Multifunctional Actions of Approved and Candidate Stroke Drugs

Jens Minnerup and Wolf-Rüdiger Schäbitz

Department of Neurology, University of Münster, Albert-Schweitzer–Straße 33, 48149 Münster, Germany

Summary: Ischemic stroke causes brain damage by multiple pathways. Previous stroke trials have demonstrated that drugs targeting one or only a few of these pathways fail to improve clinical outcome after stroke. Drugs with multimodal actions have been suggested to overcome this challenge. In this review, we describe the mechanisms of action of agents approved for secondary prevention of ischemic stroke, such as antiplatelet, antihypertensive, and lipid-lowering drugs. These drugs exhibit considerable properties beyond their classical mechanisms, including neuroprotective and neuroregenerative properties. In addition, candidate stroke drugs currently studied in clinical phase III trials are described. Among these, albumin, hematopoietic growth factors, and citicoline have been identified as promising agents with multiple mechanisms. These drugs offer hope that additional treatment options for the acute phase after a stroke will become available in the near future. **Key Words:** Stroke, drug therapy, mechanisms, neuroprotection, neuroregeneration.

INTRODUCTION

Despite the tremendous mortality and morbidity of stroke, treatment options remain limited. Many pathophysiological key mechanisms of cerebral ischemia have been identified in recent years, but drug treatment targeting one or a few of these mechanisms has failed to improve clinical outcome after stroke. The most plausible reason for this failure is the multiplicity of mechanisms involved in causing neuronal damage during ischemia. Drugs targeting a multimodal mode of action could potentially overcome this dilemma, and have recently been shown to provide a remarkable benefit in preclinical studies.

The only drug approved for the acute phase of ischemic stroke is recombinant tissue plasminogen activator (rtPA)—which, however, predominantly acts by targeting a single mechanism, the lysis of the intravascular clot. Antiplatelet, antihypertensive, and lipid-lowering therapies are approved for secondary stroke prevention. In contrast to rtPA, additional effects of these drugs beyond their classical mechanisms are suggested, particularly because prestroke use of drugs for secondary stroke prevention was found to be associated with less severe deficits when a stroke occurred. Moreover, animal experimental studies confirmed that antiplatelet, antihypertensive, and lipid-lowering drugs exert multimodal actions including neuroprotective and neuroregenerative properties. We review these approved stroke drugs with a focus on their multimodal modes of action. In addition to drugs for secondary prevention of stroke, further agents were identified that interfere with various pathophysiological mechanisms in cerebral ischemia. Some of these drugs were extensively tested in preclinical stroke studies and showed promising results regarding infarct size reduction and functional recovery enhancement. Those candidate stroke drugs with multiple mechanisms in acute or chronic stroke that were successfully translated into clinical phase III trials are also described in this review.

Address correspondence and reprint requests to: Jens Minnerup, University of Münster, Department of Neurology, Albert-Schweitzer-Straße 33, 48149 Münster, Germany. E-mail: minnerup@uni-muenster.de. Or Wolf-Rüdiger Schäbitz; e-mail: schabitz@uni-muenster.de.

APPROVED DRUGS FOR SECONDARY STROKE PREVENTION

Antiplatelet agents

Platelet aggregation is important in stroke development, both in the pathogenesis of atherosclerosis and in the occurrence of acute cerebral artery occlusions.[1] The initial step is platelet adhesion to the arterial wall, a result of endothelial damage.[2] Platelet adhesion is promoted by several factors, including as von Willebrand factor, fibrinogen, and subendothelial collagen.[2] Afterwards, adhesion platelets are activated mainly by thromboxane

Table 1. *Mechanisms of Action of Approved and Candidate Drugs for Stroke*

Drug	Status	Vascular Effects	Neuroprotection	Neuroregeneration	Systemic Effects
Aspirin	Approved for secondary stroke prevention.		Reduction of brain glutamate release. Reduction of oxidative stress. Inhibition of mitochondrial energy metabolism.		Inhibition of platelet aggregation by COX inhibition. Reduction of inflammatory response post stroke.
Dipyridamole	Approved for secondary stroke prevention.	Vasodilatation. Scavenger of free radicals. Proangiogenic effects.	Cytoprotection in neuronal cell culture. Antioxidative effects.		Anti-inflammatory properties. Inhibition of platelet aggregation by increasing plasma adenosine.
Clopidogrel	Approved for secondary stroke prevention.	Reduction of intimal proliferation. Reduction of contraction of vascular smooth muscle cells.	Increased neuronal integrity in hippocampal slices *in vitro*.		Inhibition of platelet aggregation by blocking adenosine receptors.
Angiotensin II receptor antagonists	Approved for secondary stroke prevention.	Antiatherogenic effects by inhibition of the inflammatory component of atherosclerosis. Inhibition of vascular smooth muscle cell proliferation.	Blocking of brain AT 1 receptors. Increasing activity of the BDNF/TrkB system. Inhibition of superoxide production in the cortex.	Upregulation of repair-associated proteins (MAP-2, GAP-43).	Blood pressure reduction by blocking angiotensin I receptors.
Statins	Approved for secondary stroke prevention.	Atherosclerotic plaque stabilization by inhibition of MMPs and inhibition of macrophage proliferation. Induction of smooth muscle cell apoptosis.	Increasing cerebral blood flow. Improving endothelial function. Upregulation of eNOS. Protection of NMDA-induced cell damage.	Enhancing neurogenesis, synaptogenesis, and angiogenesis.	Lipid-lowering effects. Anti-inflammatory effects. Antioxidant effects.
Albumin	In clinical trial phase III, acute stroke; time window 5 hours.		Reduction of brain swelling after ischemia.	Replenishing fatty acid lost from neural membranes.	Reduction of adherence of thrombotic material. Anti-inflammatory effects.
G-CSF	A clinical trial phase IIb/IIIa will start in the second quarter of 2009, time window 9 hours.		Antiapoptotic effects (STAT-3, ERK, and PI3K-Akt pathway). Reduction of glutamate-induced neuronal cell death in culture.	Enhancing neurogenesis and angiogenesis.	Immunomodulation. Mobilization of bone marrow stem cells.
EPO	In clinical trial phase III, acute stroke; time window 6 hours.		Antiapoptotic effects (JAK2-PI3K-Akt pathway). Reduction of glutamate-induced neuronal cell death in culture.	Enhancing neurogenesis and angiogenesis.	Modifies inflammatory response. Proliferation and maturation of erythroid precursors.
Citicoline	In clinical trial phase III, acute stroke; time window 24 hours.		Indirect antiapoptotic activity. Reduced oxidative stress. Maintaining membrane integrity.		

AT 1 = angiotensin type 1; BDNF = brain-derived neurotrophic factor; COX = cyclooxygenase; eNOS = endothelial nitric oxide synthase; EPO = erythropoietin; ERK = extracellular signal-regulated kinase; G-CSF = granulocyte colony-stimulating factor; MMP = matrix metalloproteinase; PI3K = phosphatidylinositol 3-kinase; STAT-3 = transcription factor (signal transducer and activator of transcription type 3); TrkB = tyrosine receptor kinase type B.

A2, thrombin, and adenosine diphosphate.[3] This process requires the cyclooxygenase (COX)–mediated metabolism of arachidonic acid to prostaglandin H2 (PGH2), which in turn is processed to thromboxane A2.[3] Platelet activation results in platelet aggregation due to the conversion of the glycoprotein IIb/IIIa receptor to a form that binds fibrinogen and other adhesion molecules.[1] Based on the relevance of platelet aggregation in stroke pathogenesis, its inhibition became a target for therapeutic intervention. The results of many trials that evaluated antiplatelet agents in stroke led to recommendations that all stroke patients not requiring anticoagulation should receive antiplatelet therapy.[4] Aspirin, clopidogrel, and the combination of aspirin and extended-release dipyridamole are accepted treatment options.[4]

Aspirin. Aspirin was the first antiplatelet drug used for secondary prevention in ischemic stroke.[5] Within the last 20 years, the effectiveness of aspirin for recurrent ischemic stroke prevention has been shown in several trials.[6,7] The best-characterized effects of aspirin in the prevention of cardiovascular diseases are based on its platelet inhibitory functions.[8] After diffusion through the cell membranes of platelets, aspirin first binds and then acetylates a specific serine residue of COX, thereby preventing the conversion of arachidonic acid to PGH2 and thus also preventing the formation of thromboxane A2.

Apart from the antiplatelet effects, there is growing evidence for direct neuroprotective properties of aspirin (Table 1).[9–14] In animal models of focal cerebral ischemia, both pretreatment and administration of aspirin after the onset of ischemia is neuroprotective, as indicated by infarct size reduction for aspirin doses between 15 mg/kg and 80 mg/kg body weight.[9,13,15] Reduction of brain glutamate release after ischemia appears to be an important mechanism by both NF-κB dependent and independent pathways.[9,11,12] Glutamate toxicity may additionally be reduced by aspirin-mediated inhibition of prostaglandin (PG) formation. Prostaglandins synthesized by COX enhance the release of glutamate from astrocytes. Its decreased composition may therefore abate glutamatergic neurotoxicity.[16] *In vitro* experiments showed that aspirin also prevents neurotoxicity by inhibition of the atypical protein kinase Cζ, which is a downstream signal in NMDA-induced neuronal cell death.[17] Moreover, aspirin exerts neuroprotective properties through reduction of oxidative stress and by inhibitory effects on mitochondrial energy metabolism.[18,19] Neuroprotection by aspirin was also suggested by clinical studies showing that prestroke use of aspirin reduced stroke severity.[20,21] Increasing evidence suggests that inflammation plays a role both in atherosclerosis with subsequent stroke and in poststroke morbidity and mortality.[22–24] Aspirin was found to be associated with lower values of inflammatory parameters after stroke.[25,26] To date, however, the relevance of these anti-inflammatory effects for stroke prevention remains unknown.

Dipyridamole. Dipyridamole is available in immediate-release form, usually given in doses between 50 and 100 mg three times per day, or as a fixed combination of 25 mg aspirin and 200 mg extended-release dipyridamole (ER-dipyridamole), given two times per day. In comparison of placebo to three active treatment groups (aspirin alone, ER-dipyridamole alone, and the combination of aspirin and ER-dipyridamole) in secondary prevention of ischemic stroke, the single use of either aspirin or ER-dipyridamole was superior to placebo, and the combination of aspirin and ER-dipyridamole was superior to either drug alone.[27] A meta-analysis of six trials comparing the combination of dipyridamole and aspirin (also including the immediate-release formulation) to aspirin alone confirmed these findings, revealing a significantly lower risk of serious vascular events when aspirin was combined with dipyridamole.[28]

Dipyridamole exerts antiplatelet effects by inhibition of the adenosine uptake in erythrocytes and the attenuation of adenosine catabolism, both of which result in increasing plasma concentrations of adenosine, an inhibitor of platelet aggregation. Dipyridamole is also known to act via adenosine as a potent vasodilator, mainly in coronary arteries.[29] The effect of dipyridamole on cerebral blood flow is, however, not fully understood.[30,31] Dipyridamole possesses antithrombotic properties in the vessel wall by enhancing the production of the endogenous platelet inhibitor prostacyclin, thereby preventing platelet adhesion to the endothelium.[32,33] Damage caused by hydrophilic and hydrophobic radicals can be prevented by the scavenger properties of dipyridamole.[34] Dipyridamole inhibits oxidation of low density lipoprotein (LDL) and may prevent the development of atherosclerosis.[35] In various neuronal cell culture models dipyridamole was cytoprotective.[36,37] Dipyridamole also exerted neuroprotective properties in an embolic stroke model.[38] The underlying mechanisms of neuroprotection remain unknown. Both adenosine effects and antioxidative effects are assumed to be relevant.[36,39] Further potential mechanisms of dipyridamole in stroke pathophysiology include anti-inflammatory actions and proangiogenic effects.[40,41]

Clopidogrel. In a large clinical trial, clopidogrel reduced the composite endpoint of stroke, myocardial infarction, or vascular death compared with aspirin in high-risk patients.[42] In patients with peripheral arterial disease, clopidogrel is superior to aspirin in terms of stroke prevention.[42] As recently shown, there were no significant differences in secondary stroke prevention when clopidogrel was compared with the combination of aspirin and ER-dipyridamole.[43]

Clopidogrel is a second-generation thienopyridine.

Clopidogrel irreversibly blocks the P2Y12 receptor, an adenosine receptor located on the surface of platelets, thereby preventing platelet aggregation by inhibition of the glycoprotein IIb/IIIa complex.[44] Clopidogrel itself has no antiplatelet activity and needs to be oxidated by the hepatic cytochrome P-450 system to an active metabolite.[45] Beyond the inhibition of thrombus formation, animal experimental studies suggested direct effects of clopidogrel on arterial vessels, such as a reduced intimal proliferation after arterial injury and reduced serotonin and endothelin-1-mediated contraction of vascular smooth muscle cells (Table 1).[46–48] *In vitro* studies measuring the population spike amplitude in murine hippocampal slices after a hypoxic episode found that pretreatment with clopidogrel increased neuronal integrity, compared with control treatment, taken as indication of a neuroprotective capacity of clopidogrel.[49]

Antihypertensive drugs

High blood pressure is well known as an important risk factor for ischemic stroke. Lowering an elevated blood pressure is recommended for primary prevention, as well as for prevention of recurrent stroke.[4] Several classes of antihypertensive drugs were found to be effective in secondary stroke prevention, and to date there is no recommendation clearly favoring a special antihypertensive drug.[4,50] Angiotensin II receptor antagonists were, however, found to be superior to a beta-blocker (losartan vs atenolol) in primary prevention and to a calcium channel blocker (eprosartan vs nitrendipine) in secondary prevention of ischemic stroke.[51,52] Because blood pressure reduction was similar in both comparisons, other mechanisms were assumed. Angiotensin II receptor antagonists reduce blood pressure by inhibition of the renin angiotensin system. Renin catalyzes the cleavage of angiotensinogen to angiotensin I, which in turn is cleaved by the angiotensin converting enzyme to its active form angiotensin II.[53,54] The appropriate stimulus for renin production by the kidney is glomerular hypoperfusion.[53] Angiotensin II acts via the specific angiotensin II receptors AT1 and AT2.[55] The latter is less well characterized, whereas AT1 is well known to mediate vasoconstriction, aldosterone-mediated sodium and water retention, left ventricular hypertrophy, and growth in the arterial wall.[56] Angiotensin II receptor antagonists counteract these mechanisms by blocking the angiotensin I receptor, which results in a lowered blood pressure.[57]

Beyond lowering blood pressure, other mechanisms of AT1 receptor blockers in stroke pathophysiology have been suggested (Table 1). In various animal experimental studies of focal cerebral ischemia, neuroprotective properties have been demonstrated.[58–60] Effects of AT1 receptor blockers on brain AT1 receptors were suggested to mediate these neuroprotective characteristics.[58,61] The ability of different AT1 receptor blockers to cross the blood–brain barrier is controversial, however.[62,63] Candesartan was shown to enhance the TrkB receptor of brain-derived neurotrophic factor (BDNF), a neurotrophin well known for its neuroprotective effects.[64] Additionally, candesartan inhibited the increase of superoxide production in the cerebral cortex after cerebral ischemia in mice.[65] Expression of the proinflammatory cytokine tumor necrosis factor α (TNFα) and the proinflammatory marker gene CXC ligand 1 were downregulated by candesartan, thereby decreasing infarct size.[60]

In addition to neuroprotection, candesartan exhibits poststroke neuroregenerative properties, as indicated by an upregulated expression of repair-associated proteins, such as MAP-2 and GAP-43.[65] Because a substantial role of AT1 receptors in atherogenesis was uncovered, vasculoprotective properties of angiotensin II receptor antagonists independent of blood pressure effects are suggested.[66] Indeed, the AT1 blocker losartan was found to exhibit antiatherogenic effects in nonhuman primates, as indicated by the inhibition of fatty-streak formation.[67] Assumed underlying mechanisms gained from animal model and *in vitro* studies include a reduction of oxidative stress, inhibition of the inflammatory component of atherosclerotic lesion formation, inhibition of vascular smooth muscle cell proliferation, and a reduced intimal thickening.[68–71]

Lipid-lowering drugs

Hyperlipidemia is a well-established risk factor for myocardial infarction, whereas the effect of high serum cholesterol levels on stroke incidence is less clear.[72,73] This might explain why statins, in contrast to other lipid-lowering drugs, clearly prevent stroke recurrence in patients with prior stroke or other vascular diseases, irrespective of blood lipid levels at treatment initiation.[74,75] These findings strongly suggest a multimodal mode of action.

Statins, or hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are similar to HMG-CoA, a precursor of cholesterol. Statins competitively inhibit HMG-CoA reductase, the rate-limiting step in the synthesis of cholesterol.[76] Reduced intrahepatic cholesterol leads to an upregulated low-density lipoprotein (LDL) receptor activity with an increased clearance of LDL from the circulation.

Statins non–lipid-lowering effects, which are relevant in stroke pathophysiology, can be divided into two main groups: effects on the arterial vessels (including antiatherothrombotic and anti-inflammatory actions, as well as improvement of endothelial function) and direct neuroprotective and neuroregenerative effects on the brain (Table 1).

Atherosclerotic plaque-stabilizing effects of statins have been shown in both animal and human studies.[77,78] Statins were found to mediate plaque stabilization by

various mechanisms, including inhibitory effects on matrix metalloproteinases, inhibition of macrophage proliferation in atherosclerotic plaque, and induction of smooth muscle cell apoptosis.[78,79] The so-called pleiotropic effects of statins include additional anti-inflammatory and antioxidant properties. C-reactive protein (CRP), which is a marker for inflammation associated with atherosclerosis, is reduced by statin therapy.[80] Underlying mechanisms of the anti-inflammatory effects of statins include reduced leukocyte adhesion by integrin inhibition and inhibition of MHC-II-mediated T-cell activation.[81,82] In addition, statins promote systemic antioxidant effects through suppression of distinct oxidation pathways, such as the formation of myeloperoxidase-derived and nitric oxide–derived oxidants.[83]

The neuroprotective properties of statins have been shown in animal stroke models, in which statin therapy leads to reduced infarct volumes.[84] The mechanisms of neuroprotection are presumably indirect effects, related to increased cerebral blood flow and improved endothelial function rather than to direct neuronal effects. The upregulation of brain endothelial nitric oxide synthase (eNOS) by statins with subsequent NO synthesis appears to be of particular importance.[85,86] Statins suppress the isoprenoid production by HMG-CoA reductase inhibition and thereby prevent the isoprenylation of the small GTPase-ρ, a negative regulator of eNOS expression.[87] In addition, statins act via post-translational modification on eNOS activity by activation of the phosphatidylinositol 3-kinase–protein kinase Akt (PI3K–Akt) pathway.[88] Statins reduced the association of NMDA receptors to lipid rafts of neuronal cells *in vitro*, thus suggesting neuroprotective effects of statins mediated by protection from NMDA-induced neuronal damage.[89]

In addition to their neuroprotective effects, statins appear to have neuroregenerative properties post stroke.[90,91] Atorvastatin improved functional recovery in a rat stroke model independent from infarct size reduction by enhancing neurogenesis, synaptogenesis, and angiogenesis.[91]

CANDIDATE DRUGS FOR ACUTE AND CHRONIC STROKE TREATMENT

Albumin

Human albumin is in widespread use for fluid resuscitation in shock patients, although convincing evidence for this indication is lacking.[92] Recently, high-dose human albumin (doses between 0.63 g/kg and 2.5 g/kg) was studied in animal models of focal cerebral ischemia.[93–96] Albumin reduced infarct size and improved functional recovery after permanent and temporary cerebral ischemia.[94,97] Preclinical studies included a dose–response relationship evaluation and a time window investigation (up to 4 hours).[93,94] Despite a reasonable number of preclinical albumin studies, the significance of the results is in some aspects weak. The studies showing efficacy of albumin in stroke animals were conducted in only one laboratory, only one species (rat) was tested, and only animals without comorbidities (e.g., diabetes or hypertension due to age) were included in the studies.[98]

Based on the preclinical results, a phase I dose-escalation study, the Albumin in Acute Stroke (ALIAS) Pilot Trial, was conducted with 82 stroke patients. The results showed that albumin is safe in stroke patients. The authors of that study interpreted the results as a sign for efficacy, based on comparison of higher doses with lower doses and in comparison with a historical control group.[99,100] A randomized, multicenter, double-blind, placebo-controlled trial (ALIAS Phase III Trial, www.clinicaltrials.gov; NCT00235495) is currently being conducted.

Animal stroke studies have suggested various mechanisms by which albumin provides an improved outcome after stroke. After middle cerebral artery occlusion (MCAO), albumin significantly reduced brain swelling in rats.[94,101] Intravascular mechanisms (e.g., reduced adherence of thrombotic material and an improved erythrocyte perfusion) may also contribute to a beneficial outcome.[95,102] Albumin mobilizes systemic fatty acids and may contribute to neural cell integrity post stroke by replenishing fatty acid lost from neural membranes during ischemia.[103,104] In addition, albumin was suggested to exert anti-inflammatory effects by binding the proinflammatory lysolipid lysophosphatidylcholine.[105] To date, however, convincing findings on the neuroprotective mode of action of albumin are lacking. The ALIAS pilot trial suggested a favorable outcome when thrombolysis was combined with albumin.[100] Synergistic effects of albumin with thrombolysis were also demonstrated in a study using a rat stroke model in which the combination therapy improved microvascular hemodynamics.[96]

G-CSF

The granulocyte colony-stimulating factor (G-CSF) is approved for the prevention and treatment of chemotherapy-induced neutropenia.[106] Within the last several years, a number of reports showed efficacy of G-CSF in animal models of focal cerebral ischemia.[107–111] To obtain an overall impression of the efficacy of G-CSF in preclinical studies and to evaluate conditions under which maximum efficacy can be achieved, we performed a meta-analysis and meta-regression analysis of G-CSF in animal stroke models.[112] We showed that G-CSF reduced both infarct volumes and sensorimotor deficits.[112] Our meta-regression analysis revealed a clear dose–response relationship of the efficacy of G-CSF.[112] When administered within the first 6 hours after the induction of ischemia, G-CSF reduced infarct sizes by 0.8% per 1

μg/kg body weight and by 2.1% per 1 μg/kg body weight when applied later than 6 hours.[112]

Based on these promising preclinical studies, a number of clinical studies were initiated to test the efficacy of G-CSF in stroke patients.[113] We conducted a multicenter, randomized, double-blind, placebo-controlled, dose-escalating phase IIa trial (AXIS), which was recently completed.[114] In this trial, which included 45 patients who had suffered an acute stroke, G-CSF was shown to be safe and well-tolerated. In addition, G-CSF showed signs of clinical efficacy in patients with larger baseline lesions evidenced with diffusion MRI.[114] A phase IIb/IIIa trial will start in the second quarter of 2009.

It cast doubt on the perception that G-CSF's natural function of mobilizing bone marrow stem cells is the most important mechanism, particularly since both G-CSF and its receptor were found to be expressed in the brain (Table 1).[107] *In vitro* and *in vivo* experiments confirmed that G-CSF acts antiapoptotically on neurons by at least three different antiapoptotic pathways: the signal transducer and activation of transcription 3 pathway (STAT-3), the extracellular signal-regulated kinase pathway (ERK), and the phophatidylinositol 3-kinase–Akt pathway (PI3K-Akt).[107,108,111]

Beyond the acute phase of stroke, G-CSF facilitates regeneration by enhancing endogenous neurogenesis.[107] Another mechanism underlying long-term functional recovery appears to be angiogenesis, which was increased by G-CSF post stroke even when administered as late as 7 days after the induction of ischemia.[109] G-CSF is well known for its immunomodulative properties.[115] Although the exact effect of the G-CSF–mediated systemic immunomodulation on stroke outcome remains unknown, G-CSF obviously affects the local brain immune response post stroke. G-CSF treatment reduced infiltration of neutrophils and microglia in the ischemic hemisphere, and G-CSF lowered interleukin-1β upregulation.[108,110,115]

Erythropoietin

Erythropoietin (EPO) is in broad clinical use for the treatment of cancer-related anemia.[116] EPO is the second hematopoietic growth factor besides G-CSF to be extensively tested in preclinical stroke studies.[117,118] A small clinical trial showed that EPO is safe and might be beneficial in acute ischemic stroke.[119] The results of a randomized, double-blind, placebo-controlled multicenter study of EPO treatment in acute stroke has been completed, and results are expected in the near future (http://www.clinicaltrials.gov; NCT00604630).

Erythropoietin parallels G-CSF in many respects with regard to the mode of action in ischemic stroke. Like G-CSF, EPO and its receptor are expressed in the brain.[120] Notably, the EPO receptor that mediates neuroprotection seems to be distinct from those expressed by erythroid precursors.[121] EPO also exerts antiapoptotic properties *in vitro* and *in vivo*. The JAK2–PI3K pathway was shown to be a key regulator in EPO-mediated neuroprotection.[122] In addition to its neuroprotective effects, EPO has neuroregenerative properties by stimulating neuronal differentiation and neurogenesis, as evidenced *in vitro* and *in vivo*.[117] After experimental stroke in mice, EPO restored local cerebral blood flow as measured by laser scanning imaging.[123] Concordantly, histological examination showed enlarged vascular perimeters and an increased vascular density around the ischemic lesion, indicating an enhancement of angiogenesis after EPO treatment.[117] EPO also modifies the inflammatory response after cerebral injury, as shown by reduced leukocyte infiltration after neonatal cerebral ischemia and reduced production of TNFα and IL-6.[118,124]

Citicoline

Citicoline is a dietary supplement. In some countries, it is in clinical use for the treatment of acute ischemic stroke. A meta-analysis of four clinical trials found that citicoline administration in stroke patients within the first 24 hours after symptom onset increases the probability of functional recovery at 3 months.[125] A randomized, double-blind, placebo-controlled, multicenter phase III trial is currently recruiting patients to support this evidence (http://www.clinicaltrials.gov; NCT00331890).

Citicoline is an essential intermediate in the synthesis of the key membrane phospholipid phosphatidylcholine.[126] Its role in maintaining membrane integrity was suggested as being a relevant mechanism in post-stroke recovery. After experimental stroke in rats, phospholipases (phosphatidylcholine degrading enzymes) are activated and CTP:phosphocholine cytidylyltransferase (CCT), the rate-limiting enzyme in phosphatidylcholine synthesis, is inactivated, both of which lead to neuronal cell death due to reduced phosphatidylcholine concentrations.[127] Citicoline restores phosphatidylcholine levels by attenuating phospholipase activity and by enhancing CCT activity.[127] Similar mechanisms were suggested for the preserving effects of citicoline on sphingomyelin and cardiolipin.[128] Excessive neuronal stimulation with acetylcholine release after cerebral ischemia leads to choline depletion, which in turn induces apoptosis.[129] Citicoline provides choline for the synthesis of acetylcholine and may thereby prevent apoptotic cell death in cholinergic neurons. The relevance of this mechanism was challenged, however, because acetylcholine concentrations were not significantly increased by citicoline treatment.[130] In addition, citicoline was shown to reduce oxidative stress.[128,131]

CONCLUSION

Currently, multifunctional actions of stroke therapies are limited to drugs used for secondary stroke prevention. These drugs exhibit considerable properties beyond their classical mechanisms. In particular, statins show a broad range of pleiotropic effects that may be as important in stroke pathophysiology and recurrent stroke prevention as their lipid-lowering effects. In contrast to several drugs for secondary prevention of ischemic stroke, treatment options for the acute phase of stroke are limited to thrombolysis. Ongoing phase III clinical trials raise hope that additional treatment options for this acute phase will be available in the near future. This includes, in particular, drugs with multimodal modes of action that act on the brain itself, as well as on other systems such as the vasculature or the immune system.

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