Neuroprotection in Acute Stroke

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Abstract

Purpose: To highlight recent advances in neuroprotection in acute stroke. Data Sources: A MEDLINE search was conducted from January 1985 to November 2000. Key words included neuroprotection, cerebrovascular, subarachnoid haemorrhage, perioperative stroke, hypothermia and apopotosis. All articles in English were considered for review. Additional articles were identified from the references of the retrieved articles and cross-referencing selected articles. Data Extraction: All clinical studies and review articles and abstracts were reviewed. Data Synthesis: The neuronal cells of the central nervous system are susceptible to various forms of insult such as ischaemia and haemorrhage. Each step along the ischaemic cascade is a potential target for therapeutic intervention. Neuroprotective agents are designed to minimise cellular injury and salvage brain tissue. In cerebral ischaemia, only thrombolysis had been shown to improve clinical outcome. Neuroprotective therapy has definite benefits in animals but not in humans. It may potentially extend the time window for thrombolysis. In aneurysmal subarachnoid haemorrhage, the only agent with proven efficacy is nimodipine. Research is ongoing in the development of new drugs. Currently several phase III trials are in progress. Conclusion: There is substantial optimism in the development of neuroprotective therapy to improve outcome in stroke patients.

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Key words: Apoptosis, Cerebrovascular, Hypothermia, Perioperative stroke, Subarachnoid haemorrhage

Introduction

The neuronal cells of the central nervous system are especially susceptible to various forms of insult such as trauma and ischaemia. Once the ischaemic cascade is set into motion by the initiating injury, the resultant damage is traditionally considered to be unavoidable, untreatable and permanent. Because ischaemia is clearly a process and not an instantaneous event, the potential exists for modifying the process after the clinical ictus and altering the final outcome. Arterial occlusion, either from artery-to-artery embolism or _in situ_ thrombosis, produces a central core of necrosis surrounded by an ischaemic penumbra. The penumbra is an area in which metabolic activity is suppressed but destruction not inevitable. In any individual patient with acute cerebral occlusion, it is difficult to be certain how large the ischaemic penumbra is likely to be, how long it is likely to remain in this state, how important it is functionally and how much recovery is likely if flow is restored. One of the main reasons is that accurate information about the ischaemic penumbra can only be obtained from functional neuroimaging.[1,2] Nonetheless, recognition of the penumbra in which ischaemic damage may be prevented has prompted treatments to minimise or even reverse cellular injury, especially if started within a short period of time after occlusion. This therapeutic window is divided into 2 partly overlapping components—the reperfusion window and the neuroprotection window. Restoration of blood flow during the reperfusion window limits the extent of neuronal injury and forms the underlying rationale for thrombolysis. Since the 1970s numerous trials on thrombolysis had been carried out. All trials showed no benefit until the publication of the National Institute of Neurological Disorders and Stroke (NINDS) rt-PA Study in 1995, which showed an improved functional outcome at 3 months despite a higher rate of symptomatic haemorrhage.[3] In the original NINDS trial, patients were deemed suitable for thrombolysis if they presented within 3 hours of stroke onset and computed tomographic (CT) scan of the head did not show any haemorrhage. Advances in neuroimaging [diffusion-weighted imaging (DWI) and perfusion-imaging (PI)] in recent years had revolutionised the concept of a rigid 3-hour window.[4] Both DWI and PI have improved acute stroke diagnosis and may impact on patient selection for thrombolysis and neuroprotective therapy (Table I).[5] The neuroprotection window is currently the subject of immense laboratory and clinical research. Ideally, if a

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Pattern no.	Acute PI/DWI infarct patterns	Potential acute stroke therapy
1	PI>DWI	Reperfusion therapy (e.g. thrombolysis)
2	PI=DWI	Neuroprotective therapy
3	PI<DWI	Neuroprotective therapy
4	DWI lesion only	Neuroprotective therapy
5	PI deficit only	Reperfusion therapy
6	Neither PI nor DWI	No acute intervention

DWI: diffusion-weighted imaging; PI: perfusion imaging

* These hypotheses require further investigation in prospective clinical trials.

neuroprotectant is to work, it must be given before or at the onset of injury. When started after the onset of ischaemia but before reperfusion, neuroprotective therapy can augment the beneficial effect of reperfusion and may even extend the time window for thrombolysis. A prerequisite to the development of neuroprotective therapy is an understanding of the ischaemic cascade.

Ischaemic Cascade

A major accomplishment of *in vivo* and *in vitro* model systems of cerebral ischaemia is an understanding of the ischaemic cascade.[6,7] Each step along this cascade might be a target for therapeutic intervention. Several variables exist that may affect the cascade and subsequently the outcome, most importantly the degree of blood flow reduction, its duration, distribution (global or focal) and extent of reperfusion. Nonetheless, many steps along the cascade seem to follow a fairly predictable pattern.

First, there is reduction of blood flow, followed rapidly by inhibition of protein synthesis, depletion of intracellular energy stores, membrane depolarisation, and release of extracellular potassium. This is accompanied by an initial increase in oxygen extraction and glucose metabolism and lactic acidosis. Membrane depolarisation causes opening of voltage-gated calcium channels, allowing disruption of tightly regulated neuronal calcium homeostasis. Glutamate is released from pre-synaptic stores, and in the presence of glycine activates the N-methyl-D-aspartate (NMDA) receptor. The immediate consequence is increased sodium permeability and cellular swelling, but the more damaging event is further elevation of intracellular calcium through the NMDA-associated ion channel. Further perturbations in ion flux occur as a result of glutamate's effect on the -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and metabotropic receptors. Documented increases in other neurotransmitters such as gamma-

aminobutyric acid (GABA), dopamine and norepinephrine may also be damaging.

Increased intracellular calcium activates a large number of damaging enzymatic pathways. Calcium (through calmodulin) activates protein kinases such as Cam-kinases II and protein kinase C, which may imbalance neuronal homeostasis by causing protein phosphorylation. Other pivotal enzyme systems affected by calcium include the proteases, such as calpain, which causes cytoskeletal proteolysis; lipases such as phospholipase A, which leads to production of arachidonic acid and free radical formation; phospholipase C, which results in release of intracellular calcium stores; and nitric oxide synthase (NOS), resulting in increased nitric acid (NO). The consequences of free radical production and these enzyme perturbations are widespread, including disruption of neuronal and endothelial membrane and cytoskeletal integrity and damage to mitochondrial function.

Increased gene expression in ischaemic regions, possibly induced by spreading depolarisations after ischaemia, has many damaging consequences. Cytokines such as tumour necrosis factor (TNF) and interleukin-1 (IL-1) result in tissue inflammation. Adhesion molecules such as intercellular adhesion molecule (ICAM)-1 result in white blood cell interaction with vascular endothelium to produce blood brain barrier damage, and may also plug up the microcirculation. Growth factors such as nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) may result in increased production of NO, and had also been reported to be protective when exogenously administered.

Reperfusion Injury

A growing body of evidence, primarily from animal models of cerebral ischaemia and preliminary human studies, indicates that inflammatory mechanisms contribute to secondary neuronal injury after acute cerebral ischaemia.[8,9] Ischaemia followed by reperfusion rapidly leads to the expression of inflammatory cytokines, particularly TNF. These stimulate a complex cascade of events involving local endothelial cells, neurons, astrocytes, and perivascular cells. A secondary response includes the release of other cytokines and an increase in components of the coagulation system. Up-regulation of cell adhesion molecule expression and changes in expression of the components of the immune response occur. The net effect of these events is transformation of the local endothelium to a prothrombotic/proinflammatory state. Leukocytes accumulate in the injured region and cause tissue injury by several mechanisms: occlusion of microvasculature, generation of oxygen free radicals, release of cytotoxic enzymes, alteration of vasomotor reactivity and increase in

cytokine and chemoattractant release. Recent experimental studies showed greater histological damage particularly in the cortex after temporary middle cerebral artery (MCA) occlusion lasting 3 hours compared to permanent MCA occlusion. The effect of neuroprotective therapies on reperfusion injury deserves greater attention.[10]

Clinical Status of Neuroprotective Therapy

Neuroprotective therapy is directed at these biochemical events that occur consequent to arterial occlusion. Numerous preclinical studies in animal models of global and focal ischaemia had shown efficacy by targeting each of the steps along this ischaemic cascade. Representative studies are shown in Table II to summarise the recent history of clinical research in neuroprotective therapy.[11]

Neuroprotective Agents in Acute Ischaemic Stroke

Calcium Antagonists

The first practical pharmacologic agent to be clinically evaluated for neuroprotection in cerebral ischaemia was the calcium antagonist. As ischaemia is associated with increased intracellular calcium ions, inhibiting this increase could protect neurones and is thought to reduce neurological impairment and disability. The most extensively evaluated calcium channel antagonist is nimodipine, a dihydropyridine calcium channel antagonist. Oral nimodipine had been investigated in ischaemic stroke in several randomised trials.[12-14] These studies enrolled patients with time windows ranging from 12 to 48 hours, used nimodipine doses between 60 to 240 mg/day, and treated for periods of 14 to 28 days. Early trials revealed positive results with reduction in mortality and improved neurological outcome at 3 months.[12] Nearly every clinical trial since then had not demonstrated benefit from nimodipine or any other calcium antagonist.[13-15] The Intravenous Nimodipine West European Stroke Trial (INWEST) showed a better outcome in the placebo-treated patients, a finding attributed to hypotension induced by the drug.[15] In summary, calcium antagonists have no role in acute ischaemic stroke.

Glutamate Antagonists

NMDA antagonists were the first class of acute stroke therapeutic agents to proceed from development in the laboratory to testing in humans employing modern principles of clinical trial design and, most importantly, relatively early treatment. The potential utility of NMDA

TABLE II: REPRESENTATIVE NEUROPROTECTIVE AGENTS IN ADVANCED CLINICAL TRIALS

Drug	Proposed mechanism of action	Dosing time and route*	Status of phase III trial
Lubeluzole	Inhibits NO synthesis	6-8 hours/IV	US trial encouraging but European trial negative; third trial terminated prematurely
Enlimomab	Antibody neutralisation of ICAM	6 hours/IV	Negative
Citicoline	Phospholipid precursor or free radical scavenger	24 hours/PO	First trial inconclusive and second trial negative
Fosphenytoin	Membrane stabiliser	6 hours/IV	Terminated prematurely
Aptiganel	NMDA antagonist	6 hours/IV	Terminated prematurely
Eliprodil	NMDA antagonist	6 hours/IV	Terminated prematurely
CGS 19755 (Selfotel)	NMDA antagonist	6 hours/IV	Terminated prematurely
Nalmefene (Cervene)	Opiate antagonist	6 hours/IV	Unknown
Tirilizad	Free radical scavenger	6 hours/IV	Negative
Nimodipine	Calcium antagonist	24-48 hours/IV	Negative
GM ganglioside	Ganglioside	?	Negative
Basic fibroblast growth factor	Promoter of neuronal growth and differentiation	6 hours/IV	Terminated prematurely
Clomethiazole	GABA agonist	12 hours/IV	Ongoing
GVS150526	Glycine antagonist	6 hours/IV	International trial negative; enrollment in US trial complete and data analysis underway
BMS-204352	Potassium channel modulator	6 hours/IV	Ongoing
NXY059	Spin trap agent	NA	Proposed

GABA: gamma-aminobutyric acid; ICAM: intercellular adhesion molecule; NA: not available; NMDA: N-methyl-D-aspartate; NO: nitric oxide

* Time and route of drug administration after onset of stroke symptoms

antagonists in stroke was first recognised when it was observed that a hypoxic or ischaemic insult results in elevation of brain levels of excitatory neurotransmitter glutamate.[6] Excessive stimulation of postsynaptic NMDA receptors allows entry of calcium and sodium ions into the cell leading to excitotoxic neuronal death. NMDA receptor activation requires concomitant membrane depolarisation and activation by glycine. Antagonists of the NMDA receptor are furthest advanced in clinical development for stroke. Competitive NMDA antagonists (selfotel, d-CPPene) bind directly to the glutamate site of the NMDA receptor to inhibit the action of glutamate. Phase IIa data on selfotel proved promising.[16] Two phase III trials followed but were terminated after 509 patients were enrolled: the mortality at termination was 24% in the treatment group and 19% in the placebo group ($P = 0.15$). Mortality attributed to brain injury was significantly greater in the treatment group (13%) compared with the placebo group (5%).[17] Non-competitive antagonists (dextrorphan, aptiganel hydrochloride) block the NMDA-associated ion channel in a use-dependent manner. Dextrorphan appeared well tolerated in a phase I/II study,[18] but a subsequent phase III trial was terminated because of adverse side effects. These include hypotension during loading, agitation, confusion and hallucinations.[19] Aptiganel hydrochloride (cerestat) is currently undergoing clinical evaluation after preliminary results suggest possible efficacy.[20] It appears to have less severe neuropsychiatric side effects than solfotel or dextrorphan.[21]

Other modulatory sites on the NMDA receptor complex, notably the polyamine and magnesium ion sites, are also the subject of clinical trials. Glycine site antagonists are in the clinical development stage. A recent study of glycine antagonists (gavestinel) failed to show any benefit.[22,23] Magnesium sulfate was found to be safe, improved functional outcome and slightly reduced mortality in a small study.[24,25] A multicentre phase III study recruiting patients is currently underway. Non-NMDA glutamate receptor antagonists remain in preclinical study. Other glutamate release blockers such as lamotrigine and riluzole may have neuroprotective potential and require further evaluation.[26]

Lubeluzole

Lubeluzole is a sodium-channel blocker which inhibits the release of glutamate from ischaemic neurons, thus reducing postsynaptic excitotoxicity.[27] It may also inhibit postsynaptic nitric-oxide-synthetase activity, thereby reducing intracellular ischaemic damage. It was found to be effective in animal models without apparent neuropsychiatric side effects. A phase II trial in Europe[28] and a phase III trial in America[29] showed positive clinical effects; yet a confirmatory phase III trial in Europe had

completely negative results.[30] Use of this drug must be delayed until there is further evidence of its efficacy.

Piracetam

The neuroprotective properties of the nootropic agent piracetam together with reported haemorrheologic and antithrombotic effects provided the rationale for the evaluation of piracetam in acute stroke. Pilot studies showed an increase in compromised regional cerebral blood flow and improvement in motor function, aphasia and level of consciousness.[31] The Piracetam in Acute Stroke Study[32] showed no difference in functional outcome and mortality between the placebo and piracetam groups. Post hoc analysis in the early treatment subgroup showed improved neurologic outcome ($P = 0.07$), better functional recovery ($P = 0.02$) and a greater recovery rate from aphasia ($P = 0.02$).[33] This was particularly so in patients with moderate and severe strokes. Available evidence at this point in time does not support routine administration of piracetam in acute ischaemic stroke.[34] Piracetam had been shown to be beneficial in recovery from aphasia and improvement of cerebral blood flow.[31,35] PASS II is currently in progress; its primary aim is to evaluate the effect of piracetam on aphasia.

Other Agents for Acute Ischaemic Stroke

Treatment with anti-ICAM-1 antibody reduces infarct volumes and neurological deficits after embolic stroke in animal models.[36] However, this treatment failed to show benefit in the Enlimomab Acute Stroke Trial.[37] An increased understanding of inflammation and immunologic mechanisms offers great potential for decreasing acute stroke injury.[8]

Citicoline (CDP-choline) is a key intermediary in the biosynthesis of phosphatidylcholine, an important component of the neural cell membrane. It alters phospholipid metabolism and prevents membrane breakdown into free radical-generating lipid by-products. Animal studies showed beneficial results when administered singly or in combination with thrombolytic agents.[38] A phase IIb trial of citicoline administered within 24 hours of stroke suggested efficacy[39] but a phase III trial[40] failed to confirm this effect.

Tirilazad is a non-glucocorticoid 21-aminosteroid that inhibits lipid peroxidation. Studies in experimental models of ischaemic stroke suggested it had neuroprotective properties.[41] However, human studies failed to show any benefit.[42] A systematic review showed a just-significant increase in death and disability as assessed by either the Barthel Index or Glasgow Outcome Scale.[43]

Chlormethiazole potentiates GABA function and decreases glutamatergic activity in the brain. Since increased

glutamatergic activity is the major primary event that results in cell death, GABAmimetic drugs might be expected to be neuroprotective. A phase II study[44] showed that chlormethiazole is safe and practical but a phase III trial [chlormethiazole acute stroke study (CLASS)][45] failed to show any benefit. Vigabatrin, another GABAmimetic drug, protects against kainic acid-induced neuronal damage in the rat hippocampus and may be neuroprotective.[46] Lamotrigine inhibits glutamate release and had beneficial effects in animal models of cerebral ischaemia.[26]

GM-1 ganglioside had been shown to reduce ischaemic damage in animal models of cerebral ischaemia.[47] However, two large trials in humans [Early Stroke Trial (EST)[48] and the Syngen Acute Stroke Study (SASS)][49] did not show improved outcome with GM-1 treatment. There was a non-significant trend towards greater patient recovery in the EST.

Aneurysmal Subarachnoid Haemorrhage (SAH)

Following aneurysm rupture, approximately two-thirds of cases will develop angiographic vasospasm. Fifty per cent of cases will develop symptoms and signs of delayed ischaemic neurological deficits. The onset of vasospasm following aneurysmal SAH usually begins on day 3 or 4 and peak between day 7 and day 11.[50] The delay in onset of ischaemia offers an opportunity not seen in other stroke types for the administration of putative neuroprotective agents before the actual cessation/reduction in cerebral blood flow.

Neuroprotective Agents for SAH

Calcium Antagonists

Nimodipine is the best studied neuroprotective agent in aneurysmal SAH. Several randomised controlled trials proved that it reduced the proportion of patients with CT scan evidence of infarction, poor outcome and ischaemic neurological deficits after aneurysmal SAH.[51-53] There was no difference in angiographic vasospasm between the nimodipine group and control group.[51] The exact mechanism by which nimodipine reduces delayed ischaemic neurological deficits and infarction remains unknown.

AT877 (or fasudil hydrochloride), a calcium antagonist, was reported in one randomised trial to reduce angiographic and symptomatic vasospasm following aneurysmal SAH.[54] The AT877 group also had significantly less hypodensities on CT scan and poor outcome compared to the placebo group.

Nicardipine, another dihydropyridine calcium antagonist, was studied in phase III trials and was associated with a reduced incidence of symptomatic vasospasm in patients with recent aneurysmal SAH. However, it was not associated with an improvement in overall outcome at 3 months when compared to standard management.[55]

Tirilazad

This agent had been evaluated in a multicentre randomised controlled trial in Europe, Australia, New Zealand and North America. In the study conducted in Europe, Australia and New Zealand,[56] patients receiving 6 mg/kg per day of tirilazad had reduced mortality and a greater frequency of good recovery on the Glasgow Outcome Scale 3 months after SAH than similar patients treated with vehicle. The benefits of treatment with tirilazad were predominantly shown in men rather than in women. In the North American setting,[57] tirilazad mesylate at dosage levels of up to 6 mg/kg per day for 8 to 10 days following SAH did not improve the overall outcome in patients with aneurysmal SAH. The investigators postulate that the different outcomes may be a result of differences in admission characteristics of the patients and/or differences in management protocols, including the use of anticonvulsants. The observed differential benefits which predominate in the male patients were attributed to gender-related pharmacokinetic differences. In 1999, the results of a randomised vehicle controlled trial in women using higher dose (15 mg/kg/day) tirilazad were published. In the study in Europe, Australia, New Zealand and South America,[58] there was no difference in the mortality rates and overall outcome (assessed using the Glasgow Outcome Scale at 3 months post-SAH) between the two groups despite a significantly lower incidence of delayed cerebral ischaemia in patients given tirilazad. In the North American study,[59] there was no difference in mortality for the entire group. However, sequential analysis revealed a statistically significant difference in mortality rates, favouring the study drug, among patients who were neurological Grade IV or V at admission.

Ebselen (2-phenyl-1,2-benzisoselenazol-3[2H]-one)

This is a lipid soluble seleno-organic compound that potently inhibits lipid peroxidation through a glutathione peroxidase-like action. A multicentre phase III trial was carried out in Japan.[60] There was no significant difference in the overall outcome and no difference in the incidence of delayed neurological deficits. However, in patients who developed delayed ischaemic neurological deficits, the ebselen group had significantly better outcome compared to placebo.

Neuroprotective Agents for Perioperative Cerebrovascular Events

Cerebral ischaemic insults may occur during temporary vessel occlusion for neurosurgical procedures or cardiopulmonary bypass. The nature of these interventions allows initiation of neuroprotective strategies prior to onset

of ischaemia, which in laboratory conditions often produce superior results compared to administration of neuroprotective agents after the insults.

Hypothermia

Hypothermia for cerebral protection was first described in the 1950s. In laboratory models of brain ischaemia, mild to moderate hypothermia had been repeatedly shown to reduce neurological injuries.[61] In 1997, Marion and colleagues[62] reported that treatment with moderate hypothermia for 24 hours in patients with severe traumatic brain injury and coma scores of 5 to 7 on admission hastened neurologic recovery and may have an improved outcome. A recent meta-analysis of randomised trials in traumatic brain injury suggests a strong positive effect of therapeutic hypothermia.[63] In ischaemic stroke, mortality was lower among patients with hypothermia and higher among patients with hyperthermia.[64] However, there is currently no evidence from randomised trials to support the routine use of physical or chemical cooling therapy in acute stroke.[65]

A randomised prospective pilot trial of mild hypothermia as a protective therapy during intracranial aneurysm surgery was published in 1999.[61] In this study, patients with SAH randomised to the hypothermic group had a lower frequency of deterioration at 24 and 72 hours after surgery, a greater frequency of discharge to home, and a greater incidence of good long-term outcome (although the differences did not reach statistical significance). In a separate randomised study, mild hypothermia during aneurysm surgery was found to ameliorate cerebral blood flow impairment in patients with SAH.[66]

Hypothermic circulatory arrest is sometimes used in surgery for complex intracranial giant aneurysms.[67,68] In humans, the ratio of metabolic rates at temperatures 10°C apart was found to be 2.3.[69] The cerebral metabolic rate was 17% of baseline at 15°C. If one assumes that cerebral blood flow can safely be interrupted for 5 min at 37°C, and that cerebral metabolic suppression accounts for the protective effects of hypothermia, the predicted safe duration of hypothermic circulatory arrest at 15°C is about 29 minutes. Deep hypothermia is absolutely crucial for brain protection during this period of circulatory arrest. The morbidity and mortality remains significant at 13.3% and 8.3%, respectively.[67]

General Anaesthetics

Clinically used general anaesthetics are consistently neuroprotective in rodent models of focal cerebral ischaemia. In clinical practice, however, the neuroprotective effect of individual anaesthetics remains controversial. Thiopental, at a dose sufficient to achieve EEG silence,

was shown to significantly reduce neuropsychiatric complications following cardiopulmonary bypass in a randomised trial.[70] In patients who survive cardiac arrest, administration of thiopental was found to have no effect on the neurological outcome.[71]

In a multicentre randomised study, electro-encephalographic burst suppression with propofol during cardiac valve replacement did not significantly reduce the incidence or severity of neurologic or neuropsychologic dysfunction.[72] Etomidate, which has been used in small surgical series as a neuroprotective drug,[73] had not been evaluated in randomised trials.

Other Agents

Several putative neuroprotective agents had been studied in randomised trials for their effects on neurological outcome following cardiopulmonary bypass. GM-1 ganglioside was evaluated in a randomised placebo trial involving 29 patients. It did not lead to a favourable neurological outcome score in patients undergoing cardiac surgery.[74]

Remacemide, an NMDA receptor antagonist, was studied in a randomised, placebo-controlled trial in 171 patients who underwent cardiopulmonary bypass. The proportion of patients showing a decline in neuropsychiatric performance 8 weeks after the procedure was reduced in the treated group. This was not statistically significant. On the other hand, the overall postoperative change (reflecting learning ability in addition to reduced deficits) was more favourable in the remacemide group, which demonstrated statistically significant greater improvement.[75]

Neuroprotection and Apoptosis

Transient global ischaemia results in delayed neuronal death in selectively vulnerable brain regions such as the hippocampus.[76] Recent studies suggest that cell death in this setting involves apoptosis, an active and genetically controlled cell suicide process. Histological and biochemical characteristics of apoptosis are present in dying neurons after ischaemia,[77] and inhibition of new protein synthesis protects CA1 neurons after ischaemia.[78]

Several apoptosis regulatory genes are found to be induced in ischaemic cells. Bax, a bcl-2 homolog which is proapoptotic, is up-regulated in neurons destined to die after global ischaemia.[79] On the other hand, apoptosis suppressor gene bcl-2 is expressed in neurons that survive ischaemia.[80] Bcl-2 overexpression in rodent brain was shown to reduce ischaemic injury, and transgenic mice with overexpression of neuronal Bcl2 mitigates selective neuronal vulnerability in the hippocampus after transient global ischaemia.[81]

Cysteine proteases called caspases are mammalian homologs of the ced-3 gene product and are essential for

the execution step in apoptosis. All caspases are synthesised as proenzymes activated by proteolytic cleavage. Caspase activity had been shown to play an important role in ischaemic cell death, and caspase inhibitors had been shown to reduce cell deaths following ischaemia.[82] Ventricular infusion of Z-DEVD-FMK, a caspase-3 inhibitor, decreased caspase-3 activity in the hippocampus and significantly reduced cell death after transient global ischaemia in rat models.[82] Intracerebroventricular administration of caspase 1 inhibitor Ac-YVAD.cmk significantly reduced infarct volume in rat middle cerebral artery occlusion model.[83] These observations in laboratory settings have obvious clinical implications and antiapoptotic strategies may one day become a practical therapeutic approach for stroke.

Ethical Issues in Trials of Neuroprotective Agents for Stroke Therapy

From the above discussions it can be seen that considerable progress had been made in recent years in unravelling the first layers of the complexities of well-designed clinical trials in stroke. However with this progress, comes the realisation that the puzzle is more complex than initially expected.[84] Precisely why the numerous neuroprotective agents and strategies appeared quite effective in animal studies but failed to have proven benefits in human is unclear.[85] The plethora of failed clinical trials with neuroprotective agents for acute ischaemic stroke have raised justifiable concerns about how best to proceed for the future development of such interventions. The combination of acutely ill and vulnerable stroke patients, the use of potentially toxic drugs, and very short time frames for decision making and drug administration demand an especially careful evaluation of risk versus benefit ratios of the putative neuroprotectants. Serious life threatening disease such as stroke warrants taking some risks, especially when there is potential benefits for the patients participating in the trials. Guidelines and recommendations for standards regarding preclinical testing of neuroprotective drugs have been formulated by the Stroke Therapy Academic Industry Round Table.[85] As an alternative to slowing down the already difficult and complex process of developing better stroke treatment, scientists, clinicians and ethicists should continue to co-operate to find better protection for the patients who participate in clinical trials. Much better treatments for stroke are needed, and continued clinical investigations are the only way to develop these improvements.

Conclusion

In summary, there is currently substantial optimism in the development of new neuroprotective agents to improve outcome of stroke patients. The future holds great promises as ongoing laboratory and clinical research continues to unravel new neuroprotectants. Scientists, clinicians and ethicists must pay careful attention to and be able to critically assess preclinical studies and clinical trials to ultimately improve treatment for acute stroke.

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