Update on pharmacological strategies for stroke: Prevention, acute intervention and regeneration

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Given the few options that are currently available for patients following ischemic stroke, the search for novel therapeutic approaches becomes more critical. Pharmaceutical intervention strategies for the treatment of stroke include preventative (prophylactic or stroke pretreatment), neuroprotective (early acute post-stroke treatment) and regenerative (delayed post-stroke treatment for long-term benefit) therapeutic approaches. Experimental evidence has suggested that the majority of stroke patients have a slow evolution of brain injury that occurs over several hours. This 'evolving stroke' may ultimately be a realistic target for therapeutic intervention, with the goal of inhibiting the progression of detrimental changes that normally follow the acute ischemic event. Preventing or reducing this delayed cellular injury may improve neurological outcome and also facilitate brain recovery from injury. Significant impact on stroke can be expected as additional research is conducted on biological targets or processes important in facilitating the brain's regenerative capacity following cellular/tissue loss. This review provides updates on stroke prevention therapies (anticoagulant and antiplatelet), the advances in the development of pharmacological agents that target the acute phase of stroke (thrombolytics and neuroprotective drugs), and newly evolving approaches that may facilitate brain regeneration (ie, neurobehavioral recovery) following brain damage.

Keywords Cerebral ischemia, focal stroke, neuroprotection, prevention, regeneration, therapeutic window, treatment

Introduction

An unmet medical need

Stroke is the third largest cause of death in the US (first largest cause in Japan), ranking only behind heart disease and cancer. It is the leading cause of disability in the US (ie, highest 'disease-burden cost'), where 25 to 50% of all victims require full or partial dependence. In addition to the tremendous human suffering, the cost of medical treatment and rehabilitation per patient has been estimated at US $50,000 annually, with a net economic cost of US $30 to 50 billion to society [1,2]. Estimates indicate that there are approximately 775,000 stroke cases per year in the US, and although the incidence of stroke previously appeared to be on the decline, demographic and epidemiological analyses indicate that this is not the case [3••] and that we may even see increased stroke numbers as the human population increases in longevity [1,4].

Existing medical treatment

Currently, tissue plasminogen activator (tPA), a thrombolytic agent that has to be administered within 3 h of stroke, is the only approved therapy following stroke onset. Given that most patients on average do not arrive at the hospital until well after the initial onset of stroke (ie, at a time that typically extends beyond the 3 h window) and require highly specific computerized axial tomography (CAT) scans or magnetic resonance imaging (MRI) to rule out hemorrhagic stroke [5], only a small percentage of patients (1 to 2%) qualify for this treatment [1,2,4]. In addition to the narrow therapeutic window, the use of tPA has been associated with an increased risk of hemorrhagic transformation, which further limits its utility. Clearly, a safer and more effective therapy is needed for the treatment of acute stroke.

Over the past few years, several agents that had advanced into phase III clinical trials have failed, or clinical trials have been terminated due to interim futility analyses or safety concerns [6••]. Clearly, a better understanding of the pathophysiology of stroke is necessary in order to develop more effective therapies with a longer therapeutic window. Based on our current understanding of the pathophysiology of stroke, it is evident that there are three major treatment strategies (ie, points of intervention / approaches) for stroke: prevention or reduction in risk factors, acute intervention, or improvement in functional neurobehavioral recovery / regeneration (see Figure 1).

Risk factors for stroke

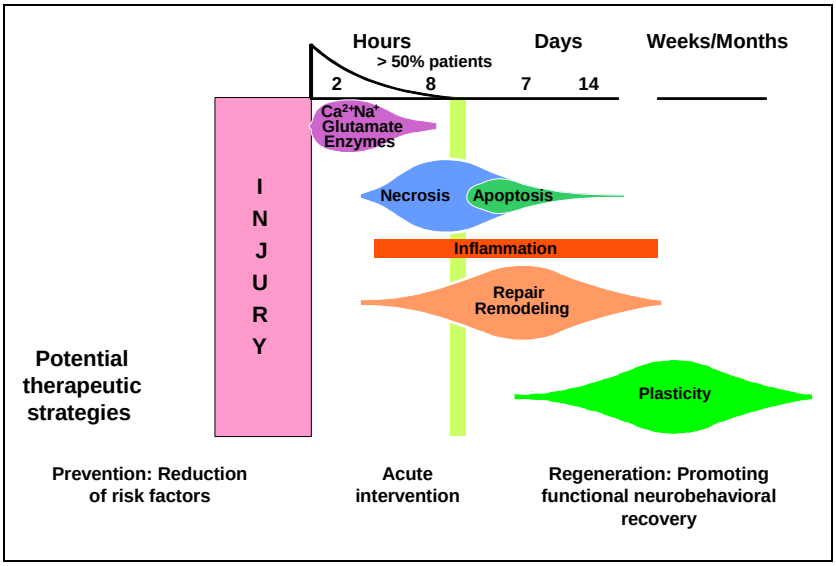
Risk factors include both genetic (predisposing) and environmental factors, which can be a function of natural processes or result from a person's lifestyle. Stroke risk factors that can be influenced include high blood pressure, heart disease, cigarette smoking, transient ischemic attacks and high red blood cell count. Risk factors for stroke that cannot be changed include increased age, gender, race, diabetes mellitus, prior stroke and a family history of strokes. Other secondary risk factors for stroke that can be controlled include those that contribute to heart disease such as high blood cholesterol and lipids, physical inactivity and obesity [7]. Both pharmacological treatment and lifestyle modifications can effectively reduce the risk for primary stroke or subsequent recurrences [8].

Stroke prevention

Update on stroke prevention

Since there are approximately four million patients who are at an increased risk of a secondary cardiovascular event, anticoagulants (eg, warfarin) and antiplatelet medications (eg, aspirin and clopidogrel) are widely utilized as preventative therapy. Prevention of stroke (initial or recurrent) has become standard procedure in patients with high-risk cardiovascular disease or those who have had a previous stroke. A recent assessment of the guidelines for prevention of ischemic stroke has suggested that the recommendations are fairly consistent and range from pharmacological interventions such as antiplatelet or antithrombotic therapy, to more aggressive surgical interventions such as carotic angioplasty or endarterectomy [9].

Figure 1. Preventative, acute intervention and regenerative pharmacological strategies for stroke.



This schematic diagram depicts the relative time sequence of the dynamic changes that occur following ischemic stroke, and current treatment strategies based on the known pathophysiology of brain injury/ischemia. Ischemic stroke reduces energy availability and therefore membrane ionic pumps rapidly fail. The rise in extracellular potassium can reach levels sufficient to release excitotoxic neurotransmitters (eg, glutamate and aspartate) to stimulate sodium/calcium channels coupled to glutamate receptors that can facilitate the development of cytotoxic edema. The significant influx of calcium through calcium channels increases free cytosolic calcium that causes mitochondrial calcium overload, cessation of already compromised ATP production, and extensive breakdown of cellular phospholipids, proteins and nucleic acids, through activation of phospholipases, proteases and endonucleases. Free radicals (eg, nitric oxide and superoxide) that contribute to membrane lipid peroxidation, protein and nuclear DNA toxic changes and cellular injury (ie, necrosis and/or apoptosis) are also produced during this process. These processes initiate neurodestructive and neuroprotective gene expression responses in the injured brain. The inflammatory response to tissue injury occurs after these initial changes but contributes to the ongoing evolution of tissue injury. Destructive gene expression (primarily inflammatory cytokines, adhesion molecules, chemokines and inflammatory proteins such as inducible nitric oxide synthase and metalloproteases) can drive brain inflammation and necrotic/apoptotic cell death. Protective gene expression includes neurotrophic and growth factors from circulating mononuclear cells that have infiltrated into damaged tissue or resident activated glial cells, and can protect cells/tissue, facilitate repair and remodeling and/or increase neuronal plasticity, and can facilitate the recovery of function for the remaining viable neurons/tissue [20•].

Although antiplatelet agents have been relatively efficacious in stroke prevention [10,11], several companies are pursuing additional antiplatelet/antithrombotic strategies to improve the safety-to-risk benefit. Included among these approaches are selective GPIIb/IIIa antagonists, thrombin inhibitors, Factor Xa inhibitors, P2Y receptor antagonists and platelet-activating factor antagonists. A select few companies are also evaluating traditional cardiovascular therapies such as verapamil and atorvastatin, and have generated some exciting results during phase II and III clinical trials. The statins are a unique class of drugs that are primarily utilized for the reduction of cholesterol. However, these 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors also have non-lipid related actions, including modification of endothelial function, immuno-inflammatory responses, smooth muscle cell activation/ proliferation/migration, thrombus formation and plaque stability [12•] that may ultimately provide an optimal preventative therapy for those at risk of stroke. The development status and details of several compounds that are being investigated for therapeutic efficacy in stroke are summarized in Table 1.

Acute stroke intervention

Although lifestyle modifying and preventative therapies appear to reduce the incidence of stroke, recent demographic and epidemiological analyses suggest that the incidence of stroke will become more prevalent in the decades ahead [3••]. Although the speed and quality of initial care post-stroke have greatly improved due to specialist training, appropriate facilities and implementation of new technologies [13], ideal pharmacological interventions are still lacking. Clearly, a better understanding of the pathophysiology of stroke is necessary to enable the development of more effective therapies with a longer therapeutic window. A summary of several of the current acute intervention strategies that are currently being evaluated is presented in Table 2. A detailed discussion of the pathophysiology of stroke can be found in recent review articles [14-17], and the potential mechanism(s) of these compounds and the possible mechanisms through which they prevent brain injury is included below.

Over the past five years, new therapeutic targets have emerged as a result of experimental evidence demonstrating prolonged gene changes [18••], comparative data from clinical genomic studies [19] and the brain's ability to mount a central and peripheral inflammatory response [20•], in addition to highly specific MRI scans that suggest that the evolution of brain injury occurs over a period of time [21•,22-25,26•,27-29]. The progressive microcirculatory disturbances caused by delayed and/or prolonged enzymatic activity, coagulation and inflammation (eg, leukocyte and platelet activation) may be attractive targets to improve brain perfusion alone, or in combination with agents designed to reduce the longer lasting thrombotic and inflammatory components of stroke (see Table 2).

Table 1. Compounds in development for the treatment of stroke.

Drug	Mechanism(s)	Comments	Reference
Phase III			
Verapamil (Johnson & Johnson/Pfizer Inc)	Controlled-release calcium channel antagonist.	The Controlled ONset Verapamil INvestigation of Cardiovascular Endpoints (CONVINCE) prospective survival trial comparing verapamil with standard therapies (diuretic or β-blocker) for reducing the risk of heart attack, stroke or death was conducted.	[110,111]
Perindopril (Servier/Daiichi Seiyaku Co Ltd/Solvay Pharmaceuticals Inc)	Angiotensin-converting enzyme inhibitor.	In the PROGRESS (Perindopril pROtection aGainst REcurrent Stroke Study) large-scale international joint clinical investigation to test the efficacy of perindopril against recurrence of cerebral stroke in 6000 normotensive or hypertensive stroke or transient ischemic patients in 10 countries including Japan, perindopril (4 mg once daily) alone or in combination with indapamide (2 or 2.5 mg) produced 28 and 26% relative risk reductions in stroke, respectively.	[112]
Atorvastatin (Pfizer Inc)	Orally active, liver-selective HMG-CoA reductase inhibitor.	Atorvastatin is currently undergoing the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. This is a double-blind, randomized, placebo-controlled, multicenter trial in 4200 patients to reduce recurrent cerebrovascular events, such as stroke or transient ischemic attack, in patients without a history of coronary heart disease. In the Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering (MIRACL) trial, patients treated with atorvastatin on hospital admission for acute coronary syndrome are 59% less likely to experience a non-fatal stroke within the following 4 months. Atorvastatin-treated acute coronary syndrome patients are also 50% less likely to experience either fatal or non-fatal stroke in comparison with placebo-treated patients.	[113,114]
Phase II			
Cromafiban (COR Therapeutics Inc)	The oral prodrug of a selective, reversible GP IIb/IIIa antagonist.	A series of phase II clinical trials investigating cromafiban for the treatment and prevention of heart disease in stroke has been completed. A further phase II trial could be required before initiation of phase III trials, but enrolment has been put on hold pending the outcome of a phase III trial with roxifiban (Bristol-Myers Squibb Pharma Co).	[115]
SB-424323 (GlaxoSmithKline plc)	Factor IIa antagonist and coagulation inhibitor.	SB-424323 is under development for deep vein thrombosis and also for secondary prevention against cerebrovascular ischemia.	[116]
Factor Xa inhibitors (2906 and FXV-673; Aventis Pharma AG)	Factor Xa inhibitor.	Phase I/IIa trials are underway with Factor Xa inhibitors that have potential for the treatment of thrombosis, stroke and myocardial infarction.	[117]
Phase I			
BGC-728 (BTG International Ltd)	GPIIb/IIIa antagonist.	BGC-728 is under development for the treatment and prophylaxis of acute myocardial infarction and stroke and is currently undergoing phase I efficacy, safety and tolerance clinical trials.	[118]
TP-9201 (Telios Pharmaceuticals Inc)	GPIIb/IIIa antagonist.	TP-9201 is a synthetic RGD sequence-containing cyclic peptide that has undergone phase I trials in the US as an antithrombotic agent for the treatment of thromboses associated with restenosis, reocclusion and ischemic stroke.	[119]
BAY-44-2041 (Bayer AG)	Adenosine uptake inhibitor.	Bayer is investigating BAY-44-2041 as a prophylactic agent for stroke.	[120]
NV-04 (Novogen Ltd)	Adrenoreceptor antagonist and antioxidant.	NV-04 is a phenolic hormone derived from plant isoflavones (plant phytoestrogens), under development by Novogen with potential for the treatment of cerebrovascular disease.	[121]
Preclinical			
Recombinant E-selectin (Novavax Inc)	E-selectin agonist.	Recombinant E-selectin protein is being developed as a tolerogen for the secondary prevention of stroke.	[122]
$P2Y_{12}$ receptor antagonists (Millennium Pharmaceuticals Inc)	Platelet ADP receptor antagonist.	Millennium is developing oral, small-molecule antagonists of the $P2Y_{12}$ ADP receptor for the prevention of arterial thrombotic disease, restenosis and stroke.	[123]
Factor Xa inhibitors (Bristol-Myers Squibb Pharma Co)	Factor Xa inhibitor.	Factor Xa inhibitors, including SR-374 and SN-429 are undergoing preclinical evaluation for the prevention and treatment of venous thromboembolic events with potential for the prevention of recurrent thrombotic stroke.	[124,125]

Development status presented is for stroke indication.

Table 2. Selection of acute stroke intervention therapies currently undergoing clinical trials.

Drug	Mechanism	Comments	Reference
Phase III			
Repinotan (Bayer AG)	5-HT_{1A} receptor agonist.	Repinotan is currently undergoing phase III clinical trials for the treatment of stroke and traumatic brain injury, and also has potential in depression and anxiety.	[126]
NXY-059 (AstraZeneca plc)	α-Phenyl-*tert*-butylnitrone-derivative free radical scavenger.	NXY-059 is a neuroprotectant, under development for the treatment of stroke. It stabilizes against ischemia-reperfusion injury, protects mitochondrial function, and blocks cytochrome C efflux and subsequent caspase activation.	[71,72]
Phase II			
Desmoteplase (PAION GmbH)	Plasminogen activator stimulant.	Desmoteplase is a recombinant fibrin-specific plasminogen activator derived from the saliva of the bat *Desmodus rotundus*, which is structurally very similar to human tPA except that it lacks the K2 domain. Its activity is 200-fold more fibrin-specific than tPA and therefore does not cause systemic plasminogen activation and fibrinogen depletion.	[127]
DP-b99 (D-Pharm Ltd)	Membrane-activated calcium chelator and apoptosis antagonist (by modulation of intracellular calcium homeostasis).	DP-b99 is under development for neuroprotection in ischemia-related conditions such as stroke, traumatic brain injury and neurological damage associated with coronary artery bypass graft.	[128]
ONO-2506 (Ono Pharmaceutical Co Ltd)	Neurotropic agent.	ONO-2506 is under development for the therapy of acute stroke and treatment of cerebral infarction. It reduces necrosis of cerebral nerve cells.	[129]
Zonampanel (Yamanouchi Pharmaceutical Co Ltd)	AMPA receptor antagonist.	Zonampanel is under development for use in acute ischemic stroke.	[44-49]
S-0139 (Shionogi-GlaxoSmithKline Pharmaceuticals LLC)	Endothelin A antagonist.	S-0139 has potential for the treatment of cardiovascular disorders and stroke.	[130]
Dexanabinol (Pharmos Corp)	Synthetic lipophilic molecule with NMDA and tumor necrosis factor-α antagonistic activity.	Dexanabinol was evaluated in a small phase II trial in patients in which no serious adverse events were observed, and has potential for the treatment of stroke.	[73]
Pamiteplase (Yamanouchi Pharmaceutical Co Ltd)	Long-acting recombinant tPA.	Pamiteplase is 4-fold more potent than native tPA in its thrombolytic effect and has a prolonged plasma half-life enabling it to be administered as a bolus injection. It is in phase II trials in Japan for acute ischemic stroke.	[131]
YM-337 (Yamanouchi Pharmaceutical Co Ltd)	GPIIb/IIIa antagonist and platelet aggregation inhibitor.	YM-337 is currently undergoing phase II clinical trials for thromboembolic disorders, including cerebrovascular ischemia.	[132]
SUN-N4057 (Daiichi Suntory Biomedical Research Co Ltd)	5-HT_{1A} agonist.	SUN-N4057 is under development for acute cerebral infarction.	[133]
Cerebril (Neurochem Inc)	Aβ-amyloid fibrillogenesis inhibitor.	Cerebril is the lead compound in a series of amyloid fibril inhibitors under development for the treatment of hemorrhagic stroke due to cerebral amyloid angiopathy.	[134]
NS-7 (Nippon Shinyaku Co Ltd)	Sodium/calcium channel blocker.	NS-7 is under development as a neuroprotective agent for cerebral ischemia for use following cerebral infarction.	[75]
Phase I			
Oxygenated fluorocarbon nutrient emulsion (OFNE; Neuron Therapeutics Inc/Thomas Jefferson University)	Oxygen carrier.	OFNE is currently undergoing phase I clinical trials for the treatment of ischemic stroke.	[135]
MLN-01 (Millennium Pharmaceuticals Inc/XOMA Ltd)	Anti-β2-integrin antagonist.	MLN-01 is a humanized monoclonal antibody, under development for the principal indication of ischemic stroke. It is in phase I clinical trials for the prevention of reperfusion injury after stroke.	[136]

Table 2. Selection of acute stroke intervention therapies currently undergoing clinical trials (continued).

Drug	Mechanism	Comments	Reference
Phase I			
BAY-38-7271 (Bayer AG)	Cannabinoid agonist	Bayer is developing a neuroprotective cannabinoid receptor agonist. The compound is currently in phase I trials as a potential therapy for traumatic brain injury and stroke. Worldwide launch was anticipated for 2006.	[137]
MLN-519 (Millennium Pharmaceuticals Inc/PAION GmbH)	Lactone/lactacystin analog-based proteasome inhibitor.	MLN-519 has a shorter duration of action than boronate inhibitors and has potential in acute inflammatory diseases, stroke and asthma.	[75]
DY-9760e (Daiichi Seiyaku Co Ltd)	Calmodulin antagonist.	DY-9760e is under development for neuronecrosis in acute cerebrovascular disorders such as cerebrovascular ischemia.	[138]

Development status presented is for stroke indication.

Update on traditional neuroprotectant strategies

Several approaches have been taken to prevent early disruptions in ion homeostasis. Among the approaches that have been evaluated experimentally and clinically are sodium channel blockers [30-32], calcium channel blockers [33-38], glutamate antagonists and NMDA antagonists [39,40]. Clinically, the glutamate receptor antagonists and calcium channel blockers have been the most widely tested, however, thus far these have all been unsuccessful. Despite these negative results, several major pharmaceutical companies have advanced similar compounds into late stage clinical trials (see Table 2). In particular, ziconotide (Elan Pharmaceuticals Inc) is a conopeptide, neuron-specific calcium channel blocker that is currently undergoing phase III clinical trials for the treatment of pain. Ziconotide was undergoing phase III trials for stroke, although no development has been reported since 2000 despite encouraging preclinical data [41-43]. A phase II clinical trial of the sodium channel blocker sipatrigine (CeNeS Pharmaceuticals plc) was halted due to adverse events and development was subsequently discontinued [30]. Dexanabinol (Pharmos Corp) is a synthetic lipophilic molecule with *N*-methyl-D-aspartate (NMDA) and tumor necrosis factor-α (TNFα) antagonistic activity that is currently undergoing phase III clinical trials for the treatment of traumatic brain injury. Dexanabinol was evaluated in a small phase II trial in stroke patients and no serious adverse events were observed. Although its ability to penetrate the blood-brain barrier penetrability and lack of reported adverse events is encouraging for the development of dexanabinol, efficacy for the treatment of stroke needs to be demonstrated. Zonampanel (Yamanouchi Pharmaceutical Co Ltd) is another agent that targets early excitotoxic changes and has exhibited dramatic preclinical neuroprotection. The compound is currently undergoing phase II clinical trials [44-49]. Other ionotropic glutamate antagonists that are currently undergoing clinical trials include NS-1209 (NeuroSearch AS) and delucemine hydrochloride (NPS Pharmaceuticals Inc), while antagonists such as remacemide and irampanel are no longer in active development [50-56]. In addition, several compounds directed at the early disruptions in ionic homeostasis have also demonstrated experimental efficacy in rodents. Based on the prevalence of past failures for using similar approaches, there appears to be a high hurdle to overcome to advance these compounds beyond clinical trials.

Update on thrombolytic therapy

Although dissolution of a thrombus with improved/extended time to treatment and reduced hemorrhagic risk profiles remains a very attractive opportunity to restore blood flow to the brain, several companies are pursuing second and third generation thrombolytics as new potential therapeutic interventions following acute stroke. Several newer generation thrombolytics are summarized in Table 2. Compounds such as monteplase, a third generation derivative of tPA [57,58] which is indicated for use in patients following acute myocardial infarction, does, however, have the same antigenicity as native tPA with similar if not worse bleeding risks, which may ultimately limit its therapeutic utility for cerebral ischemia. Recombinant *Desmodus rotundus* salivary plasminogen activator α-1 (rDSPAα-1/desmoteplase; PAION GmbH) is a plasminogen activator derived from vampire bat saliva. It has been expressed and produced in hamster ovary cells using recombinant technology and has exhibited potent fibrin-selective thrombolytic activity [59,60]. BB-10153 (TAPgen; British Biotech plc) is a genetically engineered thrombin-activatable form of plasminogen under development by British Biotech that is in phase II clinical trials for myocardial infarction [61], but has potential for the treatment of stroke [62]. However, as with other tPA activators/stimulants the risk of hemorrhagic bleeding is a major concern and therefore further work is required on this particular class of drugs.

Abbott had advanced pro-urokinase (A-74187, Prolyse) into phase III clinical trials for ischemic stroke. In a randomized, open-label clinical trial (PROACT II), 180 patients with an onset of stroke of less than 6 h were enrolled. Intra-arterial administration of pro-urokinase resulted in a 66% recanalization rate compared with 18% in the heparin-treated control group. However, intracranial hemorrhage and neurological deterioration occurred in 10% of patients treated with pro-urokinase compared with 2% in the heparin-treated control group. During phase II clinical trials (PROACT), treatment within 5.5 h resulted in significantly

better recanalization rates and no significant increases in hemorrhagic transformation or neurological deterioration (15.4 versus 7.1% placebo) [63]. The advantages of intra-arterial thrombolytic therapy for carotid and vertebrobasilar stroke is that it may result in more rapid clot lysis and higher recanalization rates compared with intravenous therapy [64]. Overall, it appears that tPA (0.9 mg/kg), administered within the 3 h period following the onset of stroke, remains the only FDA approved therapeutic option for patients with acute ischemic stroke. However, to maintain the benefit-to-risk profile of tPA, it is important to continue to adhere to strict time guidelines when instituting this therapy in patients [65]. No development of A-74187 has been reported since May 2003.

Antithrombotic and antiplatelet therapies

Since the restoration of blood flow to the brain is one of the major goals of acute stroke treatment, several companies are targeting various components of the coagulation cascade to improve efficacy and reduce risk. An alternative approach to acute stroke treatment has been the use of antithrombotic agents. To date, heparin and heparinoids administered 24 to 48 h after stroke have been thoroughly evaluated and have failed to provide significant neuroprotective benefits [8,66]. Due to the risk of intracranial hemorrhage following thrombolytic or anticoagulant therapies, more recent approaches have been directed at the GPIIb/IIIa fibrinogen-binding site [67•,68,69] or fibrinogen itself to reduce platelet aggregation with the potential for less deleterious effects on hemorrhage.

Although efficacious in stroke prevention [11,12], antiplatelet agents [70] have yet to demonstrate significant protection under acute stroke circumstances [8,71]. However, phase II trials with abciximab (c783, ReoPro), a mixed action therapeutic, have demonstrated encouraging results. Abciximab is a monoclonal antibody that blocks both the GPIIb/IIIa fibrinogen-binding site on platelets to prevent aggregation and inhibits the β2-integrin MAC-1. In a multinational phase II trial in 74 patients with acute ischemic stroke, treatment with abciximab resulted in minimal, or no residual disability in 35% of patients at 3 months compared with 20% for those on placebo. Even more encouraging was that 50% of abciximab recipients showed improved function in carrying out daily activities while none of these patients had any incidence of hemorrhagic complications from the treatment [72]. Based on these results, Johnson & Johnson/Eli Lilly are evaluating abciximab treatment in phase III trials in the US for acute stroke. Eli Lilly is also developing drotrecogin α (Xigris, Zovant), a fibrinogen antagonist (recombinant human activated protein C; rhAPC) that had undergone phase II clinical trials for stroke. Yamanouchi [73,74] has also advanced its GPIIb/IIIa antagonist YM-337 into phase II trials in both Europe and the US for the treatment of patients with acute ischemic stroke; no development has since been reported for this indication. An interesting difference between YM-337 and abciximab is that abciximab also inhibits MAC-1, which plays a role in leukocyte adhesion and activation and therefore may reduce acute inflammation, which may also contribute to the observed initial beneficial effects.

Experimental evidence has suggested that secondary cellular injury processes may be a realistic target for therapeutic intervention with the goal of inhibiting the progression of detrimental changes that normally follow ischemia to the brain. Preventing or reducing this delayed cellular injury alone may improve neurological recovery or facilitate brain tissue for future regenerative approaches that are only recently emerging.

Promoting functional neurobehavioral recovery: Regenerative approaches

Although initial brain inflammation can contribute to the degree of brain damage following injury, anti-inflammatory interventions to limit the degree of damage have been demonstrated to interfere with nerve regeneration and recovery [75]. Improved regeneration occurs in the central nervous system (CNS) associated with more marked inflammation [76] and activated macrophage and microglia facilitation of neuronal plasticity/recovery following injury is apparently associated with the secretion of neurotrophic factors. Therefore, the inflammation that does occur in response to injury in the CNS appears to serve multiple purposes. Novel strategies may be required for early intervention to reduce brain inflammation, injury and neurodegeneration, but certain aspects of the brain inflammatory process may need to be exaggerated/stimulated at later periods to facilitate repair/recovery/regeneration processes following CNS injury [77,78]. Clearly, more must be learned about these complex interactions. The timing(s) of specific intervention(s) may be critical to the development of significant neuroprotective anti-inflammatory therapy.

The relative timing of the dynamic changes that occur following stroke indicates an extension of events through repair and remodeling into neuronal plasticity and recovery of function [79,80] (see Figure 1). Of importance is the notion that brain inflammation spans all of these processes and can be expected to influence early injury and later post-injury repair processes. Our understanding of post-injury plasticity and functional recovery has been enhanced by neuro-imaging [81], which indicates that it is activity-dependent, influenced by training [82] and facilitated by growth factors [83].

Functional recovery frequently occurs spontaneously following CNS injury. Specifically, motor recovery from stroke is associated with adjacent cortical areas taking over the function of damaged areas, or the utilization and incorporation of alternative pathways to provide functional recovery, which can involve axonal regeneration, synaptogenesis and sprouting [84•]. Increased attention has focused around concepts such as 'rehabilitative pharmacology' after stroke that can be related to this new information (ie, on the ability of the CNS to functionally repair itself and a desire to understand how one can facilitate this process) [85].

Growing evidence indicates that exogenously administered neurotrophic growth factors can not only provide acute protection, but can also significantly enhance functional brain recovery following stroke [86]. A good example of this is basic fibroblast growth factor (bFGF). In preclinical studies, bFGF administered intravenously within hours post-stroke reduces infarct size [87-90]. However, if administered intracisternally even one day post-ischemia, although it does not affect infarct size, recovery of lost

sensorimotor function is increased [83,87,91,92•]. This regenerative effect is apparently due to increased neuronal sprouting and synapse formation on uninjured neurons, glial proliferation and new blood vessel growth in the stroke-injured brain [83,91]. bFGF is only one of many trophic factors that act as important signaling molecules that induce brain repair and functional reorganization following injury, thus promoting functional recovery from brain injury [92•,93-97]. Although recent trials evaluating bFGF in the clinic for acute protection were not successful [98], the delayed administration of bFGF as a regenerative-promoting agent might be expected to be successful.

In addition to neurotrophic growth factors, neural stem cells are now known to be present in normal brain, and have the potential to compensate and recover neuronal function(s) lost due to stroke [94,97]. Stem cells are also under the control of growth factors. Interestingly, even human bone marrow stromal cells administered intravenously provide significant neurological benefit in rats post-stroke [98]. Basic research into the control factors that regulate stem cell differentiation in brain injury and an increased understanding of the processes that inhibit axonal regeneration can be expected to provide important strategies for brain regeneration / recovery in the future [99,100].

It is important to emphasize that the evaluation of neurobehavioral functional recovery post-stroke will require well-defined neuroimaging strategies [81] and behavioral tests both experimentally and clinically [93,96,101,102]. Typically, only improvements on one of several scales of post-stroke independence and functionality are the primary endpoints used as an index of drug efficacy in clinical trials. One of the challenges in stroke is to identify which of the many neuro-imaging and neurophysiological changes (ie, in addition to sensitive behavioral evaluations) are important in mediating (ie, can also be used for careful monitoring of) drug-induced recovery of brain function following injury [84]. Table 3 lists some recent approaches to promote brain regeneration and neurobehavioral recovery post-stroke.

Table 3. Potential regenerative therapeutics.

Drug	Mechanism/technology	Comments	References
Phase II			
LBS-Neurons (Layton Bioscience Inc)	Unidentified.	LBS-Neurons are under development for transplantation to reverse brain damage caused by stroke. The cells originate from a human teratocarcinoma and have been treated with retinoic acid to form fully differentiated non-dividing neurons. This avoids the need to use fetal cells. In a clinical trial in 12 stroke patients with movement difficulties, LBS-Neurons transplanted into three sites within and around the damaged brain areas substantially improved movement in six patients.	[139]
SGS-111/DVD-111 (Saegis Pharmaceuticals Inc)	Unidentified.	DVD-111 is an orally-active, substituted proline-containing dipeptide with cognition-enhancing and neuroprotective effects, under development for cognitive disorders. Phase II trials have been completed for the treatment of mild cognitive impairment.	[140]
Preclinical			
Dimerized fibroblast growth factor (dFGF; Viacell Inc)	Nerve growth factor agonist.	Viacell is investigating cellular and molecular treatments for neurological diseases, including stroke, muscular dystrophy and amyotrophic lateral sclerosis.	[141]
AX-200/AX-201 (Axaron Bioscience AG)	Unidentified.	AX-200 is expected to enter phase II clinical trials towards the end of 2003. In addition to neuroprotective effects, both AX-200 and AX-201 improve functional performance after stroke and may act synergistically.	[142]
SeV-FGF2 (DNAVEC Research Inc)	Sendai virus (SeV) carrying the fibroblast growth factor (FGF).	DNAVEC is developing a gene therapy system using a SeV vector (hGDNF/SeV) for the treatment of stroke, which prevents delayed apoptosis in hippocampal CA1 pyramidal neurons.	[143]
Bcl-2 gene therapy (Selective Genetics Inc)	*Bcl-2* gene delivered using gene-activated matrices delivery technology.	Selective Genetics' gene therapy may have applications in the treatment of cardiac and peripheral ischemia, spinal cord and peripheral nerve injuries and stroke.	[144]
Neural stem cell therapy (ReNeuron Holdings plc)	Neural stem cell technology.	ReNeuron is developing undifferentiated (embryonic) neuroepithelial stem cell lines, for the treatment of neurological disorders, including stroke and Alzheimer's, Parkinson's and Huntington's diseases. In a rat model of ischemic stroke, cells from brain stem cell line MHP36 repaired sensory neglect and motor asymmetry, and significantly reduced brain lesion volume.	[145]
Axogenesis factor 1 (AF-1; Boston Life Sciences Inc)	Nerve growth factor agonist.	The CNS growth factor AF-1 is being developed as a potential treatment for stroke and spinal cord injuries.	[146]
Placental growth factor (PlGF; ThromboGenics Ltd)	Platelet growth factor.	Placental growth factor is under preclinical investigation by ThromboGenics as a potential stroke therapy.	[147]

Development status presented is for stroke indication.

Conclusions

Evidence accumulating during the last decade has shown that the CNS can mount a well-defined inflammatory reaction to a variety of insults, including trauma and ischemia. Certainly, the capacity of these insults to induce inflammation in the brain provides new areas for the discovery of novel therapeutic agents that could potentially confine the neuronal damage that follows ischemia and trauma. Other potential therapeutic targets should also focus on improving brain perfusion or collateral flow to the damaged area of the brain. Partial restoration of blood flow to the misery perfused areas of the brain may help to facilitate the development of acute neuroprotective or novel therapeutic regimes.

It is important that early acute neuronal protection strategies are not abandoned to work on 'hot topic' regenerative approaches such as stem cell research, as the ultimate determination of this success may depend on the extent of damage that occurs in the early stages following injury. Based on the disappointing results of several large-scale clinical trials evaluating the efficacy of novel neuroprotective agents for the treatment of acute stroke, it is evident that several development issues must be overcome before novel approaches can be considered a realistic therapy. Over the past few years, there have been several examples of compounds, primarily targeting excitotoxicity or ionic flux, which have failed in phase III clinical trials either due to safety concerns or lack of efficacy [34,36,103-109] that we can hopefully learn from. This lack of efficacy may be related to poor blood-brain barrier penetration and/or the very narrow therapeutic window that exists for this particular class of agents (ie, the targeting of early events). Taking into account these previous clinical trials and others, it is imperative to utilize these lessons learned to avoid similar pitfalls in an effort to help minimize future failures.

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