cytokine signaling – a unifying theme in ischemic tolerance. J Cereb Blood Flow Metab 2004;24:1288–1304.

92 Blondeau N, Widmann C, Lazdunski M, Heurteaux C: Activation of the nuclear factor-κB is a key event in brain tolerance. J Neurosci 2001;21:4668–4677.

93 Yewdell JW: Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. Trends Cell Biol 2001;11:294–297.

94 Kopito RR: Aggresomes, inclusion bodies and protein aggregation. Trends Cell Biol 2000;10:524–530.

95 Asai A, Tanahashi N, Qiu JH, Saito N, Chi S, Kawahara N, Tanaka K, Kirino T: Selective proteasomal dysfunction in the hippocampal CA1 region after transient forebrain ischemia. J Cereb Blood Flow Metab 2002;22:705–710.

96 Nishi S, Taki W, Uemura Y, Higashi T, Kikuchi H, Kudoh H, Satoh M, Nagata K: Ischemic tolerance due to the induction of HSP70 in a rat ischemic recirculation model. Brain Res 1993;615:281–288.

97 Liu Y, Kato H, Nakata N, Kogure K: Temporal profile of heat shock protein 70 synthesis in ischemic tolerance induced by preconditioning ischemia in rat hippocampus. Neuroscience 1993;56:921–927.

98 Liu Y, Kato H, Nakata N, Kogure K: Protection of rat hippocampus against ischemic neuronal damage by pretreatment with sublethal ischemia. Brain Res 1992;586:121–124.

99 Glazier SS, O'Rourke DM, Graham DI, Welsh FA: Induction of ischemic tolerance following brief focal ischemia in rat brain. J Cereb Blood Flow Metab 1994;14:545–553.

100 Ohtsuki T, Matsumoto M, Kitagawa K, Taguchi A, Maeda Y, Hata R, Ogawa S, Ueda H, Handa N, Kamada T: Induced resistance and susceptibility to cerebral ischemia in gerbil hippocampal neurons by prolonged but mild hypoperfusion. Brain Res 1993;614:279–284.

101 Nakata N, Kato H, Kogure K: Inhibition of ischemic tolerance in the gerbil hippocampus by quercetin and anti-heat shock protein-70 antibody. Neuroreport 1993;4:695–698.

102 Kato H, Liu Y, Araki T, Kogure K: MK-801, but not anisomycin, inhibits the induction of tolerance to ischemia in the gerbil hippocampus. Neurosci Lett 1992;139:118–121.

103 Kato H, Liu Y, Kogure K, Kato K: Induction of 27-kDa heat shock protein following cerebral ischemia in a rat model of ischemic tolerance. Brain Res 1994;634:235–244.

104 Yagita Y, Kitagawa K, Ohtsuki T, Tanaka S, Hori M, Matsumoto M: Induction of the HSP110/105 family in the rat hippocampus in cerebral ischemia and ischemic tolerance. J Cereb Blood Flow Metab 2001;21:811–819.

105 Kato H, Chen T, Liu XH, Nakata N, Kogure K: Immunohistochemical localization of ubiquitin in gerbil hippocampus with induced tolerance to ischemia. Brain Res 1993;619:339–343.

106 Aoki M, Abe K, Kawagoe J, Nakamura S, Kogure K: Acceleration of HSP70 and HSC70 heat shock gene expression following transient ischemia in the preconditioned gerbil hippocampus. J Cereb Blood Flow Metab 1993;13:781–788.

107 Dhodda VK, Sailor KA, Bowen KK, Vemuganti R: Putative endogenous mediators of reconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. J Neurochem 2004;89:73–89.

108 Blanco M, Aneiros A, Lema M, Castillo J: Transient ischemic attack: a clinical model of ischemic tolerance. Cerebrovasc Dis 1999;9:9.

109 Weih M, Kallenberg K, Bergk A, Dirnagl U, Harms L, Wernecke KD, Einhaupl KM: Attenuated stroke severity after prodromal TIA: a role for ischemic tolerance in the brain. Stroke 1999;30:1851–1854.

110 Moncayo J, de Freitas GR, Bogousslavsky J, Altieri M, van Melle G: Do transient ischemic attacks have a neuroprotective effect? Neurology 2000;54:2089–2094.

111 Arboix A, Cabeza N, Garcia-Eroles L, Massons J, Oliveres M, Targa C, Balcells M: Relevance of transient ischemic attack to early neurological recovery after nonlacunar ischemic stroke. Cerebrovasc Dis 2004;18:304–311.

112 Schaller B: Ischemic preconditioning as induction of ischemic tolerance after transient ischemic attacks in human brain: its clinical relevance. Neurosci Lett 2005;377:206–211.

113 Wegener S, Gottschalk B, Jovanovic V, Knab R, Fiebach JB, Schellinger PD, Kucinski T, Jungehulsing GJ, Brunecker P, Muller B, Banasik A, Amberger N, Wernecke KD, Siebler M, Rother J, Villringer A, Weih M: MRI in Acute Stroke Study Group of the German Competence Network Stroke. Transient ischemic attacks before ischemic stroke: preconditioning the human brain? A multicenter magnetic resonance imaging study. Stroke 2004;35:616–621.

114 Castillo J, Moro MA, Blanco M, Leira R, Serena J, Lizasoain I, Davalos A: The release of tumor necrosis factor-α is associated with ischemic tolerance in human stroke. Ann Neurol 2003;54:811–819.

115 Prass K, Ruscher K, Karsch M, Isaev N, Megow D, Priller J, Scharff A, Dirnagl U, Meisel A: Desferrioxamine induces delayed tolerance against cerebral ischemia in vivo and in vitro. J Cereb Blood Flow Metab 2002;22:520–525.

116 Kapinya KJ, Lowl D, Futterer C, Maurer M, Waschke KF, Isaev NK, Dirnagl U: Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke 2002;33:1889–1898.

117 Shimizu K, Lacza Z, Rajapakse N, Horiguchi T, Snipes J, Busija DW: MitoK(ATP) opener, diazoxide, reduces neuronal damage after middle cerebral artery occlusion in the rat. Am J Physiol 2002;283:H1005–1011.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):48–53
DOI: 10.1159/000091703

Published online: May 2, 2006

Estrogens as Neuroprotectants against Ischemic Stroke

María Alonso de Leciñana[a] Jose Antonio Egido[b]

[a]Servicio de Neurología, Hospital Universitario Ramón y Cajal, and [b]Unidad de Ictus, Servicio de Neurología, Hospital Clínico Universitario San Carlos, Madrid, Spain

Key Words
Estrogens · Neuroprotection · Ischemic stroke · Hormone replacement therapy · Atherosclerosis · Pathophysiology brain ischemia

Abstract
Estrogens have proven vasoprotective properties against atherosclerosis that depend on the direct effect on vascular smooth muscle and endothelium and on systemic actions that imply serum lipids, coagulation and fibrinolytic cascades, vasoactive proteins and antioxidant systems. They also have neuroprotective effects against cerebral ischemia that include antioxidant and anti-inflammatory effects, modulation of protein synthesis, inhibition of apoptosis and trophic effects and preservation of microvascular blood flow in the ischemic area. Estrogenic actions depend on activation of specific estrogen receptors that modulate gene expression and produce long-term effects on vascular endothelial and smooth muscle cells, neurons and glia, on interaction with plasma membrane sites that produce rapid non-genomic actions and also on receptor-independent mechanisms. This paper reviews what it is known about the mechanisms underlying the vaso- and neuroprotective effects of estrogens. Experimental and clinical evidences of such protective effects are also discussed. Therapeutical implications for stroke prevention and treatment derived from the available evidence are considered.

Estrogen as a Neuroprotectant in Ischemic Stroke

Observational studies suggest that estrogen exposure may decrease the risk and delay the onset of ischemic stroke and ischemic heart disease. Actually, the incidence of atherosclerotic diseases and specifically of cerebral infarction is low among premenopausal women if compared with men of the same age, but then increases after menopause and the differences with men become less marked [1–3]. This is not only attributed to older age or changes on risk factors in favor of a proatherogenic profile in postmenopausal women [4], but also to the loss of an antiatherogenic role of estrogens. Estrogens also have proven neuroprotective actions that underlie their ability to ameliorate brain damage in neurodegenerative processes such as Parkinson's disease and Alzheimer's dementia [5] as well as after brain ischemia, as demonstrated in experimental and clinical studies [6–10]. The exact mechanisms responsible for these vaso- and neuroprotective effects are not completely understood, but they may depend both on activation of specific estrogen receptors and on receptor-independent actions [11].

In this paper we will review the recent knowledge about the mechanisms for estrogen antiatherogenic effects and neuroprotection and the existing evidence for possible therapeutical applications of these effects for prevention and treatment of ischemic stroke.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0048$23.50/0

Accessible online at:
www.karger.com/ced

María Alonso de Leciñana Cases, MD, PHD
Department of Neurology, Hospital Ramón y Cajal
Ctra de Colmenar Km 9,100
ES–28034 Madrid (Spain)
Tel. +34 91 336 8144, E-Mail mariaalonsoleci@telefonica.net

Estrogenic Activity and Estrogen Receptors

Estrogen's activity is mediated through diverse signaling processes. There are two known types of intracellular estrogen receptors (ER): ERα and ERβ. These two receptors are transcription factors that modulate expression of estrogen target genes [12]. The hormone-receptor complex binds to specific DNA sequences on gene promoters sensitive to estrogens and also interacts with proteins of the transcriptional apparatus that may act as coactivators or corepressors of transcription, so modifying protein synthesis [11, 13]. Estrogens also interact with ER-like proteins at the plasma membranes that activate downstream pathways modulating rapid non-genomic actions [14, 15]. It has been demonstrated that estrogens interact with membrane receptors for neurotrophins and neurotransmitters and, as a consequence, they can modulate neurotrophin-dependent transduction pathways and neurotransmission [11]. They also have other receptor-independent actions such as antioxidant and anti-inflammatory effects [5, 11, 13]. ER are widely distributed and have been found not only in reproductive organs but also in vascular endothelial and smooth muscle cells, myocardial cells, lungs, liver, and brain, both in neurons and glia from female animals, but from males as well [13, 16]. The exact role of each ER and the other signaling pathways for estrogen activity in vaso- and neuroprotection is not completely understood and is under investigation.

ER can also be activated by synthetic estrogens and by synthetic analogues structurally modified to eliminate the feminizing actions with preservation of other effects that may increase the potential for neuroprotection [17].

Mechanisms Underlying the Vasoprotective and Antiatherogenic Effects of Estrogens

Antiatherogenic properties of estrogens depend on the direct effects on vascular smooth muscle and endothelium and also on systemic effects that imply serum lipids and lipoproteins, coagulation and fibrinolytic cascades, vasoactive proteins (as nitric oxide (NO) and prostaglandins) and antioxidant systems. The effect of estrogens on serum lipid concentration results from ER-mediated modulation of the hepatic expression of apoprotein genes [13]. It has been demonstrated that estrogen therapy in postmenopausal women reduces total serum cholesterol and LDL cholesterol concentrations while it increases HDL cholesterol [18], resulting in a more favorable profile of serum lipids similar to that observed in women during their reproductive life [19]. Furthermore, estrogens can inhibit oxidation of LDL cholesterol [20] and consequently its atherogenicity.

Hepatic expression of genes for several coagulation and fibrinolytic proteins is also regulated by estrogens through ER. Estrogenic activity provides protection against thrombotic phenomena as a consequence of the reduction of plasma concentration of fibrinogen and other coagulation proteins [21] and also increases the potential for thrombolysis due to reduction of plasminogen activator inhibitor type I concentration [22].

As has been mentioned, endothelial and vascular smooth muscle cells show both ERα and ERβ and bind estrogens with high affinity. There is experimental evidence that both types of receptors provide protection against vascular injury [23, 24]. Activation of these ER induces long-term vasodilatory effects that depend on activation of genes encoding for enzymes such as prostacyclin synthase and nitric oxide synthase (NOS) [25]. It also increases local expression of endothelial growth factor that may respond to the re-endothelialization after vascular injury [26]. Through ER activation, estrogens also inhibit apoptosis of endothelial cells [27], migration and proliferation of smooth muscle cells [28] and expression of adhesion molecules [29]. These actions together with the vasodilatory effects of prostacyclin and NO may be responsible for the antiatherogenic role of estrogens. But there are other rapid and direct effects of estrogens on vascular cells that are not ER-mediated and consequently not dependent on changes in gene expression. At physiologic concentrations, estrogens stimulate the opening of calcium-activated potassium channels of vascular smooth muscle cells through a NO and cGMP-dependent pathway, thus conditioning relaxation of smooth muscle and vasodilatation [30].

Clinical Evidence about the Efficacy of Estrogens in Stroke Prevention

The evidence about the vasoprotective effects of estrogens together with observational data from epidemiological studies led to the hypothesis that, after the menopause, the risk of atherothrombotic stroke and ischemic heart disease (IHD) would increase due, at least in part, to deprivation of estrogenic activity. As a consequence, hormone replacement therapy (HRT) was implemented in postmenopausal women in the hope of restoring protection against atherosclerosis [31, 32]. Some studies demonstrate slowing of progression [33–35] or even regression [36] of carotid atheroma among women treated with HRT, but others do not confirm these findings [37, 38]. Observational studies have reported reduction of risk of ischemic stroke [39], while others have not found any effect [40, 41] or even shown an increased risk associated

with combined therapy with estrogens and progestagens or high doses of estrogens alone [42]. Randomized placebo-controlled clinical trials have failed to demonstrate any protective effect of HRT. The Heart and Estrogen-Progestin Replacement Study (HERS) shows that combined estrogen/progestagen long-term therapy does not influence the incidence of IHD or ischemic stroke in postmenopausal women with prior IHD, and during the first year of treatment the incidence of events increased among treated women [43, 44]. The Women's Health Initiative (WHI) trial concludes that combined HRT increases the absolute risk of ischemic stroke and IHD in previous healthy postmenopausal women [45] and the same conclusion arises from a meta-analysis of studies of HRT for primary prevention of stroke and IHD [46]. Secondary prevention of stroke is not influenced by estrogen replacement therapy in postmenopausal women as shown in the Women's Estrogen for Stroke Trial (WEST) [47]. A more recent meta-analysis concludes that HRT increases the risk of ischemic stroke both in healthy postmenopausal women and in women with prior stroke [48]. These results discourage the use of HRT for stroke or IHD prevention after menopause, but contradict previous epidemiological and research data that strongly suggest beneficial effects of estrogens on vascular disease and do not answer the question about the effect, whether protective or not, of endogenous estrogens on the risk of stroke. HRT consists of a different combination of synthetic estrogens and progestagens or estrogens alone at non-physiological doses and at a non-physiological time during a woman's life. We might consider that the effects of exogenous hormones after menopause on atherogenesis and stroke risk could be different from those of endogenous estrogens during ovarian activity. The lack of beneficial effects of HRT shown in randomized trials could be explained also by the fact that none of them distinguish between etiological subtypes of ischemic stroke and the particular risk for each one associated with HRT [43, 45–48]. If estrogens protect against atherosclerosis, they would prevent atherothrombotic stroke and possibly lacunar infarction but not cardioembolic stroke. Including strokes of cardiac origin in the analysis might mask a possible protective effect against atherosclerosis and atherothrombotic stroke. We have recently found in a multicentric observational paired case-control study that longer exposure to endogenous estrogens is a protective factor against non-cardioembolic ischemic stroke, as earlier onset of menarche (<13 years) and longer duration of ovarian activity (>34 years) are inversely associated with the risk of having a stroke among postmenopausal women [49] (final results not yet published). This data would support the hypothesis that endogenous estrogens may prevent ischemic stroke due to atherothrombosis or small vessel disease and may help to identify those women at higher risk after cessation of ovarian activity who would benefit from more intense prevention programs.

Mechanisms of Estrogen-Mediated Neuroprotection against Brain Ischemia

As already mentioned, there is experimental evidence that estradiol and other estrogens or estrogen-like compounds enhance viability and favor recovery of neuronal cells in animal models of global and focal cerebral ischemia in males and females [6–9, 17]. Mechanisms underlying this protection are not completely understood. Both types of ER are implicated, as well as the interaction with membrane-binding sites and ER-independent pathways [5, 11, 13, 50]. As a result of these actions, estrogens are responsible for trophic effects, protein synthesis modulation, regulation of mechanisms of cell death, and anti-inflammatory and antioxidant effects.

Estrogens modulate expression of genes implicated in the control of cell death and more specifically in regulation of apoptosis. For example, estradiol enhances transcription of antiapoptotic genes of the Bcl-2 family proteins, such as Bcl-x, via activation of the transcription factor CREB (cyclic AMP response element-binding protein) [51], while it inhibits transcription of proapoptotic BAD [52–54] and blocks mitochondrial release of cytochrome c to the cytosol, thus inhibiting the mitochondrial pathway for apoptosis [55]. Even more, estrogens determine activation of the serine/threonine kinase Akt that is a downstream effector of the phosphoinositide-3 kinase. When Akt is activated it phosphorylates, and as a consequence inhibits various mediators of apoptosis such as BAD and caspase-9 [51, 56]. Finally, there is experimental evidence that estradiol reduces caspase-3 activation and DNA fragmentation in experimental models of global and focal cerebral ischemia [57, 58] and that it can inhibit expression of calpain, a calcium-dependent protease activated after ischemia that is implicated in direct caspase-3 activation [59]. All these effects lead to inhibition of apoptosis.

It has been demonstrated that estrogens interact with neurotrophins in the nervous system and such interactions have been involved in the mechanisms of estrogen-mediated neuroprotection. Estrogens enhance expression of brain-derived neurotrophic factor and neural growth factor [60], and regulate expression of neurotrophin receptors [57]. Genes encoding for ER and neurotrophin

receptors are colocalized in various neuronal populations and this observation led to the hypothesis that estrogens and growth factors may cooperate in regulation of specific expression of genes involved in cell survival or in restoration mechanisms [11].

The anti-inflammatory effect of estrogens has been documented in various studies. Estradiol down-regulates expression of inflammatory factors and reduces inflammatory cells migration into the central nervous system and leukocyte adhesion after brain ischemia and reperfusion [61]. This is associated with an attenuation of TNF-α expression [62] and modulation of synthesis of matrix metalloprotease-9 and receptor for fraction C3 of complement [63]. Anti-inflammatory effects also depend on inhibition of microglial activation and phagocytic activity by blockade of the inducible isoenzyme of the NOS (iNOS) and of nuclear factor-κB activation [64, 65]. Some of these actions appear to be related to activation of ER in microglial cells. Leukocyte adhesion into microvessels is enhanced after brain ischemia in ovariectomized female rats compared with intact rats with normal estrogen concentration [66].

Other neuroprotective effects depend on activation of constitutive NOS both in endothelium (eNOS) and in neurons (nNOS) via an ERα-mediated and calcium-dependent non-genomic mechanism [67, 68]. Increased synthesis of NO in endothelium improves intraischemic cerebral perfusion [69], but although there is increasing evidence demonstrating the involvement of NO in estrogen-mediated neuroprotection, its exact mechanisms of action should be further investigated.

Neuroprotective effects of estrogens are also related to modulation of neurotransmission and neuronal excitability via interaction with neurotransmitter receptors. Estrogens appear to inhibit glutamate NMDA receptor function, thus attenuating intracellular calcium increase after glutamate release. Maintenance of intracellular calcium homeostasis seems to contribute to neuroprotection provided by estrogens after cerebral ischemia [70].

Steroids also have an antioxidant activity although it is observed with supraphysiologic concentrations of the hormone, so this effect may not represent a significant mechanism of neuroprotection provided by estrogens in vivo [11].

Evidence about the Protective Effect of Estrogens against Acute Cerebral Ischemia

Estrogens have been shown to protect brain from cerebral ischemia as treatment improves histological, physiological and behavioral outcomes in animal models of stroke after transient or permanent middle cerebral artery occlusion, in models of global ischemia and in models of subarachnoid hemorrhage [16]. Beneficial effects have been demonstrated in animals of both sexes and in young and senescent rodents. Most of the experiments investigate the neuroprotective effect of chronic estrogen exposure. The conclusions of these works indicate that the therapeutic range for estrogen therapy is narrow and that any benefit disappears when plasma concentration of estrogen is beyond the physiological range [16]. When studied as treatment during the acute phase, no protection is seen if administered at low physiological levels soon after ischemia [71], but neuroprotective effects of estradiol are clearly demonstrated if initiated at the time or just before the ischemic event [9, 72]. When higher doses are used, the window of opportunity for acute estrogen treatment is widened up to 6 h after ischemia [73, 74]. Synthetic analogues of estrogens with chemical modifications that eliminate the feminizing effects can reduce infarct size in rats subjected to focal cerebral ischemia [17].

To our knowledge, up until now, no clinical trials have investigated the therapeutical effects of estrogens as being neuroprotective during the acute phase of ischemic stroke.

In conclusion, endogenous estrogens might protect against atherothrombosis and subsequently against atherothrombotic or lacunar stroke. Those women with shorter exposure to ovarian estrogens (later age at menarche and shorter duration of ovarian activity) could be at higher risk of stroke after menopause, thus prevention therapies would be implemented among these women. Estrogens also have neuroprotective effects against ischemic brain damage, and it is these effects that should be further investigated and verified in the clinical setting. Synthetic analogues of estrogens with no feminizing effects might be investigated in clinical trials of acute ischemic stroke.

References

1 Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU: Epidemiology of ischemic stroke subtypes according to TOAST criteria. Incidence, recurrence and long-term survival in ischemic stroke subtypes: a population-based study. Stroke 2001;32:2735–2740.

2 Sacco RL, Boden-Albala B, Gan R, Kargman DE, Paik M Shea S, Hauser WA: Stroke incidence among white, black and Hispanic residents of an urban community. Am J Epidemiol 1998;147:259–268.

3 Stegmayr B, Asplund K, Kuulasmaa K, Rajakangas AM, Thorvaldsen P, Tuomiletho J: Stroke incidence and mortality correlated to stroke risk factors in the WHO MONICA Project. An ecological study of 18 populations. Stroke 1997;28:1367–1374.

4 Sutton-Tyrrell K, Lassila HC, Meilahn E, Bunker C, Matthews KA, Kuller LH: Carotid atherosclerosis in premenopausal and postmenopausal women and its association with risk factors measured after menopause. Stroke 1998;29:1116–1121.

5 García Segura LM, Azcoitia I, Don Carlos LL: Neuroprotection by estradiol. Prog Neurobiol 2001;63:29–60.

6 Zhang YQ, Shi J, Rajakumar G, Day AL, Simpkins JW: Effects of gender and estradiol on focal brain ischemia. Brain Res 1998;784:321–324.

7 Fukuda K, Yao H, Ibayashi S, Nakahara T, Uchimura H, Fujishima M, Hall ED: Ovariectomy exacerbates and estrogen replacement attenuates photothrombotic focal ischemic brain injury in rats. Stroke 2000;31:155–160.

8 Sudo S, Wen TC, Desaki J, Matsuda S, Tanaka J, Arai T, Maeda N, Sakanaka M: Beta-estradiol protects hippocampal CA1 neurons against transient forebrain ischemia in gerbil. Neurosci Res 1997;29:344–345.

9 Toung TJ, Traystman RJ, Hurn PD: Estrogen-mediated neuroprotection after experimental stroke in male rats. Stroke 1998;29:1666–1670.

10 Paganini-Hill A, Ross RK, Henderson BE: Postmenopausal oestrogen treatment and stroke: a prospective study. BMJ 1988;297:519–522.

11 Amantea D, Russo R, Bagetta G, Corasiniti MT: From clinical evidence to molecular mechanisms underlying neuroprotection afforded by estrogens. Pharmacol Res 2005;52:119–132.

12 Muramatsu M, Inoue S: Estrogen receptors: how do they control reproductive and nonreproductive functions? Biochem Biophys Res Commun 2000;270:1–10.

13 Mendelsohn ME, Karas RH: The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999;340:1801–1811.

14 Kelly MJ, Levin ER: Rapid actions of plasma membrane estrogen receptors. Trends Endocrinol Metab 2001;12:152–156.

15 Levin ER: Cellular functions of plasma membrane estrogen receptors. Steroids 2002;67:471–475.

16 Hurn PD, Brass LM: Estrogen and stroke. A balanced analysis. Stroke 2003;34:338–341.

17 Simpkins JW, Yang SH, Liu R, Perez E, Cai ZY, Covey DF, Green PS: Estrogen-like compounds for ischemic neuroprotection. Stroke 2004;35(suppl I):2648–2651.

18 Writing Group for the PEPI Trial: Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. JAMA 1995;273:199–208.

19 Matthews KA, Meilahn E, Kuller LH, Kelsey SF, Caggiula AW, Wing RR: Menopause and risk factors for coronary heart disease. N Engl J Med 1989;321:641–646.

20 Sack MN, Rader DJ, Cannon RO III: Oestrogen and inhibition of oxidation of low density lipoproteins in postmenopausal women. Lancet 1994;343:269–270.

21 Writing Group for the Estradiol Clotting Factors Study: Effects on haemostasis of hormone replacement therapy with transdermal estradiol and oral sequential medroxyprogesterone acetate: a one-year, double-blind, placebo-controlled study. Thromb Haemost 1996;75:476–480.

22 Gebara OCE, Mittleman MA, Sutherland P, Lipinska I, Matheney T, Xu P, Welty FK, Wilson PWF, Levy D, Muller JE, Tofler GH: Association between increased estrogen status and increased fibrinolytic potential in the Framingham Offspring Study. Circulation 1995;91:1952–1958.

23 Iafrati MD, Karas RH, Aronovitz M, Kim S, Sullivan TR Jr, Lubahn DB, O'Donnell TF Jr, Korach KS, Mendelsohn ME: Estrogen inhibits the vascular injury response in estrogen receptor α-deficient mice. Nat Med 1997;3:545–548.

24 Lindner V, Kim SK, Karas RH, Kuiper GG, Gustafsson JA, Mendelsohn ME: Increased expression of estrogen receptor-β mRNA in male blood vessels after vascular injury. Circ Res 1998;83:224–229.

25 Binko J, Majewski H: 17β-Estradiol reduces vasoconstriction in endothelium-denuded rat aortas through inducible NOS. Am J Physiol 1998;274:H853–H859.

26 Krasinski K, Spyridopoulos I, Asahara T, van der Zee R, Isner JM, Losordo DW: Estradiol accelerates functional endothelial recovery after arterial injury. Circulation 1997;95:1768–1772.

27 Spyridopoulos I, Sullivan AB, Kearney M, Isner JM, Losordo DW: Estrogen-receptor-mediated inhibition of human endothelial cell apoptosis. Estradiol as a survival factor. Circulation 1997;95:1505–1514.

28 Sullivan TR Jr, Karas RH, Aronovitz M, Faller GT, Ziar JP, Smith JJ, O'Donnell TF Jr, Mendelsohn ME: Estrogen inhibits the response-to-injury in a mouse carotid artery model. J Clin Invest 1995;96:2482–2488.

29 Caulin-Glaser T, Watson CA, Pardi R, Bender JR: Effects of 17β-estradiol on cytokine-induced endothelial cell adhesion molecule expression. J Clin Invest 1996;98:36–42.

30 Chen Z, Yuhanna IS, Galcheva-Gargova ZI, Karas RH, Mendelsohn ME, Shaul PW: Estrogen receptor-α mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. J Clin Invest 1999;103:401–406.

31 Mosca L, Collins P, Herrington DM, Mendelsohn ME, Pasternak RC, Robertson RM, Schenck-Gustafsson K, Smith SC Jr, Taubert KA, Wenger NK: Hormone replacement therapy and cardiovascular disease: a statement for healthcare professionals from the American Heart Association. Circulation 2001;104:499–503.

32 LM Brass: Hormone replacement therapy and stroke. Clinical trials review. Stroke 2004;35(suppl 1):2644–2647.

33 Baron YM, Galea R, Brincat M: Carotid artery wall changes in estrogen-treated and untreated postmenopausal women. Obstet Gynecol 1998;91:982–986.

34 Jonas HA, Kronmal RA, Psaty BM, Manolio TA, Meilahn EN, Tell GS, Tracy RP, Robbins JA, Anton-Curver H: Current estrogen-progestin and estrogen replacement therapy in elderly women: association with carotid atherosclerosis. CHS Collaborative Research Group: Cardiovascular Health Study. Ann Epidemiol 1996;6:314–323.

35 Espeland MA, Applegate W, Furberg CD, Lefkowitz D, Rice L, Hunninghake D: Estrogen replacement therapy and progression of intimal-medial thickness in the carotid arteries of postmenopausal women. ACAPS investigators. Asymptomatic Carotid Atherosclerosis Progression Study. Am J Epidemiol 1995;142:1011–1019.

36 Akkad A, Harsthorne T, Bell PR, al-Azzawi F: Carotid plaque regression on oestrogen replacement: a pilot study. Eur J Vasc Endovasc Surg 1996;11:347–348.

37 Nabulsi AA, Folsom AR, Szklo M, White A, Higgins M, Heiss G: No association of menopause and hormone replacement therapy with carotid artery intima-media thickness. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Circulation 1996;94:1857–1863.

38 Herrington DM, Reboussin DM, Brosnihan KB, Sharp PC, Shumaker SA, Snyder TE, Furberg CD, Kowalchuk GJ, Stuckey TD, Rogers WJ, Givens DH, Waters D: Effects of estrogen replacement on the progression of coronary artery atherosclerosis. N Engl J Med 2000;343:522–529.

39 Petitti DB, Sidney S, Quesenberry CP, Bernstein A: Ischemic stroke and use of estrogen and estrogen/progestogen as hormone replacement therapy. Stroke 1998;29:23–28.
40 Falkeborn M, Persson I, Terent A, Adami HO, Lithell H, Bergström R: Hormone replacement therapy and the risk of stroke. Arch Intern Med 1993;153:1201–1209.
41 Pedersen AT, Lidegaard O, Kreiner S, Ottesen B: Hormone replacement and risk of non-fatal stroke. Lancet 1997;350:1277–1283.
42 Groodstein F, Manson JE; Colditz GA, Willett WC, Speizer FE: A prospective observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease. Ann Intern Med 2000;133:933–941.
43 Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Wittinghoff E and the HERS Research Group: Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/Progestin Replacement Study (HERS) Research Group. JAMA 1998;280:605–613.
44 Simon JA, Hsia J, Cauley JA, Richards C, Harris F, Fong J, Barret-Connor E, Hulley SB for the HERS Research Group: Postmenopausal hormone therapy and risk of stroke. The Heart and Estrogen-Progestin Replacement Study (HERS). Circulation 2001;103:638–642.
45 Writing Group for the Women's Health Initiative Investigators: Risks and benefits of estrogen plus progestin in healthy postmenopausal women. Principal results from the Women's Health Initiative randomized controlled trial. JAMA 2002;288:321–333.
46 Nelson HD, Humphrey LL, Nygren P, Teustch SM, Allan JD: Postmenopausal hormone replacement therapy. JAMA 2002;288:872–881.
47 Viscoli CM, Brass LM, Kernan WN, Sarrel PM, Suissa S, Horwitz RI: Estrogen replacement after ischemic stroke: report of the Women's Estrogen for Stroke Trial (WEST). N Engl J Med 2001;345:1243–1249.
48 Bath PM, Gray LJ: Association between hormone replacement therapy and subsequent stroke: a meta-analysis. BMJ 2005;330:342–345.
49 Alonso de Leciñana M, Egido JA, Fernández C, Martínez-Vila E, Mostacero E, Morales A, Gil-Peralta A, Pareja A, Álvarez-Sabín J, Casado I, on behalf of the PIVE Study Investigators: The PIVE Study: risk of ischemic stroke and duration of estrogenic life. Cerebrovasc Dis 2005;19(suppl 2):1.
50 Wise PM: Estrogens and neuroprotection. Trends Endocrinol Metab 2002;13:229–230.
51 Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, Reusch JE: Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem 2000;275:10761–10766.
52 Zhao L, Wu T, Brinton RD: Estrogen receptor subtypes α and β contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res 2004;1010:22–34.
53 Dubal DB, Shughrue PJ, Wilson ME, Merchenthaler I, Wise PM: Estradiol modulates Bcl-2 in cerebral ischemia: a potential role for estrogen receptor. J Neurosci 1999;19:6385–6393.
54 Alkayed NJ, Goto S, Sugo N, Joh HD, Klaus J, Crain BJ, Bernard O, Traystman RJ, Hurn PD: Estrogen and Bcl-2: gene induction and effect of transgene in experimental stroke. J Neurosci 2001;21:7543–7550.
55 Bagetta G, Chiappetta O, Amantea D, Iannone M, Rotiroti D, Costa A, Nappi G, Corasaniti MT: Estradiol reduces cytochrome c translocation and minimizes hippocampal damage caused by transient global ischemia in rat. Neurosci Lett 2004;368:87–91.
56 Choi YC, Lee JH, Hong KW, Lee KS: 17β-Estradiol prevents focal cerebral ischemic damages via activation of Akt and CREB in association with reduced PTEN phosphorylation in rats. Fundam Clin Pharmacol 2004;18:547–557.
57 Jover T, Tanaka H, Calderone A, Oguro K, Bennett MV, Etgen AM, Zukin RS: Estrogen protects against global ischemia-induced neuronal death and prevents activation of apoptotic signaling cascades in the hippocampal CA1. J Neurosci 2002;22:2115–2224.
58 Rau SW, Dubal DB, Bottner M, Gerhold LM, Wise PM: Estradiol attenuates programmed cell death after stroke-like injury. J Neurosci 2003;23:11420–11426.
59 Sribnick EA, Ray SK, Nowak MW, Li L, Banik NL: 17β-Estradiol attenuates glutamate-induced apoptosis and preserves electrophysiologic function in primary cortical neurons. J Neurosci Res 2004;76:688–696.
60 Jezierski MK, Sohrabji F: Region- and peptide-specific regulation of the neurotrophins by estrogen. Brain Res Mol Brain Res 2000;85:77–84.
61 Santizo RA, Anderson S, Ye S, Koenig HM, Pelligrino DA: Effects of estrogen on leukocyte adhesion after transient forebrain ischemia. Stroke 2000;31:2231–2235.
62 Liao SL, Chen WY, Chen CJ: Estrogen attenuates tumor necrosis factor-α expression to provide ischemic neuroprotection in female rats. Neurosci Lett 2002;330:159–162.
63 Vegeto E, Belcredito S, Etteri S, Ghisletti S, Brusadelli A, Meda C, Krust A, Dupont S, Ciana P, Chambon P, Maggi A: Estrogen receptor-α mediates the brain anti-inflammatory activity of estradiol. Proc Natl Acad Sci USA 2003;100:9614–9619.
64 Bruce-Keller AJ, Keeling JL, Keller JN, Huang FF, Camondola S, Mattson MP: Anti-inflammatory effects of estrogen on microglial activation. Endocrinology 2000;141:3646–3656.
65 Wen Y, Yang S, Liu R, Perez E, Yi KD, Koulen P, Simpkins JW: Estrogen attenuates nuclear factor-κB activation induced by transient cerebral ischemia. Brain Res 2004;1008:147–154.
66 Santizo R, Pellegrino DA: Estrogen reduces leukocyte adhesion in the cerebral circulation in female rats. J Cereb Blood Flow Metab 1999;19:1061–1065.
67 McNeill AM, Kim N, Duckles SP, Krause DN, Kontos HA: Chronic estrogen treatment increases levels of endothelial nitric oxide synthase protein in rat cerebral microvessels. Stroke 1999;30:2186–2190.
68 Grohe C, Kann S, Fink L, Djoufack PC, Paehr M, van Eickels M, Vetter H, Meyer R, Fink KB: 17β-Estradiol regulates nNOS and eNOS activity in the hippocampus. Neuroreport 2004;15:89–93.
69 Prorock AJ, Hafezi-Moghadam A, Laubach VE, Liao JK, Ley K: Vascular protection by estrogen in ischemia-reperfusion injury requires endothelial nitric oxide synthase. Am J Physiol 2003;284:H133–H140.
70 Chen J, Adachi N, Liu K, Arai T: The effects of 17β-estradiol on ischemia-induced neuronal damage in the gerbil hippocampus. Neuroscience 1998;87:817–822.
71 Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM: Estradiol protects against ischemic injury. J Cereb Blood Flow Metab 1998;18:1253–1258.
72 Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL: Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. J Neurosurg 1997;87:724–730.
73 Yang SH, Shi J, Day AL, Simpkins JW: Estradiol exerts neuroprotective effects when administered after ischemic insult. Stroke 2000;31:745–749.
74 Yang SH, Liu R, Wu SS, Simpkins JW: The use of estrogens and related compounds in the treatment of damage from cerebral ischemia. Ann NY Acad Sci 2003;1007:101–107.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):54–63
DOI: 10.1159/000091704

Published online: May 2, 2006

Neurorepair versus Neuroprotection in Stroke

Olivia Hurtado[a] Jesus M. Pradillo[a] David Alonso-Escolano[a]
Pedro Lorenzo[a] Tomás Sobrino[b] José Castillo[b] Ignacio Lizasoain[a]
María Angeles Moro[a]

[a]Departamento de Farmacología, Facultad de Medicina, Universidad Complutense de Madrid, Madrid, and
[b]Departamento de Neurología, Division de Neurología Vascular, Laboratorio de Investigación Neurovascular, Hospital Clínico Universitario, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Key Words
Stroke, neurorepair vs. neuroprotection · Brain plasticity · Stroke, recovery · Neurogenesis · Cerebral ischemia

Abstract
Stroke is the second to third leading cause of death and the main cause of severe, long-term disability in adults. However, treatment is almost reduced to fibrinolysis, a therapy useful in a low percentage of patients. Given that the immediate treatment for stroke is often unfeasible in the clinical setting, the need for new therapy strategies is imperative. After stroke, the remaining impairment in functions essential for routine activities, such as movement programming and execution, sensorimotor integration, language and other cognitive functions have a deep and life-long impact on the quality of life. An interesting point is that a slow but consistent recovery can be observed in the clinical practice over a period of weeks and months. Whereas the recovery in the first few days likely results from edema resolution and/or from reperfusion of the ischemic penumbra, a large part of the recovery afterwards is due mainly to brain plasticity, by which some regions of the brain assume the functions previously performed by the damaged areas. Neurogenesis and angiogenesis are other possible mechanisms of recovery after stroke. An understanding of the mechanisms underlying functional recovery may shed light on strategies for neurorepair, an alternative with a wide therapeutic window when compared with neuroprotective strategies.

Mechanisms of Functional Recovery

Several mechanisms have been proposed to underlie functional recovery found after stroke.

Restitution of Penumbral Zones

First, the *restitution of penumbral zones* implies that the affected tissue has just enough energy to survive for a short period of time but not enough to function. Since penumbral neurons are still capable of re-functioning, they are believed to get involved in the acute phase in order to limit the infarcted area. Indeed, the penumbra constitutes the main target for medical intervention applied shortly after ischemic onset; although there is a great variability regarding the duration of the time period in

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0054$23.50/0

Accessible online at:
www.karger.com/ced

Dra. María Angeles Moro
Departamento de Farmacología, Facultad de Medicina
Universidad Complutense de Madrid
ES–28040 Madrid (Spain)
Tel. +34 91 394 1478, Fax +34 91 394 1463, E-Mail neurona@med.ucm.es

which the penumbral neurons remain viable, due to factors such as the location and the extent of the lesion, the ischemic penumbra can be regarded as salvageable if reperfusion occurs in time [1]. Theoretically, reperfusion may be a plausible explanation for spontaneous recovery during the first days after the stroke onset. At this time, neuroprotective strategies could be useful to enhance the spontaneous recovery.

Brain Plasticity

An important mechanism that can contribute to recovery after stroke is the *brain plasticity* caused by anatomical and functional reorganization of the central nervous system (CNS). In this context, plasticity, initially described as the ability of neuronal cortical connections to be strengthened and remodeled by our experience [2], could now be defined as the events that regulate the capacity of the CNS to change in response to injury or to physiological demands [reviewed in 3, 4]. This plastic changes also take place following a brain injury such as stroke. Indeed, plastic changes after stroke may involve different mechanisms, such as (a) redundancy of brain circuitry with parallel pathways performing similar functions such that an alternative pathway may take over when another has been damaged, (b) unmasking of functionally silent pathways, and (c) sprouting of fibers from the surviving neurons with formation of new synapses [5–9].

Plasticity may appear at the level of *map plasticity*, affecting sensory and/or motor cortical representation, or as a neuronal or *synaptic plasticity*, at the neuronal level [10]. The temporal profile of changes very likely reflects different mechanisms: short-term changes are probably due to functional enforcement in existing neural circuits due to unmasking of silent synapses, through modulation of GABAergic inhibition, whereas long-term changes involve other processes apart from unmasking of underlying synapses such as axonal regeneration and sprouting with changes in shape, number, size and type of synapses [5–7]. When damage to a system is partial, recovery inside this system may occur; however, after a complete damage, the only alternative is the recruitment of a functionally related system [11].

At the level of *map plasticity*, a large piece of evidence demonstrates that cortical representation of body parts is being continuously modulated in response to activity, behavior and skill acquisition [12, 13]. Indeed, CNS functional anatomy appears to be organized so that damage may be, at least partially, functionally compensated [reviewed in 3, 14]. Acute lesions such as stroke are known to result in reactive depressions of brain areas remote but anatomically connected to the infarcted zones, due to an interrupted functional input from the injured region, a phenomenon defined as *diaschisis* [reviewed in 6]. In this context, a possible mechanism of recovery after stroke is the resolution of diaschisis. Indeed, there is evidence indicating that cortical injury immediately after stroke results in hypometabolism and inhibition of regions remote from the infarcted area. This down-regulated neural activity is considered to be due to excitotoxicity from glutamate through NMDA receptors. Additionally, a decrease in afferent input received from the area of infarction may contribute to the suppression. Recovery is thought to take place as a result of the gradual reversal of this functional suppression of viable neuronal tissue [15, 16]. Resolution of diaschisis is likely to be important in adaptations occurring in the corresponding cortical maps not only of the involved but also of the non-involved hemisphere [17, 18]. Moreover, it seems that diaschisis is not restricted to the hemispheres, but involves the spinal cord and the cerebellum as well [19–23]. Studies of brain metabolism after cortical injury confirm that resolution of diaschisis is likely to be involved in these processes of cerebral recovery [20, 23–25].

At the level of *neuronal plasticity*, unmasking silent synapses may result when cortical areas, which are probably kept 'masked' by active tonic GABAergic inhibition, allow for a rapid change of the neuronal network by removing or modifying this synaptic inhibition [8, 26–28]. Fast functional reinforcement or weakening of synapses could also result from processes related to long-term potentiation (LTP) and long-term depression (LTD) [28–32], which have been described to induce changes in the morphology of dendritic spines, an increase in the number of perforated synapses and an increase in the number of new spines. LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites [33]. After brain ischemia, both LTP and an increase in the number of spines are induced, suggesting that they are interconnected [6]. Neuronal sprouting and synaptogenesis are probably a main determinant in long-term CNS plasticity [34]. Dendritic spines are the primary post-synaptic targets of excitatory glutamatergic synapses in the mature brain and have been proposed as primary sites of synaptic plasticity, a mechanism which may underlie cortical map reorganizations [35–38]. Dendrites and dendritic spines are subjected to constant remodeling [39] under the possible influence of the release of local neurotransmitter and neurotrophic factors, the synthesis of synaptic proteins, such as growth-associated proteins and synaptophysin proteins [40, 41], as well as a modified brain

gene expression [42–45]. In addition, training may cause plastic changes, e.g. synaptogenesis in enriched environments [46].

Neurogenesis

The discovery of adult *neurogenesis* has been considered as a possible new component of recovery that could change or create new treatments for stroke [47]. In 1962, Joseph Altman [48] was the first to find new neurons produced in adulthood, suggesting 1 year later that in mammals, neurogenesis continued post-natally [49]. In 1977, Kaplan and Hinds [50] confirmed this hypothesis by electron microscopy, and they found new neurons in the dentate gyrus (DG) and olfactory bulb of adult rats. In recent years it has been demonstrated that neurogenesis exists in the adult nervous system [51–53], and that it can be increased after brain injuries such as seizures or stroke [54, 55].

The regulation of neurogenesis is beginning to be understood: it appears to be influenced by factors such as aging, environmental stimulation, exercise, genetic background, stress and afferent inputs to the dentate granule cell layer [56, 57] and also by pathological factors such as cerebral ischemia [58, 59], where newborn neurons can replace neurons that have died after an insult and thus contribute to function recovery [60].

In 1998, Liu et al. [61] were the first to find an increased birth of dentate progenitor cells after global ischemia in adult rodent. These authors, using immunohistochemistry to detect the incorporation of 5-bromodeoxyuridine, found increased cellular proliferation in the subgranular zone (SGZ) of the DG 1–2 weeks after global ischemia.

Regions Implicated in Neurogenesis. Neurogenesis produces a generation of neurons from neural stem cells (NSCs) and occurs in two specific regions of the adult brain of many animals: the SGZ of the hippocampal DG and the forebrain subventricular zone (SVZ) of the lateral ventricle (fig. 1) [62–64].

NSCs are present during embryonic and post-natal development and persist during adult life. NSCs are cells that have the ability to (1) regenerate exact copies of themselves, (2) produce exponential cell growth and division, (3) give rise to all of the mature neurons and glia, and (4) possess neuronal and glial lineage commitment, migration, and progressive cellular maturation in response to a variety of injury signals (including ischemia). It has been reported that stem cells can also be isolated from spinal cord [65], diencephalon [65] and other brain regions [66].

In the SGZ, stem and progenitor cells are located at the border between the dentate hilus and the inner margins of the upper and the lower blades of the dentate granule cell layer [67]. In normal hippocampal DG, the newborn cells migrate into the granule cell layer where the majority of the cells generate new granule cells (fig. 1). The NSCs in hippocampus are present in the brain of rodents [68], primates [69], and man [52]. The neurogenesis in the SVZ lines the lateral ventricles and gives rise to new interneurons that reach the olfactory bulb via the rostral migratory stream. Once in the bulb they integrate into the granule and glomerular layers, where they differentiate into local interneurons (fig. 1) [56]. It has been described that cerebral ischemia increases neurogenesis in the SGZ of adult DG [70] and in the SVZ [71].

Recent evidence indicates that the forebrain SVZ of humans does not show neurogenesis, suggesting that in the human brain, neurogenesis may happen only in the hippocampal DG [72]. In other brain zones, like cortex, the existence of neurogenesis remains controversial [73]. Although some authors have found a small proportion of proliferation after a photothrombotic lesion [74] and after middle cerebral artery occlusion (MCAO) [75, 76], others studies did not detect neurogenesis in the injured cortex following MCAO [59, 77].

Regulation of Neurogenesis. The process of neurogenesis consists of three main steps with different mechanisms of regulation: (1) In the first step, *precursor proliferation*, there are growth factors involved such as basic fibroblast growth factor (bFGF) [78], epidermal growth factor (EGF) [79], and brain-derived neurotrophic factor (BDNF) [80, 81]; other molecules involved include neurotransmitters [70, 82], hormones [82, 83], stem cell factor [84], erythropoietin (EPO) [85], caspase inhibitors [86] and anti-inflammatory drugs [86]. (2) In the second step, *migration*, there are chemotropic factors, such as integrin subunits, ephrins and reelin [87–90]. (3) And finally, in the molecular regulation of precursor *differentiation, integration and survival*, several studies suggest that astrocytes cues act in these steps [91–93].

Under pathological circumstances, other regulatory events may be involved. The proliferation of the progenitor cells in the injured brain can be enhanced by external stimulators like environmental enrichment [94], activation of cAMP-response-element-binding protein [95] and inducible nitric oxide synthase induction [96].

The molecular mechanisms that regulate *ischemia-induced neurogenesis* are only partly understood. It is known that neurogenesis after stroke is mediated by molecules such as growth factors, EPO and glutamater-

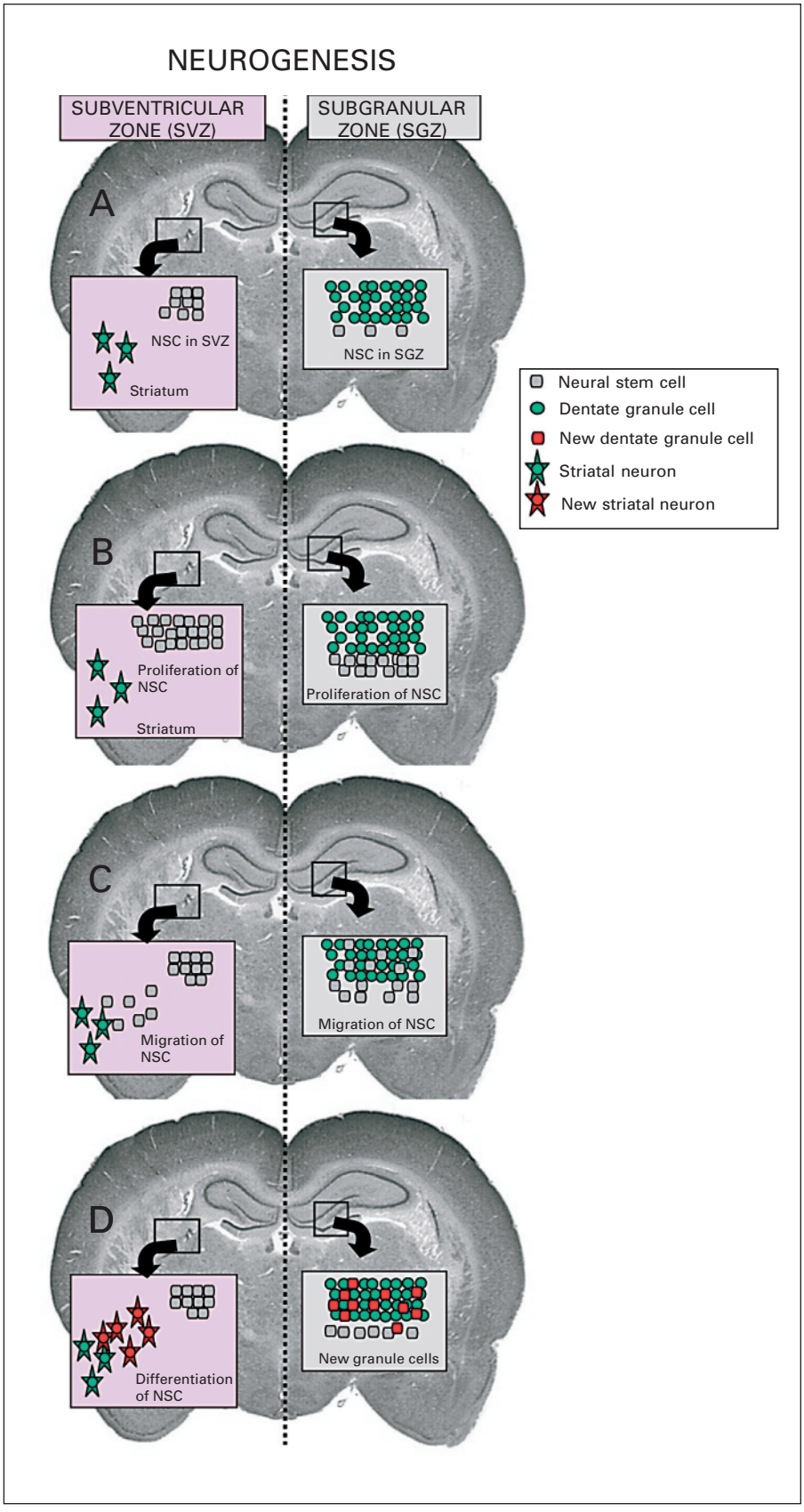


Fig. 1. Neurogenesis. **A** Neural stem cells (NSC) are located in the subventricular zone (SVZ) and in the subgranular zone (SGZ) of the dentate gyrus. **B** Proliferation of neural stem cell induced by ischemia. **C** Migration of new formed cells into the striatum or into the granule cell layer. **D** Differentiation of new striatal neurons or new granule cells. Modified from Kokaia and Lindvall [54].

gic mechanisms. In the case of *growth factors*, ischemia increases the expression of several growth factors: EGF, bFGF, BDNF and vascular endothelial growth factor (VEGF) [97–99]. These growth factors are known to induce neurogenesis after cerebral ischemia. For example, external infusion of bFGF and EGF into the lateral ventricle of the rat forebrain increases neurogenesis after global ischemia [60]. In the case of BDNF, only in the SVZ area, viral vector-mediated delivery of BDNF to the striatum leads to an increased number of new striatal neurons formed in this zone after stroke [80], but BDNF does not influence either basal or ischemia-induced proliferation of NSCs in the DG [100]. And also, administration of VEGF promotes neurogenesis in the SVZ in the ischemic penumbra region following stroke [101].

Another factor that probably modulates neurogenesis following ischemia is EPO. Intraventricular infusion of EPO leads to increased neurogenesis of olfactory bulb neurons from SVZ [85].

Glutamatergic mechanisms are also involved in the regulation of SGZ neurogenesis after global [82, 102] and focal ischemia [70]. In this context, when there is blockade of both NMDA and AMPA receptor at the time of global ischemia, the increase of neurogenesis is prevented. In focal ischemia, this blockade is prevented only with the NMDA receptor.

In spite of the new generation of neurons, it has been demonstrated that more than 80% of the new neurons die during the first weeks after stroke, resulting in a replacement of only 0.2% of the cells lost as a result of ischemia in rats [77]. Therefore, in order to improve the functional recovery, it is important not only to study the proliferation of precursors but also their survival rate.

In addition, it is important to know whether the new neurons formed after stroke are functional or not. In this context, evidence for functional neuronal replacement in the ischemically damaged brain has been reported from a model of global forebrain ischemia [60], in which new generated CA1 neurons after administration of growth factors form functional synapses and are integrated into the existing brain circuitry.

Neurogenesis and Angiogenesis. It is known that adult hippocampal neurogenesis is closely associated with angiogenesis from endothelial cell precursors, because adequate blood supply is necessary for survival and development of the news neurons [103]. Angiogenesis usually occurs in the human brain after stroke, but may have to be further stimulated to increase the number of surviving new neurons. VEGF has an important role in the vascular response to cerebral ischemia, because ischemia stimulates its expression [104] and VEGF promotes the formation of new cerebral blood vessels [105]. It is known that administration of VEGF promotes both neurogenesis in the SVZ and angiogenesis in the ischemic penumbra region following stroke [101]. The neurogenic effect of VEGF could occur through the establishment of a vascular niche that favors the proliferation and differentiation [103], or by the release of BDNF from endothelial cells [106]. Moreover, VEGF may exert a direct mitogenic effect on neuronal precursor [97].

Therapeutical Strategies

Stroke is a leading cause of death and long-lasting disability with a high socioeconomic burden; among 30-day survivors of first-ever stroke, about half survive 5 years; of survivors, one third remain disabled, and 1 in 7 are in permanent institutional care [107]. Taking into account that hemiparesis is the most common cause of disability after stroke [108], it seems essential to develop effective therapies to improve the motor recovery of these patients. In the absence of effective pharmacological therapies, *rehabilitation* is the most common treatment to improve life quality after stroke [reviewed in 109]. Most advances in this context are related to therapies combining elements of intensity and task specificity, such as constraint-induced movement therapy and body-weight-supported treadmill training [reviewed in 6, 109].

Even with optimized strategies of rehabilitation, other manipulations may be needed to increase its benefits. Activity-dependent plasticity and skill learning involve the major neurotransmitters. Interestingly, after stroke, neurotransmitters such as dopamine, acetylcholine, serotonin and noradrenaline may be interrupted or downregulated in their projections from brainstem to cortex, a mechanism that may contribute to diaschisis, described above. These 'low-aminergic' patients may then benefit from drugs affecting the noradrenergic system. In this context, drugs that increase the availability of noradrenaline such as *amphetamine* [109–113], and *methylphenidate* [114], and dopaminergic drugs such as *levodopa* [115] have shown some efficacy in stroke-rehabilitation trials when combined with physical or language therapy [reviewed in 109]. Drugs that act on these neurotransmitter receptors improve task-specific signaling [116]. Selective *serotonin reuptake inhibitors* have also shown modest efficacy in some studies [117, 118]. On the other hand, *activation of NMDA receptors*, by driving LTP, may op-

timize activity-dependent learning after stroke [119]. *Acetylcholinesterase inhibitors* may also be helpful [120, 121].

Other treatments which have shown efficacy on dendritic plasticity stimulation in animal models are *growth factors*, such as NGF or BDNF [122–124]. On the other hand, one important factor restricting brain plasticity after brain lesions is that myelin in the CNS contains proteins that inhibit axonal outgrowth [125, 126]. *Myelin inhibitors* share a common receptor, Nogo-A, and its blockage promotes CNS repair and functional recovery [127]. Some promising results with antibodies against this receptor have been reported after brain ischemic lesions [128–130].

Inosine, a naturally occurring metabolite, has likewise been reported to induce axonal rewiring and promote behavioral outcome after focal brain ischemia [131]. On the other hand, some authors have demonstrated that some efficacy of *botulinum toxin-A* for the treatment of spasticity after stroke [132].

We have recently investigated the effects of a chronic treatment with *CDP-choline*, a safe and well-tolerated drug which is known to stabilize membranes, on functional outcome and neuromorphological changes after stroke. The treatment with CDP-choline, initiated 24 h after the MCAO and maintained during 28 days, improved forelimb function in both the staircase test and the elevated body swing test, corresponding to sensorimotor integration and asymmetrical motor function, respectively [133]. Given the limitations of the previous approaches regarding restricted bioavailability or adverse effects, these results have the advantage of the fact that CDP-choline is a drug commercially available as a treatment of stroke and which it is safe and well tolerated [134].

Cell Therapy. The aim of cell therapy is to restore brain function by replacing dead neurons with new neurons through transplantation or stimulation of neurogenesis from endogenous stem/precursor cells. In theory, neurons and other cells useful for brain repair in stroke could derive from stem cells of different sources, such as embryonic stem cells from the blastocyst, NSCs from the embryonic or adult brain, or stem cells from other tissues, e.g. bone marrow [135, 136]. In animals, the transplants of different stem cells have been reported to partly reverse some behavioral deficits [137–144].

Some results that suggest that neuronal replacement induces symptomatic improvement in patients after stroke but the results are not convincing. In the only clinical trial reported so far, 12 patients with stroke affecting the basal ganglia received implants of neurons generated from the human teratocarcinoma cell line NTera-2 into the infarcted area [145]. This study revealed an increase in the European Stroke Scales score suggesting that neuronal cell transplantation could be a therapeutic option for stroke patients with a motor deficit. In a follow-up study the improvements in some affected individuals correlated with increased metabolic activity at the graft site [146]. This finding could be interpreted as graft function but might as well reflect inflammation or increased activity in host neurons [reviewed in 135, 136].

Perhaps the most favorable strategy is to combine transplantation of NSCs close to the damaged area with stimulation of neurogenesis from endogenous NSCs; also, to afford the NSCs a platform so that they can reform appropriate brain structure and connections [135, 136]. A study in neonatal mice [147] shows that if NSCs are seeded on synthetic extracellular matrix and implanted into the ischemia-damaged area, then new parenchyma composed of neurons and glia is formed and becomes vascularized. From a clinical perspective, the development of therapies for brain diseases based on neuronal self-repair is still at a very early stage. We need to understand how to control proliferation and differentiation of endogenous of NSCs into specific cell types, induce their integration into existing neural networks, and optimize the functional recovery in animal models closely resembling the human disease.

Conclusion

Considering the limitations of the potential for intervention after a stroke, the development of strategies to enhance plasticity and improve long-term outcome seems fundamental. The elucidation of the mechanisms regulating long-term recovery of post-stroke neurological sequelae may help to prompt new therapeutic strategies for this pathology.

References

1 Phan TG, Wright PM, Markus R, Howells DW, Davis SM, Donnan GA: Salvage the ischemic penumbra: more than just reperfusion? Clin Exp Pharmacol Physiol 2002;29:1–10.
2 Hebb DO (ed): The Organization of Behavior: A Neuropsychological Theory. New York, Wiley, 1949.
3 Rossini PM, Dal Forno G: Neuronal post-stroke plasticity in the adult. Restor Neurol Neurosci 2004;22:193–206.
4 Johansson BB: Brain plasticity in health and disease. Keio J Med 2004;53:231–246.
5 Lee RG, van Donkelaar P: Mechanisms underlying functional recovery after stroke. Can J Neurol Sci 1995;22:257–263.
6 Kwakkel G, Kollen B, Lindeman E: Understanding the pattern of functional recovery after stroke: facts and theories. Restor Neurol Neurosci 2004;22:281–299.
7 Calabresi P, Centonze D, Pisani A, Cupini LM, Bernardi G: Synaptic plasticity in the ischemic brain. Lancet Neurol 2003;2:622–629.
8 Sanes JN, Donoghue JP: Plasticity and primary motor cortex. Annu Rev Neurosci 2000;23:393–415.
9 Seitz RJ, Bütefisch CM, Kleiser R, Hömberg V: Reorganization of cerebral circuits in human ischemic brain disease. Restor Neurol Neurosci 2004;22:207–229.
10 Boroojerdi B, Ziemann U, Chen R, Bütefisch CM, Cohen LG: Mechanisms underlying human motor system plasticity. Muscle Nerve 2001;24:602–613.
11 Seitz RJ, Freund HJ: Plasticity of the human motor cortex; in Freund HJ, Sabel BA, Witte OW (eds): Brain Plasticity. Advances in Neurology. Philadelphia, Lippincott-Raven, 1997, vol 73, pp 321–333.
12 Kaas JH: Plasticity of sensory and motor maps in adult mammals. Annu Rev Neurosci 1991;14:137–167.
13 Donoghue JP, Hess G, Sanes JN: Motor cortical substrates and mechanisms for learning; in Bloedel JR, Ebner TJ, Wise SP (eds): Acquisition of Motor Behavior in Vertebrates. Cambridge, MIT, 1996, pp 363–386.
14 Wall JT: Development and maintenance of somatotopic maps of the skin: a mosaic hypothesis based on peripheral and central contiguities. Brain Behav Evol 1998;31:252–368.
15 Takasawa M, Watanabe M, Yamamoto S, Hoshi T, Sasaki T: Prognostic value of subacute crossed-cerebellar diaschisis: single-photon emission CT study in patients with middle cerebral artery territory infarct. Am J Neuroradiol 2002;23:189–193.
16 Carmichael ST, Tatsukawa K, Katsman D, Tsuyuguchi N, Kornblum HI: Evolution of diaschisis in a focal stroke model. Stroke 2004;35:758–763.
17 Andrews R: Transhemispheric diaschisis. Stroke 1991;22:943–949.
18 Cao Y, D'Olhaberriague L, Vikingstad EM, Levin SR, Welch KMA: Pilot study of functional MRI to assess cerebral activation of motor function after stroke hemiparesis. Stroke 1998;29:112–122.
19 Twitchell TE: The restoration of motor function following hemiplegia in man. Brain 1951;74:443–480.
20 Feeny DM, Baron J: Diaschisis. Stroke 1986;17:817–830.
21 Weiller C, Ramsey SC, Wise RJS, Friston KJ, Frackowiak RSL: Individual patterns of functional reorganization in the human cerebral cortex after capsular infarction. Ann Neurol 1991;29:63–71.
22 Atkinson PP, Atkinson JLD: Subject review: spinal shock. Mayo Clin Proc 1996;71:384–389.
23 Nudo RJ, Plautz EJ, Frost SB: The role of adaptive plasticity in recovery of function after damage to motor cortex. Muscle Nerve 2001;24:1000–1019.
24 Seitz RJ, Azari NP, Kmorr U, Binkofsky F, Herzog H, Freund HJ: The role of diaschisis in stroke recovery. Stroke 1999;30:1844–1850.
25 Nudo JR: Recovery after damage to motor cortical areas. Curr Opin Neurobiol 1999;9:740–747.
26 Jacobs KM, Donoghue JP: Reshaping the cortical motor map by unmasking latent intracortical connections. Science 1991;251:944–947.
27 Hess G, Aizenmann C, Donoghue JP: Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. J Neurophysiol 1996;75:1765–1778.
28 Hess G, Donoghue JP: Long-term depression of horizontal connections in rat motor cortex. Eur J Neurosci 1996;8:658–665.
29 Bliss TV, Lomo T: Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331–356.
30 Sakamoto T, Porter LL; Asanuma H: Long-lasting potentiation of synaptic potentials in the motor cortex produced by stimulation of the sensory cortex in the cat: a basis for motor learning. Brain Res 1987;413:360–364.
31 Feldman DE, Nicoll RA, Malenka RC: Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. J Neurobiol 1999;41:92–101.
32 Luscher C, Nicoll RA, Malenka RC, Muller D: Synaptic plasticity and dynamic modulation of the postsynaptic membrane. Nat Neurosci 2000;3:545–550.
33 Frick A, Magee J, Johnston D: LTP is accompanied by an enhanced local excitability of pyramidal neuron dendrites. Nat Neurosci 2004;7:126–135.
34 Toni N, Buchs PA, Nikonenko CR, Bron CR, Muller D: LTP promotes formation of multiple spine synapses between a single axon terminal and a dendrite. Nature 1999;402:421–425.
35 Calverley RK, Jones DG: Contributions of dendritic spines and perforated synapses to synaptic plasticity. Brain Res Brain Res Rev 1990;15:215–249.
36 Eilers J, Konnerth A: Dendritic signal integration. Curr Opin Neurobiol 1997;7:385–390.
37 Buonomano DV, Merzenich MM: Cortical plasticity: from synapses to maps. Annu Rev Neurosci 1998;21:149–186.
38 Hickmott PW, Merzenich MM: Local circuit properties underlying cortical reorganization. J Neurophysiol 2002;88:1288–1301.
39 Fisher M, Kaech S, Knutti D, Matus A: Rapid actin-based plasticity in dendritic spines. Neuron 1999;20:847–854.
40 Stroemer RP, Kent TA, Hulsebosch CE: Neocortical neural sprouting, synaptogenesis, and behavioural recovery after neocortical infarcts in rats. Stroke 1995;26:2135–2144.
41 Klintsova AY, Greenough WT: Synaptic plasticity in cortical systems. Curr Opin Neurobiol 1999;9:203–208.
42 Akins PT, Liu PK, Hsu CY: Immediate early gene expression in response to cerebral ischemia. Friend or foe? Stroke 1996;27:1682–1687.
43 Kawamata T, Dietrich WD, Schallert T, Gotts JE, Cocke RR, Benowitz LI, Finklestein SP: Intracisternal basic fibroblast growth factor enhanced functional recovery and upregulates the expression of a molecular marker of neuronal sprouting following focal cerebral infarction. Proc Natl Acad Sci USA 1997;94:8179–8184.
44 Koistinaho J, Hokfelt T: Altered gene expression in brain ischemia. Neuroreport 1997;8:i–viii.
45 Kolb B, Cote S, Ribeiro Da Silva A, Cuello AC: Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. Neuroscience 1997;76:1139–1151.
46 Johansson BB, Ohlsson AL: Environment, social interaction, and physical activity as determinants of functional outcome after cerebral infarction in the rat. Exp Neurol 1996;139:322–327.
47 Nadareishvili Z, Hallenbeck J: Neural regeneration after stroke. N Engl J Med 2003;348:2355–2356.
48 Altman J: Are new neurons formed in the brains of adult mammals? Science 1962;135:1127–1128.
49 Altman J: Autoradiographic investigation of cell proliferation in the brains of rats and cats. Anat Rec 1963;145:573–591.
50 Kaplan MS, Hinds JW: Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 1977;197:1092–1094.
51 Gage FH: Mammalian neural stem cells. Science 2000;287:1433–1438.

52 Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH: Neurogenesis in the adult human hippocampus. Nat Med 1998;4:1313–1317.
53 Gage FH: Mammalian neural stem cells. Science 2000;287:1433–1438.
54 Kokaia Z, Lindvall O: Neurogenesis after ischaemic brain insults. Curr Opin Neurobiol 2003;13:127–132.
55 Parent JM: Injury-induced neurogenesis in the adult mammalian brain. Neuroscientist 2003;9:261–272.
56 Gage FH, Kempermann G, Palmer TD, Peterson DA, Ray J: Multipotent progenitor cells in the adult dentate gyrus. J Neurobiol 1998;36:249–266.
57 Gould E, Beylin A, Tanapat P, Reeves A, Shors TJ: Learning enhances adult neurogenesis in the hippocampal formation. Nat Neurosci 1999;2:260–265.
58 Kee NJ, Preston E, Wojtowicz JM: Enhanced neurogenesis after transient global ischemia in the dentate gyrus of the rat. Exp Brain Res 2001;831:238–287.
59 Parent JM, Vexler ZS, Gong C, Derugin N, Ferriero DM: Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. Ann Neurol 2002;52:802–813.
60 Nakatomi H, Kuriu T, Okabe S, Yakamoto S, Hatano O, Kawahara N, Tamura A, Kirino T, Nakafuku N: Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. Cell 2002;110:429–441.
61 Liu J, Solway K, Messing RO, Sharp FR: Increased neurogenesis in the dentate gyrus after transient global ischemia in gerbils. J Neurosci 1998;18:7768–7778.
62 Cameron HA, McKay R: Stem cells and neurogenesis in the adult brain. Curr Opin Neurobiol 1998;8:677–680.
63 Doetsch F, García-Verdugo JM, Alvarez-Buylla A: Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. J Neurosci 1997;17:5046–5061.
64 Palmer TD, Takahashi J, Gage FH: The adult rat hippocampus contains primordial neural stem cells. Mol Cell Neurosci 1997;8:389–404.
65 Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, Reynolds BA: Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 1996;16:7599–7609.
66 Johe KK, Hazel TG, Muller T, Dugich-Djordjevic MM, McKay RD: Single factors direct the differentiation of stem cells from fetal and adult central nervous system. Genes Dev 1996;10:3129–3140.
67 Sharp FR, Liu J, Bernabeu R: Neurogenesis following brain ischemia. Dev Brain Res 2002;134:23–30.
68 Gould E, Tanapat P: Lesion-induced proliferation of neuronal progenitors in the dentate gyrus of the adult rat. Neuroscience 1997;80:427–436.
69 Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E: Hippocampal neurogenesis in adult Old World primates. Proc Natl Acad Sci USA 1999;96:5263–5267.
70 Arvidsson A, Kokaia Z, Lindvall O: N-methyl-*D*-aspartate receptor-mediated increase of neurogenesis in adult rat dentate gyrus following stroke. Eur J Neurosci 2001;14:10–18.
71 Jin K, Minami M, Lan JQ, Mao XO, Batteur S, Simon RP, Greenberg DA: Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal cerebral ischemia in the rat. Proc Natl Acad Sci USA 2001;98:4710–4715.
72 Sanai N, Tramontin AD, Quiñones-Hinojosa A, Barbaro NM, Gupta N, Kunwar S, Lawton MT, McDermott MW, Parsa AT, García Verdugo JM, Berger MS, Alvarez-Buylla A: Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 2004;427:740–744.
73 Kornack DR, Rakic P: Cell proliferation without neurogenesis in adult primate neocortex. Science 2001;294:2127–2130.
74 Gu W, Brännström T, Wester P: Cortical neurogenesis in adult rat after reversible photothrombotic stroke. J Cereb Blood Flow Metab 2000;20:1166–1173.
75 Jiang W, Gu W, Brannstrom T, Rosqvist R, Wester P: Cortical neurogenesis in adult rats after transient middle cerebral artery occlusion. Stroke 2001;32:1201–1207.
76 Zhang RL, Zhang ZG, Zhang L, Chopp M: Proliferation and differentiation of progenitor cells in the cortex and subventricular zone in the adult rat after focal cerebral ischemia. Neuroscience 2001;105:33–41.
77 Arvidsson A, Collin T, Kirik D, Kokaia Z, Lindvall O: Neuronal replacement form endogenous precursors in the adult brain after stroke. Nat Med 2002;8:963–970.
78 Yoshimura S, Teramoto T, Whalen MJ, Irizarry MC, Takagi Y, Qiu J, Harada J, Waeber C, Breakefield XO, Moskowitz MA: FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. Proc Natl Acad Sci USA 2001;98:5874–5879.
79 Teramoto T, Qiu J, Plumier JC, Moskowitz MA: EGF amplifies the replacement of parvalbumin-expressing striatal interneurons after ischemia. J Clin Invest 2003;111:1125–1132.
80 Gustafsson E, Andsberg G, Darsalia V, Mohapel P, Kirik D, Mandel RJ, Lindvall O, Kokaia Z: Anterograde delivery of brain-derived neurotrophic factor to striatum via nigral transduction of recombinant adeno-associated virus increases neuronal death but promotes neurogenic response following stroke. Eur J Neurosci 2003;17:2667–2678.
81 Chmielnicki E, Benraiss A, Economides AN, Goldman SA: Adenovirally expressed noggin and brain-derived neurotrophic factor cooperate to induce new medium spiny neurons from resident progenitor cells in the adult striatal ventricular zone. J Neurosci 2004;24:2133–2142.
82 Cameron HA, Tanapat P, Gould E: Adrenal steroids and NMDA receptor activation regulate neurogenesis in the dentate gyrus of adults rats through a common pathway. Neuroscience 1998;82:349–354.
83 Tanapat P, Hastings NB, Reeves AS, Gould E: Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. J Neurosci 1999;19:5792–5801.
84 Jin K, Mao XO, Sun Y, Xie L, Greenberg DA: Stem cell factor stimulates neurogenesis in vitro and in vivo. J Clin Invest 2002;110:311–319.
85 Shingo T, Sorokan ST, Shimazaki T, Weiss S: Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. J Neurosci 2001;21:9733–9743.
86 Ekdahl CT, Claasen JH, Bonde S, Kokaia Z, Lindvall O: Inflammation is detrimental for neurogenesis in adult brain. Proc Natl Acad Sci USA 2003;203:13632–13637.
87 Conover JC, Doetsch F, García-Verdugo JM, Gale NW, Yancopoulos GD, Alvarez-Buylla A: Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. Nat Neurosci 2000;3:1091–1097.
88 Hack I, Bancila M, Loulier K, Carroll P, Cremer H: Reelin is a detachment signal in tangential chain migration during postnatal neurogenesis. Nat Neurosci 2002;5:939–945.
89 Jacques TS, Relvas JB, Nishimura S, Pitela R, Edwards GM: Neural precursor cell chain migration and division are regulated through different β1 integrins. Development 1998;125:3167–3177.
90 Murase S, Horwitz AF: Deleted in colorectal carcinoma and differentially expressed integrins mediate the directional migration of neural precursors in the rostral migratory stream. J Neurosci 2002;11:4357–4366.
91 Doetsch F, Caillé I, Lim DA, García-Verdugo JM, Alvarez-Buylla A: Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell 1999;97:703–716.
92 Lois C, García-Vallejo JM, Álvarez-Buylla A: Chain migration of neuronal precursors. Science 1996;271:978–981.
93 Mason HA, Ito S, Corfas G: Extracellular signals that regulate the tangential migration of olfactory bulb neuronal precursors: inducers, inhibitors and repellents. J Neurosci 2001;21:7654–7663.
94 Komitova M, Perfilieva E, Mattsson B, Eriksson PS, Johansson BB: Effects of cortical ischemia and postischemic environmental enrichment on hippocampal cell genesis and differentiation in the adult rat. J Cereb Blood Flow Metab 2002;22:852–860.
95 Zhu DY, Lau L, Liu SH, Wei JS, Lu YM: Activation of cAMP-response-element-binding protein after focal cerebral ischemia stimulates neurogenesis in the adult dentate gyrus. Proc Natl Acad Sci USA 2004;101:9453–8457.

96 Zhu DY, Liu SH, Sun HS, Lu YM: Expression of inducible nitric oxide synthase after focal cerebral ischemia stimulates neurogenesis in the adult rodent dentate gyrus. J Neurosci 2003;23:223–239.
97 Jin K, Zhu Y, Sun Y, Mao XO, Xic L, Greenberg DA: Vascular endothelial growth factor stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci USA 2002;99:11946–11950.
98 Kokaia Z, Zhao Q, Kokaia M, Elmer E, Metsis M, Smith ML, Siesjo BK, Lindvall O: Regulation of brain-derived neurotrophic factor gene expression after transient middle cerebral artery occlusion with and without brain damage. Exp Neurol 1995;136:73–88.
99 Takami K, Iwane M, Kiyota Y, Miyamoto M, Tsuda R, Shiosaka S: Increase of basic fibroblast growth factor immunoreactivity and its mRNA level in rat brain following transient forebrain ischemia. Exp Brain Res 1992;90:1–10.
100 Larsson E, Mandel RJ, Klein RL, Muzyczka N, Lindvall O, Kokaia Z: Suppression on insult-induced neurogenesis in adult rat brain by brain-derived neurotrophic factor. Exp Neurol 2002;117:1–8.
101 Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA: VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest 2003;111:1843–1851.
102 Bernabeu R, Sharp FR: NMDA and AMPA/kainate glutamate receptors modulate dentate neurogenesis and CA3 synapsin-I in normal and ischemic hippocampus. J Cereb Blood Flow Metab 2000;20:1669–1680.
103 Palmer TD, Willhoite AR, Gage FH: Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000;425:479–497.
104 Zhang ZG, Zhang L, Tsang W, Soltanian-Zadeh H, Morris D, Zhang R, Goussev A, Powers C, Yeich T, Chopp M: Correlation of VEGF and angiopoietin expression with disruption of blood-brain barrier and angiogenesis after cerebral ischemia. J Cereb Blood Flow Metab 2002;22:379–392.
105 Krum JM, Mani N, Rosenstein JM: Angiogenic and astroglial responses to vascular endothelial growth factor administration in adult rat brain. Neuroscience 2002;110:589–604.
106 Louissaint A, Rao S, Leventhal C, Goldman SA: Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 2002;34:945–960.
107 Hankey GJ, Jamrozik K, Broadhurst RJ, Forbes S, Anderson CS: Long-term disability after first-ever stroke and related prognostic factors in the Perth Community Stroke Study, 1989–1990. Stroke 2002;33:1034–1040.
108 Duncan PW, Goldstein LB, Matchar D, Divine GW, Feussner J: Measurement of motor recovery after stroke: outcome assessment and sample size requirements. Stroke 1992;23:1084–1089.
109 Dobkin BH: Strategies for stroke rehabilitation. Lancet Neurol 2004;3:528–536.
110 Gladstone D, Black S: Enhancing recovery after stroke with noradrenergic pharmacotherapy: a new frontier. Can J Neurol Sci 2000;27:97–105.
111 Mattay V, Goldberg T, Fera F, Mattay V, Goldberg T: Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc Natl Acad Sci USA 2003;100:6186–6191.
112 Walker-Batson D, Smith P, Curtis S, Unwin H, Greenlee R: Amphetamine paired with physical therapy accelerates motor recovery after stroke. Stroke 1995;26:2254–2259.
113 Sawaki L, Cohen L, Classen J, Davis B, Bütefisch C: Enhancement of use-dependent plasticity by *d*-amphetamine. Neurology 2002;59:1262–1264.
114 Grade C, Redford B, Chrostowski J, Toussaint L, Blackwell B: Methylphenidate in early post-stroke recovery: a double-blind, placebo-controlled study. Arch Phys Med Rehabil 1998;79:1047–1050.
115 Scheidtmannn K, Fries W, Muller F, Koenig E: Effect of levodopa in combination with physiotherapy on functional motor recovery after stroke: a prospective, randomized, double-blind study. Lancet 2001;358:787–790.
116 Bao S, Chan V, Merzenich M: Cortical remodelling induced by activity in ventral tegmental dopamine neurons. Nature 2001;412:79–83.
117 Pariente J, Loubinoux I, Carel C, Albucher JF, Leger A, Manelfe C, Rascol O, Chollet F: Fluoxetine modulates motor performance and cerebral activation of patients recovering from stroke. Ann Neurol 2001;50:718–729.
118 Loubinoux I, Pariente J, Boulanouar K, Carel C, Manelfe C, Rascol O, Celsis P, Chollet F: A single dose of the serotonin neurotransmission agonist paroxetine enhances motor output: double-blind, placebo-controlled, fMRI study in healthy subjects. Neuroimage 2002;15:26–36.
119 Dinse H, Ragert P, Pleger B, Schwenkreis P, Tegenthoff M: Pharmacological modulation of perceptual learning and associated cortical reorganization. Science 2003;301:91–94.
120 Sawaki L, Boroojerdi B, Kaelin-Lang A, Burstein AH, Butefisch CM, Kopylev L, Davis B, Cohen LG: Cholinergic influences on use-dependent plasticity. J Neurophysiol 2002;87:166–171.
121 Conner J, Culberson A, Packowski C, Chiba A, Tuszynski M: Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. Neuron 2003;38:819–829.
122 Mervis RF, Pope D, Lewis R, Dvorak RM, Williams LR: Exogenous nerve growth factor reverses age-related structural changes in neocortical neurons in the aging rat. A quantitative Golgi study. Ann NY Acad Sci 1991;640:95–101.
123 Kolb B, Cote S, Ribeiro-da-Silva A, Cuello C: Nerve growth factor treatment prevents dendritic atrophy and promotes recovery of function after cortical injury. Neuroscience 1996;76:1139–1151.
124 Tolwani RJ, Buckmaster PS, Varma S, Cosgaya JM, Wu Y, Suri C, Shooter EM: BDNF overexpression increases dendrite complexity in hippocampal dentate gyrus. Neuroscience 2002;114:795–805.
125 Schwab ME: Myelin-associated inhibitors of neurite growth and regeneration in the CNS. Trends Neurosci 1990;13:452–456.
126 Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA, Christ F, Schwab ME: Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 2000;403;434–439.
127 Watkins TA, Barres BA: Nerve regeneration: regrowth stumped by shared receptor. Curr Biol 2002;12:R654–656.
128 Papadopoulos CM, Tsai SY, Alsbiei T, O'Brien TE, Schwab ME, Kartje GL: Functional recovery and neuroanatomical plasticity following middle cerebral artery occlusion and IN-1 antibody treatment in the adult rat. Ann Neurol 2002;51:433–441.
129 Papadopoulos CM, Tsai SY, Cheatwood JL, Bollnow MR, Kolb BE, Schwab ME, Kartje GL: Dendritic plasticity in the adult rat following middle cerebral artery occlusion and Nogo-A neutralization. Cereb Cortex 2005;Doi:10.1093/cercor/bhi132.
130 Wiessner C, Bareyre FM, Allegrini PR, Mir AK, Frentzel S, Zurini M, Schnell L, Oertle T, Schwab ME: Anti-Nogo-A antibody infusion 24 hours after experimental stroke improved behavioral outcome and corticospinal plasticity in normotensive and spontaneously hypertensive rats. J Cereb Blood Flow Metab 2003;23:154–165.
131 Chen P, Goldberg DE, Kolb B, Lanser M, Benowitz LI: Inosine induces axonal rewiring and improves behavioral outcome after stroke. Proc Natl Acad Sci USA 2002;99:9031–9036.
132 Hesse S: Recovery of gait and other motor functions after stroke: novel physical and pharmacological treatment strategies. Restor Neurol Neurosci 2004;22:359–369.
133 Lizasoain I, Cárdenas A, Hurtado O, Pradillo JM, Fernández-López D, Castillo J, Dávalos A, Secades JJ, Lozano R, Moro MA: CDP-choline improves sensorimotor recovery after ischemia. 35th Annual Meeting of the Society for Neuroscience, Washington, Nov 2005.
134 Dávalos A, Castillo J, Alvarez-Sabín J, Secades JJ, Mercadal J, Lopez S, Cobo E, Warach S, Sherman D, Clark WM, Lozano R: Oral citicoline in acute ischemic stroke. An individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.

135 Lindvall O, Kokaia Z: Recovery and rehabilitation in stroke, stem cells. Stroke 2004;25:2691–2694.
136 Lindvall O, Kokaia Z, Martínez-Serrano A: Stem cell therapy for human degenerative disorders – how to make it work. Nat Med 2004;10(suppl):42–50.
137 Modo M, Stroemer RP, Tang E, Patel S, Hodges H: Effects of implantation site of stem cell grafts on behavioural recovery from stroke damage. Stroke 2002;33:2270–2278.
138 Borlongan CV, Tajima Y, Trajanowski JQ, Lee VM, Sanberg PR: Transplantation of cryopreserved human embryonal carcinoma-derived neurons (NT2N cells) promotes functional recovery in ischemic rats. Exp Neurol 1998;149:310–321.
139 Chen J, Li Y, Wang L, Lu M, Zhang X, Chopp M: Therapeutic benefit of intracerebral transplantation of bone marrow stromal cells after cerebral ischemia in rats. J Neurol Sci 2001;189:49–57.
140 Li Y, Chopp M, Chen J, Wang L, Gautam SC, Xu YX, Zhang Z: Intrastriatal transplantation of bone marrow nonhematopoietic cells improves functional recovery after stroke in adult mice. J Cereb Blood Flow Metab 2000;20:1311–1319.
141 Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, Sanchez-Ramos J, Chopp M: Intravenous administration of human umbilical cord blood reduces behavioural deficits after stroke in rats. Stroke 2001;32:2682–2688.
142 Taguchi A, Soma T, Tanaka H, Kanda T, Nishimura H, Yoshikawa H, Tsukamoto Y, Iso H, Fujimori Y, Stern DM, Naritomi H, Matsuyama T: Administration of $CD34^+$ cells after stroke enhances neurogenesis via angiogenesis in a mouse model. J Clin Invest 2004;14:330–338.
143 Alvarez-Dolado M, Pardal R, Garcia-Verdugo JM, Fike JR, Lee HO, Pfeffer K, Lois C, Morrison SJ, Alvarez-Buylla A: Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes. Nature 2003;425:968–973.
144 Weimann JM, Johansson CB, Trejo A, Blau HM: Stable reprogrammed heterokaryons form spontaneously in Purkinje neurons after bone marrow transplant. Nat Cell Biol 2003;5:959–966.
145 Kondziolka D, Wechsler L, Goldstein S, Meltzer C, Thulborn KR, Gebel J, Jannetta P, DeCesare S, Elder EM, McGrogan M, Reitam MA, Bynum L: Transplantation of cultured human neuronal cells for patients with stroke. Neurology 2000;55:565–569.
146 Meltzer CC, Kondziolka D, Villemagne VL, Wechsler L, Goldstein S, Thulborn KR, Gebel J, Elder EM, DeCesare S, Jacobs A: Serial [^{18}F]fluorodeoxyglucose positron emission tomography after human neuronal implantation for stroke. Neurosurgery 2001;49:586–591.
147 Park KI, Teng YD, Snyder EY: The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat Biotechnol 2002;20:1111–1117.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):64–70
DOI: 10.1159/000091705

Published online: May 2, 2006

The Ischemic Penumbra: A New Opportunity for Neuroprotection

Marc Fisher

Department of Neurology, University of Massachusetts Medical School, Worcester, Mass., USA

Key Words
Acute stroke · Ischemic penumbra · Penumbral imaging · Stroke therapy

Abstract
The development of acute stroke therapies has yielded only limited success and many failures in multiple clinical trials. The target of acute stroke therapy is that portion of the ischemic region that is still potentially salvageable, i.e. the ischemic penumbra. Neuroprotective drugs have the potential to prevent a portion of the ischemic penumbra from evolving into infracted tissue and designing trials that target neuroprotective drugs at patients with persistent penumbra should enhance the likelihood of a positive outcome. Currently, diffusion and perfusion MRI has the potential to approximate the location and persistence of the ischemic penumbra and can be used in clinical trials to select appropriate patients for inclusion and to evaluate a meaningful treatment effect. Perfusion CT may also have similar capabilities. Use of these imaging modalities in clinical trials and ultimately in clinical practice will likely help in the development and utilization of novel neuroprotective drugs.

The development and approval for neuroprotective drugs for acute ischemic stroke has proven to be a difficult task. There have been many neuroprotective drugs that were evaluated in clinical trials and until recently none demonstrated a positive outcome on prespecified outcome measures [1]. There have been many potential reasons to explain the lack of success observed previously with neuroprotection in acute ischemic stroke (table 1). Many lessons have been learned from these prior neuroprotection trials that should help future trials to have a better chance for achieving a successful outcome. One of the most important lessons from prior neuroprotection trials is that the time from stroke onset to initiating therapy should not be too long. It is clearly apparent from the combined analysis of the rt-PA clinical trials that this drug's benefit is substantially greater the earlier after stroke onset it is initiated [2]. The observation that earlier initiation of rt-PA therapy is associated with a greater propensity for improved outcome is directly related to the concept of the ischemic penumbra and its evolution. Both thrombolytic and neuroprotective therapeutic approaches to acute stroke therapy are predicated on the concept that these therapies are designed to save a portion of the ischemic penumbra from evolving into infarction and that smaller infarcts on average should be associated with an improved clinical outcome as measured on various outcome scales [3]. Therefore, targeting these therapies at patients with evidence of a persistent ischemic penumbra should improve the likelihood that they will have demonstrable clinical efficacy both in treatment trials and ultimately in clinical practice. The recently reported results of the SAINT-1 trial also support this concept that early initiation of therapy should enhance the

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0064$23.50/0

Accessible online at:
www.karger.com/ced

Marc Fisher, MD
Department of Neurology, University of Massachusetts Medical School
119 Belmont Street
Worcester, MA 01605 (USA)
Tel. +1 508 334 6641, Fax +1 508 334 6695, E-Mail fisherm@ummhc.org

probability for detecting a beneficial outcome [4]. In this trial, the free radical scavenger, NXY-059 was compared to vehicle in a large, phase III clinical trial. The time to initiation of therapy was carefully controlled in the trial and the mean time to the start of therapy was less than 4 h. The primary endpoint of the trial was a shift in the Rankin Score towards a more favorable outcome not a responder analysis as in prior neuroprotection trials. This primary outcome measure demonstrated a statistically significant improvement with NXY-059, but improvement in another prespecified outcome measure, the mean NIH Stroke Scale Score, did not show any obvious difference among the two treatment groups. The results of the SAINT-1 trial are encouraging, but the results of a second trial will need to be evaluated before regulatory approval can be obtained. Subscribing to the concept that the ischemic penumbra is indeed the target of neuroprotection raises several important questions concerning the design and implementation of future neuroprotective drug development. These questions include: (1) How is the ischemic penumbra defined and how does it evolve into infarcted tissue? (2) Can the ischemic penumbra be identified in routine clinical practice and does it matter? (3) How can neuroprotective drug trials incorporate penumbral imaging and will this expedite the drug development process and drug approval?

The concept of the ischemic penumbra in acute ischemic stroke is more than 20 years old and has been extensively studied in both animal stroke models and stroke patients [5]. It is familiar to most investigators who work in the stroke field, so the concept will only briefly be reviewed. The initial definition of the ischemic penumbra by Astrup et al. [6] was that portion of the ischemic zone with absent electrical activity but with preserved ion homeostasis and transmembrane electrical potentials. Several revised definitions of the ischemic penumbra appeared over time that focused on thresholds of cerebral blood flow (CBF) decline, energy metabolism and protein synthesis (table 2). From the clinical perspective, a relatively simple and straightforward definition of the ischemic penumbra initially proposed by Hakim [7] is that portion of the ischemic region destined for infarction that is currently potentially salvageable with appropriate intervention. This definition provides a framework for approaching the important concepts of evolution of penumbra into infarcted tissue, the time window over which this evolution occurs (i.e. the therapeutic time window) and the use of imaging modalities to identify at least an approximation of this vital ischemic region.

Table 1. Potential reasons to explain why neuroprotection trials have failed

1	An appropriate time window was not used based upon preclinical data
2	Adequate drug levels were not achieved because of toxicity
3	The mechanism of drug action was not considered in trial design, i.e. drugs with no effect on white matter injury included patients with lacunar stroke (most drugs in preclinical studies showed efficacy on gray matter lesions)
4	Outcome assessment of a therapeutic response was not adjusted for baseline severity
5	The outcome assessment was not adapted to the mechanism of drug action or too great a treatment effect was expected
6	The trial included too many patients with too mild or severe neurological deficits
7	Most trials did not utilize imaging methods which could demonstrate the presence of ischemic penumbra
8	Many clinical trials were initiated on the basis of insufficient preclinical data
9	Insufficient statistical power
10	Protocol violations

Table 2. Definitions of the ischemic penumbra

1	An ischemic zone with absent electrical activity and preserved ion homeostasis and transmembrane potentials
2	An ischemic zone with reduced cerebral blood flow and preserved high-energy metabolism
3	An ischemic region with impaired protein synthesis and preserved ATP levels
4	The ischemic region with abnormal perfusion on MRI that is normal on diffusion imaging
5	The ischemic region with abnormal CBF and normal CBV on perfusion CT
6	The ischemic region with an increased oxygen extraction fraction and reduced CBF on positron emission tomography
7	A potentially salvageable ischemic region with timely initiation of therapy

In focal brain ischemia the existence and evolution of the ischemic penumbra primarily relates to varying degrees of CBF reduction within the ischemic zone [5]. That portion of the ischemic zone with little or no residual CBF, the ischemic core, evolves rapidly and should not be considered a target for acute therapy. The penumbral region has a moderate reduction of CBF and evolves over a period of time into irreversible injury, i.e. infarction, unless therapy is initiated. Within the penumbral region many mechanisms have been identified that could contribute to this evolution towards infarction, i.e. the ischemic cascade [8]. The components of the ischemic cas-

cade have expanded substantially over the past decade as knowledge about the basic science of ischemic cell injury has provided additional insights. Trying to impede components of the ischemic cascade forms the basis of neuroprotective therapy. The basic concept is that the residual CBF in the penumbral region, although modest, can deliver adequate amounts of drugs designed to block one or more components of the ischemic cascade and that such a blockade will prevent a substantial portion of the penumbra from evolving into infarction. In animal studies this hypothesis appears to be valid because many neuroprotective agents have substantially reduced infarct volumes when initiated up to several hours after the onset of experimental stroke [9]. Blockade of only one aspect of the ischemic cascade is likely a suboptimal approach to neuroprotection because of the multiplicity of mechanisms that can promote ischemic cell death. Therefore, neuroprotective drugs that impact upon multiple components of the ischemic cascade or drug combinations are likely to have better therapeutic efficacy. Obviously, combining neuroprotection with a reperfusion strategy should maximize therapeutic benefits.

Any approach to the treatment of acute focal brain ischemia should be targeted at patients with the greatest amounts of persistent ischemic penumbra because this tissue is the target of acute stroke therapy regardless of what means are employed to salvage it. Over time, the proportion of ischemic tissue that remains in the penumbral region diminishes. This shrinkage of the penumbra over time forms the basis of the therapeutic time window for treating acute ischemic stroke. The combined analysis of the rt-PA trials provides clear support for this concept [2]. In patients treated with rt-PA intravenously who were selected by clinical criteria and CT exclusion of hemorrhage but not penumbral imaging, improved outcome at 90 days was much better in patients treated earlier in the 3-hour window. Another way to approach the identification of patients most likely to benefit from acute stroke therapy is to use imaging to identify an approximation of the ischemic penumbra [10]. With very early initiation of therapy within 3 h after stroke onset, the vast majority of stroke patients have been shown to have a penumbra on imaging, so it is not surprising that intravenous rt-PA was shown to be beneficial during this time period. However, over time the percentage of stroke patients with a reasonable proportion of the ischemic zone that remains in the penumbra decreases [11]. Therefore, the most obvious way to extend the therapeutic time window for any acute stroke therapy would be to identify patients with a persistent ischemic penumbra by using advanced brain imaging and to include only these patients in a clinical trial. Extending the therapeutic time window beyond 3 h for potent reperfusion and neuroprotective therapy is dependent upon identifying and treating patients who can still respond to the therapy.

Using imaging to approximate the ischemic penumbra is an important next step in extending the therapeutic time window and for likely providing acute stroke therapy to those patients most likely to respond. Currently, diffusion/perfusion MRI and perfusion CT are the imaging modalities available that can be utilized to provide an approximation of the ischemic penumbra. Abnormalities detected as regions of hyperintensity on diffusion-weighted MRI (DWI) identify ischemic regions where high-energy metabolism has failed and loss of ion homeostasis has occurred [12]. Early after stroke onset these abnormal regions on DWI may in part be reversible and both animal and human examples of the reversibility of DWI abnormalities with early reperfusion have appeared [13, 14]. With perfusion MRI (PWI) disturbances of CBF in the microvasculature can be identified. PWI is currently performed in clinical practice with the bolus contrast technique and this methodology can only provide qualitative measurements of CBF and not absolute values [15]. The deconvolution approach to PWI measurements provides added precision but even with this addition to correct for inflow delays absolute CBF cannot be measured. The best approach for defining abnormalities on bolus contrast PWI remains contentious, but most investigators develop perfusion maps based upon mean transit time delays or time to peak delays as compared to the normal hemisphere [16]. Currently, the most widely accepted method for approximating the ischemic penumbra is to look for a mismatch in the regions of DWI and PWI abnormality with the region of PWI abnormality without a DWI abnormality assumed to represent the penumbral region. Identifying such a mismatch on screen is relatively easy and reliable (fig. 1) [10]. This approach is now widely used in centers that perform acute stroke MRI. The DWI/PWI mismatch only approximates the ischemic penumbra because as mentioned early after stroke onset not all of the DWI abnormality is irreversibly damaged and a portion of the PWI abnormality has only a modest CBF reduction, representing oligemic tissue [17]. Further refinements of DWI and PWI imaging will likely occur that will provide more quantitative estimates of individual pixel characteristics, leading to probability maps as to whether individual pixels have a low or high chance of recovery if therapy is initiated shortly after imaging is performed [18].

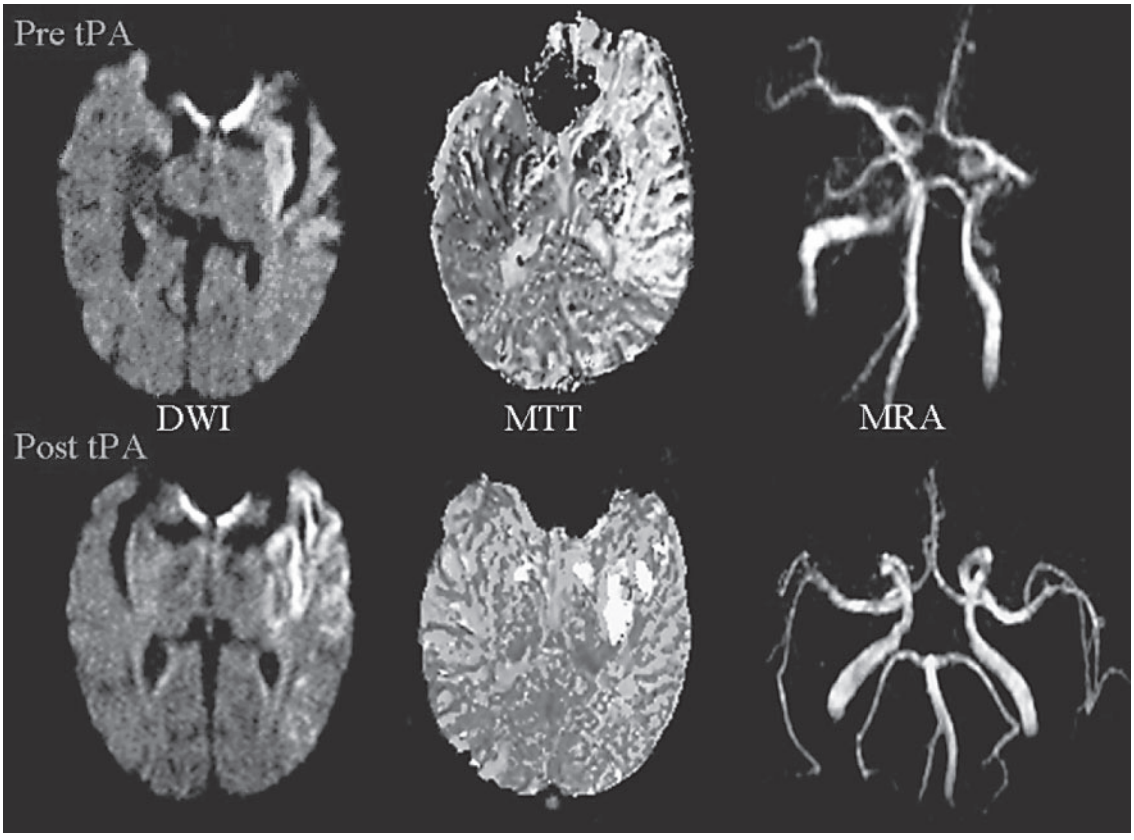


Fig. 1. The patient has evidence of a large diffusion/perfusion mismatch and an occluded proximal middle cerebral artery on an MRI battery obtained prior to intravenous rt-PA. 24 h later the perfusion abnormality has largely resolved and there is little diffusion abnormality. Courtesy of Dr. Italo Linfante, Worcester, Mass., USA.

The other imaging modality that is showing promise for penumbral imaging is perfusion CT. Wintermark et al. [19] have shown perfusion CT can identify ischemic regions with reduced CBF and also identify regions where cerebral blood volume has collapsed and autoregulation has been lost as an indicator of irreversible injury. Regions with a CBF abnormality that do not show CBV collapse are presumed to approximate the ischemic penumbra (fig. 2). The CBF/CBV mismatch on perfusion CT correlates well with regions of DWI/PWI mismatch when both studies are obtained in a close temporal window [20]. The validation of the thresholds required to identify the abnormal CBF and CBV regions remains to be proven. Other problems with perfusion CT include the need to inject iodinated contrast to obtain the images and the relatively restricted tissue volume that can be imaged currently. DWI/PWI MRI can essentially image the whole brain and is not restricted to several slices as perfusion CT currently is. Another problem with perfusion CT as compared to DWI/PWI is the tracking of presumed infarct volumes over time. An approach to assess the effect of neuroprotective treatment has been to compare pretreatment ischemic lesion volume on DWI pretreatment with the ultimate infarct at a delayed time point on T2-weighted imaging to determine if the therapy impedes the natural growth of ischemic lesion volumes over time [21]. With perfusion CT this will be more difficult but also potentially possible when various technical hurdles have been surmounted. Ultimately, it may be that DWI/PWI is more useful in proof of concept clinical trials and perfusion CT is more useful in large clinical outcome pivotal clinical trials and routine practice. It is likely that the two imaging approaches for identifying the ischemic penumbra will both have substantial and complementary utility.

Preliminary case series provide supportive evidence that penumbral imaging with both MRI and CT can impact the time window for the identification of patients

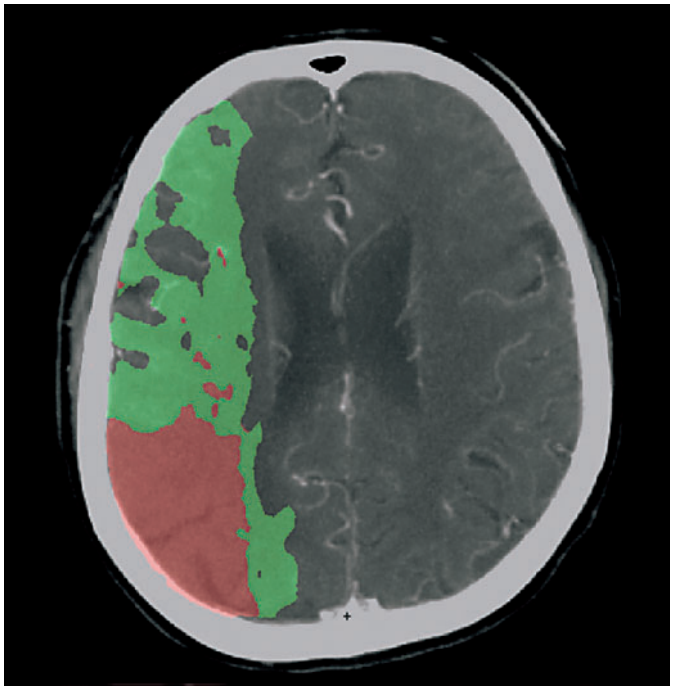


Fig. 2. This perfusion CT shows a large region with reduced CBF without a collapse of CBV (green region) and a relatively small region with both reduced CBF and CBV collapse (red region). Dr. Julien Bogousslavsky, Lausanne, Switzerland, supplied the figure.

who will benefit from intravenous Rt-PA. Several groups have shown that patients with a DWI/PWI mismatch treated with intravenous Rt-PA beyond 3 h after stroke onset had a favorable outcome rate that was similar to patients treated within 3 h after stroke onset who were not imaged to confirm the presence of an ischemic penumbra [22, 23]. The identification of stroke patients with a DWI/PWI mismatch beyond 3 h after stroke onset is now being performed in several ongoing trials to hopefully validate the hypothesis that penumbral imaging can indeed identify patients more likely to respond to intravenous thrombolysis at delayed time points. Perfusion CT identification of the ischemic penumbra has also been used to identify patients who are candidates for intra-arterial. Thrombolysis beyond 3 h and these patients had a reasonable response to treatment [24].

The use of imaging to identify an approximation of the ischemic penumbra will provide new opportunities for the evaluation and development of neuroprotective therapies. Patients for inclusion in clinical trials can be identified based upon the existence of a DWI/PWI mismatch on MRI or a CBF/CBV mismatch on perfusion CT. This approach will allow for the inclusion of stroke patients up to 9 h or longer after onset because several groups have reported that a substantial mismatch is present during this time period in a reasonable percentage of patients. Currently, MRI has the advantage over CT that the full breadth of ischemic lesion evolution can be assessed over time and the effects of treatment on this evolution determined. The use of imaging in neuroprotective drug development has a short but informative history. In several other drug development programs an MRI substudy was included in the main development program to determine if either of these neuroprotective agents affected ischemic lesion evolution [25]. No such effect was observed on MRI and no effect on clinical outcome occurred either. Two trials with citicoline utilized an MRI endpoint. In a small preliminary study, a trend towards a reduction in lesion size growth was seen in the active treatment group and a second larger study a significant effect on lesion evolution was detected [21, 26]. None of these studies required a DWI/PWI mismatch for inclusion. In the thrombolysis field, an important MRI-based study provides important lessons for future neuroprotection trials. The Desmoteplase in Acute Stroke (DIAS) study enrolled patients with a DWI/PWI mismatch of 20% or more and included patients up to 9 h from stroke onset [27]. The effects of desmoteplase on reperfusion efficacy several hours after treatment, as determined by PWI and magnetic resonance angiography, was the primary approach to evaluating treatment effects in a dose-escalation study. Late, 90-day effects on T2-determined infarct size and clinical outcome were also assessed. The study with a small number of patients per treatment group showed significant effects on early reperfusion with an apparent increase of efficacy observed with higher weight-adjusted dosing. The safety profile was quite favorable and a trend towards beneficial effects on late ischemic lesion size and clinical outcome was observed. A second similar study demonstrated similar although less robust effects [28]. Combining the results from the two desmoteplase studies provides substantial evidence for enhanced reperfusion early and evidence for later clinical and ischemic size reduction benefits. These studies are important because they demonstrate that patients can be identified up to 9 h after stroke onset who can potentially benefit from an intravenous thrombolytic therapy and that a biologically relevant treatment effect, i.e. reperfusion efficacy can be determined with a very modest sample size.

For the future development of neuroprotective drugs, imaging identification of the ischemic penumbra will likely assume an increasingly important role. A phase IIB

study with a novel neuroprotective drug should be designed as a proof of concept study to confirm a biologically relevant treatment effect. Patients can be included in the trial up to 9 h after stroke onset who have evidence of a reasonably sized DWI/PWI mismatch on a baseline MRI study and appropriate clinical inclusion criteria, as exemplified by the DIAS trial. Therapy should then be initiated as rapidly as possible. Follow-up imaging should be performed at 30 days or more after the index stroke for evaluation of a potential treatment effect on ischemic lesion evolution. An approach that has been used to assess treatment efficacy with MRI has been to measure the mean increase in ischemic lesion size from baseline, pretreatment lesion size on DWI to delayed ischemic lesion size on T2 imaging [21]. Another novel approach that will likely require a smaller sample size is to define a treatment success as those patients demonstrating no increase or shrinkage in ischemic lesion size when comparing the baseline lesion volume to that on the delayed imaging. With this so-called 'responder analysis' approach, a two-stage design to the data analysis can be used. If no difference is observed after a modest number of treated and placebo patients are compared, then the study can be terminated for futility. Conversely, if this initial analysis demonstrates that the treated group has a modestly increased number of responders, the trial should then continue to complete enrollment of the prespecified number of patients to potentially demonstrate a statistically significant difference in responders. A positive imaging-based trial in phase IIB that shows an effect of the neuroprotective agent on ischemic lesion evolution provides evidence of a biologically relevant treatment effect and should strongly support further assessment in a clinical outcome phase III trial. If a neuroprotective drug shows no effect on the evolution of ischemic lesions with either of these assessment approaches in phase IIB, then the development program should be discontinued because such a drug is unlikely to have a clinically meaningful treatment effect. The phase III trial if initiated should include penumbral imaging with MRI or CT to maximize enrollment of patients most likely to respond to the therapy, especially if a prolonged time window to treatment is desired. In the United States, it is likely that a neuroprotective drug demonstrating a positive treatment effect in one phase IIB imaging endpoint trial and one phase III clinical endpoint trial would be sufficient for registration filing [29].

The future of neuroprotection appears much brighter today than it did several years ago. The SAINT-1 trial demonstrated that a neuroprotective drug with a robust preclinical assessment package and initiated early after stroke onset can shift the modified Rankin Scale in a favorable and apparently clinically meaningful direction. This approach of a shift in the Rankin Scale should provide a more sensitive measure of treatment effects in future phase III neuroprotection trials. Penumbral imaging will enhance phase IIB and phase III clinical trials by identifying stroke patients more likely to respond to treatment and should also help to extend the time window for successful therapy. As mentioned, in phase IIB trials an imaging-based primary outcome should be employed to provide evidence of a drug's effects on ischemic lesion evolution. The combination of imaging-based phase IIB and phase III development of neuroprotective drugs should streamline and enhance the process.

References

1 Kidwell CS, Liebeskind DS, Starkman S, Saver JL: Trends in acute ischemic stroke trials through the 20th century. Stroke 2001;32:1349–1359.

2 The ATLANTIS, ECASS and NINDS rt-PA Study Group Investigators: Association of outcome with early stroke treatment: pooled analysis of ATLANTIS, ECASS and NINDS rt-PA stroke trials. Lancet 2004;363:768–774.

3 Fisher M: Characterizing the target of acute stroke treatment. Stroke 1997;28:866–872.

4 Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, Grotta J, Lyden P, Shuaib A, Hardemark HG, Wasiewski WW, Stroke-Acute Ischemic NXY Treatment (SAINT I) Trial Investigators: NXY-059 for acute ischemic stroke. N Engl J Med 2006;354:588–600.

5 Fisher M: The ischemic penumbra: identification, evolution and treatment concepts. Cerebrovasc Dis 2004;17(suppl 1):1–6.

6 Astrup J, Siesjo BK, Symon L: Thresholds in cerebral ischemia: the ischemic penumbra. Stroke 1981;12:723–725.

7 Hakim AM: The cerebral ischemic penumbra. Can J Neurol Sci 1987;14:557–559.

8 Fisher M, Ratan R: New perspectives on developing acute stroke therapy. Ann Neurol 2003;53:10–20.

9 Cheng YD, Al-Khoury L, Zivin JA: Neuroprotection for ischemic stroke: two decades of success and failure. NeuroRx 2000;1:36–45.

10 Schlaug G, Benfield A, Baird AE, et al: The ischemic penumbra: operationally defined by diffusion perfusion MRI. Neurology 1999;53:1528–1537.

11 Parsons MW, Barber A, Chalk J, et al: Diffusion and perfusion-weighted MRI response to thrombolysis in stroke. Ann Neurol 2002;51:28–37.

12 Van der Toorn A, Sykova E, Dijkhuizen RM, et al: Dynamic changes in water ADC, energy metabolism, extracellular space volume and tortuosity in neonatal rat brain during global ischemia. Magn Res Med 1996;36:52–60.

13 Li F, Liu KF, Silva, et al: Secondary decline in apparent diffusion coefficient and neurological outcome after a short period of focal brain ischemia in rats. Ann Neurol 2000;48:236–244.

14 Kidwell CS, Saver JL, Mattiello J, et al: Thrombolytic reversal of acute human cerebral ischemic injury shown by diffusion/perfusion magnetic resonance imaging. Ann Neurol 2000;47:462–469.
15 Calamante F, Thomas DL, Pell GS, Wiersma J, Turner R: Measuring cerebral blood flow using magnetic resonance imaging techniques. J Cereb Blood Flow Metab 1999;19:701–735.
16 Butcher KS, Parsons M, MacGregor L, et al: Refining the perfusion-diffusion mismatch hypothesis. Stroke 2005;36:1153–1159.
17 Kidwell CS, Alger JR, Saver JL: Beyond mismatch: evolving paradigms in imaging the ischemic penumbra with multimodal magnetic resonance imaging. Stroke 2003;34:2729–2735.
18 Shen Q, Fisher M, Sotak CH, Duong TQ: Effect of reperfusion on ADC and CBF pixel-by-pixel dynamics in stroke: characterizing tissue fates using quantitative diffusion and perfusion imaging. J Cereb Blood Flow Metab 2004:24:280–290.
19 Wintermark M, Bogousslavsky J, Thiren JP, et al: Prognostic accuracy of admission cerebral blood flow measurements by perfusion CT in acute stroke patients. Ann Neurol 2002;51:417–432.
20 Schramm P, Schellinger PD, Klotz E, et al: Comparison of perfusion computed tomography angiography source images with perfusion-weighted imaging and diffusion-weighted imaging in patients with acute stroke less than 6 hours' duration. Stroke 2004;35:1652–1658.
21 Warach S, Pettigrew LC, Dashe JF, Pullicino P, Lefkowitz DM, Sabounjian L: Effects of citicoline on ischemic lesions as measured by diffusion-weighted magnetic resonance imaging. Citicoline 010 Investigators. Ann Neurol 2000;48:713–722.
22 Neumann-Haeflin T, de Rochemont M, Fiebach JB, et al: Effect of incomplete (spontaneous and postthrombolytic) recanalization after middle artery occlusion. Stroke 2004;35:109–115.
23 Ribo M, Molina CA, Rovira A, et al: Safety and efficacy of intravenous tissue plasminogen activator in the 3- to 6-hour window using multimodal transcranial Doppler/MRI selection protocol. Stroke 2005;36:602–606.
24 Lev MH, Segal AZ, Farkas J, et al: Utility of perfusion-weighted CT imaging in acute middle cerebral artery stroke treated with intra-arterial thrombolysis: prediction of final infarct volume and clinical outcome. Stroke 2002;33:2426–2432.
25 Warach S: Use of diffusion and perfusion magnetic resonance imaging as a tool in acute stroke clinical trials. Curr Control Trials Cardiovasc Med 2001;2:38–44.
26 Warach S: Sabounjian LA: ECCO 2000 study of citicoline for treatment of acute ischemic stroke: effects on infarct volume measured by MRI (abstract). Stroke 2000;31:42.
27 Hacke W, Albers G, Al-Rawi Y, et al: The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase. Stroke 2005;36:66–73.
28 Hacke W for the DIAS and DEDAS Steering Committees and Investigators: The results of the joint analysis of two phase II trials of desmoteplase in acute ischemic stroke with treatment 3–9 hours after stroke onset. 15th European Stroke Conference, Bologna, May 2005.
29 Katz R: Biomarkers and surrogate endpoints: an FDA perspective. NeuroRx 2004;1:189–195

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):71–79
DOI: 10.1159/000091706

Published online: May 2, 2006

Evolving Paradigms for Neuroprotection: Molecular Identification of Ischemic Penumbra

Mar Castellanos[a] Tomás Sobrino[b] José Castillo[b]

[a]Department of Neurology, Hospital Universitari Doctor Josep Trueta, Girona, and
[b]Department of Neurology, Division of Vascular Neurology, Neurovascular Research Laboratory, Hospital Clínico Universitario, University of Santiago de Compostela, Santiago de Compostela, Spain

Key Words
Ischemic stroke · Neuroprotection · Penumbra · Energy metabolism

Abstract
Ischemic penumbra defines the existence of tissue at risk of infarction and which is, hence, potentially salvageable and the target for current stroke reperfusion and neuroprotective therapies. Penumbral tissue evolves toward irreversibly damaged tissue at different rates in individual stroke patients yielding different therapeutic windows depending on the individual duration of risk of infarction of this tissue. An accurate identification of the penumbra is then necessary in order to individualize the window of opportunity for therapeutic interventions. Imaging techniques, although helpful, may not give the most accurate information as to the existence of penumbra given that the threshold for identification of penumbra varies depending on the technique used. A better identification of the true penumbral tissue might be based on the cascade of molecular events that are responsible for the evolution of the penumbra toward infarcted tissue. Multiple penumbras can be defined in molecular terms taking into account which vessel is occluded, the time of evolution of the ischemia, the degree of the ischemia, and the sensitivity to ischemia of the different cells. Future studies are necessary to clarify whether the enhancement of cytoprotective mechanisms, and/or the block of cytotoxic mechanisms confirming the existence of penumbra at different times of ischemic evolution, are effective neuroprotective strategies.

Cerebral ischemia is a dynamic process that can result in progressive cell death with a subsequent increase of infarct volume in the hours immediately following stroke onset. The increase of infarct volume is the result of the recruitment of the ischemic penumbra, a region of brain tissue where blood flow is so reduced as to result in hypoxia of a severity that results in the cessation of physiological function but not so complete as to cause irreversible failure of energy metabolism and cellular necrosis [1]. This ischemic region at risk of infarction but potentially salvageable is a therapeutic target in the acute stroke clinical setting as it can be saved by using reperfusion and/or effective neuroprotective therapies [2].

The duration of the penumbra in humans varies substantially from person to person depending on a variety of factors such as the time from onset of ischemia, the location of vessel occlusion, the efficacy of collateral circulation, and the location of the ischemic lesion (e.g. the different susceptibility to ischemia of gray and white matter). This varying duration of ischemic penumbra means

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0071$23.50/0

Accessible online at:
www.karger.com/ced

Prof. José Castillo
Department of Neurology, Hospital Clínico Universitario
Travesa da Choupana, s/n
ES–15706 Santiago de Compostela (Spain)
Tel. +34 981 951 348, Fax +34 981 951 098, E-Mail mecasti@usc.es

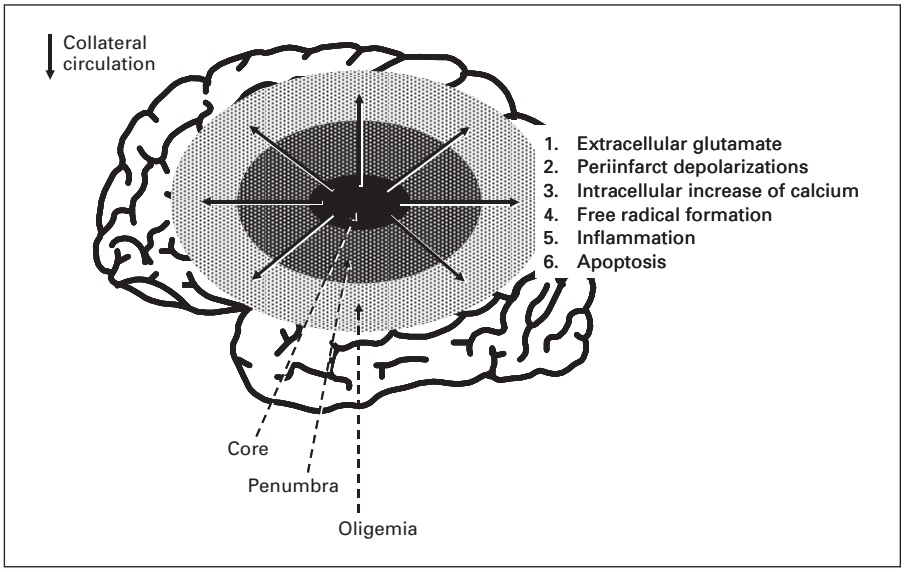


Fig. 1. Molecular mechanisms related to the evolution of the penumbra to irreversible damaged tissue.

that the window for therapeutic intervention is different for each patient and may often be longer than the currently accepted 3-hour window for the administration of reperfusion therapies [3].

Whereas reperfusion therapies with recombinant tissue plasminogen activator (rt-PA) have been shown to be effective in the ischemic acute stroke phase in humans [4, 5], most of the neuroprotective drugs have been found to be effective only in experimental models of cerebral ischemia [6]. However, clinical trials testing neuroprotective drugs have often been limited by inappropriately long time windows, insufficient statistical power, insensitive outcome measures, inclusion of protocol violators, failure to target specific stroke subtypes, and failure to target the ischemic penumbra [7]. In fact, as neuroprotection is by definition an intervention aimed at limiting the infarct volume by avoiding the death of vulnerable cells within the penumbra [8], the identification of such tissue, together with the knowledge of the molecular mechanisms related to the penumbra recruitment toward the irreversibly damaged tissue that neuroprotection seeks to prevent, are mandatory for a neuroprotective drug to be effective.

Different neuroimaging techniques, including positron emission tomography, diffusion/perfusion magnetic resonance imaging and computed tomography perfusion, are currently used to visualize the penumbra. However, the areas identified as penumbra by these imaging techniques do not completely coincide with one another [9–11] so other tools are necessary in order to accurately identify the tissue at risk of infarction. A better identification of the true penumbral tissue might rely on the cascade of molecular events started after vascular occlusion that have been the objective of extensive research in recent years [12].

Depending on the duration of the vascular occlusion, the location of the ischemia and the time of reperfusion, different cell-specific molecular penumbras may exist and so, a variety of ischemic penumbras can be described in molecular terms [12, 13]. The different molecular mechanisms related to the progression of penumbra to irreversibly damaged tissue, as well as the molecular and genetic response that occurs in areas not irreversibly damaged, are reviewed in the present article.

From Ischemic Penumbra to Irreversible Injury

The cascade of molecular mechanisms started as a result of cerebral ischemia that might contribute to the evolution of the penumbra to irreversibly damaged tissue is multiple and of increasing complexity (fig. 1). The two major contributory mechanisms to the evolution of the ischemic penumbra are the status of local cerebral blood blow (CBF) and the cellular consequences of this CBF decrease [2]. The decrease in the CBF leads to a marked reduction in ATP with Na^+/K^+ pump failure and an increase in extracellular glutamate which activates glutamate-mediated channels (NMDA and AMPA) and results in an intracellular increase of calcium. Both excito-

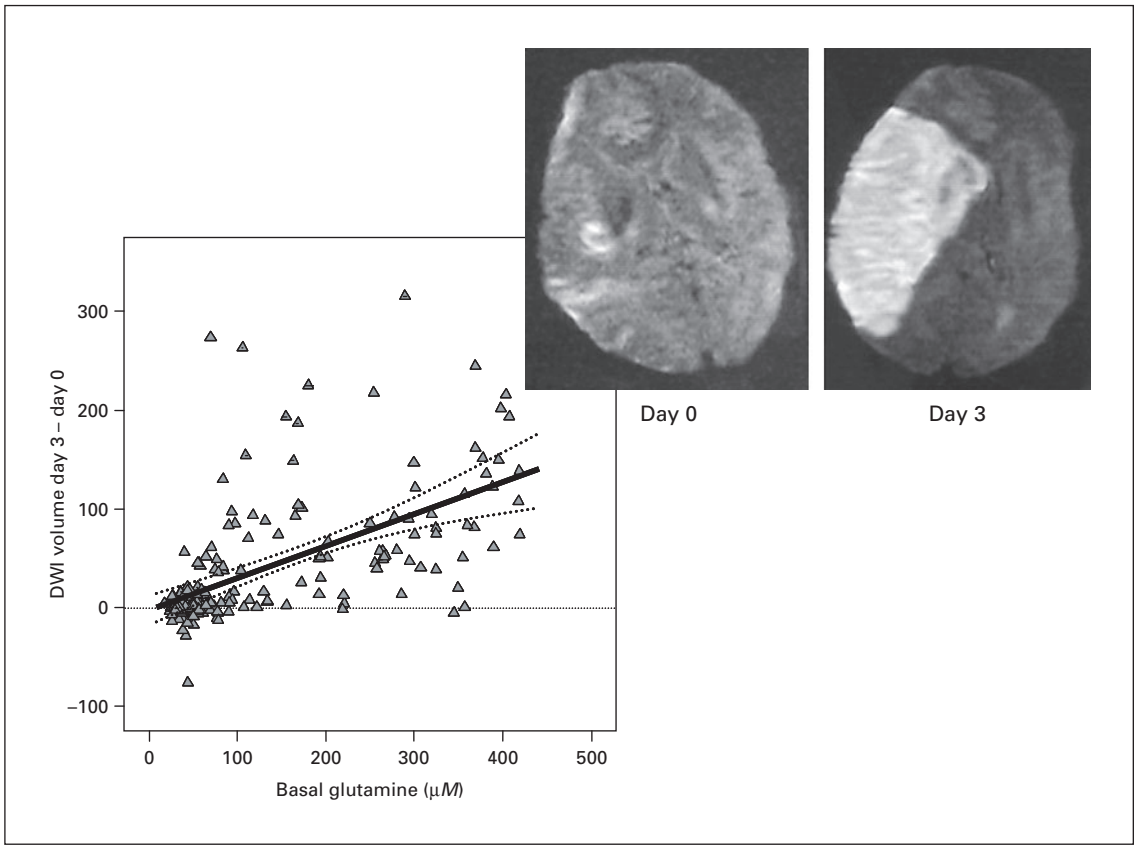


Fig. 2. Association between glutamate levels on admission and diffusion-weighted imaging lesion growth in 208 patients with hemispheric ischemic stroke of less than 12 h from onset of symptoms.

toxicity and calcium are known to be major contributors to the evolution of the ischemic lesion [14]. The intracellular calcium participates in free radical formation through the activation of nitric oxide synthase that promotes nitric oxide formation and the subsequent synthesis of the highly toxic peroxynitrite radical. The inhibition of neuronal nitric oxide synthase reduces infarct size in experimental models of cerebral ischemia [15].

The spread of glutamate from the ischemic core to the peripheral lesion is an additional mechanism that may induce irreversible injury in the penumbra. In fact, glutamate has been shown to be a mediator in the occurrence of periinfarct depolarizations which originate at the infarcted core and propagate towards the periphery of the lesion causing an increase in the infarcted volume [16]. In experimental studies, a correlation between the number of periinfarct depolarizations and infarct volume has been found [17, 18] and the pharmacological suppression of periinfarct depolarizations by glutamate and glycine antagonists reduces infarct volume [19, 20]. Moreover, in a clinical setting, a high correlation has been demonstrated between glutamate levels and infarction volume [21], and early neurological deterioration [22, 23] in patients with acute ischemic stroke. A strong association has also been found between glutamate levels on admission and diffusion-weighted imaging lesion growth in patients with acute hemispheric infarction [24] (fig. 2). These periinfarct depolarizations result in ischemic enlargement presumably by the increased energetic demand within an already energetically compromised tissue due to the low CBF in the penumbral zone.

Later, oxygen free radicals and inflammation participate in the evolution of the ischemic lesion as mediators of the reperfusion injury. Inflammatory molecules such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-6 promote the expression of adhesion molecules such as the intercellular molecule adhesion-1 and the vascular cellular adhesion molecule, facilitating leukocyte adher-

ence and migration from capillaries into the brain with subsequent microvessel occlusion and a progressive reduction in blood flow that might result in cell death and an increase in the infarct volume [25–27]. In fact, the administration of antiadhesion molecules has been shown to reduce infarct volume in experimental models of transient focal cerebral ischemia [28].

Apoptosis, or programmed cell death, has also been suggested as being a contributor to the evolution of the penumbra to necrotic tissue. As protein synthesis is required for apoptosis to occur, it is likely that this mechanism only contributes to the progression of the ischemic lesion in areas of modest ischemia or after reperfusion [2]. In fact, in experimental models of cerebral ischemia, apoptosis only appeared to contribute to delayed ischemic damage after 30 min of transient ischemia whereas no effect was observed after 90 min [29, 30].

Molecular Markers of Ischemic Penumbra

The energy failure secondary to the decrease in CBF originates from metabolic disturbances with changes in the oxygen levels, glucose metabolism and depletion of energy metabolites including ATP, phosphocreatine, lactate and *n*-acetyl aspartate (NAA), among others. The concentration of these metabolites, which can be estimated using magnetic resonance spectroscopy, differs between the ischemic core and the penumbral tissue where the metabolite concentration decrease is less severe [31] so the use of this imaging technique might be helpful to characterize the ischemic penumbra in individual patients. In fact, a differential elevation of intracellular lactate in striatum and cortex has been found during 1 h of transient middle cerebral artery occlusion (MCAO) and after 1 h of reperfusion in rats, although a recurrent lactate elevation was observed when the imaging was repeated at 24 and 72 h after reperfusion, probably as a result of delayed ischemic injury. NAA levels were not found to be a useful marker of penumbra in the acute phase of ischemia because NAA depletion was not detected until at least 2 h after the onset of ischemia [32]. In an experimental monkey transient MCAO model, extracellular glucose levels were demonstrated to be limited by the regional CBF whereas lactate concentration was more associated with the cerebral metabolic rate of oxygen. Although near-zero levels of glucose during MCAO probably indicates near-complete stop of CBF, lactate concentration was found to be a better marker of irreversible ischemia than glucose concentration, so in situations with elevated lactate levels, glucose concentration might be helpful to differentiate between penumbra and severe ischemic tissue [33].

Glutamate levels are persistently lower in the zone of ischemic penumbra than in the core. The reduction in glutamate concentration in the penumbral zone might be the result of upregulation of the proteins that are responsible for its transport from the intersynaptic space to the astrocytes [34]. This mechanism may be promoted by a change in the phosphorylation of the presynaptic proteins in the zone of penumbra, which results in a failure in the presynaptic release of glutamate [35]. A maintained activity of the GABAergic system, probably counteracting the glutamate-induced excitotoxicity, has also been demonstrated in the cortical penumbra [36].

The reduction in protein synthesis is one of the earliest and most sensitive metabolic consequences of cerebral ischemia [37]. This effect, which appears with CBF reduction of only 50% and so is not the result of energy failure as ATP levels do not decrease until the CBF falls to 20% [38], may be reversible in the penumbral tissue but not in the ischemic core. CBF reduction seems to interfere with ribosomal protein synthesis by inactivating the initiation factor 2 (elF2), the guanine nucleotide exchange factor (elF-2-GTP complex factor), and the eukaryotic elongation factor (eEF-2) whose phosphorylation is inhibited by the increased glutamate levels secondary to the ischemia [35, 39].

Synthesis of protein continues in those cells that survive the ischemic process. In fact, stress proteins such as heat shock protein 70 (HSP70) are upregulated as a result of protein denaturation secondary to ischemia [40]. The increase in HSP70 represents an endogenous protective mechanism by attempting protein renaturation that occurs in the penumbra but not in irreversibly injured cells because HSP70 mRNA is not expressed when ATP is decreased [41]. This protective effect against cerebral ischemia has been demonstrated in transgenic mice overexpressing HSP70 [42]. The neuronal expression of HSP70 can be interpreted as a molecularly defined penumbra of protein denaturation because HSP70-stained neurons extend beyond the zone of neuronal cell death [43]. Other heat shock proteins such as HSP27, αβ-crystallin (α-BC) and heme oxygenase-1 (HO-1) are also induced after brain ischemia [44–46], presumably as a response to spreading depression. Whereas HSP27 and α-BC are expressed mainly in astrocytes of the penumbral area [44, 45], HO-1 is induced in vessels in the core of an infarct as well as in microglia, scattered neurons, and astrocytes at the edges of the infarction [46]. After transient focal

cerebral ischemia in rodents, α-BC transcript and protein were transiently upregulated in pyramidal neurons within few hours after reperfusion, and this was followed by a gradual and sustained induction in reactive astrocytes localized in the penumbra for several days, suggesting that α-BC induction may play an important role in the postischemic brain injury [45]. The expression of HSP72 mRNA has been found to be induced only in the penumbral cortex but not in the necrotic core or normal brain 3 h after MCAO in mice [47]. A correlation has been found between the region of HSP expression after permanent MCAO in experimental models of cerebral ischemia and the penumbral area defined as a region of cerebral protein synthesis suppression associated with the preservation of ATP levels [48].

Other proteins such as neuregulin-1 are also expressed in the ischemic penumbra. This protein blocks apoptosis, preventing infiltration of macrophages and of microglia cells, as well as astrocyte activation, and interferes with the synthesis of some proinflammatory cytokines. Administration of neuregulin-1 has been found to reduce infarct volume between 40 and 98% in an experimental model of focal ischemia [49].

Spreading depression seems also to be responsible for the expression of other molecules released as a result of the ischemic insult and whose expression is not limited just to the necrotic core. Cyclooxygenase-2 (COX-2) which metabolizes arachidonic acid to prostaglandins is expressed throughout the whole hemisphere after focal cerebral ischemia. In fact, COX-2 expression has been shown to be induced far beyond the region of ischemia in rodents [50] and in stroke patients [51]. It has been found that COX-2 inhibitors decrease infarct volume in some experimental studies, an effect that seems to be related to inducible nitric oxide synthase-mediated injury [52]. Interleukins including IL-1 and IL-6, as well as growth factors such as basic fibroblast growth factor and brain-derived neurotrophic factor are also diffusely induced in the brain after ischemia, probably as a result of the spreading depression mechanism [13]. Spreading-like depolarizations have been shown to be blocked by the administration of NMDA receptor antagonists. However, in conditions of moderate energy depletion, as it happens in the ischemic penumbra, NMDA receptor antagonists' inhibition may not be sufficient to block these depolarizations, an effect that seems to be related with the increased extracellular K^+ concentrations in the penumbral tissue [53].

TNF-α is induced in the core and in the region adjacent to the infarction in neurons, astrocytes, and endothelial cells. TNF-α has often been thought to mediate injury and apoptotic cell death, although more recent studies suggest that it can also be protective [54, 55]. Stimulation of TNF-α receptors leads to the activation of the nuclear factor-κB (NF-κB). NF-κB and TNF-α might promote cell injury or cell protection depending upon the cells and the circumstances of their induction [13] (fig. 3).

Hypoxia inducible factor-1 (HIF-1) is a transcription factor expressed in response to hypoxia but not to inhibitors of mitochondrial respiration, which suggests that HIF-1 is activated by a molecular oxygen sensor [56]. Both HIF-1t and HIF-1 mRNA are present in the normal brain but only HIF-1 mRNA is induced in the cingulated cortex adjacent to an infarct area after suture-induced MCAO in rats [57]. Given that CBF is decreased in this region of HIF-1 expression outside the infarcted zone, it has been suggested that this region of HIF-1 expression after a stroke might be interpreted as the region of chronic hypoxia around the region of infarction [13]. HIF-1a and HSP70 expression is induced in the same penumbral areas, although HIF-1 is also expressed in more peripheral areas with presumably less severe hypoxia but persistent CBF reduction, such as in the cingulated cortex where HSP70 is rarely induced [57]. The role of HIF-1 after acute cerebral ischemia is not clear, as both harmful and protective effects have been described depending on the model, timing, and mode of the HIF induction in the brain [13].

The chemokine stromal-derived factor-1 (SDF-1), also known as CXCL12, and its receptor, CXCR4, have been involved in the recruitment of bone marrow-derived cells to sites of ischemic injury in a mice stroke model. SDF-1 is mainly upregulated and expressed for up to 30 days after MCAO in the penumbral area and at later time points in the ischemic core when new blood vessels are appearing. In fact, SDF-1 expression was mainly associated with reactive perivascular astrocytes, which suggest that SDF-1 may play a role in enhancing plasticity and recovery after ischemic injury [58].

Other proteins such as prostacyclin synthase (PGIS) are also mainly expressed in the ipsilateral penumbral area. This enzyme regulates the synthesis of prostacyclin (PGI_2) which is a potent vasodilator and an inhibitor of platelet aggregation and leukocyte activation. The overexpression of PGIS by adenoviral gene transfer at 72 h before transient ischemia has been demonstrated to reduce infarct volume in an experimental rat focal cerebral ischemia-reperfusion model [59].

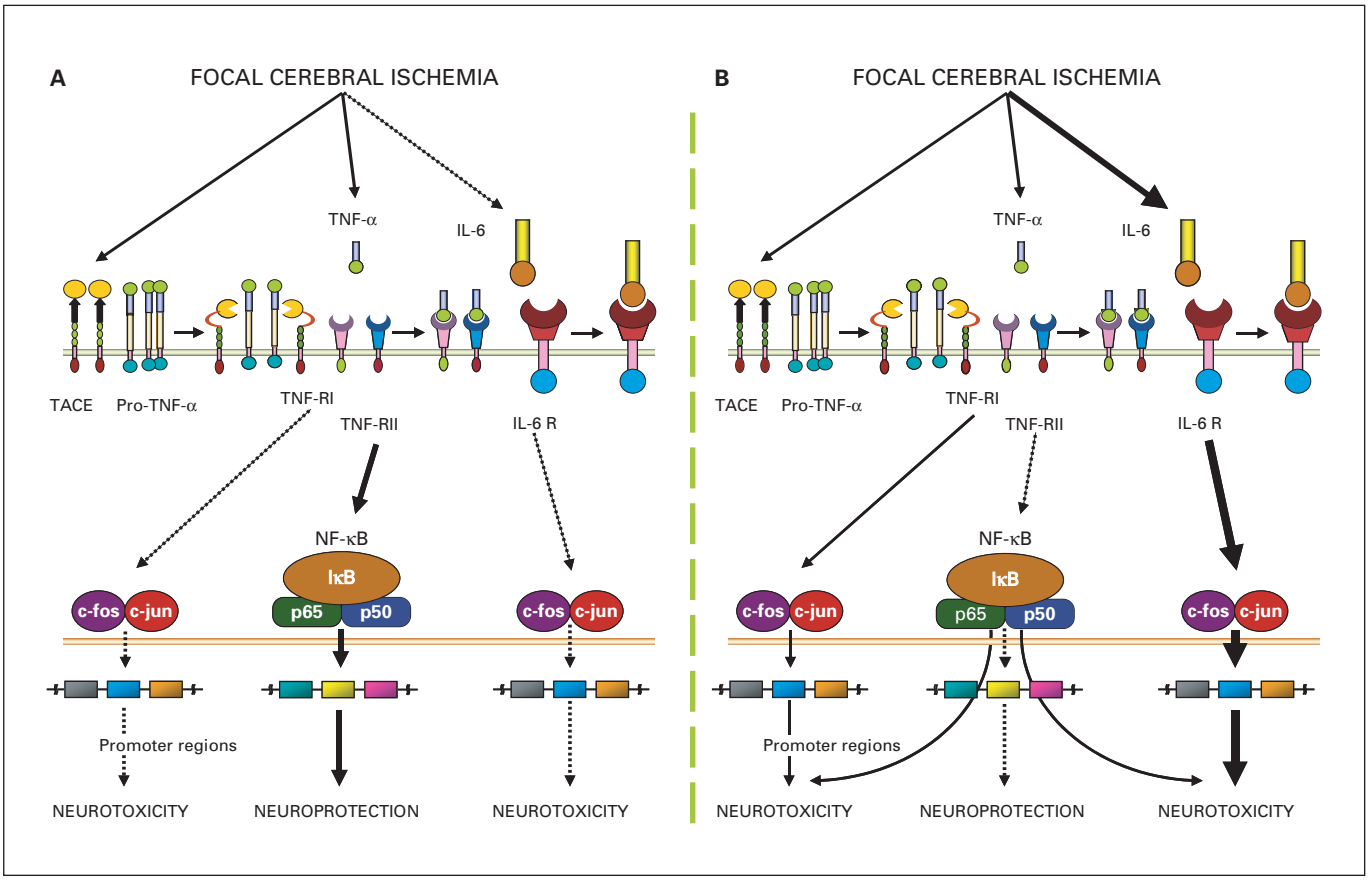


Fig. 3. TNF-α and NF-κB may condition cell protection (**A**) or cell injury (**B**) depending on the type of the stimulated receptor and the coexistence of other factors, such as the presence of raised concentrations of IL-6.

Genetics of Molecular Penumbra

DNA microarray studies have in recent years identified a large number of genes that are either up- or down-regulated after temporary MCAO in rats [60]. As a general pattern, DNA repair proteins decrease in cells expected to die in the core and are induced in cells immediately adjacent to the core that appear to survive the ischemia. Among them, the expression of early immediate genes (EIG) such as c-fos and c-jun throughout regions of the nonischemic ipsilateral hemisphere has been shown to be induced as a result of MCAO in experimental models of cerebral ischemia [61]. In fact, it has been found that c-fos mRNA expression occurs in the penumbra and the normal cortex but not in the ischemic core [47].

Spreading depression seems to be related to the expression of EIG in the penumbral tissue as the administration of NMDA antagonists inhibits this response [62]. Moreover, spreading depression secondary to cortical potassium chloride application also stimulates the expression of EIG throughout the entire hemisphere [63]. Other genes, including junB, Zac1 and pituitary adenylate cyclase-activating polypeptide (PACAP), nerve growth factor-induced (NGFI) A, B, and C, egr, Rheb, Arc and other EIGs, are also induced after focal cerebral ischemia, probably as a result of spreading depression or repeated ischemic depolarizations [13]. The expression of Bcl-2 and Bcl-xl, the anti-apoptotic genes, tends to be induced in cells that are immediately adjacent to an infarct and probably survive the ischemia. Moreover, Bcl-2 can be induced in the entire MCA territory with less severe degrees of ischemia [64].

The role of EIG and other genes whose expression is induced by ischemia has not been clearly elucidated, although they may play a role in enhancing plasticity and

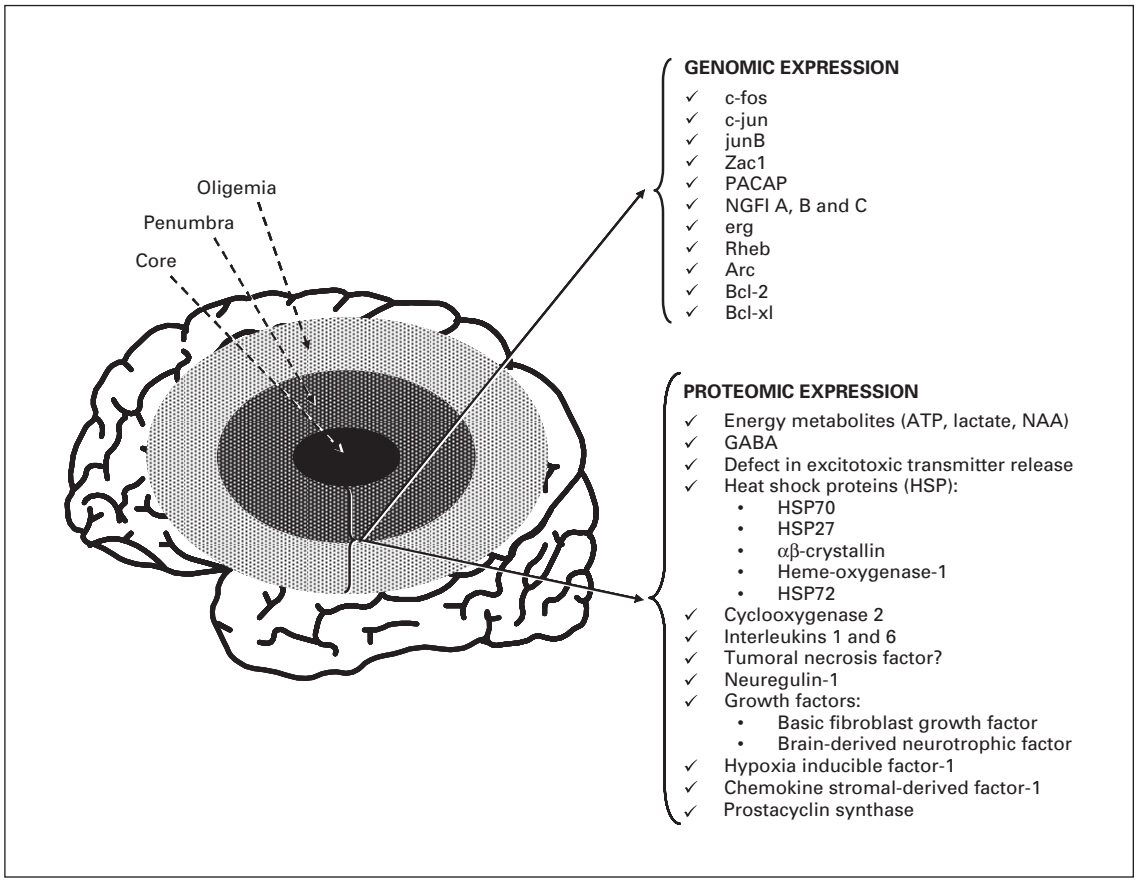


Fig. 4. Genomic and proteomic expressions in ischemic penumbra. PACAP = Pituitary adenylate cyclase-activating polypeptide; NGFI = nerve growth factor-induced; NAA = *n*-acetyl aspartate.

behavioral recovery after ischemic damage [13] even in the contralateral hemisphere. In fact, the expression of some genes including c-fos and Arc has also been found to occur in the contralateral hemisphere after MCAO [13].

Figure 4 summarizes the genomic and proteomic expression in the ischemic penumbra.

Conclusions

As the target for therapeutic intervention after stroke is the penumbra, this potentially salvageable at-risk tissue must be present in order for therapeutic interventions to be effective. Although neuroimaging techniques might be useful for the visualization of ischemic penumbra, the cascade of molecular mechanisms at which the neuroprotective strategies are directed does not take place at the same time in all patients and so it is reasonable to suppose that the length of the therapeutic window for the same neuroprotective drug will vary from one patient to another. Multiple penumbras can be defined in molecular terms and multiple therapeutic windows might exist, determined by the molecular events taking place at the moment the treatment has to be given. The enhancement of cytoprotective mechanisms and/or the blocking of cytotoxic mechanisms taking place within this tissue at risk of infarction should be considered as therapeutic molecular targets. Further studies are necessary in order to find out whether these molecular targets can be used to improve the neuronal tolerance to ischemia in the penumbral tissue, an effect that would also help to extend the reperfusion therapeutic window.

References

1 Ginsberg MD: Adventures in the pathophysiology of brain ischemia: penumbra, gene expression. Neuroprotection: the 2002 Thomas Willis Lecture. Stroke 2003;34:214–223.
2 Fisher M: Characterizing the target for acute stroke treatment. Stroke 1997;28:866–872.
3 Baron JC, von Kummer R, del Zoppo GJ: Treatment of acute ischemic stroke: challenging the concept of a rigid and universal time window. Stroke 1995;26:2219–2222.
4 NINDS rt-PA Stroke Group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581–1587.
5 Hacke W, Kaste M, Fieschi C, von Kummer R, Dávalos A, Deier D, Larrue V, Bluhmki E, Davis S, Donan J, Scheneider D, Díez-Tejedor E, Trovillas P, for the Second European-Australian Acute Stroke Study Investigators: Randomised double-blind, placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischemic stroke (ECASS II). Lancet 1998;352:1245–1251.
6 Silver B, Weber J, Fisher M: Medical therapy for ischemic stroke. Clin Neuropharmacol 1996;19:101–128.
7 Gladstone DJ, Black SE, Hakim AM, for the Heart and Stroke Foundation of Ontario Centre of Excellence in Stroke Recovery: Toward wisdom from failure. Lessons from neuroprotective stroke trials and new therapeutic directions. Stroke 2002;33:2123–2136.
8 Wahlgren NG, Ahmed N: Neuroprotection in cerebral ischaemia: facts and fancies – the need for new approaches. Cerebrovasc Dis 2004; 17(suppl 1):153–166.
9 Heiss WD, Sobesky J, Hesselmann V: Identifying thresholds for penumbra and irreversible tissue damage. Stroke 2004;35(suppl I):2671–2674.
10 Kidwell CS, Alger JR, Saver JL: Evolving paradigms in neuroimaging of the ischemic penumbra. Stroke 2004;35(suppl I):2662–2665.
11 Dávalos A, Blanco M, Pedraza S, Leira R, Castellanos M, Pumar JM, Silva Y, Serena J, Castillo J: The clinical-DWI mismatch. A new diagnostic approach to the brain tissue at risk of infarction. Neurology 2004;62:2187–2192.
12 Weinstein PR, Hong S, Sharp FR: Molecular identification of the ischemic penumbra. Stroke 2004;35(suppl I):2666–2670.
13 Sharp FR, Lu A, Tang Y, Millhorn DE: Multiple molecular penumbras after focal cerebral ischemia. J Cereb Blood Flow Metab 2000;20: 1011–1032.
14 Kogure T, Kogure K: Molecular and biochemical events within the brain subjected to cerebral ischemia. Clin Neurosci 1997;4:179–183.
15 Samdani AF, Dawson TM, Dawson VI: Nitric oxide synthase in model of focal ischemia. Stroke 197; 28:1283–1288.
16 Hossman KA: Periinfarct depolarizations. Cerebrovasc Brain Metab Rev 1996;8:195–208.
17 Mies G, Iijima T, Hossmann KA: Correlation between periinfarct DC shifts and ischemic neuronal damage in rat. Neuroreport 1993;4: 709–711.
18 Mies G, Kohno K, Hossmann KA: Prevention of periinfarct direct current shifts with glutamate antagonist NBQX following occlusion of the middle cerebral artery in the rat. J Cereb Blood Flow Metab 1994;14:802–807.
19 Gill R, Andine P, Hillered L, Persson L, Hagberg H: The effect of MK-801 on cortical spreading depression in the penumbral zone following focal ischemia in the rat. J Cereb Blood Flow Metab 1992;12:371–379.
20 Tatlisumak T, Takano K, Meiler MR, Fisher M: A glycine site antagonist, ZD9379 reduces the number of spreading depressins and infarct size in rats with permanent middle cerebral artery occlusion. Stroke 1998;29:190–195.
21 Castillo J, Davalos A, Naveiro J, Noya M: Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. Stroke 1996;27:1060–1065.
22 Castillo J, Dávalos A, Noya M: Progression of ischaemic stroke and excitotoxic amino acids. Lancet 1997;349:79–83.
23 Serena J, Leira R, Castillo J, Pumar JM, Castellanos M, Dávalos A: Neurological deterioration in acute lacunar infarctions. The role of excitotoxicity and inhibitory neurotransmitters. Stroke 2001;32:1154–1161.
24 Castellanos M, Blanco M, Pedraza S, García M, Silva Y, Leira R, Dávalos A: Molecular markers of progressing stroke are associated with diffusion-weighted MRI lesion growth in patients with acute ischemic stroke. Stroke 2004;35:247.
25 Castillo J, Leira R: Predictors of deteriorating cerebral infarct: role of inflammatory mechanisms. Would its early treatment be useful? Cerebrosvasc Dis 2001;11(suppl 1):40–48.
26 Vila N, Castillo J, Dávalos A, Chamorro A: Proinflammatory cytokines and early stroke progression. Stroke 2000;31:2325–2329.
27 Castellanos M, Castillo J, García MM, Leira R, Serena J, Chamorro A, Dávalos A: Inflammation-mediated damage in progressing lacunar infarctions. Stroke 2002;33:982–987.
28 Chen H, Chopp M, Zhang RL, Bodzin G, Chen Q, Rusche JR, Todd RF: Anti-CD11b monoclonal antibody reduces ischemic cell damage after transient focal cerebral ischemia in rat. Ann Neurol 1994;35:458–463.
29 Du C, Hu R, Csernansky CA, Hsu CY, Choi DW: Very delayed infarction after mild focal cerebral ischemia: a role for apoptosis? J Cereb Blood Flow Metab 1996;16:196–201.
30 Nakase T, Söhl G, Theis M, Willecke K, Naus CCG: Increased apoptosis and inflammation after focal brain ischemia in mice lacking connexin-43 in astrocytes. Am J Pathol 2004;164: 2067–2075.
31 Gadian DG, Frackowiak RS, Crockard HA, Proctor E, Allen K, Williams SR, Russell RW: Acute cerebral ischemia: concurrent changes in cerebral blood flow, energy metabolites, pH, and lactate measured with hydrogen clearance and ^{31}P and ^{1}H nuclear magnetic resonance spectroscopy. I. Methodology. J Cereb Blood Flow Metab 1987;7:199–206.
32 Higuchi T, Fernandez EJ, Maudsley AA, Shimizu H, Weiner MW, Weinstein PR: Mapping of lactate and *n*-acetyl-*l*-aspartate predicts infarction during acute focal ischemia: in vivo ^{1}H magnetic resonance spectroscopy in rats. Neurosurgery 1996;38:121–130.
33 Frykholm P, Hillered L, Langstrom B, Persson L, Valtysson J, Enblad P: Relationship between cerebral blood flow and oxygen metabolism, and extracellular glucose and lactate concentrations during middle cerebral artery occlusion and reperfusion: a microdialysis and positron emission tomography study in nonhuman primates. J Neurosurg 2005;102:1076–1084.
34 Bruhn T, Christensen T, Diener NH: Uptake of glutamate is impaired in the cortical penumbra of the rat following middle cerebral artery occlusion: an in vivo microdialysis extraction study. J Neurosci Res 2003;71:551–558.
35 Bolay H, Gürsoy-Özdemir Y, Sara Y, Onur R, Can A, Dalkara T: Persistent defect in transmitter release an synapsis phosphorylation in cerebral cortex after transient moderate ischemia injury. Stroke 2002;33:1369–1375.
36 Sommer C, Kollmar R, Schwab S, Kiessling M, Schabitz WR: Exogenous brain-derived neurotrophic factor prevents postischemic downregulation of [^{3}H]muscimol binding to $GABA_A$ receptors in the cortical penumbra. Brain Res Mol Brain Res 2003;111:24–30.
37 Rebel A, Koehfer RC, Martin LJ: In situ immunoradiographic method for quantification of specific proteins in normal and ischemia brain regions. J Neurosci Methods 2005;143: 227–235.
38 Mies G, Ishimaru S, Xie Y, Seo K, Hossmann KA: Ischemic thresholds of cerebral protein synthesis and energy state following middle cerebral artery occlusion in rat. J Cereb Blood Flow Metab 1991;11:753–761.
39 Massa SM, Swanson RA, Shark FR: The stress gene response in brain. Cerebrovasc Brain Metab Rev 1996;8:95–158.
40 Nowak TS Jr, Jacewiwick M: The heat shock/stress response in focal cerebral ischemia. Brain Pathol 1994;4:67–76.
41 Kobayashi S, Welsh FA: Regional alterations of ATP and heat-shock protein-72 mRNA following hypoxia-ischemia in neonatal rat brain. J Cereb Blood Flow Metab 1995;15:1047–1056.
42 Tsuchiya D, Hong S, Matsumori Y, Kayama T, Swanson RA, Dillman WH, Liu J, Panter SS, Weinstein PR: Overexpression of rat heat shock protein 70 reduces neuronal injury after transient focal ischemia, transient global ischemia, or kainic acid-induced seizures. Neurosurgery 2003;53:1179–1188.

43 States BA, Honkaniemi J, Weinstein PR, Sharp FR: DNA fragmentation and HSP70 protein induction in hippocampus and cortex occurs in separate neurons following permanent middle cerebral artery occlusions. J Cereb Blood Flow Metab 1996;16:1165–1175.

44 Plumier JC, Armstrong JN, Wood NI, Babity JM, Hamilton TC, Hunter AJ, Robertson HA, Currie RW: Differential expression of c-fos, Hsp70 and Hsp27 after protothrombotic injury in the rat brain. Brain Res Mol Brain Res 1997;45:239–246.

45 Piao CS, Kim SW, Kim JB, Lee JK: Co-induction of αB-crystallin and MAPKAPK-2 in astrocytes in the penumbra after transient focal cerebral ischemia. Exp Brain Res 2005;163:421–429.

46 Nimura T, Weinstein PR, Massa SM, Panter S, Sharp FR: Heme oxygenase-1 (HO-1) protein induction in rat brain following focal ischemia. Brain Res Mol Brain Res 1996;37:201–208.

47 Hata R, Mies G, Wiessner C, Hossmann KA: Differential expression of c-fos and hsp72 mRNA in focal cerebral ischemia of mice. Neuroreport 1998;9:27–32.

48 Hata R, Maeda K, Herman D, Mies G, Hossmann KA: Evolution of brain infarction after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 2000;20:937–946.

49 Xu Z, Jiang J, Ford G, Ford BD: Neuregulin-1 is neuroprotective and attenuates inflammatory responses induced by ischemic stroke. Biochem Biophys Res Commun 2004;322:440–446.

50 Koistinaho J, Pasonen S, Yrjanheikki J, Chan PH: Spreading depression-induced gene expression is regulated by plasma glucose. Stroke 1999;30:114–119.

51 Sairanen T, Ristimaki A, Karjalinen-Lindsberg ML, Paetau A, Kaste M, Lindsberg PJ: Cyclooxygenase-2 is induced globally in infarcted human brain. Ann Neurol 1998;43:738–747.

52 Nagayama M, Niwa K, Nagayama T, Ross ME, Iadecola C: The cyclooxygenase-2 inhibitor NS-398 ameliorates ischemic brain injury in wild-type mice but not in mice with deletion of the inducible nitric oxide synthase gene. J Cereb Blood Flow Metab 1999;19:1213–1219.

53 Petzold GC, Windmuller O, Haack S, Major S, Buchheim K, Megow D, Gabriel S, Lehmann TN, Drenckhahn C, Peters O, Meierkord H, Heinemann U, Dirnagl U, Dreier JP: Increased extracellular K^+ concentration reduces the efficacy of N-methyl-*D*-aspartate receptor antagonists to block spreading depression-like depolarizations and spreading ischemia. Stroke 2005;36:1270–1277.

54 Castillo J, Moro MA, Blanco M, Leira R, Serena J, Lizasoain I, Dávalos A: The release of tumor necrosis factor-α is associated with ischemic tolerance in human stroke. Ann Neurol 2003;54:811–819.

55 Pradillo JM, Romera C, Hurtado O, Cárdenas A, Moro MA, Leza JC, Dávalos A, Castillo J, Lorenso P, Lizasoain I: TNFR1 upregulation mediates tolerance after brain ischemic preconditioning. J Cereb Blood Flow Metab 2005;25:193–203.

56 Bunn HF, Poyton RO: Oxygen sensing and molecular adaptation to hypoxia. Physiol Rev 1996;76:839–885.

57 Bergeron M, Yu AY, Solway KE, Semenza GL, Sharp FR: Induction of hypoxia-inducible factor-1 and its target genes following focal ischemia in rat brain. Eur J Neurosci 1999;11:4159–4170.

58 Hill WD, Hess DC, Martin-Studdard A, Carothers JJ, Zheng J, Hale D, Maeda M, Fagan SC, Carroll JE, Conway SJ: SDF-1 (CXCL12) is upregulated in the ischemic penumbra following stroke: association with bone marrow cell homing to injury. J Neuropathol Exp Neurol 2004;63:84–96.

59 Fang YC, Wu JS, Chen JJ, Cheung WM, Tseng PH, Tam KB, Shyue SK, Chen JJ, Lin TN: Induction of prostacyclin/PGI_2 synthase expression after cerebral ischemia-reperfusion. J Cereb Blood Flow Metab 2005. Adv online publ, Aug 10, 2005.

60 Schmidt-Kastner R, Zhang B, Belayev L, Khoutorova L, Amin R, Busto R, Ginsberg MD: DNA microarray analysis of cortical gene expression during early recirculation after focal brain ischemia in rat. Brain Res Mol Brain 2002;108:81–93.

61 An G, Lin TN, Liu JS, Xue JJ, He YY, Hsu CY: Expression of c-fos and c-jun family genes after focal cerebral ischemia. Ann Neurol 1993;33:457–464.

62 Collaco-Moraes Y, Aspey BS, de Belleroche JS, Harrison MJ: Focal ischemia causes an extensive induction of immediate early genes that are sensitive to MK-801. Stroke 1994;25:1855–1861.

63 Herrera DG, Robertson HA: Application of potassium chloride to the brain surface induces the c-fos proto-oncogene: reversal by MK-801. Brain Res 1990;510:166–170.

64 Chen J, Graham SH, Chan PH, Lan J, Zhou RL, Simon RP: Bcl-2 is expressed in neurons that survive focal ischemia in the rat. Neuroreport 1995;6:394–398.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):80–88
DOI: 10.1159/000091707

Published online: May 2, 2006

Deterioration in Acute Ischemic Stroke as the Target for Neuroprotection

Joaquín Serena[a] Manuel Rodríguez-Yáñez[b] Mar Castellanos[a]

[a]Department of Neurology, Hospital Universitari Doctor Josep Trueta, Girona, and [b]Department of Neurology, Hospital Clínico Universitario, Universidad de Santiago de Compostela, Santiago de Compostela, Spain

Key Words

Progressing stroke · Early neurological deterioration · Glutamate · Excitotoxicity · Acute ischemic stroke

Abstract

The prevention and treatment of progressing stroke should be one of the main therapeutic targets of neuroprotective therapies. Despite the high prevalence of progressing stroke in acute stroke (25–35%) and its importance as a predictor of poor outcome, no treatment capable of preventing early neurological deterioration (END) or of reducing its impact has yet been developed. It is essential that our understanding of END's underlying mechanisms be improved as it is currently not possible to predict its occurrence accurately. Published studies to date have been unable to identify a clinical profile which reliably predicts those patients likely to suffer neurological deterioration in the very early acute phase of ischemic stroke. In the following pages, we will discuss the present situation with regard to neurological worsening in general, paying special attention to END given the prognostic and therapeutic implications of this common condition. Factors associated with neurological deterioration and the potential mechanisms, particularly excitotoxic theory, are discussed.

Between 25 and 35% of patients who suffer an ischemic stroke experience further neurological deterioration in the following 48–72 h [1–17]. The concept of progressing stroke has seen many different interpretations since Millikan and Siekert [18] first described it in 1955 with no temporal definition having been accepted as standard and without even agreement as to how it should be called [19].

The relevance of progressing stroke lies not only in the high prevalence of early neurological deterioration (END) but also in its association with worse outcome [4, 5, 7, 10, 12, 15, 20]. The fact that until now no treatment has been developed that is capable of preventing END or of reducing its impact when it occurs makes it essential that we further investigate the underlying mechanisms in the hope of being able to learn how to predict its occurrence accurately and so open the way to better handling of these cases. To do this, clinical trials with neuroprotective agents should widen their scope to include END as a surrogate endpoint.

In spite of the variations in the definition of progressing stroke, it seems clear that the time interval between stroke onset and the occurrence of progressing stroke allows us to identify two distinctive patterns, early and late neurological deterioration, which have different pathophysiological mechanisms and hence potentially require different therapeutic handling. Acute-phase reactants

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0080$23.50/0

Accessible online at:
www.karger.com/ced

Dr. Joaquín Serena
Stroke Unit, Neurology Department, Hospital Universitari Doctor Josep Trueta
Avenida de Francia s/n
ES–17007 Girona (Spain)
Tel. +34 972 940 262, Fax +34 972 228 296, E-Mail nrl.jserena@htrueta.scs.es

and hemodynamic, inflammatory and molecular intracerebral mechanisms are involved in END while in late neurological deterioration systemic factors such as infectious diseases, metabolic disorders, bronchoaspiration, etc., seem more relevant. In this review we will focus on END as the potential target of neuroprotective therapies.

END unfortunately is also a term without an agreed definition. About 50% of all clinical deterioration occurs in the first 24 h after stroke onset outcome [4, 6, 7, 9]. Somewhat arbitrarily, the most frequently used intervals to define END have been either 48 or 72 h after onset – temporal windows in which patients should be managed in acute stroke units and where experimental treatments may have a significant role in the handling.

An additional difficulty in the identification of END is that there is a lack of consensus as to when neurological worsening should be defined as progressive. The fact that the Canadian Stroke Scale (CSS) and NIH Stroke Scale (NIHSS) have been the two most widely used scales in published studies and that they have been shown to have a high predictive value of worse outcome, a reasonable definition might be to consider progression as a decrease of ≥ 1 point in the CSS and/or a decrease of ≥ 4 points in the NIHSS.

This prevalence of END and the definition given above have been found to be valid not only for cortical and subcortical brain infarction but also for lacunar brain infarction [15, 16].

Although no definitive stroke scale has been agreed upon, the CSS and the NIHSS are both extremely useful scales. The CSS is a simple and validated stroke scale for END that may be used by paramedics, nursing staff and doctors in the emergency department. On the other hand, the NIHSS has the advantage of greater completeness and is widely used by neurologists both in stroke units and stroke trials and should perhaps be taken as the standard scale until further refinement is achieved.

Predictors of Progressing Stroke

Since Britton and Roden's [4] first report in 1985 on patient outcome in non-selected hospitalized stroke patients, none of those clinical factors identified as being associated with deterioration have managed to predict END accurately and those variables which have been identified as predictors in some studies are often found not to coincide with the variables of others (table 1).

As our knowledge of the mechanisms involved in stroke progression has advanced, promising results in both the experimental and clinical understanding of biochemical markers involved in END have been achieved. This knowledge may well result in the development of new therapeutic strategies in the handling of acute ischemic stroke.

Biochemical markers of END may prove to be a promising tool for use in clinical trials and in daily clinical practice if confirmation of the results described in this review is obtained. Furthermore, since END anticipates poor outcome in a high proportion of patients, it warrants use as an early surrogate endpoint in therapeutic trials (fig. 1).

Main Predictors of END

It is not clear whether identified systemic factors such as hyperthermia, high or low blood pressure, and high serum glucose levels are causes of END or are merely factors associated with the serious disease that they are suffering from.

Blood Pressure

The role of blood pressure (BP) is controversial as high BP [10] and low BP [6] as well as a relevant drop in BP [13] have been found to be related to progressing stroke in some studies whereas other studies have failed to identify BP as a predictor of END [6, 8]. As suggested by Castillo et al. [13], these opposite findings may be partially explained by a U-shaped relationship between BP levels and outcome measures, being the fall in BP during the first day after admission are detrimental for patients with acute ischemic stroke. In analyzing results of the ECASS-I study, Dávalos et al. [8] found that whilst 37.5% of patients experienced deterioration within the first 24 h after inclusion, neither SBP or DBP at entry, nor changes in SBP within the first 24 h, were associated with END. These results suggest that high SBP does not contribute to END in patients in whom significant changes in BP during the acute phase are avoided. These results suggest that other factors must be involved in END.

Glycemia and Poor Collateral Blood Flow

Although some studies have identified hyperglycemia as a predictor of neurological worsening [7, 10], when adjusted in the ECASS-I study for the concomitant history of diabetes, initial serum glucose levels were not found to be a predictor of progressing stroke but rather the diabe-

Table 1. Predictors of END at admission

Study (first author)	Time from stroke onset to admission, h	Definition of END, h	Independent predictors of END
Britton, 1985 [4]			No predictors were found
Dávalos, 1990 [10]	<8	<48	High blood pressure High blood glucose level Carotid territory involvement
Dávalos, 1997 [8]	<8	<24	Fibrinogen High body temperature
Dávalos, 1999 [14]	<6	<24	Focal hypo- and hyperdensity of MCA on baseline CT Longer delay until treatment (rtPA) History of coronary heart disease History of diabetes
Jorgensen, 1994 [6]	–	<36	History of diabetes Lower blood pressure Stroke severity at admission (predict LND ≥ 36 h)
Toni, 1995 [7]	<5	<48	High blood glucose level Early focal hypodensity on CT with cortical and subcortical locations Carotid siphon occlusion on angiography
Toni, 1998 [11]			Abnormal TCD (asymmetry plus no-flow)
Castillo, 1997 [22]	<24	<48	Plasma glutamate >200 μmol/l CSF glutamate >8.2 μmol/l
Yamamoto, 1998 [12]	–	–	Stroke subtype Age <65 years Hypertension Lesion outside the superficial anterior circulation No transient ischemic attack Reduced level of consciousness
Nakamura, 1999 [20]	<24	<48	Diabetes mellitus Severity of motor deficit on admission
Castillo, 2000 [21]	<24	<48	Nitric oxide metabolite concentrations >5.0 μmol/ml in CSF
Dávalos, 2000 [23]	<24	<48	Plasma and CSF ferritin concentrations
Serena, 2001 [15]	<24	<48	Plasma glutamate >200 μmol/l GABA concentration <240 nmol/l Blood glucose levels at 24 and 48 h after admission Basal ganglia topography of lacunar infarction
Castellanos, 2002 [16]	<24	<48	TNF-α >14 pg/ml ICAM-1 >208 pg/ml History of hypertension
Leira, 2002 [17]	<24	<48	Headache at stroke onset
Vila, 2003 [49]	<24	<48	IL-6 >21.5 pg/ml in plasma IL-6 >6.3 pg/ml in CSF Temperature at admission Serum glucose at admission Admission CSS score Early infarct signs on brain CT scan
Álvarez, 2004 [31]	<24	<72	History of arterial hypertension Cerebrovascular reactivity (CO_2 inhalation) impairment within the first 24 h
Castillo, 2004 [13]	<24	<48	Fall in SBP >20 mm Hg during the first day Both high and low SBP or DBP within first 24 h of stroke onset
Blanco, 2005 [50]	<24	<48	Neuroprotective effect of increased plasma 15-dPGJ2 (PPAR-γ agonists) in atherothrombotic stroke

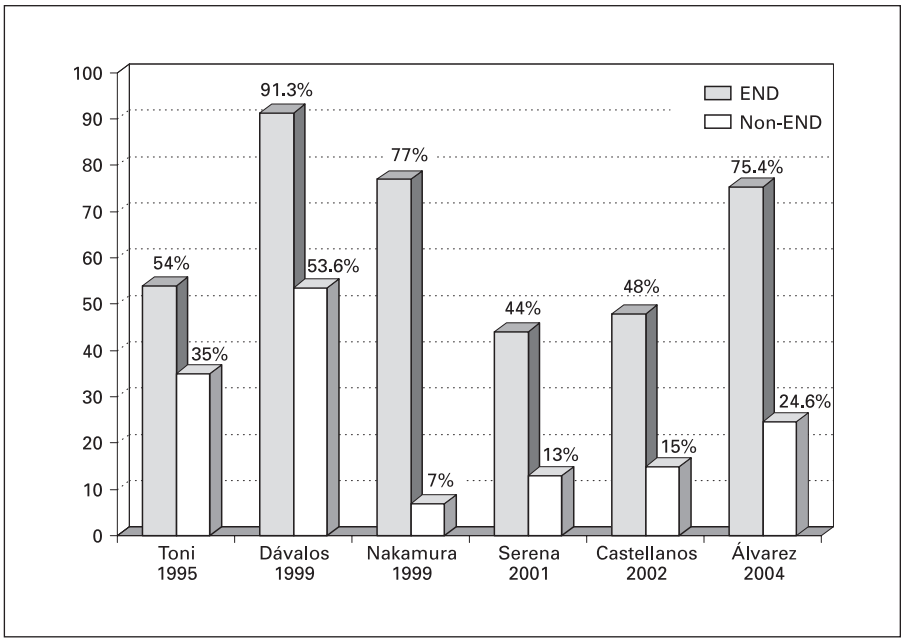


Fig. 1. Poor outcome in patients suffering from END in comparison with non-END.

tes itself [14]. Diabetic microangiopathy leading to chronic impairment of cerebral autoregulation and insufficient cerebral perfusion pressure might partly explain this finding. Moreover, diabetic microangiopathy could be responsible for an inadequate collateral blood supply after arterial occlusion which aggravates cellular damage due to the enhancement of brain edema and free radical damage [24, 25]. This mechanism involving poor collateral blood supply is also supported by the fact that coronary artery disease, which is a marker of severe extra- or intracranial atherosclerotic disease [26], was a predictor of END in this study (table 1).

Hyperthermia

Hyperthermia aggravates neuronal damage and has been described as an independent predictor of END [8, 40, 49]. This factor probably aggravates cerebral injury in the penumbral area through intracerebral mechanisms such as excitotoxicity, which is probably the main cause of neurological worsening. This hypothesis is supported by the results of Castillo et al. [22] showing that the apparent effect of body temperature on progression almost disappeared when the effect of glutamate is included in the multivariate analysis.

CT Findings at Admission

Given that early hypodensity and other CT findings have been identified as predictors of early deterioration [6, 7, 14], a CT scan performed shortly after stroke onset might be considered to be a useful tool in predicting progression. However, and as with other clinical predictors, the positive predictive value of this variable is unsatisfactory; 38% in the case of hypodensity.

Mechanisms of END

The mechanisms of END have not been completely elucidated. Hemodynamic factors, development of brain edema, stroke recurrence or biochemical mechanisms have been proposed as potential mechanisms.

Hemodynamic Factors in END

It has been suggested that hemodynamic factors may be one of the main mechanisms in clinical neurological deterioration. According to this hypothesis, failure of the collateral blood flow could provoke END due to insufficient brain perfusion after an acute proximal arterial occlusion. The risk of this occurring would be especially present where the basal situation is not optimal due to the existence of macro- or microvascular diseases such as the previously mentioned diabetes mellitus or coronary arterial disease.

Hemodynamic factors such as mean middle cerebral artery blood flow velocity, measured by transcranial Doppler, cerebral blood flow, determined either by positron

emission tomography or by single photon emission computed tomography, and cerebrovascular reactivity (CVR) have been related to the final infarct volume and long-term outcome [27–29]. Furthermore, the analysis of flow patency by TCD has recently been evaluated in detail and validated in acute cerebral ischemia with the finding that MCA recanalization is the main predictor of good outcome [11, 30]. In spite of these numerous studies, only two have focused on the influence of hemodynamic factors in progressing stroke [11, 31]. The high relevance of collateral blood flow supply in this setting was demonstrated in an angiographic study by Toni et al. [11], who found that the presence of collaterals was an independent predictor of early improvement whereas the absence of collateral blood supply was an independent predictor of END. However, this pathogenetic mechanism only manages to predict deterioration in 46% of patients.

CVR estimates the additional cerebral blood flow that can reach the distal territory of a brain artery when required. This parameter depends particularly on the presence of a proximal vessel stenosis, effective collateral circulation, and blood viscosity [27]. In patients with carotid artery stenosis, below normal values of CVR is an indication of the loss of autoregulation and the failure of collateral circulation and, hence, supposes an increased risk of stroke [32]. It has recently been found that when CVR values are below normal within 24 h of stroke onset there is an approximately eightfold increase in the likelihood of subsequent END [31]. Impaired CVR at stroke onset probably indicates that the vasodilator compensatory mechanisms are exhausted and, as a consequence, that there is a high risk of recruitment of the oligemic ischemic tissue into the infarcted area. In contrast, normal CVR may suggest that recanalization has taken place or that sufficient collateral blood flow is preventing the growth of the infarcted area. However, although we found that when CHR was normal 93% of patients remained stable or improved (negative predictive value), its positive predictive value for END was only 38%. It therefore seems clear that a further mechanism must be involved in patients who suffer END.

Brain Edema

Although brain edema plays a role in late neurological deterioration and especially in END [14], its relevance has probably being overestimated. It has been hypothesized that arterial occlusion with an insufficient collateral blood supply leads to early brain edema, which is ultimately responsible for END [7]. However, brain edema as the cause of neurological deterioration only accounts for a small proportion of END and event in those with massive MCA infarction neurological deterioration seems to be associated with intracerebral molecular mechanisms rather than brain edema itself [33]. In a recently published study, our group found that 18% of stroke patients suffered a massive MCA infarction but that only about half of these suffered malignant MCA with neurological deterioration due to brain edema. Of the remaining patients suffering massive MCA infarction, we found that 26% deteriorated in spite of non-significant brain edema suggesting that other mechanisms of END must be involved. In this same study it was found that c-Fn, a marker of blood-brain barrier disruption, was a highly sensitive and specific predictor of brain edema in acute ischemic stroke. In analyzing EAAs and inflammatory molecules, we confirmed their previously reported association with END, increased infarct volume, and poor outcome but not with brain edema infarction [33].

Excitotoxic Theory

Despite the importance of early clinical course on stroke outcome, published studies have not identified a clinical profile which is able to reliably predict those patients likely to suffer neurological deterioration in the very early acute phase of ischemic stroke. Older and younger patients, history of diabetes and arterial hypertension, stroke severity, hyperglycemia within the first 2 days after admission, and larger infarct volume amongst other factors have been associated with progressing stroke (see table 1). However, the ability to predict progression based on these clinical factors has been estimated at less than 60%. A different approach that has been receiving increasing attention is the analysis of potential molecular mechanisms of END. Excitatory amino acids, specifically glutamate, have been found to participate in the pathophysiology of cerebral ischemia [37, 38] and to be powerful predictors of END. Glutamate is found correctly to predict 86–95% of cases that will suffer END in the following hours and when adjusted in a multivariate analysis for concomitant predictors of stroke progression it still remains as the main independent predictor of END [15, 22, 39, 40].

Glutamate is released in high concentrations by the presynaptic neurons in the core of the cerebral infarction and the penumbral cortex and leads to the prolonged and intense activation of specific receptors which in turn results in a massive influx of calcium that activates a variety of catabolic processes subsequently producing cell death [41, 43].

The release of glutamate is the first stage in a cascade of molecular reactions that act in a sequential manner provoking the delayed death of those cells that are both adjacent to and distant from the area of the infarction. These reactions include the generation of nitric oxide and the activation of various proteases that participate in the inflammatory and cytotoxic mechanism leading to neuronal death so increasing the initial infarct area.

The importance of glutamate in the physiopathology of tissular necrosis has been demonstrated in different experimental models of focal cerebral ischemia. In 1996, we found high concentrations of glutamate in the CSF and plasma of stroke patients with <24 h of onset [44, 45]. Plasma glutamate concentrations of >200 μmol/l within the first 24 h of stroke onset were found to have a 97% positive predictive value for the subsequent progression of cortical, subcortical and lacunar ischemic strokes independently of the infarct volume and stroke severity at admission [22]. Plasma glutamate levels in CSF remained abnormally high for at least 24 h in those patients who suffered END whereas they returned to normal levels in <6 h in patents who did not suffer from progressing stroke [39]. Recently, in a study focused on patients with hemispheric stroke of <12 h evolution, we again found higher concentrations of glutamate in patients with END and that glutamate levels mediate DWI lesion growth in these patients [45]. Beyond the penumbral area, tissue is still at risk and there is a high correlation between baseline and 24 h glutamate levels and the volume of the peripenumbral area which subsequently becomes infracted [46]. It should be noted that only 30% of patients with peripenumbral infarction had mismatch at admission, so neuroprotective therapies with glutamate antagonists could well be useful in limiting DWI lesion growth even in those cases without initial DWI/PWI mismatch.

The extracellular accumulation of glutamate in the cerebral tissue is not directly related to the initial magnitude of the ischemic tissue suggesting the hypothesis of individual susceptibility to excitotoxic damage. Although GABA neurotransmission, which results in increased chloride flow across the postsynaptic membrane and hyperpolarization, partially counterbalances the toxic effects of glutamate during ischemia, the main neurotoxic effect is due to the total extracellular accumulation of glutamate that is essentially due to a disorder in the system of uptake by the neurons and especially by the glia cells [47]. The Na^+-dependent carriers of excitatory amino acids capture this extracellular glutamate and maintain concentrations below excitotoxic levels. During ischemia these carriers, five of which are currently known, lose their normal function and the concentrations of glutamate reach excitotoxic levels. Immunohistochemical studies have revealed that two of these carriers, EAAT1 and EAAT2, are located in the astrocytes and that EAAT2 is responsible for the uptake of more than 90% of the glutamate in the adult brain.

Our group has recently described a new functional polymorphism in the EAAT2 gene promoter associated to higher concentrations of plasmatic glutamate and greater neurological deterioration in stroke patients. This polymorphism alters the functioning of the carriers and contributes to an imbalance between the release and the uptake of glutamate with the corresponding excitotoxic damage in situations of stroke but not of normal flow. In studies performed with astrocyte cultures, we have found that the basal activity of the mutated promoter was 30% less than the wild-type promoter.

Inflammation and END

Several studies support the hypothesis that inflammation may also play an important role in progressing stroke [16, 49]. An independent association of high levels of inflammatory molecules in blood with END and poor outcome has been observed in both territorial [49] and lacunar infarctions [16].

Increased levels of cytokines such as interleukin (IL)-1, tumor necrosis factor-α (TNF-α), and IL-6, as well as adhesion molecules such as ICAM-1, have been observed in the peripheral blood and CSF of ischemic stroke patients. Several facts support the idea that plasma levels of IL-6, TNF-α, and ICAM-1 within the first 24 h of acute stroke reflect the total release of these molecules in the ischemic brain tissue rather than an acute-phase reaction or a systemic cause.

Cytokines have been found to be involved in several mechanisms that may potentiate ischemic brain injury including the release of the inducible form of nitric oxide synthase by astrocytes; the promotion of a local procoagulant state, and the regulation of apoptotic programmes. TNF-α promotes the expression of adhesion molecules such as ICAM-1 on the endothelium, facilitating leukocyte adherence and migration from capillaries into the brain, microvessel occlusion, and, subsequently, a progressive reduction in blood flow. In these studies inflammatory molecules contributed to END after adjustment for glutamate and GABA concentrations in blood. The results suggest that inflammation in END may not just have an excitotoxic role. A significant correlation has been found between glutamate and GABA concentrations with inflammatory markers in blood and it is likely

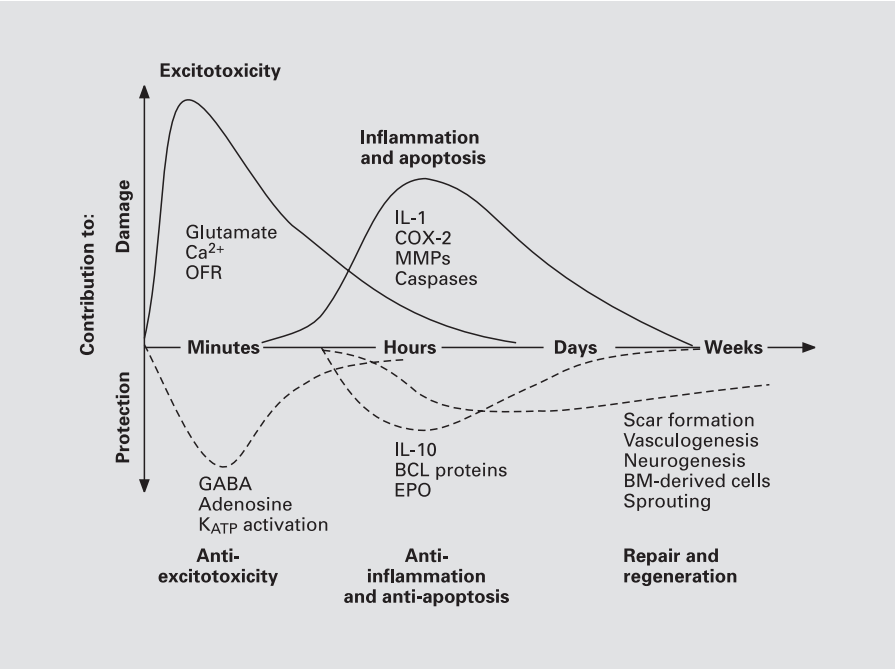


Fig. 2. x axis: the evolution of the cascades over time; y axis: the impact of each element of the destructive (top) and protective (bottom) cascades on final outcome; continuous line: major pathophysiological entities of tissue destruction in stroke (grouped by the acute mechanisms of excitotoxicity and the delayed mechanisms of inflammation and apoptosis); broken line: the corresponding protective tissue responses. Reproduced with the kind permission of Dr. Dirnagl [43].

that inflammatory and excitatory mechanisms cooperate in the progression of stroke in a sequential and interacting process [42, 43] (fig. 2).

There are few results with regard to the therapeutic implications for END. We have found that aspirin has a neuroprotective effect inhibiting glutamate release after a period of oxygen-glucose deprivation in both in vitro models and a rat model of permanent focal cerebral ischemia [34]. In acute stroke patients we have found that prior treatment with aspirin prevents END [35], an effect that has been described in series with lacunar infarction [15] as well as the patients included in the NINDS rtPA trial [36]. Our results suggest that this effect is not due to changes in the acute-phase response but rather to low glutamate concentrations. Other mechanisms also seem to participate given that after adjustment for glutamate the odds ratio of END for aspirin did not significantly change. Blanco et al. [50] have recently reported that increased plasma 15-dPGJ$_2$, a PPAR-γ agonists, is associated with good early and late neurological outcome and smaller infarct volume in atherothrombotic ischemic stroke, suggesting a neuroprotective role for 15-dPGJ2 probably resulting from the inhibition of the inflammatory cascade triggered by the ischemic event. This study provides the first evidence of the potential neuroprotective value of 15-dPGJ2, or other PPAR-γ ligands, in acute ischemic stroke.

In conclusion, the current state of our knowledge about END is that we are dealing with a multifactorial event which is only partially predictable by the clinical, laboratory, and imaging data that is used in our ordinary practice. Hence, we need to advance in the search for biochemical markers and for new neuroimaging tags of stroke progression.

Although the spread of the infarcted area due to hemodynamic factors after a proximal artery occlusion has been considered as being the main cause of END, the present pathophysiological knowledge of acute ischemic stroke suggests that the enlargement of cytotoxic edema as shown by diffusion-weighted MRI, and subsequent neurological worsening, might rather be due to a delayed propagation of neuronal death mediated by multiple molecular and cellular mechanisms such as excitotoxicity, free radicals and NO generation, inflammation and apoptosis.

The treatment of ischemic stroke must be aimed at correcting not only hemodynamic changes but also at limiting cellular changes and their consequences by blocking the inflammatory process and the neurotoxicity of excitatory amino acids, which are the main mechanisms of neurological deterioration.

Inflammation and neurotoxicity play a central role in ischemic stroke and in the mechanisms of neurological deterioration. It seems reasonable to focus therapeutic

intervention with neuroprotective agents on decreasing the neurotoxic effect of excitatory amino acids, particularly of glutamate, of proinflammatory cytokines and of cell adhesion molecules. The pharmacokinetics of these molecular mechanisms suggest that neuroprotective agents may be administered early and continuously for a period of at least 24 h. Recent findings about stroke susceptibility due to the presence of polymorphisms in the promoter region of the EAAT-2 gene, which is associated with higher glutamate plasma levels and END, opens up the possibility of using pharmacogenetics in future clinical trials with drugs that interfere with the excitotoxic pathway as a step towards the development of individualized treatment of patients with stroke.

References

1 Jones HJ, Millikan CH: Temporal profile (clinical course) of acute carotid system cerebral infarction. Stroke 1976;7:64–71.
2 Patrick BK, Ramirez-Lassepas M, Synder BD: Temporal profile of vertebrobasilar territory infarction. Prognostic implications. Stroke 1980;11:643–648.
3 Jones HR Jr, Millikan CH, Sandok BA: Temporal profile (clinical course) of acute vertebrobasilar system cerebral infarction. Stroke 1980; 11:173–177.
4 Britton M, Roden A: Progression of stroke after arrival at hospital. Stroke 1985;16:629–632.
5 Lodder J, Gorsselink EL: Progressive stroke caused by CT-verified small deep infarcts; relation with the size of the infarct and clinical outcome. Acta Neurol Scand 1985;71:328–330.
6 Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS: Effect of blood pressure and diabetes on stroke in progression. Lancet 1994;344: 156–159.
7 Toni D, Fiorelli M, Gentile M, Bastianello S, Sacchetti ML, Argentino C, Pozzilli C, Fieschi C: Progressing neurological deficit secondary to acute ischemic stroke. A study on predictability, pathogenesis, and prognosis. Arch Neurol 1995;52:670–675.
8 Dávalos A, Castillo J, Pumar JM, Noya M: Body temperature and fibrinogen are related to early neurological deterioration in acute ischemic stroke. Cerebrovasc Dis 1997;7:64–69.
9 Hachinski V, Norris JW: The deteriorating stroke; in Meyer JS Lechner H, Reivich M, Ott EO, Aranibar A (eds): 10th International Salzburg Conference on Cerebral Vascular Disease, Salzburg, Sept 1980. Amsterdam, Excerpta Medica, 1980, pp 315–318.
10 Davalos A, Cendra E, Teruel J, Martinez M, Genis D: Deteriorating ischemic stroke: risk factors and prognosis. Neurology 1990;40: 1865–1869.
11 Toni D, Fiorelli M, Zanette EM, Sacchetti ML, Salerno A, Argentino C, Solaro M, Fieschi C: Early spontaneous improvement and deterioration of ischemic stroke patients. A serial study with transcranial Doppler ultrasonography. Stroke 1998;29:1144–1148.
12 Yamamoto, H, Bogousslavsky J, van Melle G: Different predictors of neurological worsening in different causes of stroke. Arch Neurol 1998; 55:481–486.
13 Castillo J, Leira R, García MM, Serena J, Blanco M, Dávalos A: Blood pressure decrease during the acute phase of ischemic stroke is associated with brain injury and poor stroke outcome. Stroke 2004;35:520–527.
14 Dávalos A, Toni D, Iweins F, Lesaffre E, Bastianello S, Castillo J for the ECASS Group: Neurological deterioration in acute ischemic stroke. Potential predictors and associated factors in the European Cooperative Acute Stroke Study (ECASS) I. Stroke 1999;30:2631–2636.
15 Serena J, Leira R, Castillo, Pumar JM, Castellanos M, Dávalos A: Neurological deterioration in acute lacunar infarctions: the role of excitatory and inhibitory neurotransmitters. Stroke 2001;32:1154–1161.
16 Castellanos M, Castillo J, Garcia MM, Leira R, Serena J, Chamorro A, Davalos A: Inflammation-mediated damage in progressing lacunar infarctions: a potential therapeutic target. Stroke 2002;33:982–987.
17 Leira R, Dávalos A, Aneiros A, Serena J, Pumar JM, Castillo J: Headache as a surrogate marker of the molecular mechanisms implicated in progressing stroke. Cephalalgia 2002; 22:303–308.
18 Millikan CH, Siekert RG: Studies in cerebrovascular disease. I. The syndrome of intermittent insufficiency of the basilar arterial system. Mayo Clin Proc 1955;30:61–68.
19 Castillo J: Deteriorating stroke: diagnostic criteria, predictors, mechanisms and treatment. Cerebrovasc Dis 1999;9(suppl 3):1–8.
20 Nakamura K, Saku Y, Ibayashi S, Fujishima M: Progressive motor deficits in lacunar infarction. Neurology 1999;52:29–33.
21 Castillo J, Rama R, Dávalos A: Nitric oxide-related brain damage in acute ischemic stroke. Stroke 2000;31:852–857.
22 Castillo J, Dávalos A, Noya M: Progression of ischemic stroke and excitotoxic amino acids. Lancet 1997;349:79–83.
23 Dávalos A, Castillo J, Marrugat J, Fernández-Real JM, Armengou A, Cacabelos P, Rama R: Body iron stores and early neurologic deterioration in acute cerebral infarction. Neurology 2000;54:1568–1574.
24 Berger L, Hakim AM: The association of hyperglycemia with cerebral edema in stroke. Stroke 1986;17:865–871.
25 Toni D, De Michele M, Fiorelli M, Bastianello S, Camerlingo M, Sacchetti ML, Argentino C, Fieschi C: Influence of hyperglycaemia on infarct size and clinical outcome of acute ischemic stroke patients with intracranial arterial occlusion. J Neurol Sci 1994;123:129–133.
26 Craven TE, Ryu JE, Espeland MA, Kahl FR, McKinney WM, Toole JF, McMahan MR, Thompson CJ, Heiss G, Crouse JR: Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis: a case-control study. Circulation 1990;82:1230–1242.
27 Derdeyn CP, Grubb RL, Powers WJ: Cerebral hemodynamic impairment. Methods of measurement and association with stroke risk. Neurology 1999;53:251–259.
28 Kaps M, Teschendorf U, Dorndorf W: Hemodynamic studies in early stroke. J Neurol 1992; 239:138–142.
29 Rubin G, Firlik A, Levy E, Pindzola R, Yonas H: Relationship between cerebral blood flow and clinical outcome in acute stroke. Cerebrovasc Dis 2000;10:298–306.
30 Alexandrov AV, Demchuk A, Wein T, Grotta JC: The yield of transcranial Doppler in acute cerebral ischemia. Stroke 1999;30:1604–1609.
31 Álvarez FJ, Segura T, Castellanos M, Leira R, Blanco M, Dávalos A, Serena J: Cerebral hemodynamic reserve and early neurologic deterioration in acute ischemic stroke. J Cereb Blood Flow Metab 2004:2004;24:1267–1271.
32 Ringelstein EB, Sievers C, Ecker S, Schneider PA, Otis SM: Noninvasive assessment of CO_2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. Stroke 1998;19: 963–969.
33 Serena J, Blanco M, Castellanos M, Silva Y, Vivancos J, Moro MA, Leira R, Lizasoain I, Castillo J, Dávalos A: The prediction of malignant cerebral infarction by molecular brain barrier disruption markers. Stroke 2005;36: 1921–1926.

34 De Cristobal J, Moro MA, Davalos A, Castillo J, Leza JC, Camarero J, Colado MI, Lorenzo P, Lizasoain I: Neuroprotective effect of aspirin by inhibition of glutamate release after permanent focal cerebral ischaemia in rats. J Neurochem 2001;79:456–459.

35 Castillo J, Leira R, Moro MA, Lizasoain I, Serena J, Dávalos A: Neuroprotective effects of aspirin in patients with acute cerebral infarction. Neurosci Lett 2003;339:248–250.

36 Grotta JC, Lemak NA, Gary H, Fields WS, Vital D: Does platelet antiaggregant therapy lessen the severity of stroke? Neurology 1985;35:632–636.

37 Takagi K, Ginsberg MD, Globuls MYT, Dietrich WD, Martinez E, Kraydieh S, Busto R: Changes in amino acid neurotransmitters and cerebral blood flow in the ischemic penumbra region following middle cerebral artery occlusion in the rat: correlation with histopathology. J Cereb Blood Flow Metab 1993;13:575–585.

38 Butcher SP, Bullock R, Graham DI, McCulloch J: Correlation between amino acid release and neuropathologic outcome in rat brain following middle cerebral artery occlusion. Stroke 1990;21:1727–1733.

39 Dávalos A, Castillo J, Serena J, Noya M: Duration of glutamate release after acute ischemic stroke. Stroke 1997;28:708–710.

40 Dávalos A, Castillo J: Potential mechanisms of worsening. Cerebrovasc Dis 1997;7(suppl 5):19–24.

41 Rothman SM, Olney JW: Glutamate and the pathophysiology of hypoxic-ischemic brain damage. Ann Neurol 1986;19:105–111.

42 Castillo J, Leira R: Predictors of deteriorating cerebral infarct: role of inflammatory mechanisms. Would its early treatment be useful? Cerebrovasc Dis 2001;11(suppl 1):40–48.

43 Dirnagl U, Iadecola C, Moskowitz MA: Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 1999;22:391–397.

44 Castillo J, Davalos A, Naveiro J, Noya M: Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. Stroke 1996;27:1060–1065.

45 Castillo J, Dávalos A, Lema M, Serena J, Noya M: Glutamate is a marker for cerebral ischemia in cortical but not deep infarcts. Cerebrovasc Dis 1997;7:245–250.

46 Castellanos M, Blanco M, Pedraza S, García M, Silva Y, Leira R, Dávalos A: Molecular markers of progressing stroke are associated with diffusion-weighted MRI lesion growth in patients with acute ischemic stroke. Stroke 2004;35:247.

47 Seal RP, Amara SG: Excitatory amino acid transporters: a family in flux. Annu Rev Pharmacol Toxicol 1999;39:431–456.

48 Mallolas J, Hurtado O, Castellanos M, Blanco M, Rodríguez-Yáñez M, Puigdemont M, Serena J, Pedraza S, Moro MA, Lizasoain I, Castillo J, Davalos A: A novel functional polymorphism in the EAAT2 gene is associated with higher glutamate concentrations and brain injury. Stroke 2005;36:458.

49 Vila N, Castillo J, Davalos A, Esteve A, Planas AM, Chamorro A: Levels of anti-inflammatory cytokines and neurological worsening in acute ischemic stroke. Stroke 2003;34:671–675.

50 Blanco M, Moro MA, Dávalos A, Leira R, Castellanos M, Serena J, Vivancos J, Rodríguez-Yáñez M, Lizasoain I, Castillo J: Increased plasma levels of 15-deoxy δ prostaglandin j2 are associated with good outcome in acute atherothrombotic ischemic stroke. Stroke 2005;36:1189–1194.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):89–98
DOI: 10.1159/000091708

Published online: May 2, 2006

Non-Pharmacological Neuroprotection: Role of Emergency Stroke Management

Rogelio Leira[a] Miguel Blanco[a] Manuel Rodríguez-Yáñez[a] José Flores[b]
Jorge García-García[a]

[a]Department of Neurology, Division of Vascular Neurology, Stroke Unit, Hospital Clínico Universitario, and
[b]Public Emergency Health Foundation of Galicia-061, Santiago de Compostela, Spain

Key Words
Acute stroke · Non-pharmacological neuroprotection · Stroke, general care

Abstract
Acute stroke should be considered a medical emergency, where actions taken in the first hours are fundamental for achieving recovery of the damaged cerebral tissue and a better prognosis for the patient. Recanalization and neuroprotective treatment has been used with mixed results. The effectiveness observed in the first hours with thrombolytic drug treatment is only applicable to a small percentage of patients, and attempts to widen this treatment window have not yet proved fruitful. Pharmacological neuroprotective treatment has not yet demonstrated the clinical effectiveness observed in experimental models. The concept of neuroprotection in cerebral ischemia also involves a series of mechanisms that take place at the cerebral level following vascular occlusion. In this context, it should be borne in mind that a series of physiological functions usually involved in the cerebral metabolism (control of blood pressure, of temperature, of glycemia and of arterial oxygen saturation) play a key role in modulation of the ischemic process. Changes in the control of these mechanisms may aggravate the process of cerebral damage in the first hours of ischemic stroke. In this work we review the prognostic importance of the main mechanisms that may influence the acute phase of cerebral ischemic stroke, as well as their therapeutic management and control in the clinical situation.

Introduction

Ischemic stroke is a dynamic process, where a series of excitotoxic, inflammatory and microvascular mechanisms take place that lead to tissue necrosis. Recanalization with rt-PA is the only type of treatment with proven efficacy during the first 3 h. Attempts to prolong over time the efficacy of this treatment have not until now proved fruitful. Meanwhile, a large group of drugs with neuroprotective properties, and which have been shown to be effective in experimental models of ischemia, have not been proven to be effective in man [1]. Treatment with oral citicoline within the first 24 h after onset in patients with moderate to severe stroke has shown to increase the probability of complete recovery at 3 months [2]. Treatment with a free radical scavenger NXY-059 within 4.5 h showed improved outcome in a large phase III trial [3]. Methodological difficulties in patient selection and in the final objectives of studies have been used to justify differences in efficacy between results obtained in experimen-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0089$23.50/0

Accessible online at:
www.karger.com/ced

Dr. Rogelio Leira
Servicio de Neurología, Hospital Clínico Universitario
c/Travesa da Choupana, s/n
ES–15706 Santiago de Compostela (Spain)
Tel. +34 98 195 1342, Fax +34 98 195 1347, E-Mail rleiram@meditex.es

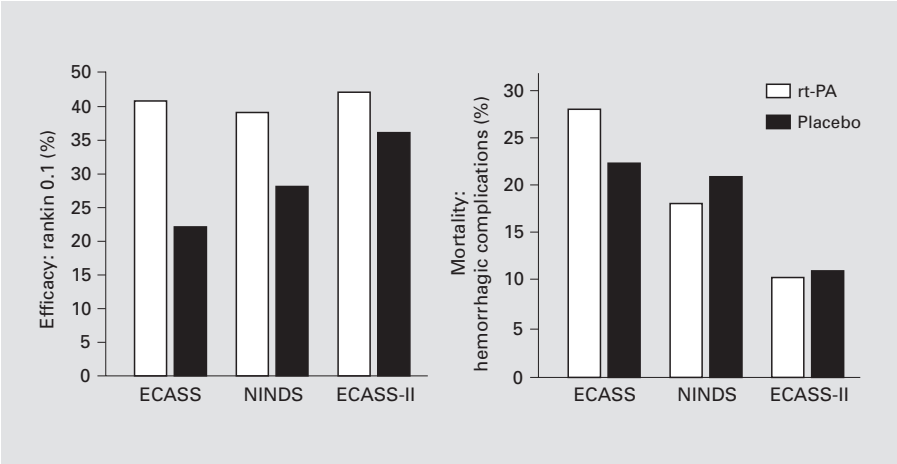


Fig. 1. Results of the clinical trials with rt-PA: progression in the improvement observed in the placebo group in the three main studies.

tal models and in clinical trials. From improved knowledge of the physiopathology of cerebral ischemia, and supported by the results obtained in clinical trials, it has been observed that fluctuations in a series of biological parameters, such as blood pressure (BP), temperature, glycemia, and tissue oxygenation, exercise a marked influence on the ischemic process. Protocolized care of patients with ischemic stroke, which is favored by controlled clinical trials and by the development of stroke units, has clearly demonstrated the effect of these parameters on stroke evolution. Progression in the improvement observed in the placebo group in the three main studies with rt-PA [4–6] suggests that protocolized care improves the evolution of the patient (fig. 1), while studies conducted to demonstrate the effectiveness and efficiency of stroke units also provide support for the positive effect on evolution of protocolized care with control of various parameters.

A meta-analysis by the Stroke Unit Trialist's Collaboration [7] showed that stroke unit care was associated with a reduction in the odds of death recorded at final follow-up (median 1 year; odds ratio 0.83; 95% CI 0.71–0.97). The odds of death or institutionalized care were lower (0.76; 95% CI 0.65–0.90), as were death or dependency at final review (odds ratio 0.75; 95% CI 0.65–0.87). Subgroup analyses showed that the observed benefits were independent of patient age, sex, stroke severity, and type of stroke unit organization.

This paper emphasizes four physiological measures that can be taken to mitigate ischemic brain damage: (1) control of body temperature, (2) control of glycemia, (3) control of the BP, and (4) control of arterial oxygen saturation.

Control of Body Temperature

A relationship between hyperthermia and ischemic stroke has not been completely demonstrated and raises questions that have yet to be resolved. In experimental models of ischemia, it has been demonstrated that hypothermia reduces cerebral damage following cerebral artery occlusion [8–11]. In this situation, the effectiveness of hypothermia appears to be related to early administration (in the first 2 h) [12] and extended duration of the same (>24 h) [13]. Hyperthermia originating after ischemia induced experimentally in animals produces greater cerebral damage [14–16]. The detrimental effect of hyperthermia on the outcome and neuropathological consequences of cerebral ischemia in experimental animals was also shown in a study demonstrating that hyperthermia increases mortality rate and the severity of histopathological damage in comparison with normothermia [17].

Although experimental studies suggest that hypothermia reduces neuronal damage, acting in various steps of the ischemic cascade, the neuroprotective mechanism of hypothermia has not been determined [18]. Hypothermia seems to counteract ischemic brain damage by several mechanisms: prevention of the blood-brain barrier disruption that happens soon after ischemic onset that allows edema formation from extravasation [19]; diminishing of oxygen-based free radical production that results from activation of microglia and other cell types [20]; reduction of the excitotoxic-neurotransmitter release that overstimulates neighboring neurons [21, 22]; lowering of metabolic rate and subsequent energy depletion [23], and anti-inflammatory action [24]. Hyperthermia is thought

to be an important event accentuating biochemical and inflammatory ischemic mechanisms within the ischemic penumbra, and thus contributing to progression of the infarct brain [18].

Numerous studies have been performed to investigate the issue of whether body temperature in stroke patients may be related to the extent of ischemic brain damage or to outcome, measured by stroke severity and poststroke mortality. These studies show a relationship between increased body temperature in stroke patients and greater brain infarct size or poorer stroke outcome [25–34]. A meta-analysis that includes a total of 3,790 patients demonstrates clearly that there is greater morbidity and mortality in patients with cerebral infarct and hyperthermia than in those without hyperthermia [35].

There are, however, discrepancies of interpretation concerning the origin and significance of hyperthemia in the acute phase of ischemic stroke. The moment of appearance of hyperthermia appears to be important: it appears that only hyperthermia that presents in the first 24 h is associated independently with greater infarct volume and with worse prognosis [31], as well as with greater mortality [36]. However, hyperthermia within the first 72 h of stroke may also predict poor outcome and is related to significantly increase poststroke mortality [27].

Another important aspect of the relationship between temperature and cerebral infarction concerns the pathogenesis of hyperthermia. A meta-analysis [35] considers that hyperthermia is a prognostic factor independent of poor evolution, while Boysen and Christensen [37] suggest that hyperthermia is determined by the severity of the ischemic stroke. A study showed that a rise in body temperature occurring in ischemic stroke patients within hours after onset was related to major, but not mild to moderate stroke, indicating that clinically measurable early poststroke hyperthermia might be a result of brain infarct itself. The influence of temperature on outcome observed in experimental models of ischemic stroke has not been proven in patients with stroke, and further studies are needed. Given that poststroke hyperthermia is associated with cerebral ischemia mechanisms related to brain infarct development within the ischemic core and the penumbra, it would be safe to say that hyperthermia following ischemic stroke seems to be an event both induced and inducing brain infarct progression [18].

Patients with acute stroke are exposed to superimposed infections (pulmonary and urinary). These infections may be an important peripheral cause of hyperthermia following stroke. The results obtained by Reith et al. [29] and Castillo et al. [31] may be suggestive of superimposed infection-induced hyperthermia not influencing stroke outcome; however it has been suggested that adverse prognosis in hyperthermic stroke patients may be associated with infective complications.

Although antipyretics are widely recommended for the treatment of hyperthermia (the European Stroke Initiative guideline recommends that the lowering of body temperature should be considered when it is >37.5°C), there is no evidence in regard to their temperature-lowering effect or to their influence on outcome in stroke.

However, despite the evidence that temperatures of 37.5°C worsen the outcome in acute stroke patients, the effect of antipyretics in stroke through the prevention of hyperthermia is also poorly studied. Therapeutic approaches with antipyretics such as paracetamol have only shown efficacy in reduction of body temperature [38], but no effect on the neurological, functional or prognostic status of the patients [39, 40]. Because of the small numbers of patients recruited into these studies, no conclusions on the use of antipyretics in acute stroke can be drawn. Until results from randomized clinical trials are available, the administration of antipyretics is recommended in all acute stroke patients with body temperature >37.5°C, in addition to rapid diagnosis and treatment of possible infections.

Hypothermia as a treatment in acute stroke is still experimental, and evidence of its efficacy is lacking. In several studies, patients with acute stroke have received hypothermic therapy for neuroprotection [41–46]. These studies are small and heterogeneous in most of variables that might determine the effect of cooling in stroke, such as time after symptom onset that therapy was started, target temperature, and duration of hypothermia. The target temperature was 32–33°C in most of them, the therapeutic window was 3–8 h, and the duration of hypothermia varied from 6 h to several days.

Feasibility and safety are far from established and serious side effects such as hypotension, cardiac arrhythmia, and pneumonia are commonly reported, especially in anesthetized patients with temperatures of 32–33°C [18]. There is currently no evidence from randomized trials to support the routine use of chemical or physical cooling therapy in acute stroke [47]. Nevertheless, since experimental studies have shown a neuroprotective effect of hypothermia in cerebral ischemia, further trials with cooling therapy in acute stroke are warranted.

Control of Glycemia

Elevated blood glucose is common in the early phase of stroke. The prevalence of hyperglycemia, defined as blood glucose level >6.0 nmol/l (108 mg/dl), has been observed in two thirds of all ischemic stroke subtypes on admission and in at least 50% in each subtype including lacunar strokes [48]. It has been stated that the presence of hyperglycemia during the acute phase of stroke is a reflection of a pre-existent and unknown diabetes mellitus. Studies to determine glycosylated hemoglobin and/or fructosamine have been carried out, showing the existence of a previous and unknown diabetes mellitus in 5.5–11.4% of patients admitted with acute stroke [49, 50].

Multiple mechanisms contribute to the detrimental effect of acute hyperglycemia. Animal models of focal cerebral ischemia suggest that type of vessel occlusion, presence of collateral blood flow, and occurrence of reperfusion are relevant and that hyperglycemia may influence neuronal damage through accentuated tissue acidosis and lactate generation [51, 52]. Moderately and severely increased blood glucose has been found to further deteriorate the metabolic state and mitochondrial function in the area of ischemic penumbra [53]. The blood-brain barrier is vulnerable to hyperglycemia, presumably through the liberation of lactic acid and free radicals [54]. In a model of middle cerebral artery occlusion, a fivefold increase in hemorrhagic infarct was observed in hyperglycemic cats compared with the normoglycemic animals [55]. Relative insulin deficiency liberates circulating free fatty acids, which, together with hyperglycemia, diminishes vascular reactivity [56]. Experimental studies have shown that the administration of insulin and glucose at the time of a focal cerebral ischemia may reduce the size of the cerebral infarction [57].

Hyperglycemia is associated with a worse stroke outcome [49, 58, 59]. There is, however, considerable debate about whether a causal relationship exists between hyperglycemia and stroke prognosis. Both prospective and case-control studies have concluded that hyperglycemia is a predictor of outcome and mortality independently of age, stroke subtype, and severity [49, 58–60]. A meta-analysis of 33 studies [61] suggests that the relative risk of death in hyperglycemic non-diabetic stroke patients is increased by 3.3 (95% CI 2.3–4.6), with a non-significant trend in hemorrhagic stroke in non-diabetics and no prognostic effect on the outcome in diabetic patients. Acute hyperglycemia also increased the risk of a poor functional recovery in non-diabetic stroke survivors with a relative risk of 1.4. Other studies have not found hyperglycemia to be an independent predictor of stroke outcome and have suggested that hyperglycemia simply reflects a catecholamine-based stress response to a more severe stroke [62–64].

The majority of the studies have used a single time-point measure of blood glucose to define glycemic control. However, animal models of focal ischemic stroke suggest that persistent elevation of blood glucose through the period during which the ischemic penumbra develops may yield a more robust measure of the influence of hyperglycemia on infarct evolution [55]. A retrospective study found an increase in the blood glucose levels in the first 12 h after stroke, which was greater in the more severe cases, and was related with early mortality, although not with outcome at 3 months [65]. However, it has been observed in a recent prospective study that blood glucose levels fall spontaneously in the first 24 h after stroke [65]. In any case, it is important to emphasize that the persistence of hyperglycemia in the first 7 days after stroke was related to a larger final infarct volume and with worse progression [66]. High blood glucose at the time of recanalization of the occluded brain artery was associated with poor outcome [67].

Although results from controlled clinical trials assessing insulin therapy in patients with stroke are still lacking, the data presented from animal studies and clinical observational studies support the need for avoiding early hyperglycemia in patients with non-lacunar stroke and global ischemia [68]. Until further data are available, it is difficult to define the optimal glucose concentration. The level of target glucose concentrations is not the same for the different current target values in the published guidelines: EUSI: <10 nmol/l, ASA: <300 mg/dl = 16.63 nmol/l [69]. Intensive treatment with insulin for maintaining glucose levels between 4 and 6 mmol/l in critical patients (of which 18% presented neurological disease) has been shown to be safe and to significantly reduce mortality and in-hospital complications [70]. The GIST demonstrates the safety of administering a glucose-potassium-insulin infusion in acute phase stroke patients with the aim of keeping glucose levels between 72 and 126 mg/dl (4 and 7 nmol/l) [71]. However, until further results prove the effectiveness of this approach, it cannot be regarded as standard practice. In some treatment guidelines for stroke, the level of glycemia beyond which the initiation of treatment with insulin is recommended seems excessively high (>200 mg/dl) [72] given that lower glucose levels (153.5 mg/dl) have been associated with a poor outcome at 3 months [73] and that a level

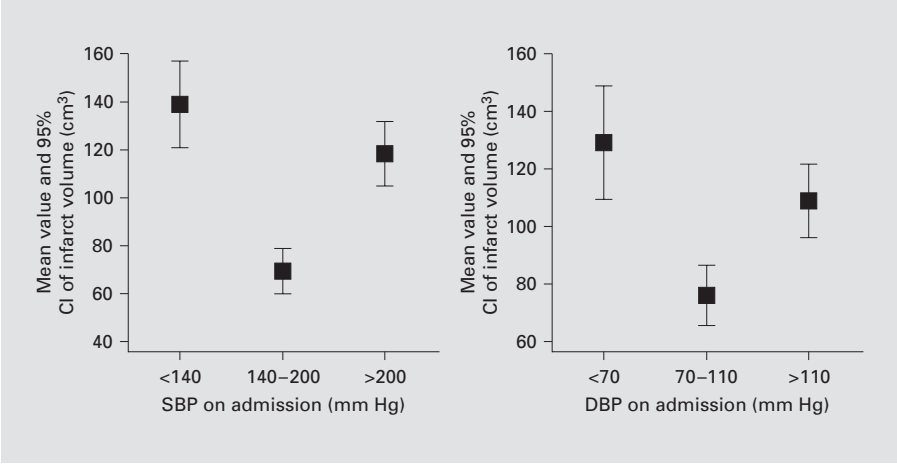


Fig. 2. High and low BPs in acute ischemic stroke are associated with higher infarct volume.

>140 mg/dl (OR 8.4; 95% CI 1.8–40.0) is an independent predictor of poor functional outcome at 3 months in patients with recanalization within 6 h, and might partially reduce the efficacy of fibrinolytic treatment and early recanalization [67].

A reasonable target in most cases of lower blood glucose levels is between 100 and 200 mg/dl (5.5 and 11 nmol/l) [74] although levels of glycemia >150 mg/dl should probably be avoided and insulin therapy should administered from a glycemia level of 150 mg/dl [75]. The decision whether to treat the individual patient intensively with insulin, aiming at normalization of blood glucose levels, also has to take into account the clinical setting.

Control of Blood Pressure

Cerebral perfusion is normally determined by local brain metabolic demands and is independent of systemic BP except at very low or high levels (cerebral autoregulation). Following acute ischemic stroke, autoregulation is lost, and perfusion becomes pressure-dependent. Observational studies suggest that approximately 75% of patients with ischemic stroke have elevated BP when measured within 24–48 h of onset. Systemic hypertension at the time of ischemic stroke is believed to be a physiological response that maintains adequate cerebral perfusion in the ischemic penumbra [76].

The prognostic influence of BP during the acute phase of ischemic stroke is still a matter of controversy. High BP may induce the formation of brain edema, hemorrhagic transformation, and further vascular damage. However, low BP may induce secondary reduction of perfusion in the area of ischemia [76]. Both high and a low BP, as well as falls in BP, have been related to a poor prognosis in patients with acute stroke (fig. 2) [77–79]. It was found that BP reduction in the first 24 h of stroke onset was independently associated with poor neurological outcome at 3 months [79, 80], and with neurological deterioration [79]. The use of sublingual nifedipine has been the subject of particular scrutiny for its tendency to cause rapid, often precipitous declines in BP [81]. A post-hoc analysis of the effect of nimodipine in acute ischemic stroke within 24 h showed that a reduction of diastolic BP (DBP) of about 15 mm Hg was associated with poor outcome [82].

Several observational studies have reported the relationship between raised BP levels and a poor prognosis [83–87], although some authors have demonstrated better outcomes in patients with high initial BP [88, 89]. A systolic pressure BP (SBP) >160 mm Hg within the first 24 h has been related to a poor outcome [87]. Data for the International Stroke Trial (IST) confirmed that the risk of early death and late death or dependency was independently associated with increasing SBP in 17,398 patients [78]. A systematic review of 32 studies involving 10,892 patients concluded that high BP in acute ischemic stroke was associated with subsequent death, deterioration or dependency, and that moderate lowering of BP might improve outcome.

Whether discontinuing a chronically administered antihypertensive medication at the time of admission would be neuroprotective and whether such benefits outweigh the short-term risks of an exacerbation is unknown. Most patients with acute ischemic stroke are treated with anti-

hypertensive drugs despite the absence of severe hypertension [90]. There is no general agreement regarding how BP should be managed in the acute phase of ischemic stroke. Current opinions vary from do not treat [91] to treat [92]. Although lowering BP seems attractive, cerebral autoregulation is lost during acute stroke, and perfusion becomes pressure-dependent, so lowering BP might be expected to worsen outcome. Mildly or moderately elevated BP frequently declines spontaneously during the first minutes or hours of focal ischemia and generally does not require urgent pharmacological treatment [93]. Observations of Mattle et al. [94] suggest that elevated BP is needed to perfuse ischemic brain tissue until recanalization takes places and after it BP declines spontaneously without treatment. It may not be appropriate to reduce BP if it is not known whether the occluded artery has recanalized. The consensus is that antihypertensive agents should be withheld unless SBP is >220 mm Hg or DBP is >120 mm Hg [95], although the recommended cutoff values for treatment are lower (SBP >185 mm Hg or DBP >110 mm Hg) in patients receiving rt-PA, because excessively high BP is associated with parenchymal hemorrhage [96, 97].

When treatment is indicated, lowering pressure should be done cautiously. Few clinical studies are available to guide clinicians [98]. Large trials assessing the effect of lowering BP on the outcome after stroke are lacking. Studies with calcium antagonists during the acute phase of ischemic stroke have shown their capacity to reduce SBP and DBP but also negative effects on stroke outcome [82, 99, 100]. Angiotensin-converting enzyme inhibitors have been shown to reduce BP without modifying cerebral blood flow, although there are no controlled clinical trials showing efficacy in stroke outcome [101]. To date, only one of the various hypotensor drugs, candesartan, has been tested in the acute phase of stroke. Administered during the first 7 days, it has been shown to be safe in these patients, and in addition has a beneficial effect on outcome and mortality at 12 months, although it had no apparent effect on BP [102].

A Cochrane Review regarding deliberate alteration of BP within 2 weeks of stroke onset found five small trials involving a total of 218 patients randomized to nimodipine, nicardipine, captopril, clonidine, glycerol trinitrate, or perindopril versus placebo or control treatment, and concluded that the limited data made it impossible to assess the relationship between BP and clinical outcome [103]. Current guidelines suggest parenteral agents such as labetalol that are easily titrated and that have minimal vasodilatory effects on cerebral blood vessels. In some cases, an intravenous infusion of sodium nitroprusside may be necessary for adequate BP control. Patients can also be treated with oral agents, such as captopril [95].

In the absence of definitive data supporting the elevation or reduction of BP in patients with acute ischemic stroke, it is clear that evidence from one or more large randomized clinical trials is now required, and it is evident that any clinical trials of vasoactive drugs in acute stroke should be paralleled by studies assessing the effect of these agents on regional cerebral perfusion [98].

Control of Arterial Oxygen Saturation

Maintaining adequate tissue oxygenation seems to be of great importance during periods of acute cerebral ischemia in order to prevent hypoxia and potential worsening of the neurological injury. Patients with acute stroke should be monitored using pulse oximetry, with a target oxygen saturation level of ≥ 95%. There is general agreement to strongly recommend supplemental oxygen to hypoxic patients (evidence of hypoxia by blood gas determination, or desaturation detected by pulse oximeter). Non-hypoxic patients with acute ischemic stroke do not need supplemental oxygen therapy [104]. There are insufficient data about the utility of hyperbaric oxygen to recommend this therapy for most patients with stroke [72, 95].

Closely related to arterial saturation is control of the volemia and viscosity of the blood. Studies investigating this issue have been discouraging. Both studies conducted by means of isovolemic hemodilution with dextran [105], and later studies of hypervolemic hemodilution by venipunction administration of pentastarch [106] were negative. In a meta-analysis of 18 control studies [107] it was demonstrated that this procedure did not significantly reduce mortality or the degree of incapacity.

Non-Pharmacological Neuroprotection: Role of Emergency Stroke Management

Non-pharmacological neuroprotection includes all interventions protecting the brain from pharmacological damage after cerebral vascular occlusion. Four physiological parameters (BP, glucose serum levels, body temperature, and oxygen saturation) are considered as independent prognostic factors in acute ischemic stroke. Although there are discrepancies and controversies concerning the real significance of some of these changes (e.g., the real

significance of hyperthermia in the acute phase of ischemic stroke, or on the behavior and prognostic importance of BP in the first hours of ischemic stroke), the current tendency is to achieve adequate control in the homeostasis of these biological parameters, independently of their pathogenic significance. As a consequence, the main treatment guidelines for acute stroke point to the advisability and need to achieve good control of these clinical parameters.

These measures should be applied at an early stage, first in the non-hospital environment and then continuing in the hospital environment within casualty departments, and later in stroke units. For this sequence to be implemented rapidly and in an organized way, there needs to be a close relationship and coordination between these three service areas. Application of the stroke code is a good example of coordination and effectiveness in immediate care for acute stroke [108]. These measures need to be protocolized, following agreed guidelines and protocols [72].

Several studies have demonstrated unequivocally the effectiveness of Stoke Units for the protocolized care of these patients. When the patient with acute stroke is cared for in a Stroke Unit, a reduction in mortality, reduced evolution and a lower hospitalization rate have all been observed [109–111]. In the meta-analysis (Stroke Trialist's Collaboration), Stroke Units were shown to be more effective than stroke teams or other forms of healthcare organization. The factors determining the benefits associated with the Stroke Unit care may be control of BP and temperature [112] and reduction of complications [113].

In a case-control study with inclusion of consecutive active stroke patients, it has been demonstrated that those who maintain physiological homeostasis showed improved outcomes at 7 days from stroke onset after being matched for other key predictors of stroke outcome [113]. Early general care, and early control of BP, glycemia, body temperature, and oxygen saturation, are the basic and best current brain-protecting measures available for all stroke patients, while the possibility of administering neuroprotective and neurorestorative drugs needs to be established. General care and homeostasis maintenance have become emergent and first-line brain-protecting treatments that must be started as soon as possible in order to save more brain tissue to obtain the best conditions for further specific stroke therapies [112].

References

1 Wahlgren NG, Ahmed N: Neuroprotection in cerebral ischaemia: facts and fancies – the need for new approaches. Cerebrovasc Dis 2004; 17(suppl 1):153–166.

2 Davalos A, Castillo J, Alvarez-Sabin J, Secades JJ, Mercadal J, Lopez S, Cobo E, Warach S, Sherman D, Clark WM, Lozano R: Oral citicoline in acute ischemic stroke: an individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.

3 Lees KR, Zivin JA, Ashwood T et al: For the SAINT-1 Study Group. Preliminary results of the SAINT-1 Trial. 14th European Stroke Conference, Bologna, May 2005.

4 The National Institutes of Neurological Disorders and Stroke rt-PA Stroke Study Group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581–1587.

5 Hacke W, Kaste M, Fieschi C, Toni D, Lesaffre E, von Kummer R, Boysen G, Bluhmki E, Höxter G, Mahagne MH, Hennerici M, for the ECASS Study Group: Intravenous thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study Group (ECASS). JAMA 1995;247:1017–1025.

6 Hacke W, Kaste M, Fieschi C, von Kummer R, Dávalos A, Meier D, Larrue V, Bluhmki E, Davis S, Donnan G, Schneider D, Díez-Tejedor E, Trouillas P: Randomised double-blind placebo-controlled trial of thrombolytic therapy with intravenous alteplase in acute ischemic stroke (ECASS II). Second European-Australasian Acute Stroke Study Investigators. Lancet 1998;352:1245–1251.

7 Stroke Unit Trialist's Collaboration: Organised inpatient (stroke unit) care for stroke. Cochrane Database Syst Review. Update Software, Issue 2. Oxford, The Cochrane Library, 2002.

8 Busto R, Dietrich WD, Globos MY, Valdes I, Schierinberg P, Ginsberg MD: Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987;7: 729–738.

9 Baron FC, Feuerstein GZ, White RF: Brain cooling during transient focal ischemia provides complete neuroprotection. Neurosci Biobehav Rev 1997;21:31–44.

10 Onesti ST, Baker CJ, Sun PP, Solomon RA: Transient hypothermia reduces focal ischemic brain injury in the rat. Neurosurgery 1991;29: 369–373.

11 Zhang Y, Wong KC, Zhang Z: The effect of intraischemic mild hypothermia on focal cerebral ischemia/reperfusion injury. Acta Anaesthesiol Sin 2001;39:65–69.

12 Colbourne F, Corbett D, Zhao Z, Yang J, Buchan AM: Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rat middle cerebral artery occlusion model. J Cereb Blood Flow Metab 2000;20:1702–1708.

13 Corbett D, Hamilton M, Colbourne F: Persistent neuroprotection with prolonged postischemic hypothermia in adult rats subjected to transient middle cerebral occlusion. Exp Neurol 2000;163:200–206.

14 Kim Y, Busto R, Dietrich WD, Kraydieh S, Ginsberg MD: Delayed postischemic hyperthermia in awake rats worsens the histopathological outcome of transient focal cerebral ischemia. Stroke 1996;27:2274–2280.

15 Cheng H, Chopp M, Welch KMA: Effect of mild hyperthermia on the ischemic infarct volume after middle cerebral artery occlusion in the rat. Neurology 1991;41:1133–1135.

16 Baena RC, Busto R, Dietrich WD, Globus MY, Ginsberg MD: Hyperthermia delayed by 24 hours aggravates neuronal damage in rats hippocampus following global ischemia. Neurology 1997;48:768–773.

17 Dietrich WD, Busto R, Valdes I, Loor Y: Effects of normothermic versus mild hyperthermic forebrain ischemia in rats. Stroke 1990;21:1318–1325.
18 Olsen TS, Weber UJ, Kammersgaard LP: Therapeutic hypothermia for acute stroke. Lancet Neurol 2003;2:410–416.
19 Huang ZG, Xue D, Preston E, Karbalai H, Buchan AM: Biphasic opening of the blood-brain barrier following transient focal ischemia: effects of hypothermia. Can J Neurol Sci 1999;26:298–304.
20 Kumura E, Yoshimine T, Takaoka M, Hayakawa T, Shiga T, Kosaka H: Hypothermia suppresses nitric oxide elevation during reperfusion after focal cerebral ischemia in rats. Neurosci Lett 1996;220:45–48.
21 Takagi K, Ginsberg MD, Globus MY, Dietrich WD, Martinez E, Kraydieh S, Busto R: Changes in amino acid neurotransmitters and cerebral blood flow in the ischemic penumbral region following middle cerebral artery occlusion in the rat: correlation with histopathology. J Cereb Blood Flow Metab 1993;13:575–585.
22 Nakashima K, Todd MM: Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. Stroke 1996;27:913–918.
23 Eguchi Y, Yamashita K, Iwamoto T, Ito H: Effects of brain temperature on calmodulin and microtubule-associated protein 2 immunoreactivity in the gerbil hippocampus following transient forebrain ischemia. J Neurotrauma 1997;14:109–118.
24 Ishikawa M, Sekizuka E, Yamaguchi SS et al: Effects of moderate hypothermia on leukocyte-endothelium interaction in the rat pial microvasculature after transient middle cerebral artery occlusion. Stroke 1999;30:1679–1686.
25 Hindfelt B: The prognostic significance of subfebrility and fever in ischemic cerebral infarction. Acta Neurol Scand 1976;53:72–79.
26 Terent A, Anderson B: The prognosis for patients with cerebrovascular stroke and transient ischemic attacks. Ups J Med Sci 1981;86:63–74.
27 Castillo J, Martinez F, Leira R, Prieto JM, Lema M, Noya M: Mortality and morbidity of acute cerebral infarction related to temperature and basal analytic parameters. Cerebrovasc Dis 1994;4:66–71.
28 Azzimondi G, Bassein L, Nonino F, Montagutiu U, Celin D, Re G, D'Alessandro R: Fever in acute stroke worsens prognosis: a prospective study. Stroke 1995;26:2040–2043.
29 Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppensen L, Olsen TS: Body temperature in acute stroke: relation to stroke severity, infarct size, mortality and outcome. Lancet 1996;347:422–425.
30 Davalos A, Castillo J, Pumar JM, Noya M: Body temperature and fibrinogen are related to early neurological deterioration in acute ischemic stroke. Cerebrovasc Dis 1997;7:64–69.
31 Castillo J, Dávalos A, Marrugat J, Noya M: Timing for fever-related brain damage in acute ischemic stroke. Stroke 1998;29:2455–2460.
32 Indredavik B, Bakke F, Slordahl SA, Roseth R, Haheim LL: Treatment in a combined acute and rehabilitation stroke unit. Which aspects are most important? Stroke 1999;30:917–923.
33 Wang Y, Lim LL, Levi C, Heller RF, Fisher J: Influence of admission body temperature on stroke mortality. Stroke 2000;31:404–409.
34 Szczudlik A, Turaj W, Slowik A, Stronjny J: Microalbuminuria and hyperthermia independently predict long-term mortality in acute ischemic stroke patients. Acta Neurol Scand 2003;107:96–101.
35 Hajat C, Hajat S, Shaarma P: Effects of poststroke pyrexia on stroke outcome: a meta-analysis of studies in patients. Stroke 2000;31:410–414.
36 Kammersgaard LP, Jorgensen HS, Rungby JA, Reith J, Nakayama H, Weber UJ, Houth J, Olsen TS: Admission body temperature predicts long-term mortality after acute stroke. Stroke 2002;33:1759–1762.
37 Boysen G, Christensen H: Stroke severity determines body temperature in acute stroke. Stroke 2001;32:413–417.
38 Dippel DWJ, van Breda EJ, van Germet HMA, van der Worp HB, Meijer RJ, Kappelle LJ, Koudstaal PJ, on behalf of the PAPAS Investigators: Effect of paracetamol (acetaminophen) on body temperature in acute ischemic stroke. A double-blind, randomized phase II clinical trial. Stroke 2001;32:1607–1612.
39 Kasner SE, Wein T, Piriyawat P, Villar-Cordova CE, Cálela JA, Krieg DW, Morgenstern LB, Kimmel SE, Grotta JC: Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. Stroke 2002;33:130–134.
40 Koennecke HC, Leistner S: Prophylactic antipyretic treatment with acetaminophen in acute ischemic stroke: a pilot study. Neurology 2001;57:2301–2303.
41 Naritomi H, Shimizu T, Oe H, Kinugawa H, Sawada T, Hirata T: Mild hypothermia is effective for the treatment of acute embolic stroke if induced within 24 hours after onset but not the later phase. J Stroke Cerebrovasc Dis 1996;6(suppl 1):193–196.
42 Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W: Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. Stroke 1998;29:2461–2466.
43 Georgiadis D, Schwarz S, Kollmar R, Schwab S: Endovascular cooling for moderate hypothermia in patients with acute stroke: first results of a novel approach. Stroke 2001;32:2550–2553.
44 Krieger DW, De Georgia MA, Abou-Chebl A, Andrefsky JC, Sila CA, Katzan IL, Mayberg MR, Furlan AJ: Cooling for acute ischemic brain damage (cool aid): an open pilot study of induced hypothermia in acute ischemic stroke. Stroke 2001;32:1847–1854.
45 Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnimo C, Mayer S: Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke 2001;32:2033–2035.
46 Kammersgaard LP, Rasmussen BH, Jorgensen HS, Reith J, Weber U, Olsen TS: Feasibility and safety of inducing modest hypothermia in awake patients with acute stroke through surface cooling, a case-control study: the Copenhagen Stroke Study. Stroke 2000;31:2251–2256.
47 Correia M, Silva M, Veloso M: Cooling therapy for acute stroke (Cochrane Review). Update Software. Oxford, The Cochrane Library, 2003.
48 Scott JF, Robinson GM, French JM, O'Connell JE, Alberti KGMM, Gray CS: Prevalence of admission hyperglycaemia across clinical subtypes of acute stroke. Lancet 1999;353:376–377.
49 Kiers L, Davis SM, Larkins R, Hopper J, Tres B, Rossiter SC, Carlin J, Ratnaike S: Stroke topography and outcome in relation to hyperglycaemia and diabetes. J Neurol Neurosurg Psychiatry 1992;5:263–270.
50 Woo J, Lam CWK, Kay R, Wong HAY, Teoh R, Nicholla MG: The influence of hyperglycaemia and diabetes mellitus on immediate and 3-month morbidity and mortality after acute stroke. Arch Neurol 1990;47:1174–1177.
51 Yip PK, He YY, Hsu CY, Garg N, Marangos P, Hogen EL: Effect of plasma glucose on infarct size in focal cerebral ischemia-reperfusion. Neurology 1991;41:899–905.
52 Prado R, Ginsberg MD, Dietrich WD, Watson BD, Busto R: Hyperglycemia increases infarct size in collaterally perfused but not end-arterial vascular territories. J Cereb Blood Flow Metab 1988;8:186–192.
53 Anderson RE, Tan WK, Martin HS, Meyers FB: Effects of glucose and PO_2 modulation on cortical intracellular acidosis, NADH redox state, and infarction in the ischemic penumbra. Stroke 1999;30:160–170.
54 Linsberg PJ, Roine RO: Hyperglycemia in acute stroke. Stroke 2004;35:363–364.
55 De Courtten-Meyers G, Myers RE, Schoolfield L: Hyperglycemia enlarges infarct size in cerebrovascular occlusion in cats. Stroke 1988;19:623–630.
56 Kawai N, Keep RF, Benz AL: Hyperglycemia and the vascular effects of cerebral ischemia. Stroke 1997;28:149–154.
57 Hamilton MG, Tranmer BI, Aver RN: Insulin reduction of cerebral infarction due to transient focal ischemia. J Neurosug 1995;82:262–268.
58 Weir JC, Murria GD, Dyker AG, Lees KR: Is hyperglycaemia an independent predictor of poor outcome after acute stroke? Results of a long-term follow-up study. BMJ 1997;314:1303–1306.
59 Jorgensen HS, Nakayama H, Raaschou HO, Olsen TS: Stroke in patients with diabetes: the Copenhagen Stroke Study. Stroke 1994;25:1977–1984.

60 Bruno A, Biller J, Adams HP, Clarke WR, Woolson RF, Williams LS, Hansen MD, for the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) Investigators. Neurology 1999; 52:280–284.

61 Capes SE, Hunt D, Malmberg K, Pathak P, Gerstein HC: Stress hyperglycemia and prognosis of stroke in nondiabetic and diabetic patients: a systematic overview. Stroke 2001;32: 2426–2432.

62 Tracey F, Stout RW: Hyperglycemia in the acute phase of stroke and stress response. Stroke 1994;25:524–525.

63 Counsell C, McDowall M, Dennis M: Hyperglycemia after acute stroke. Other models find that hyperglycemia is not independent predictor. BMJ 1997;315:810–811.

64 Christensen H, Boysen G: Blood glucose increases early after stroke onset: a study on serial measurements of blood glucose in acute stroke. Eur J Neurol 2002;9:297–301.

65 Gray CS, Hildreth AJ, Alberti GKMM, O'Connell JE, on behalf of the GIST Collaboration: Post-stroke hyperglycemia: natural history and immediate management. Stroke 2004; 35:122–126.

66 Baird TA, Parsons MW, Phanh T, Butcher KS, Desmond PM, Tress BM, Colman PG, Chambers BR, Davis SM: Persistent post-stroke hyperglycemia is independently associated with infarct expansion and worse clinical outcome. Stroke 2003;34:2208–2214.

67 Alvarez Sabín J, Molina CA, Montaner J, Arenillas JF, Huertas R, Ribo M, Codina A, Quintana M: Effects of admission hyperglycaemia on stroke outcome in reperfused tissue plasminogen activator-treated patients. Stroke 2003:34:1235–1241.

68 Kagansky N, Levy S, Knobler H: The role of hyperglycemia in acute stroke. Arch Neurol 2001;58:1209–1212.

69 Klijn CJM, Hankey GJ: Management of acute ischemic stroke: new guidelines from the American Stroke Association and European Stroke Initiative. Lancet Neurol 2003;2:698–701.

70 Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R: Intensive insulin therapy in critically ill patients. N Engl J Med 2001;345:1359–1367.

71 Scott JF, Robinson GM, French JM, O'Connell JE, Alberti KGMM, Gray CS: Glucose potassium insulin infusions in the treatment of acute stroke patients with mild to moderate hyperglycemia. The Glucose Insulin in Stroke Trial (GIST). Stroke 1999;30:793–799.

72 European Stroke Initiative (EUSI), on behalf of the European Stroke Council (ESC), The European Neurological Society (ENS), the European Federation of Neurological Societies (EFNS): Recommendations for stroke management. Cerebrovasc Dis 2000;10(suppl 3):1–34.

73 Díez Tejedor E, Fuentes B, Dávalos A, Gil Nuñez A, Vila N, Vivancos J, Egido J, Castillo J, Mostacero E: Glucose levels in acute stroke: The GLIA Study. Preliminary analysis on 250 patients. Cerebrovasc Dis 2004;17(suppl 5):2.

74 Wass CT, Lainer WL: Glucose modulation of ischemic brain injury: review and clinical recommendations. Mayo Clin Proc 1996;71:801–812.

75 Díez-Tejedor E, Fuentes B: Acute care in stroke units: the importance of early intervention to achieve better brain neuroprotection. Cerebrovasc Dis 2004;17(suppl 1):130–137.

76 Powers WJ: Acute hypertension after stroke: the scientific basis for treatment decisions. Neurology 1993;43:461–467.

77 Vemmos KN, Tsivgoulis G, Spengos K, Zakopoulos N, Synetos A: U-shaped relationship between mortality and admission blood pressure in patients with acute stroke. J Intern Med 2004;255:257–265.

78 Leonardi-Bee J, Bath PM, Philips SJ, Sandercock PA, IST Collaborative Group: Blood pressure and clinical outcomes in the International Stroke Trial. Stroke 2002;33;1315–1320.

79 Castillo J, Leira R, García MM, Serena J, Blanco M, Dávalos A: Blood pressure decrease during the acute phase of ischemic stroke is associated with brain injury and poor stroke outcome. Stroke 2004;35:520–527.

80 Oliveira-Filho J, Silva SCS, Trabuco CC, Pedreira BB, Sousa EU, Bacellar A: Detrimental effect of blood pressure reduction in the first 24 hours of acute stroke onset. Neurology 2003;61:1047–1051.

81 Grossman E, Messerli FH, Grodzicki T, Kowey P: Should a moratorium be placed on sublingual nifedipine capsules given for hypertensive emergencies and pseudoemergencies? JAMA 1996;276:1328–1331.

82 Ahmed N, Näsman P, Wahgren NG: Effect of intravenous nimodipine on blood pressure and outcome after acute stroke. Stroke 2000;131: 1250–1255.

83 Carlberg B, Asplund K, Hägg E: The prognostic value of admission blood pressure in patients with acute stroke. Stroke 1993;24:1372–1375.

84 Robinson TG, Dawson SL, Ahmed U, Manktelow B, Fotherby MD, Potter JF: Twenty-four hour systolic blood pressure predicts long-term mortality following acute stroke. J Hypertens 2001;19:2127–2134.

85 Lawes CM, Bennett DA, Feigin VL, Rodgers A: Blood pressure and stroke: a overview of published reviews. Stroke 2004;35:776–785.

86 Willmot M, Leonardo-Bee J, Bath PMW: High blood pressure in acute stroke and subsequent outcome. A systematic review. Hypertension 2004;43:18–24.

87 Jorgensen HS, Nakayama H, Christensen HR, Raaschou HO, Kampmann JP, Olsen TS: Blood pressure in acute stroke. The Copenhagen Stroke Study. Cerebrovasc Dis 2002;13: 204–209.

88 Chamorro A, Vila N, Ascaso C, Elices E, Schonewille W, Blanc R: Blood pressure and functional recovery in acute ischemic stroke. Stroke 1998;29:1850–1853.

89 Allen CMC: Predicting the outcome of acute stroke: a prognosis score. J Neurol Neurosurg Psychiatry 1984;47:475–480.

90 Lindenauer PK, Mathew MC, Ntuli TS, Pekow PS, Fitzgerald J, Benjamin EM: Use of antihypertensive agents in the management of patients with acute stroke. Neurology 2004;63:318–323.

91 Yatsu FM, Zivin J: Hypertension in acute ischemic stroke: not to treat. Arch Neurol 1985;42:999–1000.

92 Spence JD, Del Maestro RF: Hypertension in acute ischemic stroke: treat. Arch Neurol 1985;42:1000–1002.

93 Broderick J, Brott T, Barsan W, Haley EC, Levy D, Marler J, Sheppard G, Blum C: Blood pressure during the first minutes of focal cerebral ischemia. Ann Emerg Med 1993; 22:1438–1443.

94 Mattle HP, Kappeler L, Arnold M, Fisher V, Nedeltchev K, Remonda L, Jakob SM, Schroth G: Blood pressure and vessel recanalization in the first hours after ischemic stroke. Stroke 2005;36:264–269.

95 Adams HP Jr, Adams RJ, Brott TG, del Zoppo GJ, Furlan A, Goldstein LB, Grubb RL, Higashida R, Kidwell C, Kwiatkowski TG, Marler JR, Hademenos GJ; for the Stroke Council of the American Stroke Association: Guidelines for the early management of patients with ischemic stroke: a scientific statement from the Stroke Council of the American Stroke Association. Stroke 2003;34: 1056–1083.

96 The NINDS t-PA Stroke Study Group: Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. Stroke 1997;28: 2109–2118.

97 Larrue V, von Kummer R, del Zoppo G, Bluhmki E: Hemorrhagic transformation in acute ischemic stroke: potential contributing factors in the European Cooperative Acute Stroke Study. Stroke 1997;28:957–960.

98 Bath P: High blood pressure as risk factor and prognostic predictor in acute ischaemic stroke: when and how to treat it? Cerebrovasc Dis 2004;17(suppl 1):51–57.

99 Wahlgren NG, MacMahon DG, De Keyser J, Indredavik B, Ryman T for the INWest Study Group: Intravenous Nimodipine West European Stroke Trial (INWEST) of nimodipine in the treatment of acute ischemic stroke. Cerebrovasc Dis 1994;4:204–210.

100 Kaste M, Fogelholm R, Erilä T, Palomäki H, Murros K, Rissanen A, Sarna S: A randomized, double-blind, placebo-controlled trial of nimodipine in acute ischemic hemispheric stroke. Stroke 1994;25:1348–1353.

101 Dyker AG, Grosset DG, Lees K: Perindopril reduces blood pressure but not cerebral blood flow in patients with recent cerebral ischemic stroke. Stroke 1997;28:580–583.

102 Schrader J, Lüders S, Kulschewski A, Berger J, Zidek W, Treib J, Einhäupl K, Diener HC, Dominiak P, on behalf of the ACCESS Study Group: The ACCESS Study. Evaluation of acute candesartan cilexetil therapy in stroke survivors. Stroke 2003;34:1699–1703.

103 Blood Pressure in Acute Stroke Collaboration (BASC): Intervention for deliberately altering blood pressure in acute stroke. Update Software, Issue 3. Oxford, The Cochrane Library, 2003.
104 Ronning OM, Guldvog B: Should stroke victims routinely receive supplemental oxygen? A quasi-randomized controlled trial. Stroke 1999;29:779–784.
105 Strandt T: Evaluation of long-term outcome and safety after hemodilution therapy in acute ischemic stroke. Stroke 1992;23:657–662.
106 Hemodilution in Stroke Study Group: Hypervolemic hemodilution in acute stroke: a randomized multicenter trial using pentastarch. Stroke 1989;20:317–323.
107 Asplund K: Hemodilution for acute ischemic stroke. Cochrane Database Syst Rev 2002;4:CD000103.
108 Alvarez-Sabin J, Molina C, Montaner J, Arenillas J, Pujadas F, Huertas R, Mouriño J, Rubio M, Santamaria E, Quintana M: Clinical benefit following the implementation of a specialized urgent care system. Med Clin (Barc) 2004;122:528–531.
109 Indredavik B, Bakke F, Solberg R, Rokseth R, Haheim LL, Holme I: Benefit of a stroke unit: a randomized controlled trial. Stroke 1991;22:1026–1031.
110 Langhorne P, Williams BO, Gilchirst W, Howie K: Do stroke units save lives? Lancet 1993;342:395–398.
111 Stroke Unit Trialist's Collaboration: How do stroke units improve patient outcomes? Stroke 1007;28:2139–2144.
112 Diez-Tejedor E, Fuentes B: Acute care in stroke: do stroke units make the difference? Cerebrovasc Dis 2001(suppl 1):31–39.
113 Langhorne P, Tong BL, Stott DJ: Association between physiological homeostasis and early recovery after stroke. Stroke 2000;31:2518–2519.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):99–105
DOI: 10.1159/000091709

Published online: May 2, 2006

Neuroprotection in Malignant MCA Infarction

Pilar Delgado[a] Juan Sahuquillo[b] M. Antonia Poca[b] José Álvarez-Sabin[a]

Departments of [a]Neurology and [b]Neurosurgery, Vall d'Hebron Hospital, Barcelona, Spain

Key Words
MCA infarction · Hypothermia · Decompressive hemicraniectomy · Stroke

Abstract
Massive unilateral hemispheric infarction often develops progressive postischemic edema that leads to a malignant course of stroke with mortality of up to 80% with conventional medical therapies. Hypothermia and decompressive hemicraniectomy have shown neuroprotective effects in several animal models of focal transient and permanent MCA occlusion by reducing infarct size and improving neurological outcome. Our aim in this paper was to review the possible mechanisms of both therapies as well as the optimal time window and duration of application of each treatment in animal model and in human malignant MCA infarction reported in the literature.

Background

Diagnosis of malignant MCA infarction can be made in patients with a massive hemispheric unilateral infarction that clinically courses with a severe hemispheric syndrome with forced head and eye deviation, usually showing a rapid neurological deterioration within the first 2–3 days after onset. The main cause for death in these patients is the development of severe brain postischemic edema, with progressive brain herniation and raised intracranial pressure (ICP). Conventional conservative management (osmotherapy, diuretics, etc.) and even more aggressive medical therapies such as controlled ventilation, or high-dose barbiturates have shown poor results with a mortality rate of up to 80% [1]. Induced moderate hypothermia (32–33°C) and decompressive hemicraniectomy have been proposed as second-tier therapies in other neurological disorders with elevation of ICP such as traumatic brain injury (TBI) [2]. In the last few years, experimental models have shown the efficacy of these therapies in both reducing the infarct size and improving neurological outcome in focal cerebral ischemia [3–10].

A review of the neuroprotective mechanisms of moderate hypothermia and hemicraniectomy in animal models and their application in patients with a malignant middle cerebral artery infarction is the aim of this article.

Induced Hypothermia: Basic Concepts

There is much confusion regarding the use of proper terminology in thermoregulation and hypothermia clinical research. A good resource for the clinician is the glossary of terms for thermal physiology periodically revised by the Commission for Thermal Physiology of the International Union of Physiological Sciences (IUPS) [11]. According to this Commission, hypothermia is defined as 'the condition of a temperature regulator when core temperature is below its range specified for the normal active state of the species' [11]. Induced or deliberate hypothermia is defined by the same group as 'the state of hypothermia produced purposefully by increasing heat loss from the body and/or inactivation of heat conservation

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0099$23.50/0

Accessible online at:
www.karger.com/ced

Pilar Delgado Martínez
Departments of Neurology and Neurosurgery
Hospital Universitari Vall d'Hebron, Universidad Autonoma de Barcelona
Passeig Vall d'Hebron 119-129, ES–08035 Barcelona (Spain)
Tel./Fax +34 93 489 4257, E-Mail 35070pdm@comb.es

and heat production by physical and/or pharmacological means' [11]. Clinically induced or regulated hypothermia is defined by Bernard and Buist [12] as the controlled lowering of core temperature for therapeutic reasons.

Because deep hypothermia causes life-threatening arrhythmias, ventricular fibrillation, and cardiac arrest, core temperatures below 28°C can only be safely achieved by using cardiopulmonary bypass [13]. Mild-to-moderate induced hypothermia (32–34°C) has been used to achieve neuroprotection in many neurological insults such as anoxic neurological injury after cardiac arrest, severe TBI, major stroke and hepatic encephalopathy, among others. For a comprehensive review of the uses of hypothermia, the reader is referred to Bernard and Buist's review [13].

The common use of the same terminology to describe different types of hypothermia has misled clinicians. The terms mild and moderate have been used interchangeably in many papers to refer to different target core temperatures. Although agreement on terminology is lacking, it is generally accepted that, depending on the core temperature, hypothermia is classified as mild (33–36°C), moderate (28–32°C), deep (10–28°C), profound (5–10°C), and ultraprofound (0–5°C) [13]. Despite this widely used classification, the use of term 'moderate hypothermia' varies widely in neurocritical care [14] and, surprisingly, in this classification, the 32–33°C range, which is the most widely used range in neuro-ICUs, has simply been forgotten. For the sake of consistency, we will consider the usual lower core temperature used in the management of stroke and head injury (32–33°C) as moderate hypothermia.

Hypothermia is Neuroprotective in Animal Models

Hypothermia is the oldest form of brain protection. Although the neuroprotective effect of deep hypothermia has been known since the early 1950s, the fact that neuroprotection can be achieved by small changes in temperature has only been known since 1987 when Busto et al. [15] reported this finding in experimental models of brain ischemia.

There is established evidence that temperature influences stroke outcome. Different studies have shown that admission core temperature is a good predictor for both short- and long-term mortality, independently of other clinical variables associated to stroke severity, such as initial NIHSS score [16, 17]. Moderate increases in core temperature within the first 24 h are associated with greater cerebral damage [18]. Although controlled normothermia is the only accepted therapy in stroke patients [19], there is growing experimental evidence which suggests that hypothermia has a place as a neuroprotective therapy in patients with stroke and especially in those with a malignant course.

In animal models of focal ischemia, induced hyperthermia increases the severity of cerebral injury [15]. Larger lesions are provoked when hyperthermia coincides with the onset of cerebral ischemia. Several models of global and focal ischemia in animals have demonstrated the ability of hypothermia both to reduce infarct size and improve neurological outcome [20].

However, overenthusiasm with experimental results has to be tempered to avoid disappointment such as that which occurred in TBI. Enthusiasm aroused by experimental studies and encouraging results from early clinical trials reported in 1990s faded when the multicenter phase III American trial (NABISH-I) showed no significant effect of hypothermia in outcome in severe TBI [21].

Hypothermia is still the most powerful neuroprotective method in animal models of TBI and stroke because it affects a wide range of the pathophysiological processes involved. Rather that focusing on blocking a specific cascade, hypothermia has the advantage of acting at several points on the deleterious pathophysiological events triggered by TBI and stroke. Deep hypothermia unquestionably protects brain against global ischemia, but its application has been extremely limited by its side effects and it can only be used in situations that require cardiac arrest [20]. On the other hand, mild and moderate hypothermia are better tolerated and have been assayed in models of transient and permanent MCA occlusion with variable, but in general good results. Some authors [7, 8] found benefits with hypothermia only under conditions of transient (not permanent) MCA occlusion in rat. Yanamoto et al. [10] extended these results to models of permanent focal ischemia.

The optimal time window to induce hypothermia and its duration is still a matter of debate. Maier et al. [6] suggested that mild hypothermia should be maintained from the start and last at least 1–2 h to obtain optimal neuroprotection in transient focal ischemia. However, it has been shown that induced hypothermia delayed 3 h after MCA occlusion, also has neuroprotective effects, as shown in an MRI study in rats [22]. All the available data suggests a potential benefit of extending hypothermia into the late reperfusion period (postischemic hypothermia) [4, 9]. In addition, when hypothermia is induced in the postischemic period, it should be maintained for a long time [3]. If we were to extrapolate these data to clinical

practice, hypothermia should be induced early after stroke onset and may be maintained until the end of the reperfussion period.

Neuroprotective Mechanisms of Hypothermia

In most experimental studies, the effects of hypothermia are evaluated early because animals are sacrificed only after 24 h from the onset of focal ischemia. The question that arises from this approach is whether hypothermia only delays injury caused by ischemia or, to the contrary, whether its effects are maintained later on. Regarding this issue, some authors [10] have already shown that the beneficial effects of prolonged mild hypothermia remain at 21 and 30 days after the onset of ischemic insult.

Some of the suggested mechanisms reported in the literature include induction of ischemic tolerance [23], reduction the expression of early genes including c-fos [24], reduction in cerebral metabolic rate [25], reduction of neurotransmitter release during ischemia, including glutamate [26, 27], prevention of cell death by apoptosis and necrosis [6, 28], decrease of inflammatory response [29], reduction of matrix metalloproteinases activity [30] and stabilization of the blood-brain barrier [31]. However, the most important mechanisms that cause the strong neuroprotection provided by induced hypothermia are still a matter of debate and are probably multiple. In the words of Corbett and Thornhill [32]

> ... it is not possible to point to a single mechanism that underlies the robust neuroprotection provided by long duration postischemic hypothermia. Indeed, the remarkable benefit provided by mild hypothermia is likely due to a multitude of actions which make it the ultimate neuroprotective cocktail. This contrasts with neuroprotective drug therapies that typically target a single mechanism and that so far have proven ineffective in clinical trials.

Moderate Hypothermia in Malignant MCA Infarction

The first approach on moderate hypothermia in the treatment of patients with malignant middle cerebral artery infarction was reported by Schwab et al. [33]. Moderate hypothermia was induced in 25 patients within 14 ± 7 h after stroke onset by surface cooling (cooling blankets, cold infusions and cold washing). Nearly half of the patients (56%) survived with improved neurological outcome at 3 months. An important finding of this study was that in most of the patients who died, death occurred by cerebral herniation or secondary rise in ICP during rewarming.

Clinical data gathered from 50 consecutive patients from four neurocritical care units that use the same approach has been reported [34], confirming the previous results. Hypothermia was associated with considerable but reversible adverse effects such as arrhythmia or bradycardia, coagulopathy, hypotension and pneumonia. Rewarming constituted the critical phase of hypothermia therapy because of a constant secondary rise in ICP. A significantly lower mortality was associated with longer rewarming periods.

Alternative techniques for hypothermia induction have been investigated by Georgiadis et al. [35] evaluating the feasibility of inducing and maintaining moderate hypothermia using endovascular cooling devices. Six patients were included and only 1 died as a consequence of uncontrolled ICP. The target temperature was obtained in only 3.5–6.5 h.

In July 2004, De Georgia et al. [36] reported the results of a pilot randomized clinical feasibility trial of endovascular cooling (COOL AID). It included 40 patients (18 were randomized to hypothermia and 22 received conventional medical treatment). Given the small sample size, the difference was not statistically significant, however older patients with severe stroke and comorbidity developed pulmonary complications [36].

Hypothermia in MMCA infarction has also been assayed in patients with absent or minimal response to thrombolytic therapy [37]. Moderate hypothermia using surface cooling was achieved in 6–12 h time window and maintained from 12 to 72 h depending on when the vessel was recanalized. There were no hypothermia-related complications, such as coagulopathy or thrombopenia that might have caused hemorrhagic complications after thrombolysis.

Prediction of Malignant MCA Infarction

Hypothermia has been shown to be more effective as a neuroprotective measure if applied earlier in the ischemic process. However, early identification of patients at risk of developing a malignant course of cerebral infarction is not an easy task. A combination of initial clinical, laboratory or neuroimaging (CT or MRI) parameters might be used as selection criteria. A baseline NIHSS score >20 for left or >15 for right stroke hemisphere within 6 h from symptom onset, and also nausea/vomiting, have been described as good clinical predictors [38].

Among the radiological features, briefly, at early (<6 h) hypodensity at least 50% of the MCA territory has a 94% specificity for fatal outcome, with only 61% of sensitivity [39]. When it appeared later (within the first 48 h) the midline shift measured at the septum was ≥5 mm and pineal shift was ≥2 mm. Hydrocephalus, temporal lobe infarction and the involvement of additional vascular territories apart from the MCA have been associated with a poor outcome [40]. CT signs are highly specific for fatal outcome but with the limitation that their sensitivity is lower than other neuroimaging techniques such as multiparametric MRI. When MRI is performed early after stroke onset, it can accurately predict a malignant course of an acute MCA infarction as Oppenheim et al. [41] showed. MRI also yielded a good prediction even in the first 6 h [42, 43].

It has also recently been reported that laboratory data such as molecular markers of endothelial damage may be useful in predicting a malignant infarction [44].

Decompressive Hemicraniectomy

In 1905, Cushing [45] reported for the first time a detailed procedure of subtemporal and suboccipital decompression to relieve high ICP in tumoral patients with brain herniation. Usually, it was performed in TBI by removal of different areas and amounts of the skull, with or without opening the meningeal covering or augmentative duraplasty. These procedures were mainly used in the late 1960s to manage patients with high ICP or as a primary measure in the evacuation of acute subdural hematoma when the surgeon felt that the brain was tight and edematous [46]. In massive stroke, these procedures primarily allow the brain to expand and consequently provide the control of high ICP.

Decompressive Hemicraniectomy in Animal Models

Laboratory studies of hemicraniectomy for MCA occlusion in rats have shown a decrease in mortality rate, a reduction in infarct volume and also an improvement of neurological outcome, especially when the surgery was performed as soon as possible after MCA occlusion [47, 48]. In the study of Forsting et al. [48], mortality was reduced from 36% in the control group to 0% in the treated animals.

However, in clinical practice, the main problem remains to determine how long conservative treatment should be maintained before considering decompressive craniectomy. This issue has not been clarified by experimental studies. Decompressive craniectomy was effective in terms of neurological outcome whenever it was performed (1 or 24 h after vessel occlusion), but when performed again later, cortical infarct size was larger [48]. Another study, addressed to investigate the effect of reperfusion, craniectomy or the combination of both treatments, showed that even late craniectomy (4 and 12 h after occlusion) resulted in significant benefit [49]. But, on the contrary, Hofmeijer et al. [50] found no benefit in terms of infarct size histologically measured when the surgery was delayed (17 h after occlusion).

In conclusion, experimental models suggest that early craniectomy probably is associated with better results in terms of neurological outcome and infarct size.

Neuroprotective Mechanisms of Decompressive Hemicraniectomy

Brain herniations with brainstem compression and increased ICP are the most frequent causes of death and disability after massive stroke. The rationale for decompressive surgery is based on the Monro-Kellie law. According to this theory, intracranial volume should remain constant, and volumetric compensations should be achieved by shifts in CSF, in cerebral blood volume or brain herniations. Removing a variable amount of bone, with or without leaving the duramater opened or augmented by a duraplasty, is a fast and effective way for increasing intracranial volume, reducing high ICP, increasing the compliance of the intracranial space and finally, for avoiding brain herniation and brainstem compression. However, there is also some experimental evidence which shows that decompressive may be neuroprotective thanks to hemodynamic effects that consisted in an improvement of brain perfusion pressure through leptomeningeal collaterals [51, 52].

Decompressive Hemicraniectomy in Malignant MCA Infarction

Decompressive hemicraniectomy was first performed in the management of large cerebral infarction several decades ago, with many isolated cases reported in the literature. However, the first large non-randomized study

in which decompressive craniectomy was compared to controls was published in 1995 by the Heidelberg group. These authors reported that the surgically treated patients showed a significantly lower mortality rate than those who were in the control group (34 vs. 80%) [53].

The optimal time for surgery has not been defined yet. However, the mortality rate in surgically treated patients might be reduced up to 20% when decompressive craniectomy is performed earlier [54]. It must be stressed that the control group in this study was a historical group which contained patients who had been unsuitable for surgery due to comorbidity or failure in obtaining informed consent. This selection of patients introduced significant bias that was not useful to extract conclusions from this study.

The clinical factors associated with better outcome after decompressive craniectomy were investigated in a systematic review of all the cases reported in English literature from 1970 to 2004 [55]. Age was found as a crucial and independent factor in predicting not only mortality but also functional outcome. In this study, only a 36% of patients <50 years were severely disabled or dead after 4 months. But these rates increased up to 80% in older patients (≥50 years). Interestingly, the timing of surgery, the presence of signs of herniation before surgery, the hemisphere affected or the involvement of other vascular territories did not significantly affect the outcome. This age cut-off has been confirmed in other reports [56].

Moreover, decompressive craniectomy is not exempt of complications, even when conducted by an experienced group of neurosurgeons. Wagner et al. [57] described their experience with 60 patients. In their series, surgical complications such as the appearance of hemorrhages (parenchymal, subdural or epidural/subgaleal) and ischemic lesions reached 41 and 28% respectively. The occurrence of hemicraniectomy-associated bleeding was more frequently found in smaller hemicraniectomies and this complication was significantly related to an increased mortality risk. Not only the size, but other technical problems, such as the shape of edges (sharp bone defects edges associated more complications) influenced the hemicraniectomy efficacy.

Apart from mortality and functional outcome, the quality of life is a major feature that should be evaluated in the survivors of this procedure. Almost all of Walt et al.'s [58] patients achieved a good quality of life and unexpectedly, no significant differences were found between left and right hemispheric infarction. Improvement in speech function was also found, with global complete aphasia being relatively rare. However, further studies are needed to confirm these results [59].

In conclusion, there is no evidence from randomized controlled trials supporting the use of decompressive surgery in malignant MCA infarction to date [60] and no results are available in adults to confirm or refute the effectiveness of decompressive craniectomy. However, some prospective, single-center, non-randomized studies suggest that good outcomes might be expected in selected groups of patients by using this technique. The results from ongoing trials such as HAMLET (Hemicraniectomy After MCA Infarction with Life-Threatening Edema Trial) will help us in the future.

Moderate Hypothermia versus Decompressive Hemicraniectomy

Craniectomy and moderate hypothermia have been compared in a study reported by Georgiadis et al. [61]. They conducted a quasi-randomized study, in which decompressive craniectomy was performed when the non-dominant hemisphere was affected (n = 17); moderate hypothermia was used for the dominant hemisphere (n = 19). The timing of mechanical ventilation and the stay in the intensive care unit was not significantly different between both groups, although mortality in the moderate hypothermia group was higher (47%) than in the decompressive craniectomy group (12%). Most patients in the hypothermia group died as a result of rebound high ICP increase during rewarming. This study did not compare the functional outcome between both groups.

Combining Mild Hypothermia and Hemicraniectomy in Animal Models

Due to the multiple neuroprotective effects of hypothermia, it has been suggested that moderate hypothermia could increase the therapeutic benefit of other treatments such as reperfussion or craniectomy. It could also be possible that hypothermia expanded the time window for the application of other therapies by delaying brain damage caused by cerebral ischemia. In this direction, Doerfler et al. [62] designed a study to evaluate the effect of hypothermia and craniectomy separately and also when both were combined in an animal model. They found that early decompressive craniectomy significantly reduced infarct size and improved neurological outcome but hypothermia only delayed the evolution of infarction

without reducing the infarct. In addition, the highest benefit was obtained when combining both treatments.

The CoolStroke trial is an ongoing study that it is currently being performed in our institution, which combines both treatments in malignant MCA infarction. Briefly, moderate hypothermia is early induced (within the first 24 h) in patients <65 years at high risk for developing a malignant MCA infarction. All patients receive a multimodal monitoring, which includes seriated brain CT scan. Elective hemicraniectomy (fronto-temporo-parietal craniectomy with duroplasty) is performed when patients present intracranial hypertension or the midline shift increases to 5 mm or more in the following hours/days.

Conclusions

In experimental models, it has been shown that hypothermia and decompressive craniectomy improve neurological outcome in severe acute stroke. Both therapies achieve better results when applied earlier. Hemicraniectomy seems to be more effective than hypothermia in reducing mortality. The combination of both therapies may add some benefits and it is worthwhile exploring these hypotheses in pilot studies. However, although the application of these therapies in human stroke seems to offer hopeful results, the selection criteria of patients, the time window and the duration of each therapy need to be clarified in clinical trials before such therapies can be routinely recommended.

References

1 Hacke W, Schwab S, Horn M, Spranger M, De Georgia M, von Kummer R: 'Malignant' middle cerebral artery territory infarction: clinical course and prognostic signs. Arch Neurol 1996;53:309–315.

2 Marion DW: Moderate hypothermia in severe head injuries: the present and the future. Curr Opin Crit Care 2002;8:111–114.

3 Colbourne F, Corbett D, Zhao Z, Yang J, Buchan AM: Prolonged but delayed postischemic hypothermia: a long-term outcome study in the rat middle cerebral artery occlusion model. J Cereb Blood Flow Metab 2000;20:1702–1708.

4 Kawai N, Okauchi M, Morisaki K, Nagao S: Effects of delayed intraischemic and postischemic hypothermia on a focal model of transient cerebral ischemia in rats. Stroke 2000;31:1982–1989.

5 Kollmar R, Henninger N, Bardutzky J, Schellinger PD, Schabitz WR, Schwab S: Combination therapy of moderate hypothermia and thrombolysis in experimental thromboembolic stroke – an MRI study. Exp Neurol 2004;190:204–212.

6 Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK: Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. Stroke 1998;29:2171–2180.

7 Morikawa E, Ginsberg MD, Dietrich WD, Duncan RC, Kraydieh S, Globus MY, Busto R: The significance of brain temperature in focal cerebral ischemia: histopathological consequences of middle cerebral artery occlusion in the rat. J Cereb Blood Flow Metab 1992;12:380–389.

8 Ridenour TR, Warner DS, Todd MM, McAllister AC: Mild hypothermia reduces infarct size resulting from temporary but not permanent focal ischemia in rats. Stroke 1992;23:733–738.

9 Yanamoto H, Nagata I, Nakahara I, Tohnai N, Zhang Z, Kikuchi H: Combination of intraischemic and postischemic hypothermia provides potent and persistent neuroprotection against temporary focal ischemia in rats. Stroke 1999;30:2720–2726.

10 Yanamoto H, Nagata I, Niitsu Y, Zhang Z, Xue JH, Sakai N, Kikuchi H: Prolonged mild hypothermia therapy protects the brain against permanent focal ischemia. Stroke 2001;32:232–239.

11 Commission for Thermal Physiology of the International Union of Physiological Sciences: Glossary of Terms for Thermal Physiology, ed 3. IUPS Thermal Commission, 2001, vol 51, pp 245–280.

12 Bernard SA, Buist M: Induced hypothermia in critical care medicine: a review. Crit Care Med 2003;31:2041–2051.

13 Tisherman SA, Rodriguez A, Safar P: Therapeutic hypothermia in traumatology. Surg Clin North Am 1999;79:1269–1289.

14 Inamasu J, Ichikizaki K: Mild hypothermia in neurologic emergency: an update. Ann Emerg Med 2002;40:220–230.

15 Busto R, Dietrich WD, Globus MY, Valdes I, Scheinberg P, Ginsberg MD: Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cereb Blood Flow Metab 1987;7:729–738.

16 Wang Y, Lim LL, Levi C, Heller RF, Fisher J: Influence of admission body temperature on stroke mortality. Stroke 2000;31:404–409.

17 Reith J, Jorgensen HS, Pedersen PM, Nakayama H, Raaschou HO, Jeppesen LL, Olsen TS: Body temperature in acute stroke: relation to stroke severity, infarct size, mortality, and outcome. Lancet 1996;347:422–425.

18 Castillo J, Davalos A, Marrugat J, Noya M: Timing for fever-related brain damage in acute ischemic stroke. Stroke 1998;29:2455–2460.

19 Marion DW: Controlled normothermia in neurologic intensive care. Crit Care Med 2004;32:S43–S45.

20 Sutton LN, Clark BJ, Norwood CR, Woodford EJ, Welsh FA: Global cerebral ischemia in piglets under conditions of mild and deep hypothermia. Stroke 1991;22:1567–1573.

21 Clifton GL, Miller ER, Choi SC, Levin HS, McCauley S, Smith KR Jr, Muizelaar JP, Wagner FC Jr, Marion DW, Luerssen TG, Chesnut RM, Schwartz M: Lack of effect of induction of hypothermia after acute brain injury. N Engl J Med 2001;344:556–563.

22 Kollmar R, Schabitz WR, Heiland S, Georgiadis D, Schellinger PD, Bardutzky J, Schwab S: Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia: an MRI study. Stroke 2002;33:1899–1904.

23 Nishio S, Yunoki M, Chen ZF, Anzivino MJ, Lee KS: Ischemic tolerance in the rat neocortex following hypothermic preconditioning. J Neurosurg 2000;93:845–851.

24 Mancuso A, Derugin N, Hara K, Sharp FR, Weinstein PR: Mild hypothermia decreases the incidence of transient ADC reduction detected with diffusion MRI and expression of c-fos and hsp70 mRNA during acute focal ischemia in rats. Brain Res 2000;887:34–45.

25 Yager JY, Asselin J: Effect of mild hypothermia on cerebral energy metabolism during the evolution of hypoxic-ischemic brain damage in the immature rat. Stroke 1996;27:919–925.

26 Nakashima K, Todd MM: Effects of hypothermia on the rate of excitatory amino acid release after ischemic depolarization. Stroke 1996;27:913–918.
27 Huang FP, Zhou LF, Yang GY: Effects of mild hypothermia on the release of regional glutamate and glycine during extended transient focal cerebral ischemia in rats. Neurochem Res 1998;23:991–996.
28 Xu L, Yenari MA, Steinberg GK, Giffard RG: Mild hypothermia reduces apoptosis of mouse neurons in vitro early in the cascade. J Cereb Blood Flow Metab 2002;22:21–28.
29 Deng H, Han HS, Cheng D, Sun GH, Yenari MA: Mild hypothermia inhibits inflammation after experimental stroke and brain inflammation. Stroke 2003;34:2495–2501.
30 Wagner S, Nagel S, Kluge B, Schwab S, Heiland S, Koziol J, Gardner H, Hacke W: Topographically graded postischemic presence of metalloproteinases is inhibited by hypothermia. Brain Res 2003;984:63–75.
31 Karibe H, Zarow GJ, Graham SH, Weinstein PR: Mild intraischemic hypothermia reduces postischemic hyperperfusion, delayed postischemic hypoperfusion, blood-brain barrier disruption, brain edema, and neuronal damage volume after temporary focal cerebral ischemia in rats. J Cereb Blood Flow Metab 1994;14:620–627.
32 Thornhill J, Corbett D: Therapeutic implications of hypothermic and hyperthermic temperature conditions in stroke patients. Can J Physiol Pharmacol 2001;79:254–261.
33 Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W: Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. Stroke 1998;29:2461–2466.
34 Schwab S, Georgiadis D, Berrouschot J, Schellinger PD, Graffagnino C, Mayer SA: Feasibility and safety of moderate hypothermia after massive hemispheric infarction. Stroke 2001;32:2033–2035.
35 Georgiadis D, Schwarz S, Kollmar R, Schwab S: Endovascular cooling for moderate hypothermia in patients with acute stroke: first results of a novel approach. Stroke 2001;32:2550–2553.
36 De Georgia MA, Krieger DW, Abou-Chebl A, Devlin TG, Jauss M, Davis SM, Koroshetz WJ, Rordorf G, Warach S: Cooling for acute ischemic brain damage (COOL AID): a feasibility trial of endovascular cooling. Neurology 2004;63:312–317.
37 Krieger DW, De Georgia MA, Abou-Chebl A, Andrefsky JC, Sila CA, Katzan IL, Mayberg MR, Furlan AJ: Cooling for acute ischemic brain damage (COOL AID): an open pilot study of induced hypothermia in acute ischemic stroke. Stroke 2001;32:1847–1854.
38 Krieger DW, Demchuk AM, Kasner SE, Jauss M, Hantson L: Early clinical and radiological predictors of fatal brain swelling in ischemic stroke. Stroke 1999;30:287–292.
39 Von Kummer R, Meyding-Lamade U, Forsting M, Rosin L, Rieke K, Hacke W, Sartor K: Sensitivity and prognostic value of early CT in occlusion of the middle cerebral artery trunk. AJNR Am J Neuroradiol 1994;15:9–15.
40 Barber PA, Demchuk AM, Zhang J, Kasner SE, Hill MD, Berrouschot J, Schmutzhard E, Harms L, Verro P, Krieger D: Computed tomographic parameters predicting fatal outcome in large middle cerebral artery infarction. Cerebrovasc Dis 2003;16:230–235.
41 Oppenheim C, Samson Y, Manai R, Lalam T, Vandamme X, Crozier S, Srour A, Cornu P, Dormont D, Rancurel G, Marsault C: Prediction of malignant middle cerebral artery infarction by diffusion-weighted imaging. Stroke 2000;31:2175–2181.
42 Arenillas JF, Rovira A, Molina CA, Grive E, Montaner J, Alvarez-Sabin J: Prediction of early neurological deterioration using diffusion- and perfusion-weighted imaging in hyperacute middle cerebral artery ischemic stroke. Stroke 2002;33:2197–2203.
43 Thomalla GJ, Kucinski T, Schoder V, Fiehler J, Knab R, Zeumer H, Weiller C, Rother J: Prediction of malignant middle cerebral artery infarction by early perfusion- and diffusion-weighted magnetic resonance imaging. Stroke 2003;34:1892–1899.
44 Serena J, Blanco M, Castellanos M, Silva Y, Vivancos J, Moro MA, Leira R, Lizasoain I, Castillo J, Davalos A: The prediction of malignant cerebral infarction by molecular brain barrier disruption markers. Stroke 2005;36:1921–1926.
45 Cushing H: The establishment of cerebral hernia as a decompressive measure for inaccessible brain tumor: With the description of intramuscular methods of making the bone defect in temporal and occipital regions. Surg Gynecol Obstet 1905;1:297–314.
46 Britt RH, Hamilton RD: Large decompressive craniotomy in the treatment of acute subdural hematoma. Neurosurgery 1978;2:195–200.
47 Doerfler A, Forsting M, Reith W, Staff C, Heiland S, Schabitz WR, von Kummer R, Hacke W, Sartor K: Decompressive craniectomy in a rat model of 'malignant' cerebral hemispheric stroke: experimental support for an aggressive therapeutic approach. J Neurosurg 1996;85:853–859.
48 Forsting M, Reith W, Schabitz WR, Heiland S, von Kummer R, Hacke W, Sartor K: Decompressive craniectomy for cerebral infarction. An experimental study in rats. Stroke 1995;26:259–264.
49 Engelhorn T, von Kummer R, Reith W, Forsting M, Doerfler A: What is effective in malignant middle cerebral artery infarction: reperfusion, craniectomy, or both? An experimental study in rats. Stroke 2002;33:617–622.
50 Hofmeijer J, Schepers J, Veldhuis WB, Nicolay K, Kappelle LJ, Bar PR, van der Worp HB: Delayed decompressive surgery increases apparent diffusion coefficient and improves perinfarct perfusion in rats with space-occupying cerebral infarction. Stroke 2004;35:1476–1481.
51 Doerfler A, Engelhorn T, Heiland S, Benner T, Forsting M: Perfusion- and diffusion-weighted magnetic resonance imaging for monitoring decompressive craniectomy in animals with experimental hemispheric stroke. J Neurosurg 2002;96:933–940.
52 Engelhorn T, Doerfler A, de Crespigny A, Beaulieu C, Forsting M, Moseley ME: Multilocal magnetic resonance perfusion mapping comparing the cerebral hemodynamic effects of decompressive craniectomy versus reperfusion in experimental acute hemispheric stroke in rats. Neurosci Lett 2003;344:127–131.
53 Rieke K, Schwab S, Krieger D, von Kummer R, Aschoff A, Schuchardt V, Hacke W: Decompressive surgery in space-occupying hemispheric infarction: results of an open, prospective trial. Crit Care Med 1995;23:1576–1587.
54 Schwab S, Steiner T, Aschoff A, Schwarz S, Steiner HH, Jansen O, Hacke W: Early hemicraniectomy in patients with complete middle cerebral artery infarction. Stroke 1998;29:1888–1893.
55 Gupta R, Connolly ES, Mayer S, Elkind MS: Hemicraniectomy for massive middle cerebral artery territory infarction: a systematic review. Stroke 2004;35:539–543.
56 Uhl E, Kreth FW, Elias B, Goldammer A, Hempelmann RG, Liefner M, Nowak G, Oertel M, Schmieder K, Schneider GH: Outcome and prognostic factors of hemicraniectomy for space occupying cerebral infarction. J Neurol Neurosurg Psychiatry 2004;75:270–274.
57 Wagner S, Schnippering H, Aschoff A, Koziol JA, Schwab S, Steiner T: Suboptimum hemicraniectomy as a cause of additional cerebral lesions in patients with malignant infarction of the middle cerebral artery. J Neurosurg 2001;94:693–696.
58 Walz B, Zimmermann C, Bottger S, Haberl RL: Prognosis of patients after hemicraniectomy in malignant middle cerebral artery infarction. J Neurol 2002;249:1183–1190.
59 Vahedi K, Benoist L, Kurtz A, Mateo J, Blanquet A, Rossignol M, Amarenco P, Yelnik A, Vicaut E, Payen D, Bousser MG: Quality of life after decompressive craniectomy for malignant middle cerebral artery infarction. J Neurol Neurosurg Psychiatry 2005;76:1181–1182.
60 Morley NC, Berge E, Cruz-Flores S, Whittle IR: Surgical decompression for cerebral oedema in acute ischaemic stroke. Cochrane Database Syst Rev 2002;CD003435.
61 Georgiadis D, Schwarz S, Aschoff A, Schwab S: Hemicraniectomy and moderate hypothermia in patients with severe ischemic stroke. Stroke 2002;33:1584–1588.
62 Doerfler A, Schwab S, Hoffmann TT, Engelhorn T, Forsting M: Combination of decompressive craniectomy and mild hypothermia ameliorates infarction volume after permanent focal ischemia in rats. Stroke 2001;32:2675–2681.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):106–117
DOI: 10.1159/000091710

Published online: May 2, 2006

Neuroprotection in Vascular Dementia

Eduardo Martínez-Vila Manuel Murie-Fernández
Jaime Gállego Pérez-Larraya Pablo Irimia

Stroke Unit, Department of Neurology, University Clinic, Faculty of Medicine, University of Navarra, Pamplona, Spain

Key Words
Neuroprotection of vascular dementia · Vascular dementia · Vascular dementia, treatment · Vascular dementia, prevention

Abstract
Vascular dementia (VaD) is the second most common form of dementia after Alzheimer's disease (AD), and one of the major causes of mental and physical disability in developed countries. As such, the identification and implementation of strategies which prevent the development of the condition or enable improvements in patients with VaD are healthcare objectives of the first order. VaD is now regarded as a combined group of clinical-pathological entities rather than one disease, that is, multiple pathogenic mechanisms and lesion types underlie a cognitive impairment of vascular origin. The clinical diagnosis of VaD is complex and difficult because of the heterogeneous nature of its clinical presentation and progression and the low sensitivity of existing clinical criteria. Moreover, there is growing evidence of the epidemiological significance of mixed forms of dementia, and that ischemic processes may precipitate and exacerbate cognitive impairment in AD. Numerous compounds have been proposed as potentially useful in the treatment of patients with VaD, comprising vasodilatative, antithrombotic, hemorrheological, nootropic, antiserotoninergic and, most recently, antiglutamatergic and cholinergic approaches. In spite of some initially favorable reports based on the use of memantine, donepezil and galantamine, there is as yet no conclusive evidence of a definitive treatment for VaD. Unsatisfactory results from VaD drug trials may be attributed in part to the diversity of the patients included (underlying pathogenic mechanisms, number, type, and location of vascular lesions), and to methodological limitations in the design of the trials (outcome measures, end-points, size, follow-up period). The treatment of modifiable vascular risk factors – hypertension, diabetes mellitus, hypercholesterolemia and heart disease – is an important strategy for the reduction of the risk of dementia, and is likely to slow the progress of cognitive decline.

Introduction

Vascular dementia (VaD) is the second most frequent cause of dementia after Alzheimer disease (AD) in Western countries, and the most common cause in some Asian regions [1–3]. The incidence of VaD ranges from 1 to 3/1,000 persons/year, and can reach 19/1,000 persons/year if cases of mixed dementia are included [4, 5]. The prevalence of VaD ranges from 1 to 8.8% [3, 6–8], and is expected to rise further in the near future given the increase in life expectancy and the progressive aging of populations. In addition, the total costs arising from the ef-

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0106$23.50/0

Accessible online at:
www.karger.com/ced

Eduardo Martínez-Vila, MD, PhD
Stroke Unit, Departamento de Neurología, Clínica Universitaria
Facultad de Medicina, Universidad de Navarra
Avda. Pío XII 36, ES–31008 Pamplona (Spain)
Tel. +34 948 255 400, Fax +34 948 296 500, E-Mail emartinezv@unav.es

fects of dementia amount to one of the largest burdens on annual healthcare budgets in developed countries [9]. As such, the identification and implementation of therapeutic strategies focused on the prevention or improvement of the cognitive impairment associated with cerebrovascular disease (CVD) are healthcare objectives of the first order.

However, the clinical diagnosis of VaD is still a very complex procedure because of the heterogeneous nature of the condition in physiopathological terms and the diversity of individual cases [10, 11]. The clinical data collated thus far has not yielded a clear diagnostic procedure for VaD; the presence of cerebrovascular lesions must also be determined. Moreover, the existing clinical criteria for VaD are not sufficiently sensitive, use different definitions of dementia, and are not easily interchangeable [12]. At the same time, the fact that patients with dementia often suffer simultaneously from CVD and degenerative AD makes it difficult to determine whether the cause of cognitive deterioration is vascular or degenerative in origin [13, 14]. In recent years, the study of different types of mixed dementia has received special attention because of their increased prevalence and the growing evidence that CVD can precipitate or worsen the development of AD [15, 16].

Neuroprotection in VaD can be defined as the different physiological and pharmacological approaches which aim to improve core and neuropsychiatric symptoms and to slow or halt the progression of cognitive impairment. However, the current, limited understanding of the physiopathological mechanisms that produce cognitive deterioration in VaD means that a more specific therapeutic approach cannot yet be established. Several drugs have been used in the treatment of patients diagnosed with VaD, but the results from clinical trials thus far have been largely negative or have shown only very limited efficacy [10]. Unsatisfactory VaD trial results may be attributed in part to the diversity of patients included and to methodological limitations in the trials themselves [10, 17, 18]. Preliminary results from recent trials, testing some acetylcholinesterase inhibitors and the NMDA-antagonist memantine, offer some new grounds for hope in the treatment of VaD [17, 18]. Nevertheless, new clinical trials, designed specifically to evaluate the cases of patients suffering from cognitive impairment of vascular origin, are needed [18, 19]. At the same time, it is possible that vascular cognitive impairment can to some extent be improved, and VaD prevented, if vascular risk factors are brought under control and strokes do not recur [10, 18, 20].

Diagnosis of VaD and Clinical Trials

The paradigm of VaD as a condition with an abrupt start and staged progression through a series of strokes or multiple strokes in patients who register high risk vascular factors (post-stroke dementia or multi-infarct dementia) is not matched by the normal clinical presentation of VaD [11, 21]. In clinical series, around 25–30% of stroke survivors conform to the criteria for dementia 3 months after a stroke [22–24], and the possibility of delayed development of dementia remains up to nine times higher among stroke survivors than for the age-matched population for more than 5 years after the stroke [25, 26]. However, VaD can also present in patients who have not previously suffered a stroke, with a gradual onset and progressive development more indicative of a degenerative disorder [27]. Indeed, the absence of focal symptoms does not exclude the presence of cerebral vascular lesions. One neuropathological study has shown that only half of the patients with neuroradiological evidence of ischemic lesions had an indicative history of clinical stroke or motor deficits [5]. The clinical diagnosis of VaD is complex and difficult, therefore, because of the heterogeneous nature of both its clinical presentation and progression.

In the current situation, any type of dementia syndrome caused by vascular disease, whether ischemic or hemorrhagic, single or multiple, cortical or subcortical, should be classified under the term VaD [10, 28, 29]. VaD syndrome has been classified in different clinicopathological subtypes (large-vessel VaD, multi-infarct dementia and strategic infarct dementia; small-vessel VaD, subcortical and cortico-subcortical; ischemic-hypoperfusive VaD, and hemorrhagic VaD), and may present clinically in many different forms [29, 30]. Subcortical ischemic VaD, due to small-artery disease and hypoperfusion, is one the more frequent causes of VaD [30, 31]. On the other hand, although atherosclerotic and cardioembolic events are the most common causes of VaD, other less frequent etiological processes, such as amyloid angiopathy, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), vasculitis or collagen disorders, must also be taken into consideration [11]. The clinical presentation and progression of VaD can vary, depending on the etiology of the CVD and the number and location of vascular lesions among other factors [10, 11, 28, 29].

Another significant limitation on the clinical diagnosis of VaD patients arises from the definition of dementia. The current criteria for dementia require the presence of significant memory impairment because the definition is

based on an AD-like pattern. However, the memory functions of some VaD patients may be relatively uncompromised [32, 33]. Consequently, patients who present with clinically significant cognitive impairments associated with CVD frequently do not fulfill the traditional criteria of dementia, and are neither properly diagnosed nor included in epidemiological studies or clinical trials. The functional criteria for dementia must be reconsidered in order to better assess patients with cognitive impairment associated with CVD [6, 10]. Executive dysfunction may be the most significant symptom of VaD, as memory impairment is of AD [33]. In recent years, the term 'vascular cognitive impairment' (VCI) has been proposed to encompass all forms of mild to severe cognitive loss presumed to be caused by CVD, and includes VCI without dementia, mild VCI, and VaD [19].

Diagnostic Criteria for VaD

There is no single, universally-accepted set of diagnostic criteria for VaD. Several diagnostic criteria have been proposed for the clinical diagnosis of VaD, such as the Diagnostic and Statistical Manual of Mental Disorders, 4th ed (DSM-IV) [34], the National Institute of Neurological Disorders and the Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) [35], the State of California Alzheimer's Disease Diagnostic and Treatment Centers (ADDTC) criteria for VaD [36], and the International Classification of Diseases, 10th ed (ICD-10) [37]. Each of these sets of VaD diagnostic criteria is both highly specific and insufficiently sensitive [38]. Moreover, the concomitant application of different clinical criteria has shown that they overlap in less than half of cases [39–41]. Major differences between the diagnostic criteria include the requirement of focal neurological signs, an unequal distribution of cortical dysfunctions, and evidence of a relevant CVD in neuroimaging studies [12]. The diagnostic criteria for VaD, however, follow a different definition of dementia and may not describe the same population of VaD patients, a situation which is likely to cause major discrepancies in results and findings if comparative studies are carried out [10, 42].

To determine a reliable diagnostic procedure for VaD, therefore, the diagnostic criteria of VaD and AD should be followed in parallel, and combined with the evidence of neuroimaging studies. Neuroimaging techniques, mainly CT and MRI, play a key role in the diagnosis of VaD patients, because they can reveal the presence of vascular lesions. However, the evidence of neuroimaging studies is limited because neither the time at which the lesions appeared nor whether the vascular lesions are contributory or coincidental to the cognitive decline can be determined from the information it supplies [43]. A history of clinical strokes or the presence of brain infarcts in neuroimaging studies does not necessarily indicate 'pure VaD', because strokes can occur in AD subjects at the onset or during the course of the disease. Conversely, cortical and subcortical microinfarctions may be present, even though these lesions are not apparent from MRI readings [44]. Etiopathogenic diversity in VaD cases makes any comparative study of the conditions difficult because different subgroups of VaD patients may respond differently to particular drug therapies [10, 29].

Interaction of VaD and AD

Evidence of brain infarcts or a history of strokes does not necessarily rule out the presence of AD-type pathology in patients with cognitive impairment. In older demented patients, degenerative and cerebrovascular lesions are present at the same time more often than might be expected, a fact that significantly complicates the exact diagnosis of VaD and AD [2, 13, 45]. The simultaneous presence of vascular and degenerative lesions is usually referred to as 'mixed dementia' [46]. Mixed dementia is now known to be very common, although some studies suggest that it may be the single most common form of dementia [47].

Several neuropathological studies of patients who have been diagnosed with VaD found that at least half of the cases showed AD-type pathology [2, 45, 48]. Autopsy studies of patients who had been clinically diagnosed with AD showed that more than 30% of the cases that met the pathological criteria for AD also exhibited significant cerebrovascular lesions [14, 49].

Current thinking holds that vascular risk factors such as hypertension, atrial fibrillation, ischemic heart disease, diabetes mellitus, hypercholesterolemia, hyperhomocysteinemia, smoking and atherosclerosis should also be regarded as risk factors in the development of AD [50]. The convergence of risk factors for VaD and AD has led some to speculate that the underlying pathogenic mechanisms for both conditions may be in some ways similar [51, 52]. There is increasing evidence from experimental studies that the sequence of events leading to the development of AD-type pathology may be due in part to cerebral ischemia [53]. At the same time, amyloid protein precursor overexpression and Aß peptide production in AD can cause cerebrovascular dysfunction, and the microvascular alteration caused by amyloid deposition increases the risk of strokes and VaD [54]. In fact, some authors have

argued that sporadic AD is a vascular disease [53]. Vascular and degenerative processes are seen to interact in clinical presentations of cognitive impairment. Data from several clinical and neuropathological studies have emphasized the fact that the presence of ischemic lesions in the brain increased both the severity of cognitive deficits and the prevalence of dementia in patients with AD-type pathology [16, 52, 55, 56]. Moreover, strokes may precipitate or accelerate a pre-existing degenerative process or individual predisposition [57].

Treatment of VaD

In recent decades, a number of therapeutic trials have tested the effects of several drugs in the treatment of VaD, but the results have been largely negative or have shown only very limited efficacy [10, 17, 18]. The unsatisfactory results of these VaD trials may derive in part from the diversity of patients included (e.g. underlying pathogenic mechanisms, the number, type and location of vascular lesions), and from methodological limitations in the design of the trials (e.g. inadequate outcome measures and end-points, small sample sizes and short follow-up periods). Although no specific treatment has as yet been approved for VaD, preliminary results from the use of some of the drug compounds have given grounds for hope.

Symptomatic Treatment of VaD

Nimodipine, a type-L calcium channel blocker, has been proposed as a drug capable of improving cognitive function in patients with VaD because of both its vasoactive and neuroprotective effects. The Scandinavian Multi-Infarct Dementia Trial (SMDT), a 6-month, double-blind, placebo-controlled trial, tested nimodipine (30 mg, three times per day) on patients diagnosed with multi-infarct dementia (DSM-III criteria) [58, 59]. No significant effect was observed in the cognitive, social or global assessments of the patients in comparison with the results from the placebo group. However, a post-hoc subgroup analysis of the SMDT showed that VaD subcortical patients treated with nimodipine performed better on neuropsychological tests and functional scales than patients in the placebo group [59]. These results are in line with those of a previous pilot trial involving patients suffering from cognitive impairment and leukoaraiosis [60]. In a double-blind, placebo-controlled trial, nicardipine (20 mg every 8 h) had no effect on the progression of cognitive impairment in VaD patients (DSM-III-R criteria), compared to patients in the placebo group, after a year-long follow-up period.

A randomized, non-placebo-controlled trial of 325 mg/day aspirin (versus no aspirin) was conducted on multi-infarct dementia patients. After an average follow-up period of 15 months, the group of aspirin patients showed significantly higher cognitive scores than the untreated group [61]. A Cochrane review of the use of aspirin in the treatment of VaD proved inconclusive because no eligible randomized clinical trials could be found and included in the assessment [62]. Treatment with triflusal, an anti-platelet drug, in a randomized, open-label study, led to better scores in MMSE, compared to those of patients in the control group [63]. In a double-blind, randomized trial, treatment with sulodexide (50 mg bid, for 6 months) was associated with an improvement in GBS scores, compared to those of patients in the pentoxifylline group [64].

Citicoline or cytidine 5-diphosphocholine (CDP-choline) is a precursor chemical that is essential for the synthesis of phosphatidylcholine, one of the cell membrane components that are degraded during cerebral ischemia, thus freeing fatty acids and free radicals [65]. A meta-analysis of 12 double-blind, placebo-controlled, randomized trials of CDP-choline among elderly patients with cognitive impairment caused by chronic cerebral disorders provides some evidence that CDP-choline has a positive effect on memory function and behavior and an even stronger effect on the overall clinical presentation. The evidence relates predominantly to patients with cognitive impairment secondary to CVDs [66]. Further studies with more appropriate inclusion criteria and outcome measures are required.

Piracetam, a cyclic derivative of γ-aminobutyric acid, increases oxygen and glucose utilization and has rheological and antithrombotic properties. A Cochrane review of trials in AD, VaD and mixed dementia patients indicates that the evidence available does not support the use of piracetam in the treatment of patients with dementia or cognitive impairment because effects were registered only in the overall impression of change, not in specific measures [67]. Oxiracetam, an analogue of piracetam, was tested in a double-blind, placebo-controlled trial on a heterogeneous group of 307 patients who had been diagnosed with multi-infarct, primary degenerative or mixed forms of dementia. That oxiracetam produced a significant effect was observed in scores on the quality of life scale and in global clinical impression scores [68].

Hydergine or co-dergocrine mesylate was tested in a double-blind, placebo-controlled trial on patients diag-

nosed with multi-infarct dementia [69]. Treatment with a daily intravenous infusion of 3 mg hydergine over 2 weeks was associated with significant improvements in cognitive dysfunction, depression and global clinical impression scores, as compared with the results obtained from the placebo group.

Naftidrofuryl is a serotonin 5-HT_2 receptor antagonist which has been shown to inhibit serotonin-induced vascular smooth muscle contraction and platelet aggregation [70]. In a double-blind, placebo-controlled trial, patients diagnosed with VaD or mixed dementia were randomized to receive either 400 mg/day, 600 mg/day or a placebo for 6 months. Naftidrofuryl treatment was shown to have beneficial effects, measured on the ADAS-cog and SCAG scales and noted in both mild and moderate-severe (DSM-III-R criteria) patients, when compared with the placebo group [71]. This study suggests that treatment with naftidrofuryl can slow the rate of cognitive deterioration in VaD patients and confirms previous findings that naftidrofuryl is effective in the improvement of overall functioning in VaD patients [72].

The use of posatirelin, a synthetic peptide with modulatory effects in cholinergic and monoaminergic systems, was evaluated in a double-blind, placebo-controlled trial on VaD patients (NINDS-AIREN criteria). Patients treated with intramuscular posatirelin (10 mg/day) showed a significant and long-lasting improvement in cognitive functions and attention, as compared to the placebo group [73].

Gingko biloba extract performs rheological, antioxidant and free radical-scavenging functions. The evidence for the benefits of the use of *G. biloba* in the treatment of dementia is controversial. Two double-blind and placebo-controlled clinical trials on patients with multi-infarct dementia and AD found significant improvements in cognition and overall clinical impression scores in patients treated with *Gingko* [74], but the other trial was negative [75]. A Cochrane review on the use of *G. biloba* in the treatment of cognitive impairment and dementia concludes that although there is some evidence of potentially positive effects on cognition associated with the use of *G. biloba*, a larger trial using modern methodology is required [76].

Nicergoline is an ergot derivative with vasoactive, antithrombotic and antioxidant properties. A 6-month, double-blind, placebo-controlled clinical trial of nicergoline (30 mg bid) on patients with multi-infarct dementia (DSM-IIII criteria) showed nicergoline had significant positive effects on the Sandoz Clinical Assessment Geriatric scale (SCAG) and MMSE scores, as compared with results from the placebo group [77]. A pilot study on non-demented, elderly hypertensive patients, who had presented with evidence of leukoaraiosis on CT scans, showed that nicergoline administration over a 2-year period slowed the deterioration in cognitive functions [78]. A Cochrane review indicates that there is consistent evidence for the beneficial effects of the use of nicergoline in the treatment of elderly patients with cognitive impairment stemming from several causes, including CVD [79].

Argatroban, a thrombin inhibitor, was tested on Binswanger patients in a small study (without control). The group of patients treated with argatroban showed improvements in cognitive function and gait disorder, as compared with patients treated with an antiplatelet drug [80].

The heparin-mediated extracorporeal LDL/fibrinogen precipitation system (HELP system) induced a rapid and safe reduction in fibrinogen, lipids and other substances related to hemorrheology, whereby whole-blood and plasma viscosity and the aggregability of blood cells was improved [81]. The effect of two HELP applications (within 8 days) was investigated in 141 patients with multi-infarct dementia (DSM-III and NINDS-ADRDA criteria, Hachinski scale, MRI). The laboratory data and clinical symptoms were analyzed before and after treatment. A statistically significant improvement was observed in scores on the Mathew scale, MMSE, and in the Activities-of-Daily-Living-Test (ADLT). These results suggest a possible role for hemorrheology in the treatment of the symptoms of multi-infarct dementia patients [82].

Pentoxifylline is a xanthine derivative with hemorrheological and antithrombotic properties. A multicenter, placebo-controlled study compared pentoxifylline treatment (1,200 mg/day) to the use of a placebo in multi-infarct VaD patients (DSM-III criteria and evidence of infarct in CT scan). At the end of a 9-month follow-up period, intellectual and cognitive function scores had improved significantly in the active group as compared to the control group [83]. In a previous, small, double-blind, placebo-controlled trial (64 patients), pentoxifylline treatment appeared to slow the cognitive decline in patients with multi-infarct dementia [84].

Propentofylline, a combined inhibitor of adenosine reuptake and cAMP phosphodiesterases and a neuroprotective glial cell modulator, has been tested in clinical trials involving more than 800 patients with VaD. In a pooled group of VaD patients from four early phase III European trials and a European-Canadian phase III study, the beneficial effects of propentofylline (300 mg,

tid) were consistently demonstrated in the areas of cognitive and global function. The sustained beneficial results of treatment for at least 3 months after withdrawal suggest some positive effects on the progression of cognitive impairment [85, 86]. In these studies, DSM-III-R criteria, NINDS-AIREN criteria and CT/MRI scans were used to select patients with possible and probable VaD.

Memantine is a voltage-dependent, uncompetitive antagonist N-methyl-*D*-aspartate (NMDA) receptor, which appears to counteract glutamate-induced excitotoxicity after brain ischemia. Memantine (20 mg/day) was tested in two randomized, placebo-controlled trials [87, 88], which included a total of 900 patients diagnosed with mild to moderate probable VaD (NINDS-AIREN criteria) who were treated for 6 months. Both studies independently showed statistically significant cognitive benefits in the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) score in patients treated with memantine as compared to those in the placebo group. There were no differences in global functioning (CIBIC-plus) scores between the groups in either study. Indicators of the tolerance and safety of memantine were good. In a pooled subgroup analysis of these trials, the cognitive benefits of memantine were more pronounced in VaD patients with small-vessel disease than in those with large-vessel disease as registered in CT or MRI scans. In addition, cognitive decline among VaD patients with small-vessel disease in the placebo group was more severe than among those with large-vessel disease [89].

The development of acetylcholinesterase inhibitors was based on the cholinergic hypothesis of AD. However, several studies have investigated the potential use of cholinergic agents, such as donepezil hydrochloride, rivastigmine tartrate, and galantamine hydrobromide, in the treatment of VaD patients. The existence of cholinergic deficits has been demonstrated in pure VaD and AD, although they are less pronounced in VaD patients and follow a different pattern to that found among patients with AD.

Galantamine inhibits acetylcholinesterase and modulates central nicotinic receptors, thus amplifying the cholinergic response. Galantamine (24 mg/day) was tested in a randomized, parallel-group, double-blind, placebo-controlled, 6-month trial, on patients with probable VaD (NINDS-AIREN criteria) and possible Alzheimer's disease (NINCDS-ADRDA criteria) combined with radiological evidence of CVD (mixed dementia) [90]. A total of 592 patients were included in the trial (43% probable VaD). A statistically significant improvement in cognition (ADAS-cog), global functioning (CIBIC-plus), daily living activities (Disability Assessment of Dementia – DAD), and behavioral symptoms (Neuropsychiatric Inventory – NPI) was observed in the group that received treatment as compared to those in the placebo group. These results were similar to findings from previous trials in AD. However, although the trial was not structured to allow subgroup analysis, the VaD and mixed dementia groups of patients showed different patterns of response in some respects. Neither group showed significant trends in cognitive effects as a result of active treatment. Like the AD subjects in the trial, the subgroup of mixed dementia patients who received galantamine treatment showed greater improvements in cognition and global functioning than patients in the placebo group. In the subgroup of VaD patients, no differences were observed in global functioning between the placebo and galantamine groups [91]. In the galantamine trial, most of the patients with VaD had had strokes, whereas the majority of the group with mixed dementia had presented with white-matter lesions. In an open-label extension of this clinical trial, the group of galantamine patients showed similar, sustained benefits after a year [92].

Rivastigmine was used in a small open-label trial on patients with mild to moderate subcortical VaD. Mild improvements in cognition and caregiver stress were observed [93, 94]. However, the possible efficacy of rivastigmine in the treatment of patients with VaD remains to be demonstrated.

Donepezil, a piperidine derivative drug, was tested in two double-blind, placebo-controlled trials [95, 96] on patients diagnosed with possible or probable VaD (NINDS-AIREN criteria). A total of 1,219 patients were included (73% probable VaD). Patients were randomly assigned to three treatment groups: donepezil 5 mg/day, donepezil 10 mg/day or a placebo, for 6 months. At the end of the follow-up period, both clinical trials showed similar results. The group of patients treated with donepezil showed statistically significant improvement in cognition (ADAS-cog, MMSE) and global functioning (CIBIC-plus) scores, compared to results from those in the placebo group. The beneficial effects of treatment on daily living activities (Alzheimer's Disease Functional Assessment and Changes Scale – ADFACS) were higher in both groups of patients treated with donepezil as compared with patients in the placebo group, but, significantly, only in one of the trials [96]. Donepezil was well tolerated in VaD patients, although more adverse effects were reported in the 10-mg group than in the 5-mg group. The significant effect on the ADAS-cog score in both trials resulted in an improvement over baseline. 57% of patients

in the donepezil trials had had cortical or subcortical strokes, 15–18% white-matter lesions only [97]. The minimal cognitive decline observed in VaD patients in the placebo group during the follow-up period suggests that the cognitive improvement observed in patients treated with donepezil might be due to an improvement in cognitive function rather than a slowing of the progress of cognitive deterioration in those patients [95].

Prevention of VaD

Unlike forms of cognitive deterioration with a degenerative cause, VaD may be preventable to some extent, if vascular risk factors are controlled and strokes do not recur. Moreover, there is growing evidence that ischemic processes may precipitate or accelerate cognitive impairment in AD. Thus, the treatment of vascular risk factors may represent an important strategy to decrease the incidence of dementia and slow the progression of VCI [19, 53, 57, 97–101]. The prevention of VaD is centered on the early identification and control of vascular risk factors, to prevent vascular injury and CVD, and the care of stroke patients.

Stroke patients are at increased risk of both VaD and AD [102]. Not all individuals who have suffered strokes develop dementia, and the reasons for the development of dementia in stroke patients are still poorly understood. Several risk factors – arterial hypertension, cardiac disorders, diabetes, prior stroke, high hematocrit levels, alcohol abuse, pre-existing cognitive decline, age, clinical or stroke-related factors in different combinations – have all been reported to predict, in one way or another, the onset of dementia after a stroke [22, 24, 103–105]. However, the single most frequent manifestation of VaD is subcortical dementia deriving from small-vessel disease rather than multiple larger cortical infarcts. The presence of periventricular white-matter lesions and silent brain infarcts in neuroimaging studies is associated with steeper cognitive decline and identifies subjects who are at a higher risk of the development of dementia [106–108]. Various risk factors have been identified for white-matter changes and lacunae: in particular, hypertension, diabetes and orthostatic hypotension [109]. The care of acute ischemic stroke patients is based on the use of thrombolytic drugs, antiplatelet/anticoagulant agents and pharmacological and physiological neuroprotective approaches [110]. The prevention of stroke recurrence through the control of risk factors, by carotid endarterectomy as well as the use of antithrombotic drugs (warfarin or antiplatelet agents), is also likely to reduce the incidence of VaD and mixed dementia.

There is growing evidence that hypertension may contribute to the development of cognitive decline and dementia [98, 111–115]. Several studies have shown not only a connection between HTA and an increased risk of cognitive deterioration [116], but also the existence of beneficial effects from antihypertensive treatments [117]. In the Honolulu Asia Aging Study (HASS), for every 10 mm Hg increase in systolic blood pressure there was an increased risk of poor cognitive function [112]. In the ARIC cohort, the presence of hypertension or diabetes in midlife predicted a greater decline in some neuropsychological tests 6 years later [118]. In the Systolic Hypertension in Europe study (Syst-Eur), a double-blind placebo-controlled trial, the treatment of isolated hypertension in >60-year-old subjects with nitrendipine significantly reduced the incidence of dementia [119]. A 2-year open-label extension of Syst-Eur showed similar results [120]. In the SCOPE study [121], the blood pressure difference between patients treated with candesartan and patients in control groups was 3.2/1.6 mm Hg, and no effect on cognition was observed over a mean of 3.7 years. Perindopril, usually coupled with indapamide, had a significant positive effect in lowering blood pressure and reducing the risk of dementia among patients who had suffered recurrent strokes [122]. The decreased incidence of dementia associated with antihypertensive treatment probably extends to AD, VaD and mixed dementia patients.

In relation with hypercholesterolemia, several studies have noted a connection between high LDL and total cholesterol levels and a high prevalence of dementia, mainly non-AD in form [123–126]. Some studies suggest that the use of lipid-lowering agents lowers the risk of dementia and protects against cognitive decline [100, 124, 125]. In a prospective case-control study, subjects of 50 years and older to whom statins had been prescribed showed a significantly lowered risk of developing dementia, independent of plasma cholesterol levels or exposure to other lipid-lowering agents [100]. An observational study of postmenopausal women with cardiovascular disease revealed that reductions in total and LDL cholesterol levels over 4 years were associated with better cognitive functioning and an approximately 50% reduced risk of cognitive impairment [125]. It has been postulated that some of the neuroprotective effects of statins are likely to be cholesterol independent (the modulation of endothelial function, the preservation of endothelial nitric oxide synthase activity in cerebral vasculature, or anti-inflammatory and antioxidant properties) [127]. In a prospective study of elderly subjects, a higher baseline LDL cho-

lesterol level was linked to an increased risk of developing post-stroke dementia [126].

However, the possible beneficial effects of statins on cognitive decline were not supported by other observational studies [128, 129]. The results from two important randomized placebo-controlled trials [130–132] designed to assess the cardiovascular effects of statin use have not reflected a protective effect of statins on cognitive impairment. In the Heart Protection Study (HPS), no significantly decreased incidence of cognitive decline was observed in patients treated with simvastatin (40 mg/day) over a 5-year follow-up period [130, 131]. Similarly, no improvements in the incidence of cognitive impairment were observed in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) trial, which compared the use of pravastatin (40 mg/day) to a placebo [132].

Nevertheless, the growing evidence that statins can reduce the incidence of cerebrovascular and cardiovascular events has contributed to an increased prescription of these drugs which might also prevent cognitive impairment. A recent study showed that a substantial proportion of clinicians favor the use of statins in primary and secondary prevention of cognitive impairment of vascular origin, despite a lack of definitive evidence to support their use [133]. Randomized trials are needed to establish the real value of statins and other lipid-lowering agents in the prevention of dementia or the delay of its onset.

In population-based studies, the presence of type-2 diabetes was associated with an increased risk of VaD [134].

Cerebral hypoperfusion may be associated to cognitive impairment. Low blood pressure appears to predispose some subpopulations to the development of dementia [135]. A cohort study has revealed a U-shaped association between incident cognitive impairment and baseline blood pressure, with a higher risk of cognitive impairment among subjects with lower systolic blood pressure [116]. Systolic blood pressure levels below 130 mm Hg are independently associated with cognitive impairment in older subjects who suffer from heart failure [136]. The early treatment of cardiac low-output status in older people can prevent or reverse cognitive decline induced by prolonged cerebral hypoperfusion.

The role of antioxidant vitamins is unclear. A study of VaD patients (DSM-III criteria) showed low levels of plasma α-tocopherol which suggest reduced antioxidant activity [137]. In a community-based epidemiological study of older men, the use of a combined vitamin E and C supplement was associated with a reduction in the incidence of subsequent VaD [138].

Conclusions

In recent years, the focus of therapeutic trials in the field of VaD has changed. Clinical trials in VaD have tested several drugs that have different mechanisms of action. However, the results are considered to be disappointing in general terms, except for the more promising findings from some more recent studies. The explanations offered for the negative results thus far are many and various.

The greatest problems faced in VaD clinical trials derive from the difficulty in diagnosing VaD because of the heterogeneous nature of its clinical presentation and progression, and from the use of trial design models more suited to the study of AD. More often than not, VaD clinical trials follow procedures and judge their findings by the criteria of efficacy and the scales of evaluation proper to studies of AD; they do not take the specific peculiarities of cognitive decline of vascular origin sufficiently into account. Thus, the MMSE or ADAS-cog scales, for example, may not be very sensitive to changes in the conditions of patients with VaD. Future VaD clinical trials must assess cognitive functioning by means of appropriate and adequate tests, take neuropsychiatric symptoms and executive dysfunction into account, and make a clear distinction between the decline in functioning caused by the stroke and the symptoms induced by cognitive impairment [18]. At the same time, it also seems clear that VaD clinical trials should be designed for patients in the early stages of the condition, in whom the underlying pathogenic mechanisms are similar [19].

The promising results from the first trials with memantine and ChIs in the treatment of VaD suggest that the use of these drugs might be combined to cumulative effect in a synergistic form of therapy [139]. At present, a cholinesterase inhibitor (donepezil, galantamine) or an NMDA antagonist (memantine) seems to be indicated for patients diagnosed with VaD or mixed dementia; only the responsiveness of the individual to the treatment should determine whether or not it be discontinued.

References

1 Jorm AF, Korten AE, Henderson AS: The prevalence of dementia: a quantitative integration of the literature. Acta Psychiatr Scand 1987;76:465–479.
2 Knopman DS, Parisi JE, Boeve BF, Cha RH, Apaydin H, Salviati A, et al: Vascular dementia in a population-based autopsy study. Arch Neurol 2003;60:569–575.
3 Ikeda M, Hokoishi K, Maki N, Nebu A, Tachibana N, Komori K, et al: Increased prevalence of vascular dementia in Japan: a community-based epidemiological study. Neurology 2001;57:839–844.
4 Fratiglioni L, Launer LJ, Andersen K, Breteler MM, Copeland JR, Dartigues JF, et al: Incidence of dementia and major subtypes in Europe: a collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology 2000;54(suppl 5):S10–S15.
5 Knopman DS, Rocca WA, Cha RH, Edland SD, Kokmen E: Incidence of vascular dementia in Rochester, Minn. 1985–1989. Arch Neurol 2002;59:1605–1610.
6 Lopez OL, Kuller LH, Fitzpatrick A, Ives D, Becker JT, Beauchamp N: Evaluation of dementia in the cardiovascular health cognition study. Neuroepidemiology 2003;22:1–12.
7 Erkinjuntti T, Inzitari D, Pantoni L, Wallin A, Scheltens P, Rockwood K, et al: Research criteria for subcortical vascular dementia in clinical trials. J Neural Transm Suppl 2000;59:23–30.
8 Meguro K, Ishii H, Yamaguchi S, Ishizaki J, Shimada M, Sato M, et al: Prevalence of dementia and dementing diseases in Japan: the Tajiri project. Arch Neurol 2002;59:1109–1114.
9 Bonin-Guillaume S, Zekry D, Giacobini E, Gold G, Michel JP: The economical impact of dementia. Presse Méd 2005;34:35–41.
10 Lopez OL, Kuller LH, Becker JT: Diagnosis, risk factors, and treatment of vascular dementia. Curr Neurol Neurosci Rep 2004;4:358–367.
11 Bowler JV, Hachinski V: Vascular dementia; in Ginsberg MD, Bogousslavsky J (eds): Cerebrovascular Disease. Pathophysiology, Diagnosis and Management. Boston, Blackwell Sciences, 1998, pp 1126–1144.
12 Pohjasvaara T, Mantyla R, Ylikoski R, Kaste M, Erkinjuntti T: Comparison of different clinical criteria (DSM-III, ADDTC, ICD-10, NINDS-AIREN, DSM-IV) for the diagnosis of vascular dementia. National Institute of Neurological Disorders and Stroke-Association Internationale pour la Recherche et l'Enseignement en Neurosciences. Stroke 2000;31:2952–2957.
13 Neuropathology Group of the Medical Research Council Cognitive Function and Ageing Study (MRC CFAS): Pathological correlates of late-onset dementia in a multicentre, community-based population in England and Wales. Lancet 2001;357:169–175.
14 Kalaria RN, Ballard C: Overlap between pathology of Alzheimer disease and vascular dementia. Alzheimer Dis Assoc Disord 1999(suppl 3):S115–S123.
15 Heyman A, Fillenbaum GG, Welsh-Bohmer KA, Gearing M, Mirra SS, Mohs RC, et al: Cerebral infarcts in patients with autopsy-proven Alzheimer's disease: CERAD. XVIII. Consortium to establish a registry for Alzheimer's disease. Neurology 1998;51:159–162.
16 Snowdon DA, Greiner LH, Mortimer JA, Riley KP, Greiner PA, Markesbery WR: Brain infarction and the clinical expression of Alzheimer disease. The Nun study. JAMA 1997;277:813–817.
17 Bowler JV: Vascular cognitive impairment. Stroke 2004;35:386–388.
18 Erkinjuntti T, Roman G, Gauthier S, Feldman H, Rockwood K: Emerging therapies for vascular dementia and vascular cognitive impairment. Stroke 2004;35:1010–1017.
19 O'Brien JT, Erkinjuntti T, Reisberg B, Roman G, Sawada T, Pantoni L, et al: Vascular cognitive impairment. Lancet Neurol 2003;2:89–98.
20 Hachinski V: Preventable senility: a call for action against the vascular dementias. Lancet 1992;340:645–648.
21 Hulette C, Nochlin D, McKeel D, Morris JC, Mirra SS, Sumi SM, et al: Clinical-neuropathologic findings in multi-infarct dementia: a report of six autopsied cases. Neurology 1997;48:668–672.
22 Barba R, Martinez-Espinosa S, Rodriguez-Garcia E, Pondal M, Vivancos J, Del Ser T: Poststroke dementia: clinical features and risk factors. Stroke 2000;31:1494–1501.
23 Censori B, Manara O, Agostinis C, Camerlingo M, Casto L, Galavotti B, et al: Dementia after first stroke. Stroke 1996;27:1205–1210.
24 Desmond DW, Moroney JT, Paik MC, Sano M, Mohr JP, Aboumatar S, et al: Frequency and clinical determinants of dementia after ischemic stroke. Neurology 2000;54:1124–1131.
25 Ballard C, Rowan E, Stephens S, Kalaria R, Kenny RA: Prospective follow-up study between 3 and 15 months after stroke: improvements and decline in cognitive function among dementia-free stroke survivors >75 years of age. Stroke 2003;34:2440–2444.
26 Kokmen E, Whisnant JP, O'Fallon WM, Chu CP, Beard CM: Dementia after ischemic stroke: a population-based study in Rochester, Minnesota (1960–1984). Neurology 1996;46:154–159.
27 Fischer P, Gatterer G, Marterer A, Simanyi M, Danielczyk W: Course characteristics in the differentiation of dementia of the Alzheimer type and multi-infarct dementia. Acta Psychiatr Scand 1990;81:551–553.
28 Chui HC, Mack W, Jackson JE, Mungas D, Reed BR, Tinklenberg J, et al: Clinical criteria for the diagnosis of vascular dementia: a multicenter study of comparability and interrater reliability. Arch Neurol 2000;57:191–196.
29 Roman GC, Erkinjuntti T, Wallin A, Pantoni L, Chui HC: Subcortical ischaemic vascular dementia. Lancet Neurol 2002;1:426–436.
30 Brun A: The neuropathology of vascular dementia; in Chiu HC, Gustafson L, Ames D, Folstein MF (eds): Cerebrovascular Disease and Dementia. London, Dunitz, 2000, pp 69–76.
31 Inzitari D, Erkinjuntti T, Wallin A, Del Ser T, Romanelli M, Pantoni L: Subcortical vascular dementia as a specific target for clinical trials. Ann NY Acad Sci 2000;903:510–521.
32 Benson DF, Cummings JL: Angular gyrus syndrome simulating Alzheimer's disease. Arch Neurol 1982;39:616–620.
33 Looi JC, Sachdev PS: Differentiation of vascular dementia from AD on neuropsychological tests. Neurology 1999;53:670–678.
34 American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, ed 4. Washington, American Psychiatric Press, 1994.
35 Roman GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, et al: Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 1993;43:250–260.
36 Chui HC, Victoroff JI, Margolin D, Jagust W, Shankle R, Katzman R: Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's Disease Diagnostic and Treatment Centers. Neurology 1992;42:473–480.
37 World Health Organization: The ICD-10 Classification of Mental and Behavioural Disorders: Diagnostic Criteria for Research. Geneva, WHO, 1993.
38 Knopman DS, Parisi JE, Boeve BF, Cha RH, Apaydin H, Salviati A, et al: Vascular dementia in a population-based autopsy study. Arch Neurol 2003;60:569–575.
39 Wetterling T, Kanitz RD, Borgis KJ: Comparison of different diagnostic criteria for vascular dementia (ADDTC, DSM-IV, ICD-10, NINDS-AIREN). Stroke 1996;27:30–36.
40 Pohjasvaara T, Erkinjuntti T, Vataja R, Kaste M: Dementia three months after stroke. Baseline frequency and effect of different definitions of dementia in the Helsinki Stroke Aging Memory Study (SAM) cohort. Stroke 1997;28:785–792.
41 Lopez OL, Kuller LH, Becker JT, Jagust WJ, DeKosky ST, Fitzpatrick A, et al: Classification of vascular dementia in the Cardiovascular Health Study Cognition Study. Neurology 2005;64:1539–1547.
42 Lopez OL, Larumbe MR, Becker JT, Rezek D, Rosen J, Klunk W, et al: Reliability of NINDS-AIREN clinical criteria for the diagnosis of vascular dementia. Neurology 1994;44:1240–1245.
43 Korczyn AD: The underdiagnosis of the vascular contribution to dementia. J Neurol Sci 2005;229–230:3–6.

44 Vinters HV, Ellis WG, Zarow C, Zaias BW, Jagust WJ, Mack WJ, et al: Neuropathologic substrates of ischemic vascular dementia. J Neuropathol Exp Neurol 2000;59:931–945.
45 Zekry D, Duyckaerts C, Belmin J, Geoffre C, Moulias R, Hauw JJ: Alzheimer's disease and brain infarcts in the elderly. Agreement with neuropathology. J Neurol 2002;249:1529–1534.
46 Zekry D, Hauw JJ, Gold G: Mixed dementia: epidemiology, diagnosis, and treatment. J Am Geriatr Soc 2002;50:1431–1438.
47 Langa KM, Foster NL, Larson EB: Mixed dementia: emerging concepts and therapeutic implications. JAMA 2004;292:2901–2908.
48 Zekry D, Duyckaerts C, Belmin J, Geoffre C, Herrmann F, Moulias R, et al: The vascular lesions in vascular and mixed dementia: the weight of functional neuroanatomy. Neurobiol Aging 2003;24:213–219.
49 Lim A, Tsuang D, Kukull W, Nochlin D, Leverenz J, McCormick W, et al: Clinico-neuropathological correlation of Alzheimer's disease in a community-based case series. J Am Geriatr Soc 1999;47:564–569.
50 Breteler MM: Vascular risk factors for Alzheimer's disease: an epidemiologic perspective. Neurobiol Aging 2000;21:153–160.
51 Meyer JS, Rauch GM, Crawford K, Rauch RA, Konno S, Akiyama H, et al: Risk factors accelerating cerebral degenerative changes, cognitive decline and dementia. Int J Geriatr Psychiatry 1999;14:1050–1061.
52 Esiri MM, Nagy Z, Smith MZ, Barnetson L, Smith AD: Cerebrovascular disease and threshold for dementia in the early stages of Alzheimer's disease. Lancet 1999;354:919–920.
53 De la Torre JC: Alzheimer disease as a vascular disorder: nosological evidence. Stroke 2002;33:1152–1162.
54 Bugiani O: A β-related cerebral amyloid angiopathy. Neurol Sci 2004;25(suppl 1):S1–S2.
55 Goulding JM, Signorini DF, Chatterjee S, Nicoll JA, Stewart J, Morris R, et al: Inverse relation between Braak stage and cerebrovascular pathology in Alzheimer predominant dementia. J Neurol Neurosurg Psychiatry 1999;67:654–657.
56 Zekry D, Duyckaerts C, Moulias R, Belmin J, Geoffre C, Herrmann F, et al: Degenerative and vascular lesions of the brain have synergistic effects in dementia of the elderly. Acta Neuropathol (Berl) 2002;103:481–487.
57 Hachinski V, Munoz D: Vascular factors in cognitive impairment – where are we now? Ann NY Acad Sci 2000;903:1–5.
58 Pantoni L, Rossi R, Inzitari D, Bianchi C, Beneke M, Erkinjuntti T, et al: Efficacy and safety of nimodipine in subcortical vascular dementia: a subgroup analysis of the Scandinavian Multi-Infarct Dementia Trial. J Neurol Sci 2000;175:124–134.
59 Pantoni L, Bianchi C, Beneke M, Inzitari D, Wallin A, Erkinjuntti T: The Scandinavian Multi-Infarct Dementia Trial: a double-blind, placebo-controlled trial on nimodipine in multi-infarct dementia. J Neurol Sci 2000;175:116–123.
60 Pantoni L, Carosi M, Amigoni S, Mascalchi M, Inzitari D: A preliminary open trial with nimodipine in patients with cognitive impairment and leukoaraiosis. Clin Neuropharmacol 1996;19:497–506.
61 Meyer JS, Rogers RL, McClintic K, Mortel KF, Lotfi J: Randomized clinical trial of daily aspirin therapy in multi-infarct dementia. A pilot study. J Am Geriatr Soc 1989;37:549–555.
62 Williams PS, Rands G, Orrel M, Spector A: Aspirin for vascular dementia. Cochrane Database Syst Rev 2000(4):CD001296.
63 Lopez-Pousa S, Mercadal-Dalmau J, Marti-Cuadros AM, Vilalta-Franch J, Lozano-Gallego M: Triflusal in the prevention of vascular dementia. Rev Neurol 1997;25:1525–1528.
64 Parnetti L, Mari D, Abate G, Balestreri R, Cucinotta D, Coppola R, et al: Vascular dementia Italian sulodexide study (VADISS). Clinical and biological results. Thromb Res 1997;87:225–233.
65 Adibhatla RM, Hatcher JF, Dempsey RJ: Citicoline: neuroprotective mechanisms in cerebral ischemia. J Neurochem 2002;80:12–23.
66 Fioravanti M, Yanagi M: Cytidinediphosphocholine (CDP-choline) for cognitive and behavioural disturbances associated with chronic cerebral disorders in the elderly. Cochrane Database Syst Rev 2000(2):CD000269.
67 Flicker L, Grimley EJ: Piracetam for dementia or cognitive impairment. Cochrane Database Syst Rev 2000(2):CD001011.
68 Maina G, Fiori L, Torta R, Fagiani MB, Ravizza L, Bonavita E, et al: Oxiracetam in the treatment of primary degenerative and multi-infarct dementia: a double-blind, placebo-controlled study. Neuropsychobiology 1989;21:141–145.
69 Schneider LS, Olin JT: Overview of clinical trials of hydergine in dementia. Arch Neurol 1994;51:787–798.
70 Oudart N: Naftidrofuryl inhibits contractions to serotonin in intact and de-endothelialized cerebral arteries in vitro. J Cardiovasc Pharmacol 1990(suppl 3):S45–S48.
71 Moller HJ, Hartmann A, Kessler C, Rainer M, Brown T, Gamand S, et al: Naftidrofuryl in the treatment of vascular dementia. Eur Arch Psychiatry Clin Neurosci 2001;251:247–254.
72 Emeriau JP, Lehert P, Mosnier M: Efficacy of naftidrofuryl in patients with vascular or mixed dementia: results of a multicenter, double-blind trial. Clin Ther 2000;22:834–844.
73 Parnetti L, Ambrosoli L, Agliati G, Caratozzolo P, Fossati L, Frattola L, et al: Posatirelin in the treatment of vascular dementia: a double-blind multicentre study vs. placebo. Acta Neurol Scand 1996;93:456–463.
74 Le Bars PL, Katz MM, Berman N, Itil TM, Freedman AM, Schatzberg AF: A placebo-controlled, double-blind, randomized trial of an extract of *Ginkgo biloba* for dementia. North American EGb Study Group. JAMA 1997;278:1327–1332.
75 Kanowski S, Hoerr R: *Ginkgo biloba* extract EGb 761 in dementia: intent-to-treat analyses of a 24-week, multi-center, double-blind, placebo-controlled, randomized trial. Pharmacopsychiatry 2003;36:297–303.
76 Van Dongen MC, van Rossum E, Kessels AG, Sielhorst HJ, Knipschild PG: The efficacy of *Ginkgo* for elderly people with dementia and age-associated memory impairment: new results of a randomized clinical trial. J Am Geriatr Soc 2000;48:1183–1194.
77 Herrmann WM, Stephan K, Gaede K, Apeceche M: A multicenter randomized double-blind study on the efficacy and safety of nicergoline in patients with multi-infarct dementia. Dement Geriatr Cogn Disord 1997;8:9–17.
78 Bes A, Orgogozo JM, Poncet M, Rancurel G, Weber M, Bertholom N, et al: A 24-month, double-blind, placebo-controlled multicentre pilot study of the efficacy and safety of nicergoline 60 mg/day in elderly hypertensive patients with leukoaraiosis. Eur J Neurol 1999;6:313–322.
79 Fioravanti M, Flicker L: Efficacy of nicergoline in dementia and other age associated forms of cognitive impairment. Cochrane Database Syst Rev 2001(4):CD003159.
80 Tomimoto H, Akiguchi I, Ohtani R, Yagi H, Ogura S, Wakita H: Effects of an antithrombin drug in patients with subacute exacerbations of Binswanger disease. Intern Med 2000;39:966–969.
81 Schuff-Werner P, Schutz E, Seyde WC, Eisenhauer T, Janning G, Armstrong VW, et al: Improved haemorheology associated with a reduction in plasma fibrinogen and LDL in patients being treated by heparin-induced extracorporeal LDL precipitation (HELP). Eur J Clin Invest 1989;19:30–37.
82 Walzl M: A promising approach to the treatment of multi-infarct dementia. Neurobiol Aging 2000;21:283–287.
83 European Pentoxifylline Multi-Infarct Dementia Study. Eur Neurol 1996;36:315–321.
84 Black RS, Barclay LL, Nolan KA, Thaler HT, Hardiman ST, Blass JP: Pentoxifylline in cerebrovascular dementia. J Am Geriatr Soc 1992;40:237–244.
85 Kittner B: Clinical trials of propentofylline in vascular dementia. European/Canadian Propentofylline Study Group. Alzheimer Dis Assoc Disord 1999;13(suppl 3):S166–S171.
86 Rother M, Erkinjuntti T, Roessner M, Kittner B, Marcusson J, Karlsson I: Propentofylline in the treatment of Alzheimer's disease and vascular dementia: a review of phase III trials. Dement Geriatr Cogn Disord 1998;9(suppl 1):36–43.
87 Orgogozo JM, Rigaud AS, Stoffler A, Mobius HJ, Forette F: Efficacy and safety of memantine in patients with mild to moderate vascular dementia: a randomized, placebo-controlled trial (MMM 300). Stroke 2002;33:1834–1839.

88 Wilcock G, Mobius HJ, Stoffler A: A double-blind, placebo-controlled multicentre study of memantine in mild to moderate vascular dementia (MMM500). Int Clin Psychopharmacol 2002;17:297–305.
89 Mobius HJ, Stoffler A: New approaches to clinical trials in vascular dementia: memantine in small vessel disease. Cerebrovasc Dis 2002(suppl 2):61–66.
90 Erkinjuntti T, Kurz A, Gauthier S, Bullock R, Lilienfeld S, Damaraju CV: Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: a randomised trial. Lancet 2002;359:1283–1290.
91 McKeith I: Galantamine for vascular dementia. Lancet Neurol 2002;1:210.
92 Erkinjuntti T, Kurz A, Small GW, Bullock R, Lilienfeld S, Damaraju CV: An open-label extension trial of galantamine in patients with probable vascular dementia and mixed dementia. Clin Ther 2003;25:1765–1782.
93 Moretti R, Torre P, Antonello RM, Cazzato G: Rivastigmine in subcortical vascular dementia: a comparison trial on efficacy and tolerability for 12 months follow-up. Eur J Neurol 2001;8:361–362.
94 Moretti R, Torre P, Antonello RM, Cazzato G, Bava A: Rivastigmine in subcortical vascular dementia: an open 22-month study. J Neurol Sci 2002;203–204:141–146.
95 Wilkinson D, Doody R, Helme R, Taubman K, Mintzer J, Kertesz A, et al: Donepezil in vascular dementia: a randomized, placebo-controlled study. Neurology 2003;61:479–486.
96 Black S, Roman GC, Geldmacher DS, Salloway S, Hecker J, Burns A, et al: Efficacy and tolerability of donepezil in vascular dementia: positive results of a 24-week, multicenter, international, randomized, placebo-controlled clinical trial. Stroke 2003;34:2323–2330.
97 Schneider LS: Cholinesterase inhibitors for vascular dementia? Lancet Neurol 2003;2:658–659.
98 Kivipelto M, Helkala EL, Laakso MP, Hanninen T, Hallikainen M, Alhainen K, et al: Midlife vascular risk factors and Alzheimer's disease in later life: longitudinal, population-based study. BMJ 2001;322:1447–1451.
99 Haan MN, Wallace R: Can dementia be prevented? Brain aging in a population-based context. Annu Rev Public Health 2004;25:1–24.
100 Jick H, Zornberg GL, Jick SS, Seshadri S, Drachman DA: Statins and the risk of dementia. Lancet 2000;356:1627–1631.
101 Birkenhager WH, Forette F, Seux ML, Wang JG, Staessen JA: Blood pressure, cognitive functions, and prevention of dementias in older patients with hypertension. Arch Intern Med 2001;161:152–156.
102 Ritchie K, Lovestone S. The dementias. Lancet 2002;360:1759–1766.
103 Pohjasvaara T, Erkinjuntti T, Ylikoski R, Hietanen M, Vataja R, Kaste M: Clinical determinants of poststroke dementia. Stroke 1998;29:75–81.
104 Moroney JT, Bagiella E, Desmond DW, Paik MC, Stern Y, Tatemichi TK: Risk factors for incident dementia after stroke. Role of hypoxic and ischemic disorders. Stroke 1996;27:1283–1289.
105 Bornstein NM, Gur AY, Treves TA, Reider-Groswasser I, Aronovich BD, Klimovitzky SS, et al: Do silent brain infarctions predict the development of dementia after first ischemic stroke? Stroke 1996;27:904–905.
106 Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MM: Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med 2003;348:1215–1222.
107 De Groot JC, De Leeuw FE, Oudkerk M, Van Gijn J, Hofman A, Jolles J, et al: Periventricular cerebral white matter lesions predict rate of cognitive decline. Ann Neurol 2002;52:335–341.
108 Longstreth WT Jr, Bernick C, Manolio TA, Bryan N, Jungreis CA, Price TR: Lacunar infarcts defined by magnetic resonance imaging of 3660 elderly people: the Cardiovascular Health Study. Arch Neurol 1998;55:1217–1225.
109 Schmidt R, Schmidt H, Fazekas F: Vascular risk factors in dementia. J Neurol 2000;247:81–87.
110 Hack W, Kaste M, Bogousslavsky J, Brainin M, Chamorro A, Lees K, et al: European Stroke Initiative Recommendations for Stroke Management – Update 2003. Cerebrovasc Dis 2003;16:311–337.
111 Kilander L, Nyman H, Boberg M, Hansson L, Lithell H: Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. Hypertension 1998;31:780–786.
112 Launer LJ, Masaki K, Petrovitch H, Foley D, Havlik RJ: The association between midlife blood pressure levels and late-life cognitive function. The Honolulu-Asia Aging Study. JAMA 1995;274:1846–1851.
113 Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Nilsson L, et al: 15-year longitudinal study of blood pressure and dementia. Lancet 1996;347:1141–1145.
114 Skoog I: Risk factors for vascular dementia: a review. Dementia 1994;5:137–144.
115 Hebert R, Lindsay J, Verreault R, Rockwood K, Hill G, Dubois MF: Vascular dementia: incidence and risk factors in the Canadian study of health and aging. Stroke 2000;31:1487–1493.
116 Glynn RJ, Beckett LA, Hebert LE, Morris MC, Scherr PA, Evans DA: Current and remote blood pressure and cognitive decline. JAMA 1999;281:438–445.
117 Murray MD, Lane KA, Gao S, Evans RM, Unverzagt FW, Hall KS, et al: Preservation of cognitive function with antihypertensive medications: a longitudinal analysis of a community-based sample of African-Americans. Arch Intern Med 2002;162:2090–2096.
118 Knopman D, Boland LL, Mosley T, Howard G, Liao D, Szklo M, et al: Cardiovascular risk factors and cognitive decline in middle-aged adults. Neurology 2001;56:42–48.
119 Forette F, Seux ML, Staessen JA, Thijs L, Birkenhager WH, Babarskiene MR, et al: Prevention of dementia in randomised double-blind placebo-controlled Systolic Hypertension in Europe (Syst-Eur) trial. Lancet 1998;352:1347–1351.
120 Forette F, Seux ML, Staessen JA, Thijs L, Babarskiene MR, Babeanu S, et al: The prevention of dementia with antihypertensive treatment: new evidence from the Systolic Hypertension in Europe (Syst-Eur) study. Arch Intern Med 2002;162:2046–2052.
121 Lithell H, Hansson L, Skoog I, Elmfeldt D, Hofman A, Olofsson B, et al: The Study on Cognition and Prognosis in the Elderly (SCOPE): principal results of a randomized double-blind intervention trial. J Hypertens 2003;21:875–886.
122 Tzourio C, Anderson C, Chapman N, Woodward M, Neal B, MacMahon S, et al: Effects of blood pressure lowering with perindopril and indapamide therapy on dementia and cognitive decline in patients with cerebrovascular disease. Arch Intern Med 2003;163:1069–1075.
123 Reitz C, Tang MX, Luchsinger J, Mayeux R: Relation of plasma lipids to Alzheimer disease and vascular dementia. Arch Neurol 2004;61:705–714.
124 Dufouil C, Richard F, Fievet N, Dartigues JF, Ritchie K, Tzourio C, et al: APOE genotype, cholesterol level, lipid-lowering treatment, and dementia: the Three-City Study. Neurology 2005;64:1531–1538.
125 Yaffe K, Barrett-Connor E, Lin F, Grady D: Serum lipoprotein levels, statin use, and cognitive function in older women. Arch Neurol 2002;59:378–384.
126 Moroney JT, Tang MX, Berglund L, Small S, Merchant C, Bell K, et al: Low-density lipoprotein cholesterol and the risk of dementia with stroke. JAMA 1999;282:254–260.
127 Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, et al: The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1:263–276.
128 Gengo F, Cwudzinski D, Kinkel P, Block G, Stauffer L, Lines C: Effects of treatment with lovastatin and pravastatin on daytime cognitive performance. Clin Cardiol 1995;18:209–214.
129 Rea TD, Breitner JC, Psaty BM, Fitzpatrick AL, Lopez OL, Newman AB, et al: Statin use and the risk of incident dementia: the Cardiovascular Health Study. Arch Neurol 2005;62:1047–1051.
130 MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7–22.

131 Collins R, Armitage J, Parish S, Sleight P, Peto R: Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20,536 people with cerebrovascular disease or other high-risk conditions. Lancet 2004;363:757–767.
132 Shepherd J, Blauw GJ, Murphy MB, Bollen EL, Buckley BM, Cobbe SM, et al: Pravastatin in elderly individuals at risk of vascular disease (PROSPER): a randomised controlled trial. Lancet 2002;360:1623–1630.
133 Suribhatla S, Dennis MS, Potter JF: A study of statin use in the prevention of cognitive impairment of vascular origin in the UK. J Neurol Sci 2005;229–230:147–150.
134 Hassing LB, Johansson B, Nilsson SE, Berg S, Pedersen NL, Gatz M, et al: Diabetes mellitus is a risk factor for vascular dementia, but not for Alzheimer's disease: a population-based study of the oldest old. Int Psychogeriatr 2002;14:239–248.
135 Guo Z, Viitanen M, Fratiglioni L, Winblad B: Low blood pressure and dementia in elderly people: the Kungsholmen Project. BMJ 1996;312:805–808.
136 Zuccala G, Onder G, Pedone C, Carosella L, Pahor M, Bernabei R, et al: Hypotension and cognitive impairment: selective association in patients with heart failure. Neurology 2001;57:1986–1992.
137 Ryglewicz D, Rodo M, Kunicki PK, Bednarska-Makaruk M, Graban A, Lojkowska W, et al: Plasma antioxidant activity and vascular dementia. J Neurol Sci 2002;203–204:195–197.
138 Masaki KH, Losonczy KG, Izmirlian G, Foley DJ, Ross GW, Petrovitch H, et al: Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. Neurology 2000;54:1265–1272.
139 Sobrado M, Roda JM, Lopez MG, Egea J, Garcia AG: Galantamine and memantine produce different degrees of neuroprotection in rat hippocampal slices subjected to oxygen-glucose deprivation. Neurosci Lett 2004;365:132–136.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):118–126
DOI: 10.1159/000091711

Published online: May 2, 2006

Thrombolysis and Neuroprotection in Cerebral Ischemia

M. Gutiérrez[a] E. Díez Tejedor[b] M. Alonso de Leciñana[c] B. Fuentes[b]
F. Carceller[d] J.M. Roda[d]

Cerebrovascular Research Group, Hospital Universitario La Paz, Universidad Autónoma Madrid:
[a]Cerebrovascular Laboratory (Research Unit), [b]Department of Neurology, Hospital Universitario La Paz,
[c]Department of Neurology, Hospital Universitario Ramon y Cajal, [d]Department of Neurosurgery,
Hospital Universitario La Paz, Madrid, Spain

Key Words
Stroke · Thrombolytics · Neuroprotection in acute stroke · Cerebral ischemia, animal models · Thrombolysis and neuroprotection combination

Abstract
Stroke is a major cause of death and disability worldwide. The resulting burden on society grows with the increase in the incidence of stroke. The term brain attack was introduced to describe the acute presentation of stroke and emphasize the need for urgent action to remedy the situation. Though a large number of therapeutic agents, like thrombolytics, NMDA receptor antagonists, calcium channel blockers and antioxidants, have been used or are being evaluated, there is still a large gap between the benefits of these agents and the properties of an ideal drug for stroke. So far, only thrombolysis with rtPA within a 3-hour time window has been shown to improve the outcome of patients with ischemic stroke. Understanding the mechanisms of injury and neuroprotection in these diseases is important to target news sites for treating ischemia. Better evaluation of the drugs and increased similarity between the results of animal experimentation and in the clinical setting requires critical assessment of the selection of animal models and the parameters to be evaluated. Our laboratory has employed a rat embolic stroke model to investigate the combination of rtPA with citicoline as compared to monotherapy alone and investigated whether neuroprotection should be provided before or after thrombolysis in order to achieve a greater reduction of ischemic brain damage.

Physiopathologic Cerebral Ischemia

The significant advances in our understanding of the physiopathological mechanisms of cerebral ischemia are leading to a considerable development of drugs that, at various levels, can block or modify the chain of biochemical processes set off by the hypoperfusion of the cerebral parenchyma.

The ischemic cascade starts within seconds to minutes of loss of perfusion. Protein synthesis initially ceases as the ischemic neuron attempts to conserve its rapidly waning energy store. Membrane ion-transport systems fail, and the neuron becomes depolarized. Membrane depolarization results in calcium influx, which in turn causes

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218–0118$23.50/0

Accessible online at:
www.karger.com/ced

Exuperio Diez Tejedor, MD
Stroke Unit, Department of Neurology, Hospital Universitario La Paz
Paseo de La Castellana, 261
ES–28046 Madrid (Spain)
Tel. +34 72 77 444, Fax +34 91 358 1403, E-Mail ediezt@meditex.es

the release of stored neurotransmitters like glutamate, the major excitatory neurotransmitter in the brain. This release worsens the cellular insult by further increasing intracellular calcium and depolarizing other metabolically compromised neurons. The massive calcium influx stimulates several enzymes, which become unregulated and may provoke the destruction of cellular homeostatic mechanisms. Free radical formation and nitric oxide synthesis contribute to neuronal damage. Hours to days after a stroke, the ischemic territory activates specific genes and the consequent cytokine and cell adhesion molecule formation stimulates local inflammation and may further impair microcirculatory blood flow. Last, the activation of apoptotic genes may promote programmed cell death in the surviving neurons.

Ischemic Penumbra

It appears that intervention must occur very early for any substantial portion of brain tissue to be preserved. The penumbral region is fundamentally salvageable and is therefore the most important target of therapy for acute stroke. If the target of acute stroke therapy is the ischemia core, where the neurons most severely affected by oxygen starvation die rapidly, only fast and effective reperfusion strategies can reverse the blockage of the blood supply and potentially increase the flow above the critical threshold, before the cells are irreversibly damaged [1]. Bordering the core of the ischemia is the penumbra zone [2] where blood flow gradually drops below the functional threshold but is still sufficient to maintain morphological integrity for a certain time, but this depends on the degree of the residual perfusion [1]. This penumbra zone is usually considered the most promising target for acute stroke therapy because the therapeutic window can last several hours [3] and because these areas can be revealed by functional neuroimaging modalities [4]. Again the penumbra would benefit mainly from sufficient reperfusion before irreversible cell damage has occurred, but additional neuroprotective agents targeted at various steps in the pathobiochemical cascade could help, or might even be necessary, to prevent or mitigate secondary ischemic cell damage. The rate of progression of the penumbra from reversible to irreversible ischemic injury depends on many variables and may be accelerated in the presence of poor collateral circulation, hyperglycemia, and other exacerbating factors [4]. If reversible ischemia is not present at the time of treatment, then neuroprotective therapy cannot be expected to work.

Therapeutic Window

This therapeutic window of opportunity, specifically the time between the occurrence of the stroke and the time that treatment is initiated, has, until recently, often been assumed to differ in animals and humans. That is, there is the view that damage develops more slowly in human brains and that a short time window in a rat model did not preclude giving the drug after a longer interval between stroke and administration in humans. A good example of this is the clinical investigation of NMDA antagonist. Despite substantial evidence that these compounds only provide protection when given shortly after (60–90 min) the ischemic insult [5], they have nevertheless been administered to stroke patients up to 6 h after stroke onset [6]. The predominant reason is probably practicality because it is difficult to get patients to hospital and diagnosed within 90 min of stroke onset whereas 6 h is a reasonable time frame for presentation and treatment. Indeed, the problems of carrying out a clinical trial within a short time frame are substantial. However, the success of the tissue plasminogen activator (tPA) trial with a 3-hour time window [7] shows that such studies are possible. It is noteworthy that tPA is also effective in animal stroke models within the same time frame [8], supporting the idea that animals and humans may be similar in the time their window of opportunity is open. Most investigated compounds act on the early events in the neurodegenerative cascade. Consequently, one can extrapolate that these drugs ought to be given rapidly after the ischemic insult if they are to be of any value. If, as we believe, the time window of neurodegenerative events is similar in experimental animals and humans, then we must use one of two approaches: (1) administer the drug very soon after the stroke – an approach that is practically very difficult, or (2) develop a compound acting on a later part of the ischemic cascade that can be given some time after the ischemic insult, indicating its practicality for clinical practice.

Development of Acute Stroke Therapies

The two most important therapeutic approaches in acute cerebral ischemia consist of improving cerebral blood flow by early reperfusion and blocking the biochemical and metabolic changes at the ischemic cascade level. Most likely, the effective time windows for these treatments are different: rather short for effective reperfusion, probably because of the hemorrhagic complica-

tions associated with late reperfusion of ischemic brain tissue, and later for neuroprotection, and particularly prolonged in the anti-inflammatory and antiapoptotic approaches. Reperfusion induced by thrombolysis has been shown to be effective when initiated within 3 h of symptom onset [7]. In contrast, neuroprotective strategies have been disappointing clinically so far and have not improved stroke outcome [9–11], although significant reductions of infarct size were demonstrated in animal models with the use of strategies to antagonize the various steps in the excitotoxic cascade [9, 12], and inhibit free radical toxicity [13, 14], harmful secondary inflammatory mechanisms [15] and attenuate cell death due to apoptosis [16, 17]. The discrepancy between animal models results and clinical efficacy of the neuroprotective drugs is probably due to the limits of animal models in reflecting complex clinical stroke.

Animals Models of Cerebral Ischemia

The efficiency of various neuroprotective strategies is well documented in animal experiments but has thus far given disappointing results in ischemic stroke. The different causes of discrepancies between the animal models and clinical studies depend on both the drugs studied and the design of the experimental model and clinical study [18, 19].

Neuroanatomical, pathophysiological and metabolic differences exist between the rat, the animal most often used in preclinical studies of neuroprotective therapies, and humans, and these differences may help explain why the results of experimental studies are generally more favorable.

The objective of an experimental animal model is to achieve homogeneous and reproducible lesions with a minimum of variability, so as to maximize reliability and results.

The most appropriate model must be chosen when designing the experimental investigation. There are various models of focal cerebral ischemia [18], although the ones most frequently used at present are the model of middle cerebral artery ligation after craniotomy [20, 21], the middle cerebral artery intraluminal occlusion model, inserting a filament via the internal carotid artery [22] and the model of occlusion with autologous blood clot emboli [23, 24] (fig. 1a). The first produces very homogeneous cortical lesions but is traumatic and not very physiological, and is the furthest removed from clinic. It is very useful in pathophysiological studies of ischemia thanks to its regular results and can be very useful in the study of neuroprotective agents for demonstrating a certain effect on the lesion, but its results are hard to reproduce in clinical trials for all the reasons mentioned above. On the other hand, the intraluminal occlusion models, particularly the embolic method, are more similar to the cerebral infarction produced by arterial emboli in humans, and produce to very extensive lesions of widely varying size which affect basal ganglia and the cortex and cause high mortality. This model is very attractive to study neuroprotectors, particularly in combination with pharmacological thrombolysis, but it has the inconvenience of being much less cost-effective due to the variability of the resulting lesions and high mortality.

Thrombolysis for Ischemic Stroke

Once we have produced a stroke we then experiment with methods to prevent or protect against its effect. One method is thrombolysis to restore cerebral blood flow.

The aim of thrombolytic therapy is to lyse an occluding thrombus or embolus and reduce the volume of irreversibly damaged cerebral tissue. However, a major complication of thrombolysis in stroke is cerebral hemorrhage, which would offset any beneficial effects.

Restoration of cerebral blood flow after an acute vascular occlusion may be achieved by the administration of thrombolytic agents. Reperfusion plays an important role in the pathophysiology of cerebral ischemia. tPA and streptokinase are of effective in acute ischemic stroke and are the most extensively studied agents for thrombolysis in stroke. However, the results of streptokinase and tPA studies are not directly comparable. The mechanisms of action of the two agents differ substantially, and the prolonged and nonspecific systemic lytic effects of streptokinase may have contributed to the high risk of hemorrhage.

The specific choice of thrombolytic drug to treat acute stroke depends on several pharmacokinetic factors. The timing of thrombolysis is of paramount importance. Ischemic brain tissue may be salvageable if reperfusion occurs before the tissue is irreversibly damaged, and moreover, the risk of hemorrhage appears to increase once the ischemic tissue becomes edematous [7, 9]. The time between symptoms onset and initiation of medication and the dose levels of the thrombolytic agents are important determinants for the risk of cerebral hemorrhage. Thrombolysis is an effective therapy for acute stroke, but only one thrombolytic agent, tPA, has proven efficacy and

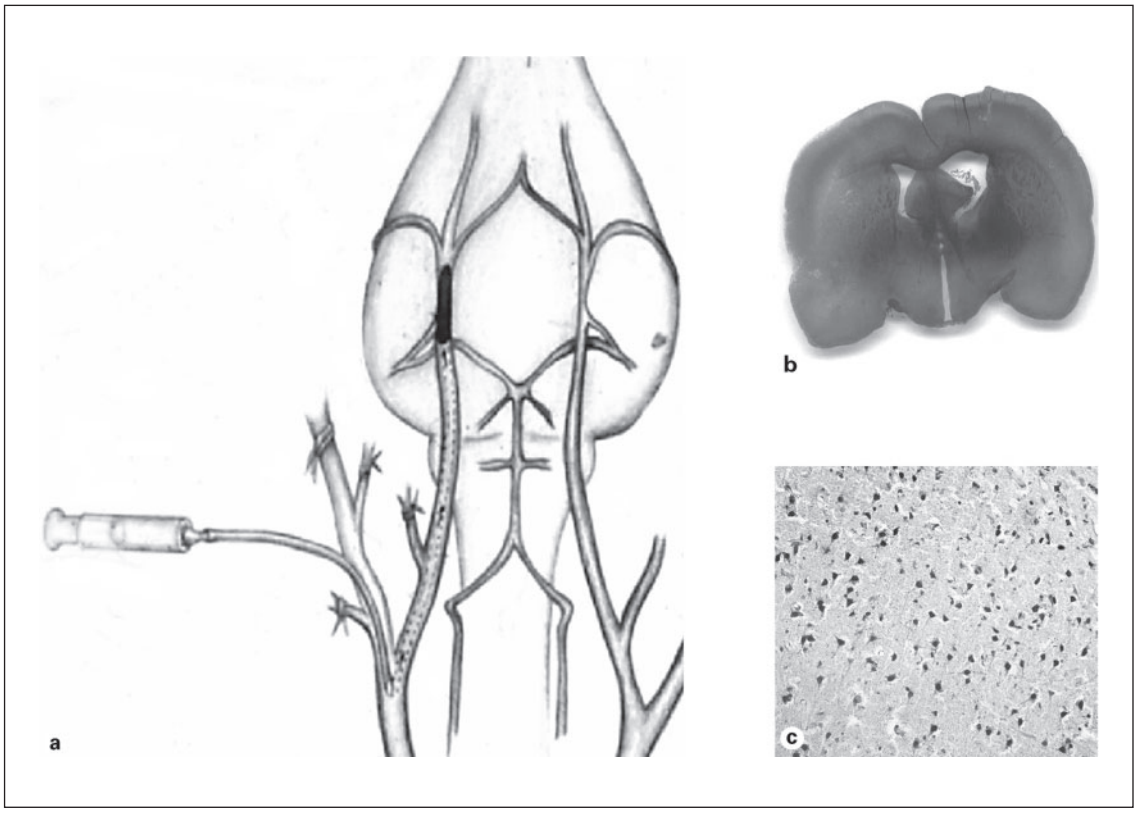


Fig. 1. a Schematic representation of experimental embolization procedure. **b** Infarct volume identified in a coronal section of brain stained with HE. **c** TUNEL-positive cells at the infarct border zone.

safety. Early and rapid assessment is essential because animal and human studies have shown that treatment must begin in the 3 h after stroke onset. A major limitation in thrombolysis for acute ischemic stroke is this restricted time window, and any method that could widen the reperfusion time window would be important. Only a small proportion of acute stroke patients are currently eligible for thrombolysis, mainly because of excessive delay in reaching the presenting at the hospital.

Thrombolysis with intravenous tPA has been demonstrated to be an effective treatment for acute ischemic stroke with unselected subtypes of vasculo-occlusive disease. Unfortunately, there is a substantial risk of cerebral hemorrhage when thrombolytic agents are used in the setting of cerebral ischemia [7, 25, 26]. This risk of hemorrhage is greatest in patients with the most severe neurologic deficits and they have the least chance for a good outcome [25]. Strategies that provide better information regarding the response to thrombolysis may help evaluate patients' identity for intravenous tPA or alternative therapies. Theoretically, patients with smaller and more distal clots represent a subset of patients with a greater probability of benefit from tPA due to less severe deficits at onset, smaller volumes of cerebral ischemia and a greater likelihood of adequate collateral circulation. These patients may also have a lower risk of intracerebral hemorrhage due to their smaller volume of tissue injury [27, 28]. The recommendation for the intravenous administration of rtPA within 3 h of stroke onset in carefully selected patients should not be changed [29, 30]. The evidence is strong that all delays in treating patients should be avoided.

Neuroprotective Therapies

At present, no agent with putative neuroprotective effects can be recommended for the treatment of patients with acute ischemic stroke [29, 30].

Neuroprotective drugs aim to salvage ischemic tissue, limit infarct size, prolong the time window for reperfu-

sion therapy, or minimize postischemic reperfusion injury or inflammation and the risk of hemorrhage. Each step along the ischemic cascade is a potential target for therapeutic intervention. In cerebral ischemia, only thrombolysis has been shown to improve clinical outcome. Neuroprotective therapies have been effective in experimental models of ischemia but, at the moment, there is no definitive evidence of its benefit in the numerous trials carried out in humans, although some subgroups of patients seem to benefit from some of them. The observed lack of efficacy from these drugs may be due to delays in the initiation of treatment, inadequate dose, inadequate penetration, adverse effects, or insufficient matching of the mode of action of the drug to the mechanism of brain injury [31, 32]. Active neuroprotection in acute stroke should include control of blood pressure within certain limits, antipyretic therapy, maintenance of blood glucose, and early feeding and fluid replacement. Manipulation of blood pressure in acute stroke may improve outcome. The design of new clinical trials with neuroprotective drugs requires adequate preclinical assessment and the use of the new magnetic resonance techniques to the select patients and assess the efficacy of the treatment. Some drugs (citicoline, clomethiazole, piracetam and ebselen) have shown a certain degree of clinical efficacy, limited to subgroups of patients, and with a narrow therapeutic window, longer lasting in the case of citicoline [33]. The ECCO 2000 study [34] involved 899 patients at 125 centers who, within 24 h of ischemic hemispheric stroke onset, were randomly assigned to receive either oral citicoline (1,000 mg twice daily) or placebo for 6 weeks. The primary outcome measure, a 7-point or greater improvement in the National Institutes of Health stroke scale score, was achieved by almost the same proportion of patients in both groups (52% citicoline, 51% placebo), suggesting no benefit of citicoline. However, approximately 5% more patients treated with citicoline had excellent outcomes (modified Rankin score </= 1) than those receiving placebo. In this study the results were not conclusive but a positive neuroprotective tendency of citicoline was evidenced. Citicoline is the only putative neuroprotectant that has shown partial positive results in all randomized, double-blind individual trials and that has demonstrated efficacy in the predefined primary end-point of a meta-analysis. The treatment with oral citicoline within the first 24 h after symptom onset in patients with moderate to severe stroke increases the probability of complete recovery at 3 months [35]. Recently, the results of SAINT-1 study have been communicated. This study included more than 1,600 subjects, outcome on the mRS was significantly improved by NXY-059 (Lees et al. for the SAINT-1 Study Group: Preliminary results of the SAINT-1 Trial presented at the 14th European Stroke Conference, Bologna, May 2005). However, one should wait for the final publication to evaluate the data and usefulness of drugs.

Effective neuroprotection may require polytherapy that combines drugs with different mechanisms of action, perhaps administered at different poststroke intervals, to maximize efficacy and/or extend the window for reperfusion, minimize reperfusion injury or hemorrhage, or inhibit delayed cell death [36–38]. Furthermore, because the failure of several neuroprotective trials has been attributed to dose-limiting toxicity [39], combination therapy may permit lower doses of each agent and minimize adverse effects.

Combination of Thrombolysis and Neuroprotection

Combined thrombolysis-neuroprotective approaches have shown promise in animal studies and are beginning to be investigated in clinical trials. The addition of neuroprotective medication may enhance effectivity of thrombolysis and reduces the incidence of hemorrhages. Synergistic effects have been demonstrated in animals when thrombolysis is combined with citicoline [40], an AMPA antagonist [41], and an NMDA antagonist [42]. Administration of antileukocytic adhesion antibodies has been shown to extend the therapeutic window for thrombolysis [43].

Animal models suggest that the combination of low doses of intra-arterial urokinase with a neuroprotective agent, topiramate, may benefit ischemic stroke treatment by improving neurologic recovery, attenuating infarction size, and reducing the risk of cerebral hemorrhage [44]. In a model of focal cerebral ischemia, citicoline may offer significant protection that may be further enhanced with the addition of urokinase. In other experimental studies, the administration of eliprodil, a neuroprotective agent which blocks both the modulatory polyamine site of the NMDA receptor and neuronal voltage-sensitive calcium channels or a thrombolytic agent (rtPA) have similarly reduced the volume of brain damage and the neurological deficit. Combined cytoprotective therapy and thrombolysis markedly improved the degree of neuroprotection and may, thus, represent a valuable approach for the treatment of stroke in humans [45].

Various experimental animal models studies show that the combination of thrombolysis with neuroprotectors (citicoline, MK-801, tirilazad, NBQX, anti-CD18) produces beneficial effects superior to those obtained with monotherapy [40, 42, 43, 46, 47]. However, clinical trials combining therapy (lubeluzole, clomethiazole) [48, 49] did not show efficacy. Combining neuroprotective drugs such as lubeluzole simultaneously with rtPA is feasible and safe. The efficacy of this strategy, using potentially more effective neuroprotective agents, should be evaluated in an adequately powered clinical trial [48]. In a pilot study, there were no safety concerns related to the combination of tPA and clomethiazole. The combination proved effective even though many patients received clomethiazole several hours after thrombolysis; future studies must require prompt administration of the neuroprotector either before or during administration of the thrombolytic. Patients with major strokes may be able to benefit from the combination tPA and clomethiazole [49]. Recently, the results of SAINT-1 study show data where the combination alteplase and NXY-059 reduced the risk of any hemorrhagic transformation and of symptomatic intracranial hemorrhage (Lees et al. for the SAINT-1 Study Group: Preliminary results of the SAINT-1 Trial presented at the 14th European Stroke Conference, Bologna, May 2005). Benefits of combined therapy are already being demonstrated in this study, but, once more, only final results would allow further conclusions for better understanding of mechanisms of this combination and to demonstrate its usefulness.

Our Experience

As above mentioned, we chose citicoline due to its proven clinical efficacy [35]. Citicoline has been demonstrated to be beneficial in several models of cerebral ischemia. The good results with citicoline are probably the result of its mechanism of action providing a neuroprotective effect against both early and delayed ischemic damage, since it inhibits different steps of the ischemic cascade simultaneously and protects the targets (membranes, nucleus, nucleic acids...). Citicoline stabilizes and repairs the membrane [50], favors the synthesis of phosphatidylcholine, nucleic acids, proteins, acetylcholine and other neurotransmitters, inhibits free fatty acid release [51] and protects against apoptosis [52]. Citicoline is used in our study for these reasons.

The treatment regimen, which, would theoretically allow us to reduce the extent and seriousness of cerebral infarction to the maximum, would be the combination of thrombolysis to restore blood flow, as soon as possible together with effective neuroprotection to the inhibit the injury-causing mediators produced by ischemia- reperfusion. To obtain the desired efficacy of combined therapy, the most effective administration regimen must be identified. Two possibilities are proposed in general: (1) administer the neuroprotector before reperfusion to delay progression to irreversible infarction in the penumbra zone and prolong the therapeutic window for thrombolysis, or (2) administer the neuroprotector once reperfusion has been carried out so as to improve neuroprotector penetration in the penumbra zone, and its protective action against injuries due to ischemia-perfusion.

Experimental studies have demonstrated the superiority of combining thrombolysis with different neuroprotectors [47, 53]. Specially, the association of citicoline with rtPA [40] and urokinase [54], with the first citicoline dose given before or simultaneous to thrombolysis, produces a greater reduction in the brain lesion than when either drug was used alone in animal models of ischemic stroke. However, to our knowledge, no evaluation of administering citicoline once reperfusion has occurred has yet been published. With the objective of investigating whether neuroprotection should be provided before reperfusion or once it is ensured, we have compared the effect of rtPA (5 mg/kg i.v.) with citicoline at low (250 mg/24 h for 3 days by the intraperitoneal route) [55] or high (1,000 mg/24 h for 3 days by the subcutaneous route) [56] doses and the combination of both treatments by two routes [57], giving citicoline before or after rtPA in a rat embolic stroke model. This experimental rat model can be useful in preclinical studies of thrombolytics and neuroprotectors. The study has considered a combination of clinical (reduction of mortality and neurological scale score), morphological (infarct volume and TUNEL) (fig. 1b, c) and biochemical markers (IL-6, TNF-α) of ischemic damage. The model also has associated high mortality rates due to the seriousness of the cerebral damage it produces (table 1). Global mortalities do not differ irrespective of whether citicoline is given before or after rtPA. Mortality due to brain damage was decreased with reperfusion and even more in the groups with a combined treatment, particularly when citicoline was administered after thrombolysis. Citicoline as a monotherapy, if anything, has an equally high mortality as rtPA monotherapy. The high scores obtained on the neurological scale illustrate the seriousness of the brain damage, but outcome was more favorable when reperfusion occurred and when the neuroprotector was associated to thrombolysis.

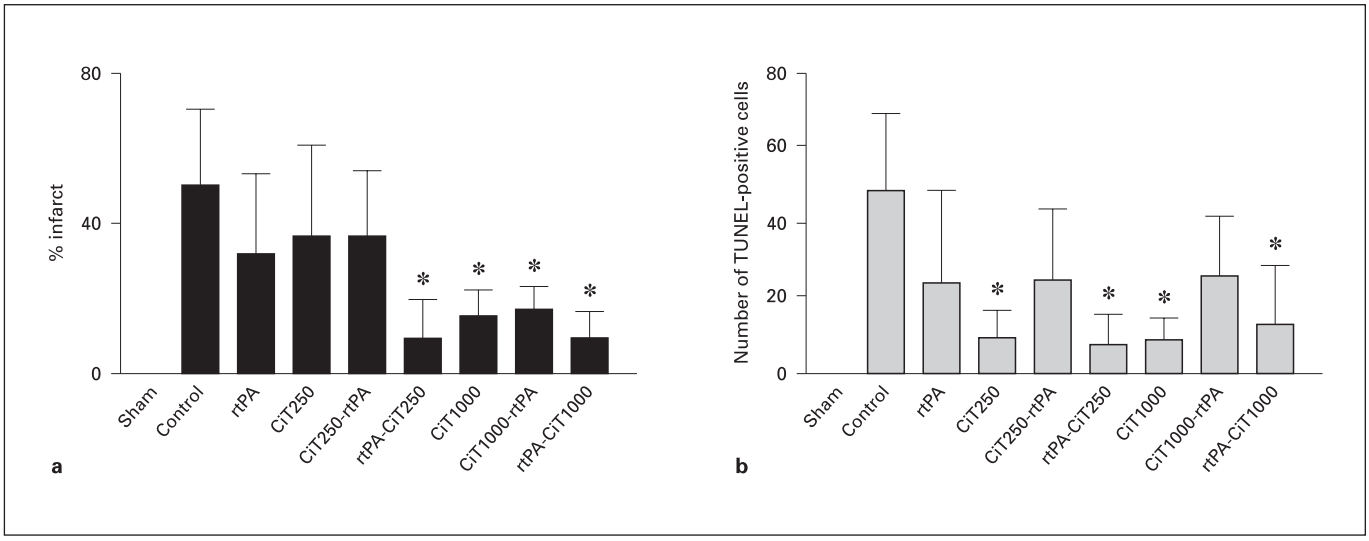


Fig. 2. a Infarct volume in each treatment group expressed as a percentage of the embolized hemisphere. Data are means ± SD. * Significant difference compared with controls (Mann-Whitney test $p < 0.05$). **b** Number of deaths and percentage are shown. * Significant difference ($p < 0.05$ χ^2 test). The groups of treatment were: Sham, Control, rtPA = recombinant tissue plasminogen activator; CiT = citicoline; CiT-rtPA = combination of citicoline before rtPA; rtPA-CiT = combination of citicoline after rtPA.

Table 1. Mortality rates and causes of mortality (number of deaths and percentage are shown)

Group	n	Global mortality	Hemorrhage	Cerebral damage	Other causes
Sham	4	0 (0)	0 (0)	0 (0)	0 (0)
Control	34	29 (85.29)	0 (0)	29 (85.29)	0 (0)
rtPA	19	15 (78.95)	8 (42.11)	7 (36.84)	0 (0)
CiT250	27	23 (85.19)	0 (0)	20 (74.7)	3 (11.11)
CiT250-rtPA	12	8 (66.67)	3 (25)	4 (33.33)	1 (8.33)
rtPA-CiT250	13	9 (69.23)	5 (38.46)	4 (30.77)	0 (0)
CiT1000	16	12 (75)	0 (0)	9 (56.25)	3 (18.75)
CiT1000-rtPA	10	6 (60)	2 (20)	4 (40)	0 (0)
rtPA-CiT1000	18	14 (77.78)	3 (16.67)	11 (61.11)	0 (0)

Figures in parentheses are percentage.

Lower doses of citicoline as a monotherapy failed to reduce lesion size significantly, but our study observed that higher doses of citicoline produced a greater reduction of brain damage than did low doses (unpubl. data). When citicoline was used in combination after rtPA therapy, there was a significant reduction in the ischemic lesion (fig. 2a). This would support the hypothesis that combined neuroprotection after thrombolysis can optimize results. Our results suggest that reperfusion enhances the supply of neuroprotector to the penumbra zone, thus increasing its inhibition of the ischemic cascade and reperfusion injury. A significant benefit of any treatment in regards to reduction of neuronal death (TUNEL) (fig. 2b) was observed when citicoline was administered after rtPA, but isolated reperfusion did not reduce cell death, probably because it failed to inhibit the mechanisms of delayed neuronal death. In summary, the combination of citicoline after reperfusion with rtPA appears to be the optimal treatment. Citicoline at a high dose is most efficacious and might be superior to thrombolysis as mono-

therapy, without the associated risk of hemorrhage (unpubl. data). Low dose of citicoline or rtPA when given alone did not significantly reduce ischemic damage.

The final consideration that most agents claimed to be neuroprotective in animal models has failed in human trials. The human data from the failed trials indicate that the neuroprotective agents were administered long after the successful administration times in animal models. In contrast, thrombolytic therapy has been reported as beneficial in animal and human stroke.

Optimization of therapeutic treatments might involve a complex series of interventions. Disruption of the ischemic cascade of events at multiple levels is likely to be more effective than disruption at any single point. A cocktail of drugs could be administered within the first few hours of illness.

With the use of multiple neuroprotective therapies, each agent or approach could be given or applied either simultaneously or in rapid succession, allowing each agent to work on different ischemic injury mechanisms. Multiple drug therapy and the use of lower doses of individual agents in the mixture thus potentially reduce side effects. The combination of neuroprotection and tPA markedly improved the degree of neuroprotection and opens a route for future studies on the management of acute ischemic stroke. The possible additive or synergistic effects of these drugs should be investigated in future leading studies.

References

1 Heiss W, Graft R: The ischemic penumbra. Curr Opin Neurol 1994;7:11–19.
2 Astrup J, Siesjö B, Symon L: Thresholds in cerebral ischemia: the ischemic penumbra. Stroke 1981;12:723–725.
3 Baron J, von Kummer R, del Zoppo G: Treatment of acute ischemic stroke: challenging the concept of a rigid and universal time window. Stroke 1995;26:2219–2221.
4 Fisher M: Characterizing the target of acute stroke therapy. Stroke 1997;28:866–872.
5 Massieu l, Thedinga K, McVey M, Fagg G: A comparative analysis of the neuroprotective properties of competitive and uncompetitive N-methyl-aspartate receptor antagonist in vivo: implications for the process of excitotoxic degeneration and its therapy. Neuroscience 1993;55:883–892.
6 Davis S, Lees K, Albers G, Diener H, Markabi S, Karlsson G, Norris J: Selfotel in acute ischemic stroke: possible neurotoxic effects of an NMDA antagonist. Stroke 2000;31:347–354.
7 National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group: Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581–1587.
8 Brinker G, Franke C, Hoehn M, Uhlenkuken U, Hossmann K: Thrombolysis of cerebral clot embolism in rat: effect of treatment delay. Neuroreport 1999;10:3269–3272.
9 Grotta J: The current status of neuronal protective therapy: why have all neuronal protective drugs worked in animals but none so far in stroke patients? Cerebrovasc Dis 1994;4:115–120.
10 Del Zoppo G, Wagner S, Tagaya M: Trends and future developments in the pharmacological treatment of acute ischaemic stroke. Drugs 1997;54:9–38.
11 Lees K: Does neuroprotection improve stroke outcome? Lancet 1998;351:1447–1448.
12 Choi D: Antagonizing excitotoxicity: a therapeutic strategy for stroke? Mt Sinai J Med 1998;65:133–138.
13 Kontos H: Oxygen radicals in cerebral vascular injury. Circ Res 1985;57:508–516.
14 Chan P: Role of oxidants in ischemic brain damage. Stroke 1996;27:1124–1129.
15 Chopp M, Zhang Z: Anti-adhesion molecule and nitric oxide protection strategies in ischemic stroke. Curr Opinion Neurol 1996;9; 68–72.
16 Barinaga M: Stroke-damaged neurons may commit cellular suicide. Science 1998;281: 1302–1304.
17 Endres M, Namura S, Shimizu-Sasamata M, Waeber C, Zhang L, Gómez-Isla T, Hyman B, Moskowitz M: Attenuation of delayed neuronal death after mild focal ischemia in mice by inhibition of the caspase family. J Cereb Blood Flow Metab 1998;18:238–247.
18 Alonso de Leciñana M, Díez Tejedor E, Carceller F: Cerebral ischemia: from animal studies to clinical practice. Should the methods be reviewed? Cerebrovasc Dis 2001;11(suppl 1):20–30.
19 Fisher M, Brott T: Emerging therapies for acute ischemic stroke. New therapies on trial. Stroke 2003;34:359–361.
20 Tamura A, Graham D, McCulloch J, Teasdale G: Focal cerebral ischemia in the rat. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J Cereb Blood Flow Metab 1981;1: 53–60.
21 Roda J, Carceller F, Díez Tejedor E, Avendaño C: Reduction of infarct size by intra-arterial nimodipine administered at reperfusion in a rat model of partially reversible brain focal ischemia. Stroke 1995;26:1888–1892.
22 Koizumi J, Yoshida Y, Nakazawa T, Oneda G: Experimental studies of ischemic brain edema. A new experimental model of cerebral embolism in rats which recirculation can be reintroduced in the ischemic area. Jpn J Stroke 1986; 8:1–8.
23 Overgaard K, Sereghy T, Boysen G, Pedersen H, Hoyer S, Diemer N: A rat model of reproducible cerebral infarction using thrombotic blood clot emboli. J Cereb Blood Flow Metab 1992;12:484–490.
24 Alonso de Leciñana M, Díez Tejedor E, Carceller F, Vega A, Roda J: Advantages of associate inhibitors of ischemia reperfusion injury to intravenous thrombolysis for treatment of focal cerebral ischemia. Description of an experimental model. Rev Neurol 1996;24:207–213.
25 NINDS t-PA Stroke Study Group: Intracerebral hemorrhage after intravenous t-PA therapy for ischemic stroke. Stroke 1997;28:2109–2118.
26 Katzan I, Furlan A, Lloyd L, et al: Use of tissue-type plasminogen activator for acute ischemic stroke: The Cleveland area experience. JAMA 2000;283:1151–1158.
27 Heo J, Kim S, Lee K, Kim E, Chu C, Nam J: Increase in plasma matrix metalloproteinase-9 in acute stroke patients with thrombolysis failure. Stroke 2003;34:48–50.
28 Montaner J, Molina C, Monasterio J, et al: Matrix metalloproteinase-9 pretreatment level predicts intracranial hemorrhagic complications after thrombolysis in human stroke. Circulation 2003;107:598–603.
29 Adams H, Adams R, Del Zoppo G, Goldstein L: Guidelines for the early management of patients with ischemic stroke. 2005 Guidelines Update. A scientific statement from the Stroke Council of the American Heart Association/ American Stroke Association. Stroke 2005;36: 916–921.

30 Toni D, Chamorro A, Kaste M, Lees K, Wahlgren N, Hacke W for the EUSI Executive Committee and the EUSI Writing Committee. Cerebrovasc Dis 2004;17(suppl 2):30–46.
31 Lee J, Zipfel G, Choi D: The changing landscape of ischaemic brain injury mechanisms. Nature 1999;399(suppl): A7–A14.
32 Fisher M, Bogousslavsky J: Further evolution toward effective therapy for acute ischemic stroke. JAMA 1998;279:1298–1303.
33 Martinez-Vila E, Sieira P: Current status and perspectives of neuroprotection in ischemic stroke treatment. Cerebrovasc Dis 2001;11: 60–70.
34 Gamman R, Sherman D: ECCO 2000 Investigators. ECCO 2000 study of citicoline for treatment of acute ischemic stroke: final results. Stroke 2000;31:278A.
35 Dávalos A, Castillo J, Álvarez-Sabín J, Secades J, Mercadal J, López S, Cobo E, Warach S, Sherman D, Clark W, Lozano R: Oral citicoline in acute ischemic stroke. An individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.
36 Stroke Therapy Academic Industry Roundtable II: Recommendations for clinical trial evaluation of acute stroke therapies. Stroke 2001; 32:1598–1606.
37 Lindsberg P, Roine R, Tatlisumak T, Sairanen T, Kaste M: The future of stroke treatment. Neurol Clin 2000;18:495–510.
38 Kaste M: Thrombolysis in ischaemic stroke: present and future: role of combined therapy. Cerebrovasc Dis 2001;11(suppl 1):55–59.
39 Dawson D, Wadsworth G, Palmer A: A comparative assessment of the efficacy and side-effect liability of neuroprotective compounds in experimental stroke. Brain Res 2001;892:344–350.
40 Andersen M, Overgaard K, Meden P, Boysen G, Choi S: Effects of citicoline combined with thrombolytic therapy in a rat embolic stroke model. Stroke 1999;30:1464–1471.
41 Meden P, Overgaard K, Sereghy T, Boysen G: Enhancing the efficacy of thrombolysis by AMPA receptor blockade with NBQX in a rat embolic stroke model. J Neurol Sci 1993;119: 209–216.
42 Zivin J, Mazzarella V: Tissue plasminogen activator plus glutamate antagonist improves outcome after embolic stroke. Arch Neurol 1991;48:1235–1238.
43 Zhang R, Zhang Z, Chopp M: Increased therapeutic efficacy with rt-PA and anti-CD18 antibody treatment of stroke in the rat. Neurology 1999;52:273–279.
44 Yang Y, Li Q, Shuaib A: Enhanced neuroprotection and reduced hemorrhagic incidence in focal cerebral ischemia of rat by low dose combination therapy of urokinase and topiramate. Neuropharmacology 2000;39:881–888.
45 Lekieffre D, Benavides J, Scatton B, Nowicki J: Neuroprotection afforded by a combination of eliprodil and a thrombolytic agent, rt-PA, in a rat thromboembolic stroke model. Brain Res 1997;776:88–95.
46 Meden P, Overgaard K, Pedersen H, Boysen G: Effect of early treatment with tirilazad (U7400F) combined with delayed thrombolytic therapy in rat embolic stroke. Cerebrovasc Dis 1996;6:141–148.
47 Overgaard K, Sereghy T, Pedersen H, Boysen G: Neuroprotection with NBQX and thrombolysis with rt-PA in rat embolic stroke. Neurol Res 1993;15:344–349.
48 Grotta J: The Combination Therapy Stroke Trial Investigators. Combination therapy stroke trial: recombinant tissue plasminogen activator with/ without lubeluzole. Cerebrovasc Dis 2001;12:258–263.
49 Lyden P, Jacoby M, Schim J, Albers G, Mazzeo P, Ashwood T, Nordlund A, Odergren T: The Clomethiazole Acute Stroke Study in tissue-type plasminogen activator-treated stroke (CLASS-T): final results. Neurology 2001;57: 1199–1205.
50 Tovarelli G, DeMedio G, Dorman R, Piccinin G, Horrocks L, Porcellati G: Effects of cytidine diphosphate choline (CDP-choline) on ischemia-induced alteration of brain lipid in the gerbil. Neurochem Res 1981;6:821–833.
51 Secades J: CDP-choline: updated pharmacological and clinical review. Methods Find Exp Clin Pharmacol 2002;24(suppl 2):1–56.
52 Krupinski J, Ferrer I, Barrachina M, Secades J, Mercadal J, Lozano R: CDP-choline reduces pro-caspase and cleaved caspase-3 expression, nuclear fragmentation, and specific PARP-cleaved products of caspase activation following middle cerebral artery occlusion in the rat. Neuropharmacology 2002;42:846–854.
53 Sereghy T, Overgaard K, Boysen G: Neuroprotection by excitatory amino acid antagonist augments the benefit of thrombolysis in embolic stroke in rats. Stroke 1993;24:1702–1708.
54 Shuaib A, Yang Y, Li O: Evaluating the efficacy of citicoline in embolic ischemic stroke in rats: neuroprotective effects when used alone or in combination with urokinase. Exp Neurol 2000;161:733–739.
55 Alonso de Leciñana M, Gutiérrez M, Roda JM, Carceller F, Díez Tejedor E: Effect of combined therapy with thrombolysis and citicoline in a rat model of embolic stroke. J Neurol Sci 2006 (in press).
56 Gutiérrez M: Tratamiento del infarto cerebral mediante trombolisis i.v. y neuroproteción aisladas o combinadas en isquemia cerebral focal en rata; thesis, Universidad Autónoma Madrid, Madrid 2005.
57 Díez Tejedor E, Gutierrez M, Carceller F, Roda JM, Alonso de Leciñana M: Treatment with reperfusion and neuroprotection with low and high dose of citicoline in an experimental model of focal cerebral ischemia. Which is the best? Cerebrovasc Dis 2004;17(suppl 5):67.

Cerebrovascular Diseases

Cerebrovasc Dis 2006;21(suppl 2):127–130
DOI: 10.1159/000091712

Published online: May 2, 2006

Other Neuroprotective Therapies on Trial in Acute Stroke

José M. Ferro[a] Antoni Dávalos[b]

[a]Department of Neurosciences and Mental Health, Hospital de Santa Maria, Lisboa, Portugal, and
[b]Department of Neurosciences, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

Key Words
Neuroprotection · Insulin · Interferon-β_{1a} · Magnesium · Zonampanel · Repinotan · Piclozotan · Cerovive · Citicoline

Abstract
New neuroprotective agents on trial may potentially offer benefit to stroke patients without the associated hemorrhagic risk of thrombolytic therapy. Clinical investigation of these drugs has been designed to obtain the highest probability of success, or concentrates on the salvageable ischemic brain and use infarct growth on MRI as a surrogate end-point. Nine substances in 10 trials are currently being tested in three therapeutical strategies in patients with acute ischemic stroke. These strategies focus on: (1) the optimal management of serum glucose with the infusion of glucose, insulin and potassium to induce and maintain euglycemia; (2) the modulation of the inflammatory response with recombinant human interferon-β_{1a}, and (3) interfering with the ischemic cascade using magnesium, albumin, the metal iron chelator DP-b99, the AMPA receptor antagonist zonampanel, the serotonin agonists repinotan and piclozotan, the free radical scavenger cerovive, and the membrane modulator citicoline. Future directions should develop neuroprotective compounds that are safe and well tolerated, are effective in a broad range of patients and can be used with or without rt-PA.

Intravenous rt-PA is the only approved treatment for acute ischemic stroke. However, only a small percent of patients are eligible for such therapy. Neuroprotective agents may potentially offer benefit to stroke patients without the associated hemorrhagic risk of thrombolytic therapy. Such agents target the ischemic penumbra and aim to reduce the risk of brain injury and long-term disability by disrupting the cellular, biochemical, and metabolic consequences of infarction following exposure to ischemia. Despite evidence of utility in animal models, to date no neuroprotectants have demonstrated unequivocal efficacy in humans. In order to address these inconsistencies, the Stroke Therapy Academic Industry Roundtable (STAIR) has produced guidelines for the preclinical and clinical evaluation of new drugs for the treatment of acute ischemic stroke [1, 2]. These guidelines outline stringent criteria (including an appropriate therapeutic window, adequate dosing and plasma level measurement, functional outcome measures, patient selection, and sample size) with the aim of optimizing clinical trial design towards the highest probability of identifying new agents for the treatment of acute ischemic stroke. Some of the new drugs under investigation follow these guidelines, while others concentrate on the salvageable ischemic brain and use infarct growth on MRI for patient selection and as a surrogate end-point.

The compounds mentioned below were identified through a search of the following sources: personal files, 'Major ongoing stroke trials' section of the journal *Stroke* [3], the trials directory of the Internet Stroke Center

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–9770/06/0218 –0127$23.50/0

Accessible online at:
www.karger.com/ced

Prof. Dr. José Ferro
Centro de Estudas, Egas Moniz
Hospital de Santa Maria
PT–1649-035 Lisboa (Portugal)
Tel./Fax +351 21 795 7474, E-Mail jmferro@fm.ul.pt

(www.strokecenter.org/trials) [4], the US National Institutes of Health Service Clinicaltrials.gov [5], the Cochrane clinical trials register [6] and a Medline search using the key words *neuroprotection*, *clinical trial* and *acute stroke*. Trials on temperature control, hypothermia and blood pressure management were not considered.

Nine substances being tested in 10 trials were retrieved. They could be grouped into three therapeutical strategies.

Strategy 1: Optimal Management of Physiological Parameters

Insulin and glucose: It is known that diabetes and hyperglycemia are associated with worse outcomes in acute ischemic stroke. Current guidelines [7] state the hyperglycemia should be treated with insulin, but the glycemia levels to initiate treatment are arbitrary and the efficacy and safety of aggressive glycemia control to maintain euglycemia is unknown. In a previous pilot trial the safety and practicability of an infusion of glucose, insulin and potassium (GIK) has been demonstrated (GIST study stroke) [8]. The United Kingdom Glucose Insulin in Stroke trial (GIST-UK) is a multicenter randomized trial that seeks to determine whether outcome from acute stroke is favorably influenced by GIK-induced and maintained euglycemia. Patients with acute ischemic stroke (with concordant CT) within 24 h form onset and admission plasma glucose of at least 6.0 mmol/l are eligible. Primary end-points are all-cause mortality and dependency or death (modified Rankin score 4–6) at 90 days.

Strategy 2: Fighting Inflammation

Interferon-β_{1a}: Inflammation plays a deleterious role in the processes that lead from ischemia to necrosis. Interferon-β_{1a} diminishes the inflammatory response and therefore is a candidate drug to limit brain damage in acute ischemic stroke. The recombinant human interferon-β_{1a} in acute ischemic stroke is a randomized, placebo-controlled sequential phase 1 dose escalation and safety trial of interferon-β_{1a} in acute ischemic stroke (<24 h). CT or MR is required. Outcome measures are toxicities graded according to the NCI criteria and to study specific predefined criteria.

Strategy 3: Interfering with the Ischemic Cascade

1. Magnesium: Magnesium is an ion channel blocker that blocks voltage-gated calcium channels and NMDA receptors. However, the IMAGES trial failed to show a benefit of magnesium in acute ischemic stroke when given intravenously within 12 h, with the possible exception of lacunar infarcts [9]. The large time window and consequent delayed administration of magnesium may be one of the causes of the failure to demonstrate a beneficial effect. The FAST-MAG (Field Administration of Stroke Therapy – Magnesium) is a multicenter, randomized, double-blind, placebo-controlled trial aims to access the potential neuroprotective efficacy of hyperacute paramedic-initiated magnesium sulfate administration (4 g i.v. over 15 min) to acute stroke patients identified in the field. Probable stroke patients, as identified by the Los Angeles Prehospital Stroke Screen, whose neurological deficits have been present for at least 15 min and who can be treated within 2 h of symptom onset, are eligible. The primary outcome is the functional outcome at 90 days, as measured by the distribution of the scores in the modified Rankin scale.

2. DP-b99: This compound is a membrane-activated metal ion chelator intended to reduce metal levels once activated. Previous phase I and II trials indicated that DP-b99 may be safely administered to stroke patients with no major side effects. The ongoing double-blind, placebo-controlled multicenter trial will aim to confirm the safety and efficacy of this compound. Patients will be included within 9 h of stroke onset and receive the medication intravenously over 4 days. Patients will be stratified into those treated within 6 and 9 h of stroke onset. Primary outcome is change in the NIHSS score from baseline to day 90.

3. Zonampanel: Zonampanel is a glutamate blocker, AMPA receptor antagonist. ARTIST+ (AMPA Receptor Antagonist Treatment in Acute Stroke Trial) is a multicenter, double-blind, placebo-controlled, parallel-group randomized trial with a planned enrolment of 600 patients that was launched in 2001. Inclusion criteria are patients with acute ischemic stroke who can be treated with tPA within 3 h of onset, a NIHSS of 7–23 and a level of consciousness of 0 or 1 in that scale. Efficacy will be measured by neurological and functional scales. A companion trial (ARTIST MRI) will include patients with acute ischemic stroke within 6 h of onset, who had an ischemic lesion volume measured by DWI MR of 5–120 cm^3 and a diffusion-perfusion mismatch of >20%. Primary outcome is T_2 weighted and FLAIR lesion volume at 90 days. By October 2005, results were not yet published.

4. Repinotan: Repinotan is a serotonin agonist of the 5-HT_{1A} receptor subtype. Its safety, tolerability and dosage were investigated in the BRAINS study [10]. Repinotan was well tolerated. The most common adverse events were headache. In a randomized, double-blind, placebo-

controlled trial including 681 ischemic stroke patients, repinotan failed to show clinical benefit [11]. This study included patients with suspected ischemic stroke admitted and treated within 4.5 h. The primary outcome was a successful response on the Barthel Index, defined as a score of 85 at 3 months.

5. Cerovive (NXY-059): Cerovive is a nitrone that acts as a potent free radical scavenger. The clinical development program consists of two large, randomized, double-blind, placebo-controlled phase IIb/III studies (SAINT I and II), and was designed in accordance with the STAIR criteria for clinical development. The primary outcome measure is the distribution of scores on the modified Rankin scale. The recently completed SAINT I study included about 1,700 patients with ischemic stroke with a maximal time from onset of 6 h. Results were presented in May 2005 during the 14th European Stroke Conference in Bologna [12]. The incidence and profile of side effects were similar in the cerovive and placebo groups. A significant reduction in disability measured by the modified Rankin scale (odds ratio 1.20, 95% confidence interval 1.01–1.42, $p = 0.038$) in patients treated with the study drug was found. However no differences in NIHSS scores could be detected between the two groups. Hopefully, these encouraging results will be confirmed by the ongoing SAINT II study. SAINT II started in 2005 in US, Canadian and European centers, and plans to include 3,200 patients with acute ischemic stroke and limb weakness within 6 h of onset.

6. Piclozotan (SUN N4057): Piclozotan is a $(5\text{-HT})_{1A}$ receptor agonist that has shown marked neuroprotective effects in animal models of middle cerebral artery occlusion. The SUN N4057 in acute ischemic stroke is a phase II RCT that will determine the efficacy of a 72-hour infusion of piclozotan in patients with acute ischemic stroke and a MRI demonstrating measurable penumbra (perfusion-weighted imaging [PWI] minus diffusion-weighted imaging [DWI] volume). A total of 112 patients with localizing cortical signs, and a moderate-to severe neurologic deficit (NIHSS score of 6–22) will receive two different doses of the study drug (80 or 120 ng/ml) or placebo less than 6 h (50% of subjects) or between 6 and 9 h after the onset of symptoms. Primary outcome will be the change in stroke lesion volume from screening to day 28.

7. Albumin: Moderate-to-high dose human albumin therapy affords consistent neuroprotection. Among its multiple actions, albumin administration after middle cerebral artery occlusion induces the systemic mobilization of *n*-3 polyunsaturated fatty acids and may help to replenish polyunsaturated fatty acids lost from neural membranes. The ALIAS trial will determine if human serum albumin at 2 g/kg given over 2 h within 5 h of symptoms onset results in improved outcome at 3 months of patients with moderate to severe ischemic stroke (NIHSS ≥ 6). Treatment infusion must be started within 60 min of the onset of the tPA bolus in patients receiving thrombolytic treatment. This phase III, multicenter RCT started in 2005, and will randomize 1,800 patients without congestive heart disease in more than 50 centers.

8. Citicoline (CDP-choline): Citicoline increases the biosynthesis of phospholipids of the neuronal membrane and has shown antiapoptotic and neuroplasticity effects in cerebral ischemia. Citicoline showed a significant 33% (odds ratio 1.33; 95% confidence interval 1.10–1.62) increase in the global odds of recovery at 3 months compared with placebo in a meta-analysis by pooling the individual patients' data from a number of clinical trials [13]. In contrast with many other drugs that have failed in the treatment of stroke within the first 6 h, citicoline proved efficacy when administered within 24 h after symptoms onset, without side effects. A new multicenter pivotal RCT is in progress to confirm these results. The ICTUS trial will compare the effects on global recovery (combined NIHSS ≤ 1, modified Rankin scale ≤ 1, and Barthel Index ≥ 95 averaged using the GEE) at 90 days of citicoline (2,000 mg/day i.v. for 3 days and orally for 6 weeks) given within 24 h from symptoms onset and placebo in patients with a moderate to severe acute ischemic stroke (baseline NIHSS ≥ 8) of the MCA territory. The study will follow a sequential analysis, with the first approach to test the efficacy with 1,000 patients and an upper limit of 2,600 patients.

For acute ischemic stroke, safe and effective treatments that offer an extended therapeutic window are urgently needed to decrease disability and aid neurological recovery. In particular, it would be beneficial if treatment could start immediately without the requirement for selection by a CT scan. This should improve patient prognosis and reduce the burden on family, carers and society. To offer new hope for the treatment of patients who experience an acute ischemic stroke, future directions should concentrate on developing a neuroprotective compound that is safe and well tolerated, is effective in a broad range of patients, and can be used with or without rt-PA.

References

1 Stroke Therapy Academic Industry Roundtable (STAIR). Stroke 1999;30:2752–2758.
2 Stroke Therapy Academic Industry Roundtable II (STAIR II). Stroke 2001;32:1598–1606.
3 Major ongoing stroke trials. Stroke 2005;36:e59–e67.
4 Trials directory of the Internet Stroke Center. www.strokecenter.org/trials/
5 US National Institutes of Health. www.clinicaltrials.gov
6 Cochrane Central Register of Controlled Trials. http://gateway.ut.ovid.com/
7 European Stroke Initiative Executive Committee and the EUSI Writing Committee: European Stroke Initiative recommendations for stroke management – update 2003. Cerebrovasc Dis 2003;16:311–337.
8 Scott JF, Robinson GM, French JM, O'Connell JE, Alberti KG, Gray CS: Glucose potassium insulin infusions in the treatment of acute stroke patients with mild to moderate hyperglycemia: the Glucose Insulin in Stroke Trial (GIST). Stroke 1999;30:793–799.
9 Muir KW, Lees KR, Ford I, Davis S, Intravenous Magnesium Efficacy in Stroke (IMAGES) Study Investigators: Magnesium for acute stroke (Intravenous Magnesium Efficacy in Stroke Trial): randomised controlled trial. Lancet 2004;363:439–445.
10 Teal P, Silver FL, Simard D: The BRAINS study: safety, tolerability, and dose-finding of repinotan in acute stroke. Can J Neurol Sci 2005;32:61–67.
11 Teal P, Lyden P, Kaste M, Davis S, Hacke W, Fierus M: Effects of repinotan in patients with acute ischemic stroke: phase IIB modified randomized exposure controlled trial. Cerebrovasc Dis 2005;19(suppl 2):68.
12 Lees KR, Zivin JA, Ashwood T, Davalos A, Davis SM, Diener HC, Grotta J, Lyden P, Shuaib A, Hardemark HG, Wasiewski W for the SAINT I Investigators: NXY-059 for Acute Ischaemic Stroke: the SAINT I Trial. N Engl J Med 2006;354:588–600.
13 Dávalos A, Castillo J, Álvarez-Sabín J, Secades JJ, Mercadal J, López S, Cobo E, Warach S, Sherman D, Clark WC, Lozano R: Oral citicoline in acute ischemic stroke: an individual patient data pooling analysis of clinical trials. Stroke 2002;33:2850–2857.

Cerebrovascular Diseases

Alonso de Leciñana, M. 48, 118
Alonso-Escolano, D. 54
Álvarez-Sabin, J. 99
Arenillas, J. 30

Blanco, M. 21, 38, 89

Carceller, F. 118
Cárdenas, A. 1
Castellanos, M. 21, 71, 80
Castillo, J. VI, 1, 21, 38, 54, 71

Dávalos, A. 127
Delgado, P. 99
Díez Tejedor, E. 118

Egido, J.A. 48

Ferrer, I. 9
Ferro, J.M. 127
Fisher, M. 64
Flores, J. 89
Fuentes, B. 118

Gállego Pérez-Larraya, J. 106
García-García, J. 89
Gutiérrez, M. 118

Hurtado, O. 1, 54

Irimia, P. 106

Kaste, M. VI

Leira, R. 89
Lizasoain, I. 1, 38, 54
Lorenzo, P. 1, 54

Mallolas, J. 1
Martínez-Vila, E. 106
Millán, M. 30
Moro, M.A. 1, 54
Mosquera, E. 21
Murie-Fernández, M. 106

Poca, M.A. 99
Pradillo, J.M. 54

Roda, J.M. 118
Rodríguez-Yáñez, M. 21, 80, 89
Romera, C. 1

Sahuquillo, J. 99
Serena, J. 80
Sobrino, T. 38, 54, 71

Vivancos, J. 38

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ced

Cerebrovascular Diseases

Subject Index Vol. 21, Suppl. 2, 2006

Acute ischemic stroke 80
– stroke 64, 88
Angiogenesis 30
Antioxidants 1
Apoptosis 9, 30
Atherosclerosis 48

Brain plasticity 54

Cerebral ischemia 30, 54
– –, animal models 118
Cerovive 127
Citicoline 127

Decompressive hemicraniectomy 99

Early neurological deterioration 80
Energy metabolism 71
Estrogens 48
Excitotoxicity 80

Focal ischemia 9

Gene expression 30
Glutamate 1, 80

Hormone replacement therapy 48
Hypothermia 99

Inflammation 1
Insulin 127
Interferon-β_{1a} 127
Ischemic penumbra 64
– preconditioning 38
– stroke 21, 48, 71
– tolerance 38

Magnesium 127
MCA infarction 99

Necrosis 9
Neurogenesis 54
Neuroprotection 1, 9, 30, 38, 48, 71, 127
– in acute stroke 118
– of vascular dementia 106
Non-pharmacological neuroprotection 88

Pathophysiology brain ischemia 48
Penumbra 71
Penumbral imaging 64
Piclozotan 127
Progressing stroke 80

Repinotan 127

Statin treatment 21
Stress response 38
Stroke 9, 99, 118
–, general care 88
–, neurorepair vs. neuroprotection 54
– recovery 54
– therapy 64
–, treatment 21

Thrombolysis and neuroprotection combination 118
Thrombolytics 118

Vascular dementia 106
– –, prevention 106
– –, treatment 106
– protection 21

Zonampanel 127

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/ced