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TeaserAs stroke rates are increasing neuroprotection becomes of paramount importance.

Foundation review: Current therapies in ischemic stroke. Part B. Future candidates in stroke therapy and experimental studies

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Stroke still remains a major healthcare problem. The growing understanding of the mechanism of cell death in ischemia leads to new approaches in stroke treatment. The aim of neuroprotection is to reduce the post-stroke impairment and the overall costs that are accompanied in patients with severe disability. Despite encouraging data from experimental animal models, almost all neuroprotective therapies have, to date, not been established in clinical routine. In this part B of our review on stroke therapies we provide an overview on future candidates in stroke therapy and neuroprotective agents that are under investigation.

Stroke remains a major healthcare problem. Its impact on human and economic toll is tremendous. According to the World Health Organization (WHO), 15 million people suffer stroke worldwide each year. Of these, 5 million die and another 5 million are permanently disabled. Each year 795 000 people in the USA experience a new or recurrent stroke. Approximately 610 000 of these are first attacks, and 185 000 are recurrent attacks [1].

On the basis of the WHO stroke findings and the population projections from the United Nations the calculated expected number of new strokes that will occur during the period 2000–2025 in Europe have been estimated. Even with stable stroke incidence rates there will be a marked increase in the number of stroke events from approximately 1.1 million per year in 2000 to 1.5 million per year in 2025 in Europe. These numbers strongly advocate for intensified prevention of stroke [2] and highlight how import primary and secondary stroke prevention are independent from acute stroke therapy (we have discussed these topics in part A of our review). According to these facts, neuroprotection is of paramount importance. The challenge is to reduce the burden is stoke for patients at high risk of stroke or after stroke occurrence. Despite successful neuroprotective therapy in animal models of stroke, the translation of

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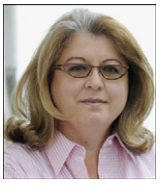

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TABLE 1

Overview of common stroke assessment scales [100–103].

Scale	Description	Range	Favorable score[a]	What the scores mean
National Institutes of Health Stroke Scale (NIHSS)	A serial measure of neurologic deficit on a 42-point scale across 11 categories – including paralysis, speech difficulty, and sensory and visual loss	0–42 (the lower the score, the better the performance)	≤1	0 = typically normal function without neurologic deficit 1 = for example, mild facial paralysis ≥22 = for example, severe stroke symptoms 25 = for example, complete right hemiplegia with aphasia, gaze deviation, visual deficit, dysarthria, and sensory loss
Barthel Index	Measures the ability to perform activities of daily living – for example, eating, bathing, walking, and using the toilet	0–100 (the higher the score, the better the performance)	95 or 100	100 = able to perform all activities of daily living with complete independence
Modified Rankin Scale	A simplified overall assessment of function	0–5 (the lower the score, the better the performance)	0 or 1	0 = absence of symptoms 5 = severe disability
Glasgow Outcome Scale	A global assessment of function – from good to vegetative state and death	0–5 (the lower the score, the better the outcome)	1	1 = good recovery 2 = moderate disability 3 = severe disability 4 = survival, but in a vegetative state 5 = death

[a] Favorable scores are associated with either normal or near-normal status.

neuroprotective benefits from bench to bedside has not yet been successful (Table 1, Box 1 and Figs 1,2).

Brain self-repair by neuronal replacement from endogenous precursors is insufficient and functional recovery remains incomplete. Amplification of this self-repair mechanism could be a promising strategy in stroke therapy [3]. Further new agents mentioned in the text are under investigation with the aim to achieve neuroprotection (Tables 2 and 3). In this part B of our review on stroke therapies, we provide an overview on future candidates in acute stroke therapy and neuroprotective agents that are under investigation.

BOX 1

Prime key points of neuroprotection

What is neuroprotection?

Neuroprotective drugs for stroke were first initiated during the 1980s and are still in development [91]. The basic aim of neuroprotection is to interfere with the events of the ischemic cascade by focusing on one or more of mechanisms of damage, blocking the pathological processes, and preventing the death of vulnerable nerve cells in the ischemic penumbra [91]. This concept involves inhibition of the pathological molecular events which eventually lead to the influx of calcium, activation of free radicals and neuronal death. That excludes, per definition, reperfusion modalities or drugs aimed to reduce the vasogenic edema surrounding the infarct [92].

Why neuroprotection is not yet established?

Despite encouraging data from experimental animal models demonstrating large reduction in pathological infarct volume in focal and global ischemia [93], almost all clinical trials of neuroprotective therapies have to date been consistently unsuccessful [91,93].

Explanations:

- Time window

In many of the animal models the neuroprotective drug was given shortly after stroke was induced, or even before vessel occlusion, in contrast with the clinical set up in which there is a substantially longer time window between onset of symptoms and drug administration [94,95].

- Differences in outcome measurement

Preclinical studies use the reduction of infarct volume, demonstrated by imaging orhistological work-up as the primary end point in comparison to the human studies, which use clinical and functional end points measured by the modified Rankin Scale and the Bartel Index [96].

- Differences in evaluation

The standard end point in clinical trials, functional outcome at 3 months, is beside efficacy of the drugs, influenced by numerous factors, including comorbidity, intensity of physical therapy, secondary complications, social and environmental factors, among others [97].

- Differences in comorbidities

Most of the experimental models use young healthy rodents which are not exposed to other medications, whereas stroke patients often suffer from several severe comorbidities (prior strokes, cardiovascular diseases, among others), are older and have premedication with other drugs [96].

- Diversity of stroke types

Most of the preclinical studies use middle cerebral artery occlusion as a model for ischemic stroke and therefore do not mimic the pathophysiological heterogenicity of different stroke types, as well as their extent, duration of ischemia and severity [98,99]. By contrast, human studies include patients with a broad pathophysiological heterogenicity.

- Differences in physiological variables

Animals are tightly controlled in laboratory parameters, blood pressure and temperature in addition to other metabolic factors in the acute phase in contrast to humans [96].

- Differences in-between gray and white matter damage

Animal studies focus on the protection of the gray matter from the tissue damage caused by ischemia with uncertain relevance to glia or white matter. By contrast, human studies include a high proportion of patients with subcortical strokes and diffused white matter damage [90,93].

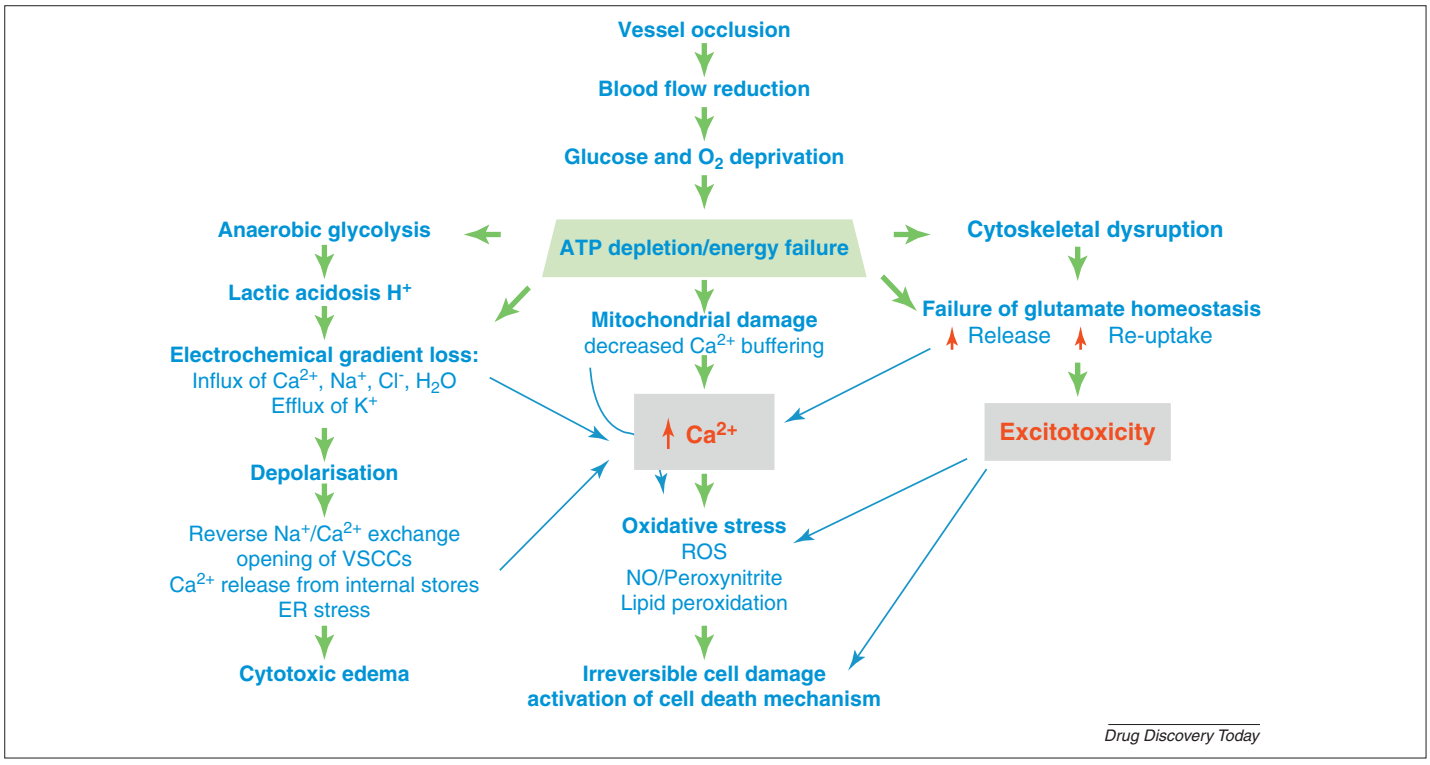

FIGURE 1

The acute neurochemical changes after ischemic stroke [89]. *Abbreviations:* ER, endoplasmatic reticulum; NO, nitric oxide; ROS, reactive oxygen species; VSCC, voltage sensitive calcium channels.

Acute future stroke treatment

Desmoteplase

Desmoteplase from the saliva of vampire bats might be more fibrin-specific, in comparison to recombinant tissue plasminogen activator (rtPA), and therefore might be a safe thrombolytic agent because it has the effect of catalyzing the conversion of plasminogen to plasmin. In comparison to rtPA, desmoteplase has a high fibrin selectivity (100 000- versus 550-fold increase in catalytic

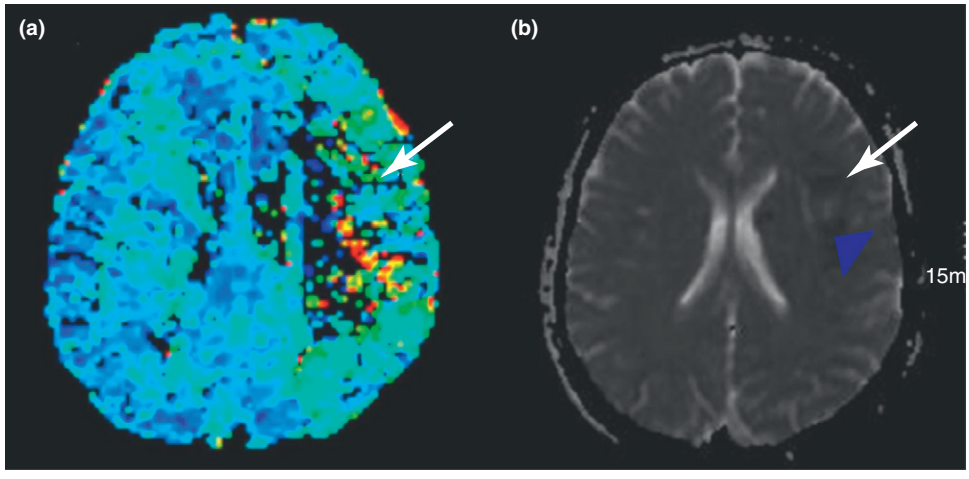

FIGURE 2

DWI and MRI following an ischemic stroke. DWI is highly sensitive to the changes occurring in the lesion. It is speculated that increases in restriction (barriers) to water diffusion, as a result of cytotoxic edema (cellular swelling), is responsible for the increase in signal on a DWI scan. The DWI enhancement appears within 5–10 min of the onset of stroke symptoms (as compared with computed tomography, which often does not detect changes of acute infarct for up to 4–6 hours) and remains for up to two weeks. According to the concept of an ischemic penumbra DWI in combination with cerebral perfusion, clinicians can highlight regions of 'perfusion and/or diffusion mismatch' that might indicate regions capable of salvage by reperfusion therapy. **(a)** DWI demonstrates an acute lesion as a region of hyperintensity (brightness – white arrow) in the left temporal lobe and a hypointense area (blue arrowhead) as a sign for infart demarcation. **(b)** In contrast to the relatively small lesion on the diffusion weighted image (DWI) 3 hours after stroke onset perfusion weighted images show a large abnormality (white arrow) on a relative MTT image. *Abbreviations:* DWI, diffusion weighted imaging; MRI, magnetic resonance imaging; MTT, mean transition time.

TABLE 2

Overview of the neurotoxic and neuroprotective effects of inflammatory markers

		Neurotoxicity	Neuroprotection
Inflammatory mediators			
Cytokines	IL-1β	Endogenous pyrogen Promoting gliosis Increase of Ca^{2+} in neurons Edema formation BBB breakdown Priming of endothelium for leukocyte adherence	Increase of survival promoting factors Induction of IL-1ra
	TNF-α	Inhibition of glutamate uptake Promoting gliosis Increase of neurotoxic mediators Increase of Ca 2^+ signaling in neurons Stimulation of apoptosis of endothelial cells Edema formation BBB breakdown Priming of endothelium for leukocyte adherence Increase NF-κ≡ activation	Increase of neutrophilic factors Control of extracellular Ca 2^+ Mediation neuronal plasticity Activation of repair processes of cerebral microvasculature Induction of anti-apoptotic factors Induction of antioxidants Ischemic tolerance induction
	IL-6	Endogenous pyrogen Attraction of T-lymphocytes	Induction of IL-1ra
	TGF-β	Increase of β-amyloid precursor Increase of glial scar formation	Reduction gliosis Less inflammatory mediators Suppressed release of ROS Less brain edema Inhibition of neutrophil adherence Reduction of apoptosis Induction of IL-1ra Promotion angiogenesis
	IL-10		Less release of cytokines and expression receptors Attenuation of astrocytic activation
High mobility group box protein family	HMGB1	Stimulation of inflammatory mediators Activation of microglia Increase NF-κB activation	
Chemokines	CINC, MCP-1, MIP-1, MRF-1, fractalkine	Regulation and migration of leukocyte trafficking Stimulation of BBB permeability Stimulation of phagocytes Increase of cytokine secretion Stimulation of apoptosis	Scavenge and repair of necrotic tissue Angiogenesis
Free oxygen radicals	ROS, NO	Lipid peroxidation Stimulation of inflammatory response Disruption of protein biochemistry	
	NO	Induction of iron loss of cells Inhibition of enzymes for DNA replication Stimulation of inflammatory mediators	Vasodilatator
Matrix metalloproteinases	MMP-9 (and -2)	BBB breakdown Stimulation of leukocyte adherence and transmigration Vasogenic edema Hemorrhagic transformation	Stimulation of plasticity, recovery and repair Clearance of necrotic cell debris
Adhesion molecules			
Selectins	E- and P-selectin	Slow down of neutrophils and monocytes Promotion of rolling over endothelium	
	P-selectin	Enhancement of platelet binding to neutrophils and monocytes	
	L-selectin	Guidance of unstimulated leukocytes	
Cellular adhesion molecules	ICAM-1 and -2, VCAM-1	Stronger attachment of leukocytes to endothelium Stimulation of diapedesis	
Integrins	LFA-1, Mac-1, CD11c	Stimulation of adhesion to endothelium Stimulation of conformational changes on leukocytes for diapedesis	

TABLE 2 (*Continued*)

		Neurotoxicity	Neuroprotection
Cellular inflammatory response			
Glia	Migroglia	Phagocytes to clear dead cells Production inflammatory and cytotoxic mediators	Production of neutrophic factors Facilitation of neurogenesis and plasticity Less release of toxic mediators Scavenge and removal of necrotic debris
	Astrocytes	Production of inflammatory and cyxtotoxic mediators Production of chmeokines Formation of glial scar tissue Enhancement of oxidative stress Release glutamate	Production of neutrophic factors Glial scar isolates damaged tissue
Endothelial cells (BBB)		BBB breakdown Hyperpermeability to macromolecules Vasogenic edema Increase of intracranial pressure Stimulation of inflammatory mediators and adhesion molecules Astrocyte detachment	
Leukocytes	Neutrophils	Release of pro-inflammatory and cytotoxic mediators Stimulation of lipid peroxidation Release of proteolytic enzymes Damage of endothelial cell membrane Increase of BBB permeability Post-ischemic edema No-reflow phenomenon	
	Monocytes	Generation of superoxide anions Release of pro-inflammatory cytokines	Removal of necrotic cell debris and neutrophils

TABLE 3

Overview of current Phase II and III trials and ongoing trials.

Agent	Trial	Phase	Ongoing or following trials
Desmoteplase	The Desmoteplase in Acute Ischemic Stroke Trial (DIAS): a Phase II MRI-based 9-hour window acute stroke thrombolysis trial with intravenous desmoteplase	Phase II	DIAS 3 and 4 ongoing
Tenecteplase	Phase IIB/III trial of tenecteplase in acute ischemic stroke: a prematurely terminated randomized clinical trial	Phase II	Statistical aspects of the TNK-S2B trial of tenecteplase versus alteplase in acute ischemic stroke: an efficient, dose-adaptive, seamless Phase II/III design.
Ancrod	Intravenous Ancrod for the treatment of acute ischemic stroke within 6 hours after onset of symptoms	Phase III	–
Eptifibatide	Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke (CLEAR Stroke) trial	Phases I and II	Study of the Combination Therapy of rt-PA and Eptifibatide to Treat Acute Ischemic Stroke (CLEAR-ER)
DP-b99	Effects of DP-b99 on neurological function in subjects with acute ischemic hemispheric stroke	Phase II	The Membrane-Activated Chelator Stroke Intervention (MACSI) trial of DP-b99 in acute ischemic stroke: a randomized, double-blind, placebo-controlled, multinational pivotal phase III study.
Granulocyte colony-stimulating factor	AXIS: a trial of intravenous granulocyte colony-stimulating factor in acute ischemic stroke	Phase II	AXIS 2: AX200 for the treatment of ischemic stroke
Edaravone	Edaravone-sodium ozagrel comparative post-marketing study on acute ischemic stroke	Phase IV	–
NXY	Safety and effectiveness of NXY -059 for the treatment of patients who have suffered from a stroke	Phase IIb/III	–
Citicoline	Navigation brain stimulation for evaluation of the neuroprotective drug efficiency in patients after ischemic stroke	Phase III	–
Citicoline	ICTUS Study: International Citicoline Trial on Acute Stroke	Drug: Placebo Phase III	–
MLC601	Clinical study to investigate the safety and efficacy of MLC601 in 150 iranian patients after stroke	Phase III	CHInese Medicine NeuroAid Efficacy on Stroke Recovery (CHIMES)

activity), shows no neurotoxicity, and no apparent negative effect on the blood–brain barrier (BBB) [4]. In the Desmoteplase in Acute Ischemic Stroke trial (DIAS), intravenous desmoteplase, administered within 3–9 hours of ischemic stroke onset, was found to be associated with a higher rate of reperfusion and better clinical outcome compared with placebo. The intracerebral bleeding rate was low when using doses up to 125 μg/kg [5]. DIAS 3 and 4 are recently ongoing trials to determine whether desmoteplase is effective and safe in the treatment of patients with acute ischemic stroke when given within 3–9 hours from onset of stroke symptoms [6].

Tenecteplase

Tenecteplase is a genetically engineered product of the alteplase molecule. Tenecteplase is a recombinant fibrin-specific plasminogen activator that is derived from native tissue plasminogen activator by modifications at three sites of the protein structure. It binds to the fibrin component of the thrombus and selectively converts thrombus-bound plasminogen to plasmin, which degrades the fibrin matrix of the thrombus. An early Phase II study with 88 patients showed that doses of 0.1–0.4 mg/kg were safe with no symptomatic intracerebral hemorrhage in patients treated within 3 hours [7]. However, a Phase IIB clinical trial was prematurely terminated owing to slow enrollment into the trial. There were no statistically persuasive differences in 3-month outcomes between the remaining tenecteplase groups and rtPA. Symptomatic intracranial hemorrhage rates were highest in the 0.4 mg/kg tenecteplase group and lowest in the 0.1 mg/kg tenecteplase group [7].

Ancrod

Ancrod (a pit viper venom) cleaves fibrinogen rather than fibrin, thereby reducing clot formation and reducing blood viscosity [8]. In the *post hoc* analysis of data from the Stroke Treatment with Ancrod Trial (STAT) it was hypothesized that an initial rapid ancrod infusion would yield superior efficacy and safety in patients with mean fibrinogen levels greater than 70 mg/dl, 9 hours after stroke onset. Those patients had statistically significant efficacy versus placebo and a marked reduction in the incidence of symptomatic intracerebral hemorrhage versus patients taking ancrod with lower maintenance fibrinogen levels. However, modifications of ancrod dosing might substantially improve efficacy while reducing the rate of symptomatic intracerebral hemorrhage [8].

The *post hoc* analysis of data from the STAT trial analyzed ancrod-related variables as potential determinants of efficacy or safety. The resulting hypotheses were then tested in the European STAT (ESTAT) database. Although the desired changes in fibrinogen level were seen in over 90% of ancrod subjects, interim analysis for futility led to the result that the study was halted owing to the lack of efficacy. It was demonstrated that intravenous ancrod starting within 6 hours after symptom onset did not improve the outcome and revealed a trend of increased bleeding despite successful efforts to achieve rapid initial defibrinogenation and to avoid prolonged hypofibrinogenemia [9].

Batroxobin

Batroxobin is a fibrinogen-depleting agent, a thrombin-like snake venom enzyme, without affecting the function of the platelets [10]. A randomized placebo-controlled study included 90 patients after stroke caused by pathology in the internal carotid artery 72 hours before the treatment and revealed significant efficacy, in particular on motor function [11].

A further study evaluated the safety and efficacy of batroxobin in treating hyperfibrinogenemia for secondary stroke prevention. A total of 112 patients with cerebrovascular events had concomitant hyperfibrinogenemia. Stroke and/or transient ischemic attack (TIA) recurrence in patients without batroxobin was higher than with batroxobin ($P \leq 0.05$), with no difference for the development of hemorrhagic stroke between the groups. Mortality rate was 9.6% in the batroxobin and 11.7% in the non-batroxobin group ($P \geq 0.05$). It was concluded that the intermittent intravenous injection of batroxobin can effectively reduce the risk for stroke and/or TIA recurrence in patients with concomitant hyperfibrinogenemia [12].

Augmentation of thrombolysis with combination therapies

Glycoprotein IIb/IIIa inhibitors prevent platelet aggregation and thrombus formation. They could provide a complementary mode of clot disruption. The combination of glycoprotein IIb/IIIa inhibitors with rtPA has been used in studies for acute coronary syndrome with improvement in reperfusion but also with increased rates of bleeding. This might be an eligible approach in stroke therapy [4].

Case series of patients with stroke comparing intra-arterial urokinase alone with a combination of intravenous abciximab followed by intra-arterial urokinase have shown a 90% recanalization in the combination group compared with 44% in the other group [4]. A further study investigated 12 patients with percutaneous coronary intervention with a high thrombus burden. In this study, intraclot administration of urokinase followed by abciximab significantly reduced the thrombotic burden with respect to baseline [13].

Eptifibatide, a glycoprotein IIb/IIIa inhibitor, is an arginin-glycin-aspartat-mimetic that reversibly binds to platelets. The combined approach to lysis utilizing eptifibatide and rtPA in stroke trial (CLEAR) assessed the safety of treating patients with acute ischemic stroke within 3 hours of symptom onset with this combination. A total of 94 patients were enrolled. However, the study was halted, because there was a trend towards increased clinical efficacy of standard-dose rtPA [14].

Experimental antithrombotic therapy

Hericenone B

Hericenone B has a strong anti-platelet activity and owing to its novel mechanism it might be a novel compound for antithrombotic therapy. *Hericium erinaceus*, an extract of several species of mushrooms potently inhibits platelet aggregation induced by collagen. Hericenone B selectively inhibits collagen-induced platelet aggregation, but it does not suppress the aggregation induced by U46619 (stable synthetic analog of the prostaglandin PGH2), adenosine diphosphate, thrombin, or adrenaline. Furthermore, hericenone B did not inhibit arachidonic acid or convulxin-induced platelet aggregation. Therefore, hericenone B was considered to block collagen signaling from integrin α2/β1 to arachidonic acid release. Hericenone B was found to inhibit collagen-induced aggregation in human platelets, similar to in rabbit platelets [15]. However, the efficacy and safety profile has to be tested in clinical trials.

Neuroprotection

Metal chelation

Iron chelators were shown to induce neuroprotection against brain injury [16]. Iron chelators prevent hydroxyl radical formation by sequestering redox-active iron. An additional neuroprotective mechanism of iron chelators is their ability to upregulate, or to stabilize the transcriptional activator, hypoxia-inducible factor-1α (HIF-1α). HIF-1α stability within the cells is under the control of a class of iron-dependent and oxygen-sensor enzymes that target HIF-1α for degradation. An emerging novel target for neuroprotection is associated with the HIF system to promote stabilization of HIF-1α and to increase transcription of HIF-1-related survival genes, which have been reported to be regulated in patients' brains afflicted with diverse neurodegenerative diseases [17].

Two iron chelators, deferoxamine and deferasirox are prolyl hydroxylase inhibitors. They are known to reduce stroke volume in an animal model when administered at high doses in the peri-stroke period [18].

Deferasirox is an orally administered and well-tolerated medication in clinical use; it has the potential to act as prophylaxis against stroke in high risk patients [18]. Recent Phase I and II studies concluded that the effect of the lipophilic iron chelator dipyridyl has curative and preventive effects and represents a favorable and effective approach to increase the ischemia-induced BBB resistance towards ischemic injury. The Membrane-Activated Chelator Stroke Intervention (MACSI) trial, a Phase III study is about to be conducted [19].

It is known that rationale Zinc is both a direct neurotoxin and signaling mediator in multiple early and late detrimental processes following ischemia. Several Phase I studies and two double-blind placebo-controlled Phase II trials analyzed DP-b99, a lipophilic moderate-affinity chelator of zinc, that is a first-in-class multi-targeted neuroprotective agent for ischemic stroke. These trials revealed a beneficial effect on post-stroke recuperation. Recently a Phase III MACSI trial is ongoing to evaluate the safety and therapeutic effects of intravenous DP-b99, initiated within 9 hours of stroke onset [20].

Granulocyte colony-stimulating factor

Growth factors are polypeptides essentially involved in regulating survival, proliferation, maturation, and outgrowth of developing neuronal cells. The effects of growth factors lead to transcription factors being phosphorylated. Cells can then be induced to grow and differentiate or to obtain enough trophic support to survive.

Recent animal studies demonstrated the beneficial effect of granulocyte colony-stimulating factor (G-CSF) as a neuroprotective and anti-inflammatory agent with an anti-apoptosis mechanism [21]. It was further analyzed that G-CSF substitution revokes detrimental effects by reducing lesion size and therefore enhancing neurological outcomes [21].

In another study 21 animal brains were analyzed in autopsy. This study revealed that in acute ischemic stroke, strong neuronal G-CSF receptor immunoreactivity was encountered in the infarct area and the peri-infarct rim as compared with the contralateral cortex. In subacute infarctions, microglial and macrophage G-CSF receptor immunoreactivity dominated, whereas chronic infarction was characterized by the presence of G-CSF receptor expressing reactive astrocytes. Neuronal G-CSF expression was encountered early upon ischemic stroke. At later time-points, an upregulation of vascular G-CSF expression in the peri-infarct area prevailed. The conclusion of this study was that the observed upregulation of G-CSF receptors and G-CSF have a role in the pathophysiology of human ischemic stroke [22].

The AXIS trial, a national, multicenter, randomized, placebo-controlled dose escalation study analyzed 44 patients with the results that G-CSF was well-tolerated even at high dosages, and that a substantial increase in leukocytes did not appear to be problematic in patients with stroke. This trial observed an interaction of drug effect with diffusion-weighted imaging (DWI) volume (14–17 cm^3) at baseline on functional outcome after 3 months with an apparent benefit for patients. As explanation for the dependence of the action of G-CSF and infarct size has been speculated that G-CSF has a better effect on cortical volumes and has therefore an increasing content in cortex of larger infarcts. AXIS-2 has been initiated for confirming safety of intravenous G-CSF in a larger population of patient with stroke [23].

Further studies are necessary to clarify the phenomenon and mechanisms of neuroprotection by G-CSF, because studies showed a significant neuroprotective effect.

Free-radical scavengers and trapping agents

Free radicals are produced in the brain during ischemia, during reperfusion and during intracranial hemorrhage. Removal of pathologically produced free radicals is therefore a viable approach to neuroprotection. It is known that free radicals have a significant pathogenetic role of cerebral tissue damage following both ischemia and reperfusion [16]. Four compounds with free radical scavenging activity (tirilazad, ebselen, edaravone) or free radical trapping properties (NXY-059) have been developed in experimental models of stroke and are evaluated clinically as neuroprotective agents.

Ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), a seleno-organic compound, has a radical-scavenging activity. A recent study observed the neuroprotective effects of ebselen. The administration of ebselen significantly reduced the neuronal death in the CA1 region induced by ischemia and/or reperfusion. These results suggest that ebselen protects neurons from ischemic damage through control of the expressions of Gamma-aminobutyric acid (GABA) shunt enzymes to enter the tricarboxylic acid cycle [24]. Yamagata *et al.* found that ebselen has a marked inhibitory effect on neuronal damage during stroke and that it might be effective in the prevention and/or treatment of neurodegenerative diseases [25].

Tirilazad is a free radical-induced lipid peroxidation inhibitor [26]. Tirilazad was without benefit in clinical trials despite of broad evidence of acting as a neuroprotective drug in animal models of stroke [27,28].

Edaravone has been clinically available in Japan since 2001. Since then it has been revealed that edaravone can improve clinical outcomes in patients exhibiting ischemic strokes. The possible mechanisms of edaravone include decreasing oxidative stress, protecting neurovascular units, and reducing the activation of microglia after ischemic stress [29]. As a neuroprotective agent edaravone can effectively reduce infarct size and improve clinical outcome. Until now further studies are needed to identify efficacy of this agent in neuroprotection after stroke.

NXY-059 is a novel neuroprotectant with free-radical-trapping [16]. The first clinical trial of NXY-059 (SAINT I) showed a small

but significant benefit on a co-primary end point. However, this benefit was not seen in the subsequent, larger SAINT II trial which failed to show substantial efficacy [27,29].

NMDA receptor antagonists

Ischemia causes the release of glutamate and many further molecules. Excess glutamate release, with activation of N-methyl-D-aspartate (NMDA) receptors, is a pivotal event in the evolution of irreversible ischemic damage in animal models of ischemia. Drugs modulating glutamate action are known to be potent neuroprotective agents. No improvement in clinical outcome of stroke has been seen with competitive NMDA antagonists (selfotel) and non-competitive NMDA antagonists (dextrorphan, GV150526, aptiganel and eliprodil) [30].

A novel postsynaptic antagonist of NMDA receptors called CP-101606-27 might attenuate the effects of focal ischemia. In current experiments, its neuroprotective effect was investigated alone and in combination with rtPA in thromboembolic focal cerebral ischemia in rats. It was concluded that post-ischemic treatment with CP-101606-27 is neuroprotective in the stroke model [31].

A novel dimeric acetylcholinesterase inhibitor, bis(12)-hupyridone (B12H), was investigated *in vitro* and *in vivo*, with results of prevention of glutamate-induced apoptosis in a rat model [32]. In an 2-hour middle cerebral artery occlusion rat model, B12H significantly attenuated ischemia-induced apoptosis in the penumbra region, improved neurological outcome, and decreased infarct volume, cerebral edema and neuronal apoptosis in the stroke model. In summary, these results show a neuroprotective effect of B12H against excitotoxic and ischemic insults *in vitro* and *in vivo*.

7,8-Dihydroxyflavone (7,8-DHF), a member of the flavonoid family, is a selective tyrosine kinase receptor B (TrkB) agonist that has neurotrophic effects. Experimental studies demonstrated that 7,8-DHF can still confer neuroprotection against glutamate-induced toxicity through its antioxidant activity [33].

Another agent, 1,2,3,4-tetrahydroisoquinolines shares neuroprotective abilities with established uncompetitive NMDA receptor antagonists, because it inhibits NMDA receptors and therefore has anti-excitotoxic activity [34].

Neu2000 is an NR2B-selective, moderate NMDA receptor antagonist with potent cell-permeable, spin trapping antioxidant action. Non-clinical human Phase I studies demonstrated that Neu2000 can be translated to treat patients with stroke with better efficacy and therapeutic time window [35].

Ifenprodil, an NR2b selective NMDAR antagonist in combination with flurbiprofen, a selective acid sensing ion channels 1a inhibitor, reduced infarct volume and neurological deficit in a rat model by significantly inhibiting apoptotic cell death [36].

Thus, it appears that the above mentioned combination therapy will be more effective as it seems to offer more effects on neuroprotection.

Citicoline

Citicoline is an essential precursor in the synthesis of phosphatidylcholine, a key cell membrane phospholipid, and is known to have neuroprotective effects in acute ischemic stroke [37] by reducing lipid metabolism [16]. Citicoline provides the brain with a source of choline and cytidine, which are efficiently used in the Kennedy cycle to generate phospholipids. It is able to attenuate the production of free radicals in ischemic conditions, while it also stimulates glutathione synthesis and the activity of glutathione reductase. A recent study reported that oral citicoline improved neurological, functional and global outcomes in patients with acute ischemic stroke without significant safety concerns [38].

Two major clinical trials with citicoline are ongoing. The citicoline brain injury treatment trial (COBRIT), conducted in the USA, is a randomized, double-blind, placebo-controlled, multicenter trial. In Europe has the International Citicoline Trial on Acute Stroke (ICTUS) recently be initiated [37]. Although the mechanisms of some of these actions remain unclear, so far citicoline seems to have a good neuroprotective effect, capable of enhancing endogenous protective pathways at the same time as preparing the scenario for plasticity.

Inflammatory markers

Inflammatory mechanisms have an important role in the risk of stroke and during the acute phase of brain ischemia, which contributes to the functional outcome of patients. Several inflammatory molecules are implicated during the acute phase of ischemic stroke, such as cytokines [interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), adhesion cell molecules (vascular cell adhesion molecule-1, intercellular adhesion molecule-1) and metalloproteinases. Inflammatory cytokines (IL-6 and TNF-α) and adhesion cell molecules are related to the presence of early neurological deterioration and infarct volume. Furthermore, metalloproteinases have been seen to have an important role in the development of hemorrhagic transformation [39]. Several studies reported that plasma levels of TNF-α and IL-6 are associated with prognosis after ischemic stroke and showed that plasma levels of cytokines, such as TNF-α, IL-1β are different in every diagnostic subtype of ischemic stroke and that plasma levels of some inflammatory markers and thrombotic–fibrinolitic markers are predictive of acute ischemic stroke diagnosis in the acute setting (Table 2) [40].

MLC901 – a traditional Chinese medicine

NeuroAiD™ (MLC601 and MLC901), a traditional Chinese medicine has been used in China in patients after stroke as drugs to facilitate recovery after stroke since 2001. These agents combine nine herbal and five animal components. *In vitro* and *in vivo* results showed that NeuroAiD made cells more resistant against glutamate aggression, increased neurite outgrowth and connectivity and reduced infarct volume [41]. In a rodent model with focal ischemia an improved survival, brain protection and decreased functional deficits were demonstrated. MLC901 also prevented neuronal death in an *in vitro* model and induced neurogenesis in rodent and human cells [41]. Chen *et al.* analyzed the improvement of neurological recovery after stroke 2009 in clinical trials [42]. A recent study showed that MLC901 can also improve functional recovery of rats after global ischemia, because it was found to have an important role in neuroprotection [3]. Overall, NeuroAID, as it has already been effective in a cohort of Chinese patients with stroke, seems to represent an interesting agent in stroke treatment.

Experimental agents for neuroprotection

Glutamate oxaloacetate transaminase

Ischemic stroke is associated with an excessive release of glutamate into the neuronal extracellular space [43]. A decrease in blood

glutamate levels could provide a mechanism to remove it from the brain tissue, by increasing the brain–blood gradient. In this regard, the ability of alanine aminotransferase (ALT) also known as glutamate oxaloacetate transaminase (GOT) to metabolize glutamate in blood could represent a potential neuroprotective tool for ischemic stroke. Campos *et al.* revealed that ALT has the capacity to remove glutamate from the brain by means of blood glutamate degradation, and suggested that the applicability of this enzyme is an efficient and novel neuroprotective tool against ischemic stroke [43].

Prostaglandin E1 and lithium

Evidence suggests that lithium might protect against the cerebral atrophy and neuronal degeneration induced by the neurochemical processes and pathways known to regulate cell death and atrophy after an ischemic event. Lithium-mediated neurotroprotective and neurotrophic effects involve mechanisms highly relevant to the post-stroke population including the increased expression of brain-derived neurotrophic factor (BDNF) and Bcl-2, and inhibition of GSK-3β. Lithium-induced increase in human gray matter have been reported [44]. The antiapoptotic effect was achieved by inhibitory signals following uptake into neurons through the prostaglandin transporter [45].

The combination of prostaglandin E1 and lithium reduced infarct volume and neurological deficits induced by focal cerebral ischemia in rats [46]. Moreover, the combination had a greater neuroprotective effect against cerebral ischemia compared with prostaglandin E1 or lithium alone. The combination was effective even when it was administered 3 hours after ischemia [46].

Sigma-1 receptor

Animal studies in rats with middle cerebral artery occlusion found increased sigma-1 receptor expression in peri-infarct areas [47]. Sigma-1 receptor agonists act through inhibition of inducible nitric oxide synthase [48]. Treatment of rats subjected to permanent or transient middle cerebral artery occlusion with an agonist of the sigma-1 receptor, starting two days after injury, enhanced the recovery of lost sensorimotor function without decreasing infarct size. Sigma-1 receptor activation increased the levels of synaptic proteins in membrane rafts in the peri-infarct area, whereas sigma-1 receptor silencing prevented sigma-1 receptor-mediated neurite outgrowth in primary cortical neuronal cultures [47]. In conclusion, sigma-1 receptor activation can stimulate recovery after stroke by enhancing cellular transport of biomolecules required for brain repair, thereby stimulating brain plasticity.

Stem cells

In animal models, transplanted stem cells have been shown to migrate into the injured regions, secrete neurotrophic compounds, promote revascularization, enhance plasticity and regulate the inflammatory response, thereby minimizing injury. Endogenous neural stem cells have also a remarkable propensity to respond to injury. Under selected conditions, subventricular zone progenitors can be mobilized to replace lost neurons. In response to focal infarcts, neuroblasts have important trophic roles to minimize neural injury [49].

The therapeutic potential of bone marrow-derived stromal stem cells has been demonstrated in different experimental models of ischemic stroke. Bone marrow-derived stromal stem cells infiltrating the post-ischemic brain exhibit persistent epigenetic changes in gene expression for numerous extracellular genes, compared with their naive counterparts. These genes are relevant for neuroprotection, regeneration and angiogenesis [50].

A recent *in vivo* study showed that intrathecal implantation of mesenchymal stromal cells induces an increase in IL-6 production as well as a decrease in apoptosis in neuronal stem cells. Direct implantation of mesenchymal stromal cell enhances neuroprotection through activation of resident neuronal stem cells NFκB activity (independent of the PI3 kinase and/or AKT pathway) leading to an increase in IL-6 production and a decrease in apoptosis [51].

Studies have demonstrated that the human placenta is a source of adult stem cells. Amniotic epithelial cells are known to express some of the neuronal and glial cell markers.

Transplantation of these human placenta-derived cells in *in vitro* and *in vivo* stroke models promoted functional recovery. Finally, analysis of trophic factors revealed that cultured human amniotic epithelial cells secreted vascular endothelial growth factor (VEGF) in the presence of melatonin [52]. Animal studies showed that VEGF is strongly implicated in mediating the vascular response to cerebral ischaemia. Expression of VEGF and its receptors is induced by focal ischaemia in the rat brain. Hypoxia-inducible expression of VEGF precedes neovascularization following cerebral ischaemia [53]. The ability of VEGF to stimulate angiogenesis and to elicit direct neurotrophic effects makes it an attractive candidate for neuroprotection [52,54]. These data indicate that melatonin, by stimulating melatonin receptor type 1A, increases cell proliferation and survival rates while enhancing neuronal differentiation of cultured human amniotic epithelial cells. Together with VEGF upregulation, rendered neuroprotection in experimental *in vitro* models of ischemic and oxidative stress injury.

Fingolimod

Fingolimod (FTY720) is a sphingosine-1-phosphate receptor agonist. It acts on G protein-coupled receptors, regulating proliferation, apoptosis, adhesion, migration, cytoskeletal organization, differentiation and/or morphogenesis, and inflammation [55].

Fingolimod has proven efficacy in Phase III multiple sclerosis clinical trials and also, decreases reperfusion injury in heart, liver and kidney [55]. Fingolimod has also been tested in several rodent models of focal cerebral ischemia. A recent study showed that in a mouse model of transient ischemia, fingolimod reduces infarct size, neurological deficits, edema, and the number of dying cells in the core and peri-infarct area [55].

Another recently published animal study was able to detect a reduction of lymphocyte brain invasion by fingolimod but could not achieve a significant reduction of infarct volumes and behavioral dysfunction [56]. This lack of neuroprotection despite effective lymphopenia was attributed to a divergent impact of fingolimod on cytokine expression and possible activation of innate immune cells after brain ischemia.

Opioid receptor agonists

Opioid receptor activation might provide neuroprotection during stroke. Animal studies suggested that opioid receptor agonists might inhibit excitatory postsynaptic potentials by attenuating presynaptic calcium influx and consequent glutamate release. Furthermore, opioid receptor agonists increased regional nitric

oxide synthase activity in mice brain under non-ischemic conditions. Another neuroprotective effect of opioid receptor agonists is the possibility to attenuate the excitotoxic effects of nitric oxide from neuronal sources. A further neuroprotective mechanism includes amelioration of cerebral edema [57]. The non-selective opioid receptor agonist biphalin exhibited a statistically significant greater effect in decreasing water content in oxygen and glucose deprivation exposed hippocampal slices, compared with μ, δ, and κ selective opioid agonists. Moreover, biphalin exhibited anti-edematous effects in a dose-responsive manner. The non-selective opioid antagonist naloxone returned the water content nearly back to original oxygen glucose deprivation values for all opioid agonist treatments, supporting that these effects were mediated by an opioid receptor pathway.

In conclusion, data show that biphalin decreased significantly edema (53%) and infarct size (48%). Biphalin also significantly decreased the cell volume increase in primary neuronal cells exposed to oxygen glucose deprivation condition [58].

Cinnamophilin

Cinnamophilin, a novel thromboxane A2 receptor antagonist, isolated from cinnamomum philippinense [59], is a potent antioxidant. It is not only radical-scavenging but also anti-inflammatory active and can reduce acute ischemic brain damage, even when it is given up to 6 hours after stroke onset. Recent animal studies revealed that administration of cinnamophilin provides long-lasting neuroprotection against gray and white matter damage and improves functional and electrophysiological outcomes in an animal model of ischemic stroke [60]. It can be assumed that cinnamophilin might be a potent drug in the therapy of ischemic stroke in future.

Hawthorn extract (Crataegus oxycantha)

Recent animal studies revealed that Hawthorn extract, which is well known as a prophylactic agent for cardiac conditions, might protect the brain against ischemia-derived reperfusion damage by its antioxidant property [61].

Hawthorn extract mechanism might be attributed to its antioxidant property which restores gluthathione levels, circumvents the increase in lipid peroxidation and nitric oxide levels thereby reducing peroxynitrite formation and free radical induced brain damage [61]. Hawthorn extract can suppress activated inflammatory cells in an experimental stroke model [62]. From our point of view this agent might gain a crucial role in neuroprotection through its immunomodulatory effect.

Dichlorobenzamil

Dichlorobenzamil is a sodium and/or calcium exchanger inhibitor. Sodium and/or calcium exchanger as a transmembrane protein has an important role in the exchange of sodium and calcium across the cell membrane. It has been implicated in various pathological conditions including hypoxia, or anoxia, white matter degeneration after spinal cord injury, among others. A significant role in cardioprotection and renoprotection has also been documented. Its neuroprotective effect has recently been revealed as it showed a significant decrease in cerebral infarct size along with reversal of ischemia-reperfusion-induced impairment of memory and motor co-ordination in an animal model [63].

As the ischemic post-conditioning-induced neuroprotective effects were significantly abolished by pretreatment with dichlorobenzamil, it might have an important role in ischemic post-conditioning-induced neuroprotection in the future.

Magnesium sulfate

Magnesium is an important cofactor in metabolism and protein synthesis and inhibits the release of excitatory neurotransmitters at the presynaptic level and blocks voltage-gated calcium channels. A large randomized trial did not show any beneficial effect of magnesium sulfate on death and disability; however, it slightly increased mortality. Interestingly, the investigators revealed a beneficial effect in a subgroup of patients with lacunar strokes [64].

Prehospital trials of magnesium sulfate are important as they demonstrate the feasibility of delivering potentially brain-protective agents in the first minutes after stroke onset [65].

Intravenous magnesium sulfate administration during the hyperacute phase of stroke was shown to be safe in a small, open-label pilot trial. Good functional outcome after 90 days was achieved by 69% of all patients and in 75% treated within 2 hours [66].

Overall, subacute neurorestoration therapies enhanced neuroplasticity and brain reorganization following stroke, but magnesium needs to be investigated in more detail as the current studies were not conclusive.

Cilostazol

Cilostazol is a potent inhibitor of type III phosphodiesterase and has been approved as an antiplatelet agent for the treatment of chronic cerebral infarction since 2001. Recently, neuroprotective effects of cilostazol have been described, such as anticytotoxic effect and have antiapoptotic effects, in focal cerebral ischemic models.

The brain-protective role of cilostazol might be explained through its antiapoptotic effect through the cyclic adenosine monophosphate (cAMP)-responsive element binding protein phosphorylation signaling pathway and subsequent activation of Bcl-2 positive cells [67].

Recent animal studies determined that cilostazol protects against ischemic brain injury and hemorrhagic transformation subjected to transient focal cerebral ischemia, but further studies are necessary [68].

Arundic acid

Arundic acid (ONO-2506) is an astrocyte-modulating compound that inhibits the synthesis of the protein S-100b in cultured astrocytes and was seen to improve neuronal survival after stroke [18,69]. A multicenter, dose-escalating, randomized, double-blind Phase I trial of arundic acid in acute ischemic stroke on 92 subjects showed a trend towards improvement in the change from baseline NIHSS. This finding needs to be confirmed in a future clinical trial [69].

An animal study of stroke-prone spontaneously hypertensive rats that have a high incidence of stroke suggests that arundic acid can prevent hypertension-induced stroke. It might inhibit the enlargement of stroke lesions by preventing the inflammatory changes caused by overproduction of the S100B protein in astrocytes [70]. Thus, arundic acid shows the potential as a neuroprotective agent for treating patients after ischemic stroke.

Repinotan

Repinotan hydrochloride is a serotonin (5-HT) 1A receptor agonist with evidence of neuroprotection in animal models of permanent and transient focal ischemia [16].

A Phase IIb study (mRECT) investigated the efficacy, safety, and tolerability of a targeted exposure to repinotan in patients with acute ischemic stroke. The study revealed that the response rate on the Barthel Index was 37.1% (127 of 342) for patients on repinotan and 42.4% (143 of 337) for patients taking the placebo. The study failed to demonstrate a clinical benefit of repinotan and therefore the development of repinotan in acute ischemic stroke was discontinued [71].

Pioglitazone

Pioglitazone is an antidiabetic agent and has a possible neuroprotective effect. This peroxisome proliferator-activated receptor (PPAR)-γ agonist has been tested on acute phase changes in a murine model of cerebral ischemia induced by Bilateral Common Carotid Artery Occlusion (BCCAO) [72].

Pioglitazone significantly reduced the plasma TNF-α levels. Pioglitazone treatment also improved all antioxidant levels showing activity against oxidative stress induced by BCCAO in this model. Pioglitazone led to neuroprotection suggesting a potential role of PPAR-γ agonists as neuroprotective agents [72].

A recent study investigated the role of pioglitazone in activation of transcription-3 (p-STAT3) after cerebral ischemia, it was further investigated whether the increase in p-STAT3 by estrogen is mediated by the estrogen receptor α. The study revealed an essential part for neuroprotection in activation of p-STAT3 in the peri-infarct region and that pioglitazone might be of benefit in post-menopausal stroke patients [73]. These findings determine the beneficial effect of pioglitazone and maybe gender-based activity difference.

Antioxidants

Antioxidant approaches have failed in previous clinical trials, and the relevant sources of oxidative stress in stroke are unknown. A recent study identified nicotinamide adenine dinucleotide phosphate (NADPH) oxidase type 4 (NOX4) as a major source of oxidative stress and as an effective therapeutic target in acute stroke. Application of the only validated low molecular-weight pharmacological NADPH oxidase inhibitor in mice, several hours after ischemia was as protective as deleting NOX4. It improved significantly neurological functions and reduced mortality [74]. However, NOX4 might represent a major source of oxidative stress and novel class of drug targets for stroke therapy.

C-Phycocyanin

The application of C-Phycocyanin (C-PC) applied either prophylactically or therapeutically in an experimental model can reduce infarct volume owing to its antioxidant effects. It could further be shown that it exhibits a protective effect against hippocampus neuronal cell death and improves functional outcome [75]. An animal model showed that treatment with C-PC prevented the lipid peroxidation and increased the ferric reducing ability of plasma. The anitioxidant effects can be explained by these mechanisms [75]. C-PC may become a modifying pharmacological agent for stroke therapy owing to its antioxidant effects.

Ginsenoside Rd

Ginsenoside Rd is one of the main active ingredients in Panax ginseng and attenuates neuronal oxidative damage *in vitro* induced by hydrogen peroxide and oxygen-glucose deprivation. Ginsenoside Rd showed an inhibitory effect on the hydroxy radical formation in a rat model. Early accumulations of DNA, protein and lipid peroxidation products were also suppressed. Furthermore, Ginsenoside Rd significantly eliminated inflammatory injury as indicated by the suppression of microglial activation, inducible nitric oxide synthethase and cyclooxygenase-2 expression [76].

These neuroprotective effects of Ginsenoside Rd were evaluated in a recent study analyzing aged mice, revealing partly enhanced endogenous antioxidant activities after stroke. Ginsenoside Rd neuroprotection might be caused by its attenuation of redox imbalance [77].

Manganese-superoxide dismutase and other reactive oxygen species are produced in mitochondria during an ischemic event. These agents generate oxidative stress and consecutive cellular damage.

Manganese-superoxide dismutase (SOD2), is an inducible antioxidant enzyme, the transcriptional activity and expression is regulated by STAT3 signal transducer and activator of transcription 3 (STAT3). Further studies revealed that ischemic reperfusion causes STAT3 inactivation, decreases SOD2 expression, and enhances brain damage. Recently published data demonstrated the neuroprotective effect against reperfusion-induced oxidative stress and cell injury by upregulation of mitochondrial SOD2. This study revealed that the inflammatory cytokine IL-6 leads to increased expression of STAT3, activation of SOD2 and subsequent neuroprotection [78].

Another recently published study investigated a focal stroke model using SOD2-deficient mice to investigate neurovascular endothelial damage that occurs during reperfusion. Following focal stroke and reperfusion, SOD2−/+ mice had delayed BBB breakdown, associated with activation of matrix metalloproteinase and increased brain hemorrhage rates, whereas a decrease in apoptosis and hemorrhage was observed in SOD2 overexpressors [79]. Induction and activation of SOD2 was discussed to be a novel strategy for neurovascular protection after ischemia or reperfusion. This study identified STAT3 as a transcription factor of the mouse *SOD2* gene. During reperfusion, activation of STAT3 and its recruitment into the *SOD2* gene were blocked, resulting in increased oxidative stress and neuronal apoptosis [79]. These studies point out the potential of antioxidant-based neurovascular protective strategies.

Recombinant human MFG-E8

MFG-E8 is a 66 kDa glycoprotein that has shown tissue protection in various models of organ injury. Levels of MFG-E8 protein in the brain are known to be reduced at 24 hours after cerebral ischemia. A recent study on rats revealed that rhMFG-E8 treatment led to significant decreases in neurological deficits, and infarct size. In conclusion, rhMFG-E8 treatment is a neuroprotective against cerebral ischemia by suppression of inflammation and apoptosis [80].

HIF-1 Inhibition by YC-1

Hypoxia-inducible factor 1 (HIF-1) is a major regulator of cellular adaptation. Data from a recently published study from an ischemic stroke model of rats demonstrated that HIF-1 inhibition ameliorates

ischemia-induced BBB disruption, although it does not affect brain edema. Further analyses show that ischemia upregulates HIF-1 and its downstream genes by 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole (YC-1). HIF-1 inhibition ameliorates erythropoietin, VEGF, and glucose transporter [81]. This data indicates that it has no potential for cerebral ischemic treatment, although YC-1 confers certain protection to the cerebral vascular system.

Gelsolin

Gelsolin (GSN) is an actin- and calcium-binding protein mediating the disassembly of actin filaments and activity of calcium channels, with further functions as a regulator of apoptosis and inflammatory responses. In a rat stroke model celsolin significantly reduced infarct volumes [82]. Gelsolin could therefore be a promising drug for protection against neurodegeneration after cerebral ischemia.

Combination strategies of acute and neuroprotective stroke agents

The complementary approach aiming at promoting cerebrovascular integrity and blocking adverse cerebral vascular events might increase the thrombolytic efficacy of rtPA and reduce rtPA-induced hemorrhagic transformation, and, thereby, might make thrombolytic therapy more accessible to the aged population. The combination of neuroprotective agents and rtPA might become a promising approach for the treatment of stroke. A recent animal study evaluated that the treatment with a selective proteasome inhibitor, Velcade®, in combination with rtPA might extend the therapeutic window of thrombolysis for up to 6 hours in young rats after stroke. In contrast to tPA alone, the treatment with Velcade significantly reduce infarct volume. The combination treatment promoted thrombolysis and did not increase the incidence of hemorrhage transformation [83].

XG-102 (formerly D-JNKI1), a TAT-coupled dextrogyre peptide which selectively inhibits the c-Jun N-terminal kinase, is known to be a powerful neuroprotectant in mouse models of middle cerebral artery occlusion with the result of infarct reduction. The additional treatment with rtPA decreased the ischemic damage both *in vitro* and *in vivo* [84]. This study confirmed compatibility of XG-102 with rtPA.

A significantly reduced infarct volume and a lower incidence of hemorrhage, as well as a neurological improvement was achieved in a recent animal study that analyzed the combination of S-0139 [a specific endothelin type A receptor (ET(A)) antagonist] with rtPA. S-0139 was seen to enhance the neuroprotective effect of rtPA by suppressing ischemia- and rtPA-triggered molecules that evoke thrombosis and BBB disruption [85].

In a rat embolic stroke model, the effects of the use of citicoline after thrombolysis were analyzed. Citicoline (Cytidine-5′-diphosphocholine) is known to have neuroprotective effects through providing choline for synthesis of neurotransmitter acetylcholine, stimulation of tyrosine hydroxylase activity and dopamine release. The combination showed the greatest reduction of mortality caused by the reduction of infarct volume, number of neuronal death positive cells and plasma levels of TNF-α [86].

The use of NXY-059, either with or without recombinant tissue plasminogen activator, was not successful in Phase III studies. However, these results could reflect its weak antioxidant capacity, poor BBB penetration, and lack of synergism with recombinant tissue plasminogen activator as well as the overly broad treatment window used in the reported trials.

The coadministration of uric acid and recombinant tissue plasminogen activator has shown to provide synergistic neuroprotection in experimental thromboembolic models and to lessen several biomarkers of oxidative stress in patients with acute stroke. A recent Phase III trial investigates the clinical efficacy of uric acid [87].

Further studies revealed that rtPA treatment increased levels of matrix metalloproteinases after embolic focal cerebral ischemia. Matrix metalloproteinases are involved in the mechanism of rtPA-associated hemorrhage. In conclusion, it seems that combination therapies with matrix metalloproteinases inhibitors might be useful for decreasing the risk and severity of this life threatening complication of thrombolytic therapy [88].

A recent study analyzed the synergic role of serum uric acid (SUA) with thrombolytic. SUA is a final enzymatic product of purine metabolism. Animal models of acute ischemic stroke have shown that SUA might be neuroprotective [3] and might reinforce the benefits of rtPA. Clinical improvement was significantly higher in patients treated with high SUA levels at admission. In conclusion, SUA might not be neuroprotective alone, but might provide an additional beneficial effect in patients receiving thrombolysis [89].

Concluding remarks

Cerebral ischemia is a multifactorial disorder which includes several pathways for progression of injury to brain cells. Over the years investigations focus more and more on neuroprotection with many new promising agents under investigation.

These novel approaches including extending penumbral survival for the later use of reperfusion therapy, reducing reperfusion injury after successful reperfusion, and using drugs with both neuroprotective and recovery enhancing effects will open new therapeutic windows in stroke therapy. To maximize the outcome after stroke, the combined use of reperfusion and neuroprotection is likely to be needed as well as the initiation of carefully designed trials. Stroke investigators become even more innovative and revert to old Chinese medicine on behalf of neuroprotection. Novel studies will confirm the superiority of the combined treatment with rtPA followed by neuroprotective agents, because early reperfusion should be followed by effective neuroprotection to inhibit ischemia-reperfusion injury and better protect the tissue at risk.

New drugs have the potential that stroke will become even more treatable, in particular these strategies have the potential to lead to a reduced permanent disability in patients with stroke.

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