Apoptosis and Acute Brain Ischemia in Ischemic Stroke

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Abstract

Apoptosis may contribute to a significant proportion of neuron death following acute brain ischemia (ABI), but the underlying mechanisms are still not fully understood. Brain ischemia may lead to stroke, which is one of the main causes of long-term morbidity and mortality in both developed and developing countries. Therefore, stroke prevention and treatment is clinically important.

There are two important separate areas of the brain during ABI: the ischemic core and the ischemic penumbra. The ischemic core of the brain experiences a sudden reduction of blood flow, just minutes after ischemic attack with irreversible injury and subsequent cell death. On the other hand, apoptosis within the ischemic penumbra may occur after several hours or days, while necrosis starts in the first hours after the onset of ABI in the ischemic core. ABI is characterized by key molecular events that initiate apoptosis in many cells, such as overproduction of free radicals, Ca^{2+} overload and excitotoxicity. These changes in cellular homeostasis may trigger either necrosis or apoptosis, which often depends on cell type, cell age, and location in the brain. Apoptosis results in DNA fragmentation, degradation of cytoskeletal and nuclear proteins, cross-linking of proteins, formation of apoptotic bodies, expression of ligands for phagocytic cell receptors and finally uptake by phagocytic cells.

This review focuses on recent findings based on animal and human studies regarding the apoptotic mechanisms of neuronal death following ABI and the development of potential neuroprotective agents that reduce morbidity. The effects of statins on stroke prevention and treatment as well as on apoptotic mediators are also considered.

Key words: apoptosis, acute brain ischemia, stroke, caspase, death receptor, statins

List of abbreviations

ABI	Acute Brain Ischemia
AIP	APAF-1-Interacting Protein
Akt	Protein kinase B
APAF-1	Apoptotic Protease Activating Factor 1
APC	Activated Protein C
ATP	Adenosine Triphosphate
BAX	Bcl-2 associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra-large
BH-3	Bcl-2 homology (BH) domain 3
BID	BH-3 Interacting Domain
BIRC5 gene	Survivin gene polymorphism
Ca^{2+}	Calcium Ion
CAD	Caspase-Activated DNase
Cu,Zn-SOD	Superoxide dismutase
Cyt *c*	Cytochrome *c*
dATP	Deoxyadenosine Triphosphate
EPO	Erythropoietin
EPOR	EPO Receptor
ER	Endoplasmic Reticulum
ERK1/2	Extracellular signal-regulated kinases 1/2
Fas	Cell surface death receptor
FasL	Fas ligand
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
IAP	Inhibitor of Apoptosis

IMM-H004	3-piperazinylcoumarin
iNOS	inducible Nitric Oxide Synthase
JAK-2	Janus kinase 2
K^+	Potassium Ion
LC3-II	Microtubule-associated protein 1A/1B-Light Chain 3
MCAo	Middle Cerebral Artery occlusion
Na^+	Sodium Ion
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NMDA	N-methyl-D-aspartate receptor
nNOS	neuronal Nitric Oxide Synthase
NOXA	NADP Oxidase Activator
NSE	Neuron-Specific Enolase
p53	Tumor suppressor
PGK	Phosphoglycerate Kinase
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PUMA	p53-Upregulated Modulator of Apoptosis
TNFα	Tumor Necrosis Factor alpha
TRAIL	TNF-Related Inducing Ligand
XIAP	X-linked Inhibitor of Apoptosis Protein

I. Introduction

Brain ischemia may lead to stroke, which is one of the main causes of long-term morbidity and mortality for both developed and developing countries [1]. The risk of stroke is about 10% in the first year following ischemic attacks and atherosclerotic cerebral thrombotic events are responsible for 80% of the acute brain ischemia (ABI) cases [1-3]. Both primary and secondary stroke prevention is of major clinical importance [4,5]. In this context, best medical treatment including lifestyle measures, antihypertensive, antidiabetic, antiplatelet and hypolipidemic drugs should be implemented in both asymptomatic and symptomatic patients with carotid artery stenosis [6,7]. Surgical intervention is also useful in some of these patients [8]. Anticoagulant therapy is essential in patients with atrial fibrillation, whereas patients with transient ischemic attacks and minor strokes should be appropriately treated both with drug therapy and surgical interventions [9]. Targeting cardiovascular risk factors such as hypertension and diabetes may also affect cognitive function after a stroke [10].

Ischemic stroke is defined as a neurological disease that occurs due to decreased or blocked blood flow to brain tissues, usually caused by a thrombus or embolus occlusion or hemorrhage due to rupture of blood vessel, thus preventing adequate delivery of oxygen and glucose [11,12]. This nutrient restriction triggers an “ischemic cascade”, a series of pathophysiological events such as energy failure, excitotoxicity, oxidative stress, inflammation and apoptosis that finally lead to neuronal cell death [11,12]. All damaging factors are dependent on the duration, severity, and location of the ischemia within the brain [1]. Moreover, there are an increasing number of reports that apoptosis may contribute to a significant proportion of neuron death following ABI, but the underlying mechanisms are not fully understood [13]. The apoptotic process has been implicated in the pathogenesis of acute as well as chronic neurodegenerative diseases [14,15].

This review focuses on recent advances from animal and human studies in understanding the apoptotic mechanisms of neuronal death following ABI and the development of potential neuroprotective agents that can improve clinical practice especially in the field of anesthesiology and finally reduce morbidity rate.

II. Search strategy

We performed a comprehensive search of the scientific literature on the pathophysiology of ischemic stroke, experimental models of brain ischemia, drug development and preclinical studies and clinical trials in ischemic attacks. A literature search for relevant articles was conducted using PubMed, Web of Science, NIH Stroke Trials Registry [1972 - July 2016] and Google Scholar. The search terms for this narrative review were: brain ischemia, stroke, cell death, apoptosis, promoters and inhibitors of apoptosis.

III. ABI and the ischemic cascade

Previous ABI research revealed two important separate areas of the brain during ABI: the ischemic core and the ischemic penumbra [16]. The ischemic core of the brain exhibits sudden reduction of blood flow, just minutes after ischemic attack, experiencing irreversible injures and subsequently cell death [17]. Furthermore, the ischemic core exhibits reduction of oxygen and glucose, which have adverse effects on the structure and function of brain [16,18,19]. Energy and ion homeostasis breakdown, lipolysis, and proteolysis lead to cell membrane fragmentation in the ischemic core and neurons death within minutes after onset of ABI [16,20,21].

The ischemic cascade triggered in the penumbra results in progressive neuronal tissue injury after onset of ABI [16]. The ischemic penumbra is an area of less severely affected tissue that surrounds the core, exposed to moderately reduced blood flow, which makes the penumbra a functionally and electrically silent but metabolically active tissue zone [17]. However, after reperfusion, neurons within the penumbra may restore their membrane potentials [16,22].

ABI is followed by glucose and oxygen loss, metabolic changes and accumulating acidic by-products in brain tissue [1,23,24]. This series of events further leads to disruption of the mitochondria electron transport chain activity, which results in a rapid decrement of adenosine triphosphate (ATP) concentration [1,25,26]. The first event that occurs in ABI is failure of energy homeostasis and due to ATP loss causes disruption of ionic pumps resulting in a rise in intracellular sodium ion (Na^+), calcium ion (Ca^{2+}) and

intracellular potassium ion (K^+) concentrations [1,27,28]. Redistribution of these ions across plasma membrane causes neuronal depolarization, leading to excess release of neurotransmitters, especially glutamate [1,16,29,30]. Glutamate further induces neuronal overload of intracellular Ca^{2+} through over-activation of its receptors, which then triggers a number of deleterious consequences, such as free radicals generation, mitochondrial permeability transition activation and neuronal excitotoxicity [1,31,32]. These changes in cellular homeostasis may trigger either necrosis or apoptosis, which often depends on cell type, cell age and location in the brain [33]. The apoptosis within the ischemic penumbra may occur after several hours or days, while necrosis starts in the first hours after the onset of ABI in the ischemic core [16,17,19,34]. This indicates that neuronal and cellular death in the penumbra and in the core are temporally and spatially different phenomena [16]. Interestingly, markers of apoptosis and necrosis can be simultaneously present in the same cell after ABI, implicating that more than one death program may occur at the same time [34].

IV. Link between apoptosis and ABI: *Evidence from animal and human studies*

Animal studies: There is considerable evidence from animal studies indicating that brain ischemia triggers the extrinsic apoptotic signaling cascade. In rat brain within 12 h after an ischemic attack, the expression of tumor necrosis factor alpha (TNFα), TNF-related inducing ligand (TRAIL) and upregulation of cell death surface receptor Fas by Fas ligand (FasL), was reported [35]. This phenomenon culminates between 24 and 48 h, which coincides with ongoing apoptotic cell death in the brain [36,37]. Interestingly, evidence shows that TNF-α knockout mice and those expressing dysfunctional FasL have significantly decreased brain infarcts after middle cerebral artery occlusion (MCAo) [36,37]. Fas/FasL system acts as apoptosis inducer and triggers pro-inflammatory cytokine production, while the hematopoietic growth factor, erythropoietin (EPO) inhibits apoptosis and protects from ischemic neuronal damage [38,39]. These findings indicate that death receptors are critically engaged in the apoptosis induction after ischemia in the adult brain and that their suppression may improve the neuronal survival after ischemic injury [17,36].

The literature reports involvement of cytochrome *c* (cyt *c*) in the apoptosis initiation by its translocation to the cytoplasm after either ABI or permanent focal brain ischemia in mice [17,40]. Once cyt *c* is released from mitochondria it interacts with apoptotic protease activating factor (APAF-1) and deoxyadenosine triphosphate (dATP)/ATP, leading to the activation of caspase-9, which then activates caspase-3. Sugawara et al. [41] demonstrated increase of cleaved caspase-9 in rat brain 12 h after brain ischemia [17,41]. Caspase-3 and caspase-9 have a crucial role in apoptosis following ischemic stroke in animal models. Also, increased levels of cleaved caspase-3 and LC3-II was reported in the penumbra within 5 h after ischemia, and after that decreased at different rates, thus implying temporal differences in the activation status of the autophagic and apoptotic pathways and a possible new therapeutic approach [42]. Ischemic preconditioning in animals triggers activation of caspase-3 downstream and upstream of its target caspase-activated DNase (CAD) to prevent neuronal death [43]. Furthermore, enhanced formation of APAF-1/caspase-9 complex is observed in the rat hippocampus 8 to 24 h after ischemia [40,44]. Cao et al. [44] have cloned a rat gene product, a specific APAF-1 inhibitor of the APAF-1/caspase-9 pathway that can be neuroprotective in ABI [17,44]. Therefore, APAF-1 signaling pathway may be a legitimate therapeutic target for the treatment of neonatal ischemic brain injury [45]. Additionally, it is reported that different concentrations of normobaric oxygen can inhibit the apoptotic pathway by reducing caspase-3 and -9 expression, thereby promoting neurological functional recovery after ABI [46].

Evidence shows that p53, a tumor suppressor and transcription factor, has been implicated in the ischemic cascade and subsequent neuronal damage [47]. p53 is rapidly upregulated in ischemic brain tissue where it initiates apoptosis through the transcription of pro-apoptotic genes such as the BH-3 only proteins B-cell lymphoma 2 (Bcl-2) associated X protein (BAX), p53-upregulated modulator of apoptosis (PUMA), and NADPH oxidase activator (NOXA) [48]. This transcription factor mediates mitochondrial dysfunction and caspases activation [48]. Jeffers et al. [49] reported that PUMA is a principal mediator of apoptosis and that PUMA could be the key target for post-stroke therapy, implicating its role in tumor suppression, evidenced by impairment of apoptosis *via* p53 in PUMA knockout mice [49]. p53 is also a promising target

for stroke therapy due to findings that p53 knock-out transgenic mice experienced reduced brain vulnerability to brain ischemia. Heterozygous mice was better protected than homozygous mice for the p53 null gene, but greater protection was afforded by reduced expression of p53, which is consistent with some beneficial actions of p53 and some gene dose effect [50]. Additionally, p53 activates anti-apoptotic agents in a rat model of transient global ischemia, where apoptosis occurred in region I of hippocampus proper (CA1) neurons after the mitochondrial translocation of p53 and its interaction with Bcl-extra-large (Bcl-xL), resulting in cyt *c* release [51]. A study by Ji et al. [52] showed reduction of p53 activation as well as mitochondrial translocation of PUMA and NOXA during post-ischemic reperfusion in hypothermia treated rat brains [17,52]. If apoptogenic stimulus is removed, cells can be rescued from the apoptotic process because DNA repair is activated early in the p53-induced apoptotic process [17,53].

BH-3 interacting domain death agonist (BID) is recognized as a critical mediator of cell death during brain ischemia [17]. BID was cleaved *in vivo* 4 h after MCAO and BID knockout mice had a significant reduction of infraction and lower release of cyt *c* [54]. Levels of Bcl-2 and Bcl-xL were decreased during the first hours of brain ischemia [55]. Mice with overexpressed Bcl-2 or Bcl-xL have significantly smaller infarcts after focal brain ischemia, as opposed to Bcl-2 knockouts [56-58]. Kilic et al. [59] reported that EPO protects against focal brain ischemia *via* activating extracellular signal-regulated kinases 1/2 (ERK1/2) and protein kinase B (Akt) signaling pathway, using transgenic mouse that expresses elevated levels EPO [59]. The same authors showed elevated expression of Bcl-xL in ischemic brain, suggesting that ERK1/2, Akt, and Bcl-xL pathways mediate the neuroprotective function of EPO [59]. Additionally, protein kinase A (PKA) can phosphorylate Bcl-2-associated death promoter (BAD) and trigger its dissociation from Bcl-xL after onset of brain ischemia [59]. A study by Chu et al. suggests that IMM-H004, a 3-piperazinylcoumarin compound derived from coumarin, contribute to survivin expression through the activation of phosphoinositide 3-kinase (PI3K)-dependent Akt, which led to the phosphorylation of forkhead box O1, and relieved the inhibiting effect on survivin promoter. Additionally, IMM-H004 also enhanced the expression of hepatitis B X-interacting protein, which formed a complex with survivin to prevent the activation of the

caspase death cascade [60]. These data suggest that Akt, ERK1/2 and PKA pathways may inhibit BAD function as well as trigger pro-survival signaling pathways after ABI [61]. All these findings suggest the great importance of anti-apoptotic Bcl-2 family members in brain ischemia and its potential in neuroprotection against stroke. Findings from animal studies in ABI are summarized in **Table 1**.

Human studies: There are a restricted number of studies investigating apoptosis following ABI in human brain. Love et al. [62] reported limited expression of caspase-3 in neurons 24 h after ischemia, with the majority of labeling found in macrophages, concluding that apoptosis made little contribution to neuronal cell death [62]. However, this can be explained with significant delay of post mortem tissue collection. Askalan et al. [63] demonstrated that apoptosis delay occurs several days after ischemic attack in the human developing brain [63]. The same authors detected continued expression of caspase-3 more than 72 h after ischemic insult [64]. Askalan et al. [63] were the first to investigate the role of x-linked inhibitor of apoptosis protein (XIAP) in the human developing brain. In developing human brain, significant upregulation of XIAP expression in the penumbra during ABI has an inhibitory effect on caspase-dependent and -independent apoptosis [63,65,66]. Another member of the inhibitor of apoptosis (IAP) family, survivin, inhibits caspase activation, leading to negative regulation of apoptosis. Interestingly, survivin is completely repressed and undetectable in normal adult tissues while abundantly expressed in the human fetus and cancer cells [67]. Hoffman et al. [68] reported that p53 can trigger apoptosis by repression of anti-apoptotic genes, such as survivin [68]. Additionally, overexpressed survivin protects cells from p53-induced apoptosis in a dose-dependent manner, suggesting that loss of survivin mediates in p53-dependent apoptosis [67]. Polymorphism screening of the survivin gene polymorphisms (BIRC5 gene) showed association with hemorrhagic transformation, which is common and serious occurrence following ABI. It was also reported that -241 C/T polymorphism increases survivin promoter activity, reinforcing the hypothesis that patients with the mutant allele may have increased survivin expression in the brain [69]. An increasing amount of evidence indicates that apoptosis contributes to neuronal cell death after ABI, suggesting that targeting

physiologic and pharmacologic inhibitors of apoptosis could be an important part of therapeutic strategy after brain ischemia [63,65,67,68].

Endoplasmic reticulum (ER) stress and apoptosis take place around ischemia-affected brain tissues and potentially protects from necrosis-induced injury and cell death [70]. Duan et al. [70] showed temporal occurrence of elevated ER chaperone glucose-regulated protein and caspase-9 in ABI patients, suggesting that ER stress, apoptosis and the resulting DNA fragmentation are developed sequentially in ischemic brain [70]. Endoplasmic reticulum stress stimulates apoptosis following ABI beyond activating a defense for affected brain tissue [70].

Cheng et al. [71] examined the role of activated protein C (APC) in the pathophysiology of apoptosis following ABI, and showed that APC as a systemic anti-coagulant and anti-inflammatory factor plays an important role in brain protection after ischemic attacks [71]. Directly acting on brain cells, APC is involved in transcriptionally dependent p53 inhibition, normalization of the BAX/Bcl-2 ratio and reduction of caspase-3 signaling thereby preventing apoptosis in ischemic brain endothelium [71]. Additionally, the over-expression of Bcl-2 in the mitochondrial outer membrane prevents cytosolic redistribution of cyt *c* [40,72,73]. It is evident that apoptotic and necrotic cell loss, with predominate apoptotic neuronal changes in the infarct periphery are key sequel of ischemic attack [74]. FasL and EPO have opposite effects on apoptosis. Therefore, EPO/EPOR signaling is proposed as a possible cellular response for survival in less severely damaged areas of the brain after ischemic stroke [74]. Findings from human studies in ABI are summarized in **Table 2**.

V. Statins, stroke and apoptosis

Statins may reduce stroke incidence as well as improve stroke outcomes [75]. In this context, statins may contribute to collateral formation and infarct volume limitation, thus preserving the ischemic penumbra [76,77]. Physicians should reinforce the use of cardiovascular drugs including statins in stroke survivors after hospital discharge in order to minimize morbidity and mortality rates, especially in the elderly [78].

Statins have been reported to exert both pro- and anti-apoptotic properties [79]. It should be noted that the data on statin anti-apoptotic effects are fewer and refer to lower drug concentrations and normal cell lines, whereas statins induce apoptosis at higher doses and in cancer cells [79]. In this context, statins were found to upregulate the expression of certain caspases, downregulate the expression of survivin and destabilize p53 [80-82]. Furthermore, statins were reported to enhance apoptosis induced by TNF-α and Fas [83]. In contrast, statins inhibited endoplasmic reticulum (ER) stress-related apoptosis [84].

There is evidence that statins may act as neuro-anti-inflammatory drugs since they were shown to reduce the expression of inflammatory mediators (such as nitric oxide and TNF-α) in the central nervous system [85]. Furthermore, statins reduced the extent of brain damage and improved neurological outcomes following an ischemic stroke via anti-inflammatory and anti-oxidant effects [86]. Cognitive function may also be preserved due to statin treatment [87]. Of note, a recent meta-analysis found that statin use was associated with a significant reduction in the risk of Parkinson's disease [88]. Statins may also be protective of Alzheimer disease and incident dementia [89].

Other drugs may also affect apoptotic pathways as well as stroke prevention and outcomes but this topic is beyond the scope of this review.

VI. Conclusions

Apoptosis is critically involved in the pathophysiology of ABI and may contribute to a significant proportion of brain cell loss. In this review we consider recent animal and human studies which focused on investigating different apoptotic mechanisms during ABI, as well as anti-apoptotic agents and inhibitors of apoptosis that may contribute to the protection of ischemic brain tissue. An understanding of apoptotic mechanisms is essential to develop neuroprotective agents that limit neuronal damage after acute brain ischemia. Statins may reduce stroke incidence and improve stroke outcomes.

Conflict of interest

NK has given talks, attended conferences and participated in trials sponsored by Amgen, Angelini, Astra Zeneca, Boehringer Ingelheim, MSD, Novartis, Novo Nordisk and Sanofi. The other authors declare that they have no conflict of interest.

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Table legends

Table 1. Summarized findings from animal studies in acute brain ischemia

MCAo- Middle Cerebral Artery Occlusion; TNF-α- Tumor necrosis factor alpha; TRAIL- TNF-related apoptosis-inducing ligand; Fas- Cell surface death receptor; FasL- Fas ligand; Cyt c- Cytohrome *c*; EPO- Erythropoietin; Cu,Zn-SOD- Superoxide dismutase; LC3-II- Microtubule-associated protein 1A/1B-Light Chain 3; NMDA- N-methyl-D-aspartate receptor; APAF-1- Apoptotic Protease Activating Factor; AIP- APAF-1-Interacting Protein; GAPDH- Glyceraldehyde 3-phosphate dehydrogenase; PUMA- p53-Upregulated Modulator of Apoptosis; NOXA- Nicotinamide Adenine Dinucleotide Phosphate Oxidase Activator; NSE- Neuron-Specific Enolase; PGK- Phosphoglycerate Kinase; Bcl-2- B-cell lymphoma 2; BAX- Bcl-2 associated X protein; Bcl-xL- B-cell lymphoma-extra-large; BID- BH-3 Interacting Domain; EPO- Erythropoietin; JAK-2- Janus kinase 2; ERK1/2- Extracellular signal–regulated kinase 1/2; Akt- Protein kinase C; nNOS- neuronal Nitric Oxide Synthase; iNOS- inducible Nitric Oxide Synthase; IMM-H004- 3-piperazinylcoumarin; PI3K- Phosphoinositide 3-kinase.

Table 2. Summarized findings from human studies in acute brain ischemia

XIAP- X-linked Inhibitor of Apoptosis Protein; p53- Tumor suppressor; ER- Endoplasmic Reticulum; APC- Activated protein C; Bcl-2- B-cell lymphoma 2; BAX- Bcl-2 associated X protein; Fas- Cell surface death receptor; FasL- Fas ligand; EPO- Erythropoietin.

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Model	Animal model	Treatment	Effect	Reference
90 min MCAo	Rat, Mice (Fas-deficient)	Death-inducing ligands	Induction of TRAIL and TNF-α expression	36
3 h MCAo	Rat, Mice (Fas-deficient)	H_2O_2	Up-regulation of Fas and FasL expression	37
1 h MCAo	Rat	EPO	EPO inhibits apoptosis	39
Permanent focal brain ischemia	Mice	Caspase inhibitor	Attenuation of cyt *c* cytosolic release	40
Mild spinal cord injury	Rat	Cu,Zn-SOD	Activation of cyt *c* mitochondrial release and cleaved caspase-9	41
Permanent MCAo	Rat	Nylon monofilament	Increased levels of cleaved caspase-3 and LC3-II	42
Four-vessel occlusion	Rat	Ischemic preconditioning	Activation of caspase-3 downstream and upstream of its target caspase-activated DNase to prevent neuronal death	43
90 min Oxygen-glucose deprivation	Rat (primary cultures of hippocampal-cortical neurons)	Staurosporin or bleomycin, NMDA receptor antagonist MK801	AIP inhibition of the APAF-1-caspase-9 pathway by directly interacting with APAF-1	44
2 h Ischemic occlusion	Rat	Normobaric oxygen (33%, 45% or 61%)	Reduction of caspase-3 and -9 expression	46
90 min MCAo	Rat	GAPDH	p53 couples to GAPDH and the disruption of this interaction inhibits glutamate-induced cell death and ischemia induced neuronal damage	47
Gamma-irradiation	PUMA knockout mice	p53 and glucocorticoids	Impairment of apoptosis via p53 in PUMA	49
MCAo	Transgenic mice	/	Moderate expression of p53 is needed for maximal protection, while over-expression of p53 results in maximal pathology	50
Transient cerebral global ischemia	Rat	Pifithrin-α	Mitochondrial translocation of p53 and its interaction with Bcl-xL, resulting in cyt *c* release	51
2 h MCAo	Rat	Hypothermia	Reduction of p53 activation as well as mitochondrial translocation of PUMA and NOXA during post-ischemic reperfusion	52
MCAo	Mice and primary cultured mouse neurons	BID	Reduction of infraction and lower release of cyt *c* in BID knockout mice	54
MCAo	Mice	NSE or PGK promoters	Bcl-2 overexpression reduced neuronal loss and Bcl-XL genes reduce lesion size after MCAo	56-58
MCAo	Transgenic mice	Selective inhibitors of ERK1/2 or Akt	EPO induced an activation of JAK-2, ERK1/2, and Akt pathways is associated with elevated Bcl-XL and decreased nNOS and iNOS levels in neurons	59
Four-vessel occlusion	Rat	IMM-H004	Induce survivin expression through the activation of PI3K-dependent Akt. Prevention of caspase death cascade activation	60
1 h MCAo	Transgenic mice	Cu,Zn-SOD	Bad pathway is mediated by PKA. Overexpression of Cu,Zn-SOD may attenuate this apoptotic cell death	61

Table 2. Summarized findings from human studies in acute brain ischemia
XIAP- X-linked Inhibitor of Apoptosis Protein; p53- Tumor suppressor; ER- Endoplasmic Reticulum; APC- Activated protein C; Bcl-2- B-cell lymphoma 2; BAX- Bcl-2 associated X protein; Fas- Cell surface death receptor; FasL- Fas ligand; EPO- Erythropoietin.

Condition	**Tissue and cell type**	**Conclusions**	**Reference**
Focal cerebral ischemic infarct	Post mortem pediatric brain	Upregulation of XIAP expression has an inhibitory effect on caspase-dependent and -independent apoptosis	63,64,65,66
Adenovirus or adriamycin induced apoptosis	Fetus and cancer ovarian cells	Survivin gene expression is repressed by wild-type p53. Overexpressed survivin protects cells from p53-induced apoptosis	67
UV-C or adriamycin induced apoptosis	Melanoma, osteosarcoma and the human lung adenocarcinoma cells	Overexpressed survivin in cells sensitive to p53-dependent cell death markedly inhibits apoptosis induced by UV light	68
Hemispheric ischemic stroke	DNA isolated from peripheral blood sample	Survivin gene polymorphisms showed association with hemorrhagic transformation. -241 C/T polymorphism increases survivin promoter activity	69
Ischemic brain infarction	Post mortem brain tissue	ER stress, apoptosis and the resulting DNA fragmentation are developed sequentially in ischemic brain	70
Hypoxic brain injury	Brain endothelial cells	APC is involved in transcriptionally dependent p53 inhibition, normalization of the BAX/Bcl-2 ratio and reduction of caspase-3 signalling	71
Staurosporine or etoposide induced apoptosis	Human acute myeloid leukemia	Overexpression of Bcl-2 prevented the efflux of cyt *c* from the mitochondria and the initiation of apoptosis	73
Fatal ischaemic stroke	Post mortem brain tissue	Fas/FasL system acts as apoptosis inducer while increased EPO signalling may be a cellular response for survival in less severely damaged areas	74