Combination of Therapeutic Hypothermia and Other Neuroprotective Strategies after An Ischemic Cerebral Insult#

Joline Goossens* and Saïd Hachimi-Idrissi

Critical Care Department and Cerebral Resuscitation Research Group, Ghent University, De Pintelaan 185, 9000 Ghent, Belgium

Abstract: Abrupt deprivation of substrates to neuronal tissue triggers a number of pathological events (the “ischemic cascade”) that lead to cell death. As this is a process of delayed neuronal cell death and not an instantaneous event, several pharmacological and non-pharmacological strategies have been developed to attenuate or block this cascade. The most promising neuroprotectant so far is therapeutic hypothermia and its beneficial effects have inspired researchers to further improve its protective benefit by combining it with other neuroprotective agents. This review provides an overview of all neuroprotective strategies that have been combined with therapeutic hypothermia in rodent models of focal cerebral ischemia. A distinction is made between drugs interrupting only one event of the ischemic cascade from those mitigating different pathways and having multimodal effects. Also the combination of therapeutic hypothermia with hemicraniectomy, gene therapy and protein therapy is briefly discussed. Furthermore, those combinations that have been studied in a clinical setting are also reviewed.

Keywords: Acute ischemic stroke, clinical, combination therapy, experimental, human, hypothermia, neuroprotection, rodent.

INTRODUCTION

Following an ischemic stroke (IS), intravenously (i.v.) injected recombinant tissue plasminogen activator (rt-PA) reduces the size of ischemic damage and salvages neuronal cells by dissolving the clot obstructing a cerebral artery [1]. Unfortunately, this drug needs to be administered within a therapeutic window of 4.5 h after symptom onset [2, 3], otherwise hemorrhagic complications increase [4]. Another therapeutic strategy is “neuroprotection”, which is defined as any strategy, or combination of strategies, that antagonizes, interrupts or slows the propagation of the pathological events that occur following an IS [5]. Abrupt deprivation of oxygen and glucose to neuronal tissue triggers a series of pathological events leading to cell death. This series of destructive events is referred to as an ischemic cascade and can be summarized as cellular bio-energetic failure due to focal cerebral hypoperfusion, followed by excitotoxicity, oxidative stress, blood-brain-barrier (BBB) dysfunction, ischemia-induced microvascular injury, hemostatic activation, post-ischemic inflammation and finally death of neurons, glial and endothelial cells [6-10]. In the last two decades, neuroprotective agents, designed to block this cascade, have been investigated in animal models of cerebral ischemia. The most promising neuroprotectant so far is therapeutic hypothermia (TH), defined as an intentionally induced, controlled reduction of the body temperature below 36 °C [11, 12]. The success of this therapy can be explained by its multifaceted neuroprotective effect [13]. It retards energy depletion by lowering the metabolic and the enzymatic rate [14], restores the neurotransmitter balance [15], reduces the intracellular calcium influx, reduces intracellular acidosis [16], suppresses reactive oxygen species (ROS) formation [17], suppresses infiltration of inflammatory cells [18], prevents BBB disruption [19], suppresses specific cell death pathways or up-regulates cell survival mechanisms [11, 20-22]. However, its beneficial effect is still not completely known [23] and researchers tend to further improve its salvageable beneficial effect by combining it with other neuroprotective strategies. This review will summarize which neuroprotective strategies have already been combined with TH in rodent models of focal cerebral ischemia. A distinction is made between those drugs interrupting only one event of the ischemic cascade and those mitigating different pathways and thereby having multimodal effects. Also the combination of TH with hemicraniectomy, gene therapy and protein therapy is briefly discussed. Furthermore, those combinations tested in a clinical setting are also reviewed and discussed.

Although there are some articles reviewing combination therapies with TH [24, 25], this review is the first to provide a complete overview of all combination strategies with TH in rodent models of IS as well as in a clinical setting. Moreover, the mechanisms involved are detailed. A summary can be found in Table 1.

THERAPEUTIC HYPOTHERMIA IN ISCHEMIC STROKE

Based on the target temperature, TH can be classified into mild (32 °C-34 °C), moderate (28 °C-32 °C), deep
Magnesium ions antagonize calcium entry. It has also an anti-excitotoxic property since essential for cell functions such as preservation of membrane integrity, protein synthesis, energy metabolism, maintenance of ionic gradients and regulation of vascular smooth muscle tone. It has also an anti-excitotoxic property since magnesium ions antagonize calcium entry via NMDA receptors. This has been the rationale for the administration of magnesium as a neuroprotective treatment following cerebral ischemia. In a permanent middle cerebral artery occlusion (MCAO) rat model, Campbell et al. [34] concluded that magnesium (i.v. loading dose of 360 µmol/kg followed by a 25 h i.v. infusion at 120 µmol/kg/h) is not neuroprotective unless it is combined with TH (35 °C). In a transient MCAO rat model, Song et al. [35] infused a 15 °C magnesium sulfate solution (120 mg/kg), which reduced the temperature of the MCA supplied territory to 33-34 °C within 5-10 min after infusion. The authors concluded that the combination of local hypothermia and magnesium is more effective in reducing acute ischemic damage than local hypothermia alone. Meloni et al. [36], on the other hand, could not confirm that a combination of TH at 35 °C and magnesium (360 µmol/kg) significantly reduces infarct size in a permanent MCAO rat model.

Furthermore, TH can also be classified based on the moment of institution, i.e. prior to the insult (protective), during the insult (preservatitve) or after the insult (resuscitative). In the case of stroke, TH will be induced after the insult (resuscitative TH).

**DRUGS INTERRUPTING ONE EVENT OF THE ISCHEMIC CASCADE**

**NMDA Antagonists**

IS starts with severe focal hypoperfusion which restricts oxygen and substrates delivery to cells leading to energy failure, loss of membrane potential and consequently depolarization of neurons and glial cells [9]. After depolarization, large amounts of excitotoxic amino acids, especially glutamate, are released into the synaptic cleft. This massive glutamate release stimulates the postsynaptic N-methyl-D-aspartate (NMDA) receptors allowing Ca²⁺ influx into neurons. This excess of glutamate release is likely to maintain the activation of these NMDA receptors resulting in further increase of the intracellular calcium concentrations and hence neuronal death [27]. NMDA receptors were therefore considered as potential neuroprotective targets. Various NMDA antagonists have been studied as they limit the subsequent neurodegradative processes through reduction of Ca²⁺ influx through the NMDA-operated Ca²⁺ channels [28]. Also their combination with TH, which reduces the ischemia induced release of glutamate [29], has been evaluated.

The first report of an NMDA antagonist combined with TH dates back to the early 90s where dextromethorphan, a non-competitive NMDA antagonist, was shown to be synergistically protective when combined with post-ischemic hypothermia (30 °C) in a hypothermic rat model of bilateral carotid artery occlusion [30]. Post-ischemic hypothermia has also been combined with the non-competitive NMDA antagonist MK-801 (dizocilpine) in a rat model of permanent focal ischemia. It appeared that hypothermia (33 °C) and MK-801, administered 30 min before MCAO at 1 mg/kg, offer similar cerebroprotective effects when administered separately but do not confer additional cerebroprotection when combined [31].

Other than drugs that antagonize the NMDA receptor, researchers also tested products that enhance the concentration of endogenous NMDA antagonists, such as magnesium. Magnesium is the fourth most abundant cation in the body, essential for cell functions such as preservation of membrane integrity, protein synthesis, energy metabolism, maintenance of ionic gradients and regulation of vascular smooth muscle tone [32].

In conclusion, the advantage of NMDA receptor antagonists resides in their excitotoxic inhibitory pathway. One caveat is that, however, it affects also other pathways necessary for normal neuronal function and survival and generates many adverse side effects necessitating dose reduction beneath the potential neuroprotective dose [42-45]. Furthermore, the protective time window for NMDA receptor antagonists is only 1 to 2 h [46], which limits its clinical use [47].

**Oxidative Stress Scavengers**

When there is an imbalance between free radical production and degradation, oxidative stress occurs. Under normal conditions, endogenous antioxidants scavenge the ROS produced. However, in ischemic conditions these defense mechanisms fail to protect tissue from oxidative damage because of overproduction of ROS and inactivation of antioxidant enzymes [48]. It was therefore suggested that the administration of therapeutic compounds that have the ability to neutralize ROS, could provide neuroprotection. Several antioxidant agents have therefore been investigated as free radical scavenging effect-providing neuroprotectants after an IS. Some of them have also been combined with TH, which is known to attenuate oxidative DNA damage and DNA damage-triggered pro-death signaling events [49]. In a rat model of transient (2 h) focal ischemia, Nito et al. [50] investigated the effect of the antioxidant 3-methyl-1-phenyl-pyrazolin-5-one (edaravone) (3.0 mg/kg), intravenously administered just prior to reperfusion, combined with TH (35 °C). It could be concluded that the combined treatment...
| Reference | Model | Therapeutic Hypothermia – Target Temperature (°C) | Second Neuroprotective Strategy | Is Combination Therapy More Neuroprotective than Monotherapy? |
|-----------|-------|-----------------------------------------------|---------------------------------|----------------------------------------------------------|
| [30]      | Hypotensive rat model of bilateral carotid artery occlusion | 30 | dextromethorphan (20 mg/kg i.p.) | yes |
| [31]      | Permanent rat MCAO model | 33 | MK-801 (dizocilpine) (1 mg/kg) | no |
| [34]      | Permanent rat MCAO model | 35 | magnesium (IV loading dose of 360 µmol/kg followed by a 25 h i.v. infusion at 120 µmol/kg/h) | yes |
| [35]      | Temporal rat MCAO model | 33-34 | magnesium sulfate solution (15 °C, 120 mg/kg) | yes |
| [36]      | Permanent rat MCAO model | 35 | magnesium (360 µmol/kg) | no |
| [41]      | Temporal rat MCAO model | 34 | N-acetyl-aspartyl-glutamate (NAAG) (10 mg/kg i.p.) | no |
| [50]      | Temporal rat MCAO model | 35 | 3-methyl-1-phenyl-pyrazolin-5-one (edaravone, 3 mg/kg) | yes |
| [53]      | Temporal mouse MCAO model | 33 | rt-PA (10 mg/kg i.v.) | yes |
| [54]      | Rat thromboembolic stroke model | 34 | rt-PA (10 mg/kg) | yes |
| [55]      | Rat model of embolic stroke | 32 | rt-PA (20 mg/kg i.v.) | no |
| [56]      | Rat model of thromboembolic occlusion | 33 | rt-PA (1 mg/100 g) | no |
| [57]      | Clinical study | 32-34 | rt-PA (0.9 mg/kg) | no |
| [58]      | Clinical study | 33 | rt-PA (0.9 mg/kg) | no |
| [65]      | Temporal rat MCAO model | 35 | argatroban (3 mg/kg) | yes |
| [69]      | Temporal rat MCAO model | 32-33 | atorvastatin pretreatment (1 mg/kg/day, for 10 days before ischemia) | yes |
| [77]      | Temporal rat MCAO model | 35 | FK506 (tacrolimus) (0.3 mg/kg) | yes |
| [78]      | Temporal rat MCAO model | 32-34 | ketoprofen (10 mg/kg) | no |
| [86]      | Temporal rat MCAO model | 34 | citicoline (400 mg/kg i.p.) | yes |
| [94, 95]  | Permanent rat focal embolic stroke model | 34 | minocycline (45 mg/kg on the first day and 22.5 mg/kg 24 h and 32 h after stroke) | no |
| [96]      | Temporal rat MCAO model | 33 | minocycline (twice daily 30 mg/kg) | yes |
| [101]     | Temporal rat MCAO model | 35 | caffeinol (ethanol 0.32 g/kg + caffeine 10 mg/kg) | yes |
| [106]     | Clinical study | 33-35 | caffeinol (8-9 mg/kg caffeine + 0.4 g/kg ethanol) + rt-PA (0.9 mg/kg i.v.) | combination could not be evaluated as no control group was included |
| [132]     | Temporal rat MCAO model | 36 | xenon (30%) | yes |
| [145]     | Temporal rat MCAO model | 33 | magnesium (1 mmol/kg) and tirilazad (3 mg/kg) | yes |
| [146]     | Permanent rat MCAO model | 33 | magnesium (2x1 mM/kg) + tirilazad (2x3 mg/kg) | yes |
significantly reduced edema volume and infarct size. When administered separately, edaravone attenuated only brain edema and TH failed to reduce post ischemic brain damage.

**Anticoagulants**

In acute IS, the endogenous fibrinolysis is usually outweighed by ongoing activation of the coagulation cascade and platelet activation [51]. Components of the coagulation cascade have therefore been attractive targets for neuroprotective agents. rt-PA is currently the only approved treatment for acute IS [52]. Since rt-PA needs to be administered within the first 4.5 h after stroke onset [2, 3], and TH is most effective when initiated as soon as possible after the insult, the question raised whether rt-PA could be combined with TH. In a study by Liu et al. [53], mice subjected to MCAO and receiving rt-PA in hypothermic circumstances (33 °C) had smaller infarcts than those receiving rt-PA in normothermic circumstances. In a rat model of thromboembolic stroke, the combination of TH (33 °C) with rt-PA treatment, both induced 1.5 h after stroke onset, was superior to thrombolysis alone and resulted in a reduction of infarct volume as well as a mitigation in the breakdown of the BBB [54]. On the other hand, Meden et al. [55] concluded that, although rt-PA and a 3-h TH (32 °C) reduced the infarct volume remarkably, the combination did not show any further protection in a rat model of embolic stroke. Similarly, Kollmar et al. [56] could not show an additive effect when rt-PA, administered 1 or 3 h after embolization, was combined with TH (33 °C), initiated 1 h after embolization and maintained for 4 h, in a rat model of thromboembolic occlusion of the middle cerebral artery. The safety and efficacy of TH combined with intravenous rt-PA were also investigated in acute IS patients. Based on the National Institutes of Health Stroke Scale (NIHSS) score and the Barthel Index (BI), it was concluded that the combination of TH (32-34 °C), induced locally on the surface of the lesion side of the head, and rt-PA (0.9 mg/kg, 10% of the dose as a bolus and the remainder given as a constant infusion over 60 min) provided no benefit compared to rt-PA alone [57]. Also Hemmen et al. [57, 58] could not demonstrate statistically significant differences between hypothermic (33 °C) and normothermic patients receiving an rt-PA treatment (0.9 mg/kg) [58]. Nevertheless, both studies indicated that it is safe to combine TH with rt-PA.

Next to rt-PA, argatroban also has been combined with TH. Argatroban directly inhibits free and clot-bound thrombin and, hence, thrombin-induced activities [59]. It has predictable anticoagulant effects [60], causes less bleeding compared to heparin for the same anticoagulant effect [61] and is well-tolerated [62]. It has been shown that argatroban is beneficial in models of acute IS [63, 64]. In an MCAO rat model, Kamiya et al. [65] investigated and confirmed that argatroban (3.0 mg/kg), continuously injected for 24 h after onset of ischemia, combined with TH (35 °C), significantly

Thus improves microcirculation \[64, 68\]. Lee smooth muscle, it increases cerebral blood flow and synthase (eNOS) \[66\]. As nitric oxide relaxes vascular effects by up-regulating endothelial nitric oxide (NO) of cholesterol biosynthesis, but also exerting neuroprotective CoA) reductase inhibitors, originally introduced as inhibitors statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-

## Statins

Improvement in the microcirculation can be achieved by statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, originally introduced as inhibitors of cholesterol biosynthesis, but also exerting neuroprotective effects by up-regulating endothelial nitric oxide (NO) synthase (eNOS) \[66\]. As nitric oxide relaxes vascular smooth muscle, it increases cerebral blood flow and thus improves microcirculation \[67, 68\]. Lee et al. \[69\] demonstrated that atorvastatin pretreatment (1 mg/kg, daily for 10 days before ischemia) enhances the efficacy of neuroprotection conferred by TH (32-33 °C) in acute IS. The simultaneous administration of atorvastatin and TH showed a greater reduction of infarct size than each treatment modality alone. Moreover, atorvastatin pretreatment also extended the therapeutic time window of TH from 3 h to 6 h after stroke. As statins are safe and effective drugs, it is an attractive strategy to combine them with TH.

## Anti-Inflammatory Agents

In ischemic brain parenchyma, inflammatory reactions are triggered which may amplify further tissue damage by triggering other deleterious mechanisms \[70, 71\]. Ischemic injury induced inflammation can be characterized by the rapid synthesis of pro-inflammatory cytokines and chemokines and the coordinated infiltration of neutrophils, T-lymphocytes and macrophages \[72\]. Because inflammation reactions occur rapidly and persist for a few days after ischemic brain injury \[73\], these responses are potential targets for human therapy.

FK506 or tacrolimus is a drug that suppresses the release of inflammatory cytokines and decreases NO synthase resulting in a reduced production of NO. It has been demonstrated that FK506 provides neuroprotective effects against transient and permanent focal ischemia \[74\], global ischemia \[75\] and chronic hypoperfusion \[76\]. In a rat model of transient MCAO, Nito et al. \[77\] combined TH (35 °C) with FK506 (0.3 mg/kg) and found that TH further reduced the infarct and edema damage. TH also expanded the therapeutic time window for FK506 administration. A low dose of FK506 (0.3 mg/kg) was shown to be neuroprotective when administered at 1 h, but not at 2 h. However, the combination of FK506 and TH significantly improved infarct size and edema volume, even when administered 2 h after MCAO. Although the precise mechanisms of this neuroprotective effect remain unclear, this combination may be useful in future treatment for acute stroke.

Another drug tested was ketoprofen, a non-steroid agent with a very potent analgesic and anti-inflammatory action. At a dose of 10 mg/kg body weight, combined with TH (32-34 °C), it was not more effective than the isolated action of these two neuroprotective therapies \[78\].

## Drugs Interrupting Multiple Ischemic Cascades

Next to those agents interrupting only one pathway of the ischemic cascade, there are several agents that have the ability, like TH, to influence numerous pathways simultaneously. Some of these agents have also been combined with TH.

## Citicoline

Citicoline is an endogenous compound, originally identified as the key intermediary in the biosynthesis of phosphatidyl-choline \[79\]. Since it was recognized as a neuroprotectant, it has been used in a plethora of experimental and clinical trials. It has some neuroprotective ability to improve phosphatidyl-choline synthesis in the injured brain \[80\]. It stabilizes membrane function \[81\], reduces free radical formation \[82, 83\], inhibits free fatty acid release \[84\] and it may also interact with the apoptotic cascade by inhibiting pro-caspase expression and caspase activation \[85\]. Sahin et al. \[86\] investigated the effect of citicoline (400 mg/kg i.p.) combined with TH (34 °C) in a rat model of transient focal cerebral ischemia. The authors concluded that the combination therapy is more effective in mitigating cerebral damage than either therapy used alone. The combination resulted in a greatly reduced expression of the tested apoptotic proteins; it also significantly affected the expression of the anti-apoptotic protein Bel-2 and reduced apoptotic cell death, thus preventing neuronal damage.

## Minocycline

Minocycline is a second-generation tetracycline which easily crosses the BBB \[87\]. Besides its antimicrobial action, it has also neuroprotective properties through inhibition of microglial activation \[88, 89\], attenuation of apoptosis \[90\], suppression of free-radical production \[91\] and inhibition of matrix metalloproteinases (MMPs) \[92, 93\]. Two research groups investigated the combination of minocycline and TH. In a permanent focal embolic stroke model, it was demonstrated that TH (34°C) combined with minocycline, administered i.p. at 1 h and 4 h after embolization on the first day (45 mg/kg body weight), and again after 24 h and 32 h (22.5 mg/kg), reduced infarct volume \[94, 95\]. However, there was no additive effect compared to the group receiving minocycline alone. Moreover, the group that received only TH, showed no reduction in infarct size compared to the control group \[94, 95\]. It was suggested this might be due to the insufficient duration of hypothermia, i.e. only 2 h. Therefore, Nagel et al. \[96\] investigated, in a transient MCAO model, the effect of a prolonged hypothermic phase (4 h, 33 °C) combined with minocycline, administered twice daily (30 mg/kg). They concluded that the combined therapy was only slightly superior to either treatment alone. Nevertheless, since minocycline has only minimal side effects and is well tolerated in humans, it could be a useful drug to treat stroke patients.

## Caffeinol

Caffeine (1,3,7-trimethylxantine) and ethanol are among the most widely and frequently used psychoactive drugs in most societies. Both are rapidly absorbed from the gastrointestinal tract achieving high concentrations in the brain via the blood stream. Caffeine is a competitive antagonist at the adenosine receptor. Its chronic administration up-regulates the brain adenosine receptor \[97\]. This receptor up-regulation, in combination with increased brain adenosine
concentrations produced in response to ATP breakdown during ischemia, could increase stimulation of inhibitory adenosine transduction pathways resulting in neuroprotection [98]. Ethanol, on the other hand, acts on the brain via GABA<sub>A</sub> receptor stimulation and inhibition of NMDA receptors [99]. It was shown to reduce neuronal death in a gerbil model of global ischemia [100].

Aronowski et al. [101] demonstrated that caffeine, a mixture of ethanol (0.32 g/kg) and caffeine (10 mg/kg), combined with intra-ischemic TH (35 °C), initiated 1 h after a 2.5 h transient MCAO, enhanced the neuroprotective effect of caffeine or TH alone by >50%. They also demonstrated that it is very important to apply ethanol and caffeine at the same time in order to achieve optimal neuroprotection. Animals that were treated with ethanol and 2 h later with caffeine showed no benefit [101]. However, despite the positive effects of caffeine, a disadvantage is the development of tolerance to its neuroprotective effect. Adenosine receptors are known to desensitize rapidly in response to repetitive activation [102]. Analogously, repetitive treatment with ethanol up-regulates N-methyl-D-aspartate (NMDA) receptor activity, down-regulates GABA<sub>A</sub> receptor function [103] and potentiates susceptibility to glutamate excitotoxicity [104]. Daily administration of caffeine, for 2 to 3 weeks, before stroke builds up a tolerance to its neuroprotective properties [105]. In a study by Martin-Schild et al. [106], it was demonstrated that it is feasible to combine a 2 h caffeine infusion (caffeine 8-9 mg/kg + ethanol 0.4 g/kg), started 4 h after symptom onset, and TH (33-35 °C, started 5 h after symptom onset) in patients with acute stroke treated with rt-PA. No adverse events were attributed to caffeine and there was no apparent increase in brain hemorrhage by linking caffeine and cooling to rt-PA. However, as no control group was included in the study, the efficacy of the combination therapy could not be evaluated.

**Xenon**

Xenon, a noble gas with anesthetic properties, provides neuroprotection in experimental models of neonatal asphyxia [107, 108], focal IS [109-111], cardiopulmonary bypass [112] and cardiac arrest [113]. It is known to interact with the adenosine triphosphate potassium channel [114, 115] and the glutamatergic NMDA receptor [116-118]. In addition, xenon may also be neuroprotective by reducing overall neurotransmitter release [119], inhibition of the α-amino-3-hydroxy-5-methyl-4-isoxazolole propionate and kainate receptors, which are 2 subtypes of glutamate receptor channels [120], reduction in cytosolic pro-apoptotic Bax protein expression and enhanced Bcl-xL expression [121-123], activation of 2-pore domain K<sup>+</sup> channels [124], inhibition of calcium/calmodulin-dependent protein kinase II [119], and increased phosphorylation of transcription factor cAMP-response element binding protein, which may, in turn, up-regulate cAMP-response element binding protein-dependent pro-survival genes [125, 126]. Since TH and xenon have been shown to have both anti-excitotoxic [127, 128] and anti-apoptotic properties [129-131], the combination has been studied in a rat model of temporary focal ischemia [132]. The results showed that xenon (30%) and TH (36 °C) exhibit synergistic neuroprotective properties when administered together. Xenon may thus be a promising agent with effective neuroprotective properties and has no adverse effects when administered at sub-anesthetic concentrations [109, 110, 112]. However, there are two drawbacks related to the use of xenon. First, it is described that xenon inhibits tPA thrombolysis and should only be administered after tPA-induced reperfusion [133]. Second, xenon delivery capability systems are currently very expensive, making their wide use less probable.

**Magnesium and Tirilazad**

Magnesium is a naturally occurring calcium antagonist that promotes vasodilation of cerebral arteries [134]. Magnesium ions (Mg<sup>2+</sup>) participate in a voltage-sensitive blockade of ion channels, resulting in a non-competitive antagonism of NMDA receptors [33, 134]; they compete with extracellular calcium ions to reduce calcium entry into cells [135] and inhibit the release of intracellular calcium ions [136] and excitatory amino acids [137]. The 21-aminosteroid tirilazad mesylate, on the other hand, is a free radical scavenger and lipid peroxidation inhibitor [138]. Both drugs have been shown to have beneficial effects in numerous animal studies of focal and global cerebral ischemia [139, 140], traumatic brain injury [141], subarachnoid hemorrhage [142] and spinal cord ischemia [143]. Also the combination of these pharmacological agents has been shown to be synergistically neuroprotective in a rat model of transient focal cerebral ischemia [144]. Schmid-Elsaesser et al. [145] investigated the effect of TH (33 °C) combined with tirilazad (3 mg/kg) and magnesium (MgCl<sub>2</sub>, 1 mmol/kg), administered both before and after ischemia, in rats subjected to MCAO for 90 min and concluded that the combination significantly reduced infarct size. Even in a model of permanent focal cerebral ischemia of 6 h, the combined therapy provided neuroprotection [146]. In a follow-up paper, Zausinger et al. [147] defined the therapeutic window of this combination and concluded that, in a temporal MCAO rat model (90 min), this therapeutic strategy is even efficient when applied up to 3 h after the ischemic insult.

**Mannitol**

Mannitol has neuroprotective properties in transient [148] and permanent MCAO models [149, 150] as well as in models of hemorrhagic stroke [151]. It is a hypertonic agent that reduces blood viscosity up to 2 h after i.v. administration [152] and thus improves cerebral blood flow in ischemic situations [153, 154]. It also reduces cerebral edema because of its osmotic effect [155] and acts as a free-radical scavenger [156]. The combination of mannitol with TH was evaluated by Karibe et al. [157] who concluded that, in a rat model of transient focal ischemia, mannitol (25%, 1 g/kg) added no significant protection to TH (33 °C). Also in a hypertensive rabbit model of temporary focal ischemia, mannitol (1 g/kg of body weight) combined with TH (33-34 °C) did not result in significantly smaller infarct volumes compared with the individual therapies [158]. The use of mannitol in combination with TH is therefore not indicated. Furthermore, as the effects of mannitol change with time after administration, it is an unsuitable agent to use. It was found that within 30 min of administration, intracranial pressure (ICP) drops...
followed by an increase in ICP secondary to a rebound effect [159, 160].

**Methohexital**

The protective properties of barbiturates include stabilization of cell membranes, improvement of blood flow into the ischemic brain tissue as well as counteraction of oxidative and excitotoxic processes, resulting in an inhibition of intracellular calcium overload [161]. Westermaier et al. [162] studied whether methohexital, a barbiturate, provides an additional protective effect under hypothermic conditions. Sodium methohexital infusion started 30 min before ischemia at a dose of 1 to 1.5 mg/kg/min until an EEG burst-suppression pattern. Next, it was kept at an infusion rate of 0.4 to 0.6 mg/kg/min. TH (33 °C) was also induced 30 min before the ischemic insult. Although the total infarct volumes were significantly smaller in TH vehicle-treated animals and in TH methohexital-treated animals, compared with normothermic vehicle-treated controls and normothermic methohexital-treated animals, the barbiturate therapy did not provide a significant additional protection under hypothermic conditions [162].

**Brain-Derived Neurotrophic Factor**

Brain-derived neurotrophic factor (BDNF) is a well-characterized neurotrophic factor which plays an important role in proliferation, differentiation, maintenance, plasticity, survival and function of neurons in the central and peripheral nervous system [163]. Considering its actions, several studies reported a neuroprotective effect in various ischemia models. In a rat model of temporary focal cerebral ischemia, administration of BDNF through an intraventricular route [164], as well as intravenously [165], has been shown to reduce infarct size and improve neurological outcome. Potential mechanisms of the neuroprotective role of BDNF in focal cerebral ischemia include counter-regulation of Bax and Bcl-2 proteins within the ischemic penumbra [165]. However, the exact mechanisms are still under debate. Berger et al. [166] investigated, in a rat model of IS, whether TH (33 °C) and intravenous administration of BDNF at 300 µg/kg/h for 2 h, applied 30 min after stroke onset, acted synergistically. They concluded that, when applied separately, TH and BDNF reduced infarct size by 20% and 19%, respectively. However, the combined strategy reduced the infarct size by approximately 40% as compared to nontreated control animals. Both therapies seem to reduce glutamate and the extracellular accumulation of endogenous BDNF. Important to notice is that the BDNF dose used in this study was rather high, i.e. 300 µg/kg/h, compared to studies where BDNF was administered intraventricularly [164] or when other growth factors were used to treat focal cerebral ischemia [167]. The authors mention to have chosen this dose since lower daily doses of 60-80 µg/d were not neuroprotective when given intravenously after transient forebrain ischemia. This may be explained by the fact that, after peripheral delivery, exogenous BDNF may encounter two major obstacles in arriving at the central nervous system, i.e. degradation during circulation in blood and the presence of the BBB which limits transport of BDNF to the brain [168]. Surprisingly, in that study, it seems that TH does not influence the level of endogenous BDNF. Also in a rat model of global ischemia [169] and a pig model of hypoxic ischemic (HI) brain injury [170], it has been shown that endogenous BDNF levels remain unaltered by TH.

**Albumin**

Albumin is an endogenous plasma protein with neuroprotective properties. It is known to protect both parenchymal and vascular elements of the brain, diminish brain edema, maintain microvascular integrity, inhibit endothelial cell apoptosis and exert antioxidant effects [171]. However, both moderate and high-dose systemic human albumin therapies have been shown to cause severe dose-related adverse effects in clinical stroke studies [172]. Therefore, Chen et al. [173] investigated the neuroprotective effect of low-dose albumin (0.5 g/kg), infused locally at the ischemic site, in a rat model of 2 h MCAO. By infusing albumin as a cold (0 °C) solution, it was furthermore possible to induce local brain hypothermia. Temperature was significantly reduced within 3 min in the cerebral cortex and the striatum (from ± 37 °C to ± 30 °C) and the reduced temperature was sustained up to 45 min. The local low-dose cold albumin infusion into the ischemic area, which offered a combination of regional brain hypothermia and albumin administration, resulted in a significant reduction in infarct volume and a significant improvement in neurological and motor function. This method has the potential to be used in a clinical setting as it has the advantage that cooled low doses of albumin can be infused, which reduces the risk of systemic adverse dose-related effects. Furthermore, it produces local TH much faster than systemically induced whole body TH. However, the local infusion of albumin in human brain is very invasive and risky as well as the long-term neuroprotective effect of this strategy in animal models remains to be investigated.

**DECOMPRESSIVE CRANIECTOMY**

Regardless of the underlying pathology, all types of stroke are accompanied with brain edema. Because the brain is encased by the walls of the bony skull, this swelling leads to an increased ICP and results in an enlarged area of brain damage. Cranietomy, a technique in which a portion of the skull is removed and the dura is opened, is one of the most effective ways to relieve the increased ICP secondary to cerebral edema following IS [174]. The effect of combined decompressive craniectomy and TH was first investigated by Doerfler et al. [175]. In a rat model of permanent MCAO, it was demonstrated that craniectomy combined with TH (32 °C, maintained for 5 h), yields a significant additional benefit as the combination resulted in additional reduction in infarct size and improvement in neurological outcome compared with controls. Using the same model, Jieyong et al. [176] demonstrated that the combined therapy up-regulates Bcl-2, an anti-apoptotic protein, and down-regulates Bax, a pro-apoptotic protein, resulting in a reduction in cell apoptosis and infarct size.

This combination strategy was also evaluated in patients with malignant supratentorial cerebral ischemia [177]. It seemed that patients who received a combination of TH (35 °C) and hemi-craniectomy performed better compared to patients who were treated with decompressive hemicraniectomy.
alone [177]. However, the difference was not statistically significant, due to the small sample size, and therefore, no definitive conclusions could be drawn.

GENE THERAPY

A novel therapeutic strategy to treat IS is gene therapy or the transfer of genetic material into host cells with the intention of expressing the protein of interest. It is known that cerebral ischemia alters the expression of many genes. Those genes possessing neuroprotective properties may thus be ideal candidates for gene therapy.

Lawrence et al. [178] showed that overexpression of the anti-apoptotic protein Bcl-2 prevents apoptosis and improves striatal neuron survival when delivered 1.5 h after stroke. When delivered 5 h after stroke, no protection was observed. However, post-ischemic TH (33 °C) prolonged the therapeutic window for Bcl-2 gene therapy from 1.5 to 5h, and Bcl-2 plus TH blocked cytochrome c release 48 h after the ischemia onset. These data demonstrate a synergistic effect of TH and Bcl-2 overexpression, suggesting a potential clinical application of TH combined with gene therapy [179].

PROTEIN THERAPY

Gene therapy may be a potential treatment strategy to improve stroke related brain damage. However, it takes considerable time to deliver a gene of interest into the brain and to express a sufficient amount of therapeutic proteins. As an alternative approach, delivering therapeutic proteins directly to ischemic brain tissue has been proposed [180]. This is possible through protein transduction domains (PTDs), which are small peptides that are able to ferry much larger molecules into cells independent of classical endocytosis [181]. PTDs have made it possible to design strategies for delivering pharmacologically potent macromolecules and even active enzymes to brain tissue by crossing the BBB and cell membranes [182]. One of these macromolecules is FNK, an artificial protein derived from the anti-apoptotic protein Bcl-xL by substituting three amino acids [183]. It is the first mutant with a gain-of-function phenotype among the mammalian anti-apoptotic factors and shows anti-necrotic as well as anti-apoptotic activity [183]. By fusing it with the human immunodeficiency virus type 1/trans-activator of transcription (HIV-TAT) protein transduction domain, it can be transduced into neuronal cells rapidly [184]. The protein transduction domain fused FNK (PTD-FNK) protein has been shown to be significantly protective against rat brain focal ischemia, even when administered 3 h after ischemia [184]. Recently, Sakurazawa et al. [185] evaluated whether PTD-FNK combined with TH (35 °C) is more effective than monotherapy in a rat model of MCAO. It could be concluded that a hypothermic PTD-FNK treatment significantly reduces infarct volume compared to a normothermic PTD-FNK treatment [185]. The combined therapy is thus an effective and safe strategy for neuronal protection against cerebral ischemia.

COMBINATION THERAPY TESTED IN NEONATAL RATS

Several of the combination treatments mentioned above were also tested in a neonatal rat model of HI brain injury. HI injury is a devastating complication in childbirth [186] that occurs at a frequency of 1-4 per 1,000 live births [187]. It causes long-term neurological and behavioral impairment in the developing brain. To date, hypothermia is the only intervention that improves outcome. As with stroke, several research groups evaluated whether a second intervention could augment the protection afforded by TH.

Ma et al. [131] demonstrated that concurrent administration of xenon (20%) and TH (35 °C) synergistically reduced long-term damage in a rat model of neonatal asphyxia. In a follow-up study, the authors also concluded that even asynchronous administration of xenon and hypothermia at a 1 h interval could produce a significant reduction in infarct volume [123]. The combination was also evaluated by Hobbs et al. [188] who concluded that xenon (50%) and TH (32°C) additively confer greater protection than either treatment alone. These findings are in accordance with the study of Sheng et al. [132], who demonstrated that xenon (30%) and TH (36 °C) exhibit synergistic neuroprotective properties when administered together in a temporal rat MCAO model.

Another combination that was tested in neonatal rats was TH plus MK-801. Ikonomidou et al. [189] concluded that lowering the body temperature by 2.5 °C together with MK-801 pretreatment provides total protection against HI brain damage in infant rat pups. Also Alkan et al. [190] demonstrated that, in a rat HI model, the combined treatment significantly reduced mortality rate and offered better protection in terms of neuron survival. However, these findings are in contrast with those of Frazzini et al. [31] who evaluated the neuroprotective effect of this combination in a rat MCAO model but could not demonstrate an additive degree of cerebroprotection. This finding demonstrates that the results depend on the model used.

CONCLUSION

A better understanding of the underlying pathophysiology of IS has led to the development of several pharmacological and non-pharmacological neuroprotective agents. Despite the promising results in experimental studies, not all success at the bench has translated to success at the bedside [191]. Therefore, the development of additional acute stroke therapies represents a large unmet need with many remaining challenges but also opportunities to incorporate novel approaches [192]. Because of the poor response to the neuroprotective monotherapy, several research groups have evaluated the neuroprotective potential of combination therapies. As TH is currently the most promising neuroprotective strategy, it is an ideal candidate to combine with other neuroprotective compounds. Of all agents summarized in this review, dextromethorphan, edaravone, argatroban, atorvastatin, tacrolimus, citicoline, BDNF, albumin and magnesium, whether or not combined with tirilazad, were found to be synergistically protective when combined with TH in rodent models of acute IS. However, their neuroprotective potential still needs to be confirmed in clinical trials. To date, only r-tPA, caffenol and decompressive hemiecraniectomy have been combined with TH in IS patients. Unfortunately, no definitive conclusions concerning the efficacy of these treatment strategies can be
drawn yet. Further researches, both experimental and clinical, are needed.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

Goossens J. drafted the manuscript. Hachimi-Idrissi S. contributed to the design as well as the correction of the manuscript.

REFERENCES

[1] Goldstein, L. B. Acute ischemic stroke treatment in 2007. Circulation, 2007, 116(13), 1504-1514. DOI: http://dx.doi.org/10.1161/CIRCULATIONAHA.106.670885

[2] Hacke, W.; Kaste, M.; Bluhmki, E.; Brozman, M.; Davalos, A.; Guidetti, D.; Larrue, V.; Lees, K. R.; Medeghri, Z.; Machin, T.; Schneider, D.; von Kummer, R.; Wahlgren, N.; Toni, D.; Investigators, E. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N. Engl. J. Med., 2008, 359(13), 1317-1329. DOI: http://dx.doi.org/10.1056/NEJMoa0804656

[3] Lees, K. R.; Bluhmki, E.; von Kummer, R.; Brot, T. G.; Toni, D.; Grotta, J. C.; Albers, G. W.; Kaste, M.; Marler, J. R.; Hamilton, S. J.; Investigators, E. Thrombolysis with recombinant tissue plasminogen activator for acute hemispheric stroke. The European Cooperative Acute Stroke Study (ECASS). JAMA, 1994, 274(13), 1017-1025. DOI: http://dx.doi.org/10.1001/jama.274.13.1017

[5] Ginsberg, M. D. Neuroprotection for ischemic stroke: past, present and future. Neuropharmacology, 2008, 55(3), 363-389. DOI: http://dx.doi.org/10.1016/j.neuropharm.2007.12.007

[8] Durukan, A.; Tatlisumak, T. Acute ischemic stroke: overview of major rodent models in neurology, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol. Biochem. Behav., 2008, 87(1), 179-197. DOI: http://dx.doi.org/10.1016/j.pbb.2007.04.015

[10] Lyden, P. D.; Krieger, D.; Yenari, M.; Dieterich, W. D. Therapeutic hypothermia for acute stroke. Int. J. Stroke, 2006, 1(1), 9-19. DOI: http://dx.doi.org/10.1111/j.1747-4949.2005.00011.x

[12] So, H. Y. Therapeutic hypothermia. Korean J. Anesthesiol., 2010, 60(2), 179-201. DOI: http://dx.doi.org/10.4097/kjae.2010.60.2.179

[13] Gupta, R.; Jovin, T. G.; Krieger, D. W. Therapeutic hypothermia for stroke: do new outfits change an old friend? Expert Rev. Neurother., 2005, 5(2), 235-246. DOI: http://dx.doi.org/10.1586/14737155.5.2.235

[14] Lanier, W. L. Cerebral Metabolic-Rate and Hypothermia - Their Relationship with Ischemic Neurologic Injury. J. Neurosurg. Anesthesiol., 1995, 7(3), 216-221. DOI: http://dx.doi.org/10.1097/00005856-199507000-00021

[15] Busto, R.; Globus, M. Y.; Dietrich, W. D.; Martinez, E.; Valdes, I.; Ginsberg, M. D. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke, 1989, 20(7), 904-910. DOI: http://dx.doi.org/10.1161/01.STR.20.7.904

[16] Busto, R.; Globus, M. Y.; Dietrich, W. D.; Martinez, E.; Valdes, I.; Ginsberg, M. D. Effect of Mild Hypothermia on Ischaemia-Induced Release of Neurotransmitters and Free Fatty Acids in Rat-Brain. Stroke, 1989, 20(7), 904-910. DOI: http://dx.doi.org/10.1161/01.STR.20.7.904

[17] Globus, M. Y.; Alonso, O.; Dietrich, W. D.; Busto, R.; Ginsberg, M. D. Glutamate release and free radical production following brain injury: effects of posttraumatic hypothermia. J. Neurochem., 1995, 65(4), 1704-1711. DOI: http://dx.doi.org/10.1006/jnca.1995.65041704x

[18] Smith, S. L.; Hall, E. D. Mild pre- and posttraumatic hypothermia attenuates blood-brain barrier damage following controlled cortical impact injury in the rat. J. Neurotrauma, 1996, 13(1), 1-9. DOI: http://dx.doi.org/10.1089/neu.1996.13.1

[19] Liu, L. P.; Yenari, M. A. Clinical application of therapeutic hypothermia in stroke. Neuro. Res., 2009, 31(4), 331-335. DOI: http://dx.doi.org/10.1159/000174313209XX440499

[20] Krieger, D. W.; Yenari, M. A. Therapeutic hypothermia for acute ischemic stroke - What do laboratory studies teach us? Stroke, 2004, 35(6), 1482-1489. DOI: http://dx.doi.org/10.1161/01.STR.0000126118.42439.Sc

[21] Zhao, H.; Steinberg, G. K.; Saposky, R. M. General versus specific actions of mild-moderate hypothermia in attenuating cerebral ischemic damage. J. Cereb. Blood Flow Metab., 2007, 27(12), 1879-1894. DOI: http://dx.doi.org/10.1038/sj.jcbfm.9600540

[22] Zgavc, T.; Ceulemans, A. G.; Sarre, S.; Michotte, Y.; Hachimi-Idrissi, S. Experimental and clinical use of therapeutic hypothermia for ischemic stroke: opportunities and limitations. Stroke Res. Treat., 2011, 2011, 689290.

[23] Tang, X. N.; Liu, L.; Yenari, M. A. Combination therapy with hypothermia for treatment of cerebral ischemia. J. Neurotrauma, 2009, 26(3), 325-331. DOI: http://dx.doi.org/10.1089/neuro.2011.689290

[24] Hemmen, T. M.; Lyden, P. Multimodal neuroprotective therapy with induced hypothermia after ischemic stroke. Stroke, 2009, 40(3 Suppl), S126-128. DOI: http://dx.doi.org/10.1161/STROKEAHA.108.533083

[25] Zeiner, A.; Holzer, M.; Sterz, F.; Behringer, W.; Schokhuber, W.; Mullner, M.; Frass, M.; Siostrzonek, P.; Ratheiser, K.; Kaff, A.; Lagner, A. M. Mild resuscitative hypothermia to improve neurological outcome after cardiac arrest. A clinical feasibility trial. Hypothermia After Cardiac Arrest (HACA) Study Group. Stroke, 2000, 31(1), 86-94.

[26] Choi, D. W. Glutamate neurotoxicity and diseases of the nervous system. Neuro, 1988, 1(I), 623-634. DOI: http://dx.doi.org/10.1010/0086-6273(88)90162-6

[27] Meldrum, B. Protection against ischaemic neuronal damage by drugs acting on excitatory neurotransmission. Cerebrovasc. Brain Metab. Rev., 1990, 2(1), 27-57. PMID: 2169834

[28] Mitani, A.; Kataoka, K. Critical levels of extracellular glutamate mediating gerbil hippocampal delayed neuronal death during hypothermia: brain microdialysis study. Neuroscience, 1991, 42(3), 661-670. DOI: http://dx.doi.org/10.1016/0306-4522(91)90035-M

[29] Ginsberg, M. D.; Globus, M. Y. T.; Busto, R.; Dietrich, W. D. The Potential of Combination Pharmacotherapy in Cerebral Ischemia. In: Krieglstein, J.; Oberpichler, H., Eds. Pharmacology Cerebral Ischemia, 1990, Stuttgart, Germany: Wissenschaftliche Verlagsgesellschaft, mbH; 1990, 499-510.

[30] Frazzini, V. I.; Winfree, C. J.; Choudhri, H. F., Prestigiacciomo, C. J.; Solomon, R. A. Mild Hypothermia and Mx-801 Have Similar but Not Additive Degrees of Cerebroprotection in the Rat Permanent Focal Ischemia Model. Neurosurgery, 1994, 34(6), 1040-1045. DOI: 10.1227/00006123-199406000-00013

[31] Meloni, B. P.; Zhu, H.; Knuckey, N. W. Is magnesium neuroprotective following global and focal cerebral ischemia? A review of published studies. Magnes. Res., 2006, 19(2), 123-137. PMID: 16955724

[32] Nowak, L.; Bregestovski, P.; Ascher, P.; Herbet, A.; Prochiantz, A. Magnesium gates glutamate-activated channels in mouse central neurones. Nature, 1984, 307(5950), 462-465. DOI: http://dx.doi.org/10.1038/307462a0

[33] Campbell, K.; Meloni, B. P.; Knuckey, N. W. Combined magnesium and mild hypothermia (35 degrees C) treatment reduces
infarct volumes after permanent middle cerebral artery occlusion in the rat at 2 and 4, but not 6 h. Brain Res., 2008, 1230, 258-264. DOI: http://dx.doi.org/10.1016/j.brainsci.2008.06.110

[35] Song, W.; Wu, Y. M.; Ji, Z.; Ji, Y. B.; Wang, S. N.; Pan, S. Y. Intracarotid cold magnesium sulfate infusion induces selective cerebral hypothermia and neuroprotection in rats with transient middle cerebral artery occlusion. Neurosci. Lett., 2013, 544, 479-486. DOI: http://dx.doi.org/10.1016/j.neulet.2013.06.057

[36] Meloni, B. P.; Cross, J. L.; Brookes, L. M.; Clark, V. W.; Campbell, K.; Knuckey, N. FAST-Mag protocol with or without mild hypothermia (35 degrees C) does not improve outcome after permanent MCAO in rats. Stroke Res. Pract., 2013, 34(2), 67-73. DOI: http://dx.doi.org/10.1155/2013/606179

[37] Coyle, J. T. The nagging question of the function of N-methyl-d-aspartate (NMDA) receptor ion channels. Pharmacol. Rev., 2005, 57(5), 1287-1297. DOI: http://dx.doi.org/10.1121/1.1475192

[38] Sistare, F. D.; Lester, H. A.; Paule, M. G.; Scallet, A. C.; Haberny, K. A.; Meloni, B. P.; Cross, J. L.; Brookes, L. M.; Clark, V. W.; Campbell, K.; Knuckey, N. W. FAST-Mag protocol with or without mild hypothermia in the endothelin-1 rat model of focal cerebral ischaemia. J. Neurochem., 2005, 93(5), 1287-1297. DOI: http://dx.doi.org/10.1111/j.1471-4159.2005.03450.x

[39] Ping, J. P.; Duvoisin, R., The Metabotropic Glutamate Receptors - Structure and Functions. Neuropharmacology, 1995, 34(1), 1-26. PMID: 7623957

[40] Van Hemelrijck, A.; Hachimi-Idrissi, S.; Sarre, S.; Ebinger, G.; Pin, J. P.; Duvoisin, R. The Metabotropic Glutamate Receptors - Structure and Functions. Neuropharmacology, 1995, 34(1), 1-26. PMID: 7623957

[41] Haberny, K. A.; Paul, M. G.; Scallet, A. C.; Sistare, F. D.; Lester, D. S.; Hanig, J. P.; Sikker, W., Jr. Ontogeny of the N-methyl-D-aspartate (NMDA) receptor system and susceptibility to neurotoxicity. Toxicol. Sci., 2002, 68(1), 9-17. DOI: http://dx.doi.org/10.1093/toxsci/68.1.9

[42] Hardingham, G. E.; Bading, H. The Yin and Yang of NMDA receptor signalling. Trends Neurosci., 2003, 26(2), 81-89. DOI: http://dx.doi.org/10.1016/S0166-2236(02)00040-4

[43] Ihn, J.; Kim, J.; Kang, H. I.; Han, S. J.; Lee, J. H.; Park, S. J. Green tea polyphenol (EGCG) reduces NMDA-induced neuronal death in vitro and protects against NMDA-induced neurodegeneration in vivo. Brain Res., 2007, 1146, 129-137. DOI: http://dx.doi.org/10.1016/j.brainres.2007.03.034

[44] Ihn, J.; Kim, J.; Kang, H. I.; Han, S. J.; Lee, J. H.; Park, S. J. Green tea polyphenol (EGCG) reduces NMDA-induced neuronal death in vitro and protects against NMDA-induced neurodegeneration in vivo. Brain Res., 2007, 1146, 129-137. DOI: http://dx.doi.org/10.1016/j.brainres.2007.03.034

[45] Kim, J.; Kang, H. I.; Han, S. J.; Lee, J. H.; Park, S. J. Green tea polyphenol (EGCG) reduces NMDA-induced neuronal death in vitro and protects against NMDA-induced neurodegeneration in vivo. Brain Res., 2007, 1146, 129-137. DOI: http://dx.doi.org/10.1016/j.brainres.2007.03.034

[46] Kim, J.; Kang, H. I.; Han, S. J.; Lee, J. H.; Park, S. J. Green tea polyphenol (EGCG) reduces NMDA-induced neuronal death in vitro and protects against NMDA-induced neurodegeneration in vivo. Brain Res., 2007, 1146, 129-137. DOI: http://dx.doi.org/10.1016/j.brainres.2007.03.034

[47] Grotta, J.; Marler, J. Intravenous rt-PA: a tenth anniversary reflection. Surgical Neurol., 2007, 68 (1), 12-16. DOI: http://dx.doi.org/10.1016/j.surneu.2007.07.079

[48] Liu, L. P.; Tang, X. N.; Yenari, M. A. Mild hypothermia reduces hemorrhage and blood brain barrier (BBB) disruption following transient focal cerebral ischemia but does not improve neurologic outcome after permanent middle cerebral artery occlusion. Neurology, 2006, 66(5), A242-A243. ISSN: 0028-3878. IDS Number: 022PM, Accession Number: WOS:000236068103009

[49] Kallmünzer, B.; Schwab, S.; Kollmar, R. Mild hypothermia of 34 degrees C reduces side effects of rt-PA treatment after thromboembolic stroke in rats. Exp. Stroke Med., 2012, 1(1), 3. DOI: http://dx.doi.org/10.1186/2046-9993-1-3

[50] Neulen, P.; Oertel, M.; Pedersen, H.; Boysen, G. Effect of hypothermia and delayed thrombolysis in a rat embolic stroke model. Acta Neuro. Scand., 1994, 90(2), 91-98. DOI: http://dx.doi.org/10.1111/j.1600-0404.1994.tb02686.x

[51] Kollmar, R.; Henninger, N.; Bardutzky, J.; Schelling, P. D.; Schabitz, W. R.; Schwab, S. Combination therapy of moderate hypothermia and thrombolysis in experimental thromboembolic stroke—an MRI study. Exp. Neuro., 2004, 190(1), 204-212. DOI: http://dx.doi.org/10.1016/j.expneurol.2004.01.007

[52] Bi, M.; Ma, Q.; Zhang, S.; Li, J.; Zhang, Y.; Lin, L.; Tong, S.; Wang, D. Local mild hypothermia with thrombolysis for acute ischemic stroke within a 6-h window. Clin. Neuro. Neurosurg., 2013, 113(S), 768-773. DOI: http://dx.doi.org/10.1016/j.clinneuro.2011.08.100

[53] Hemmen, T. M.; Raman, R.; Guluma, K. Z.; Meyer, B. C.; Gomes, J. A.; Cruz-Flores, S.; Wijman, C. A.; Rapp, K. S.; Grotta, J. C.; Lyden, P. D.; Investigators, I. C.-L. Intravenous thrombolysis plus hypothermia for acute treatment of ischemic stroke (ICTuS-L): final results. Stroke, 2010, 41(10), 2265-2270. DOI: http://dx.doi.org/10.1161/STROKEAHA.110.592295

[54] Okamoto, S.; Hiki-jaka, Okunomiya, A. Synthetic selective inhibitors of thrombin. Methods Enzymol., 1993, 222, 328-340. DOI: http://dx.doi.org/10.1006/menl.1993.1001

[55] Swan, S. K.; Hursting, M. J. The pharmacokinetics and pharmacodynamics of argatroban: effects of age, gender, and hepatic or renal dysfunction. J. Pharmacokin. Pharmacother., 2003, 30(3), 218-329. DOI: http://dx.doi.org/10.1159/2003.00040000525183.34157.51

[56] Berry, C. N.; Girard, D.; Lochot, S.; Lecoffre, C. Antithrombotic actions of argatroban in rat models of venous- 'mixed' and arterial thrombosis, and its effects on the tail transection bleeding time. Br. J. Pharmacol., 1994, 113(4), 1209-1214. DOI: http://dx.doi.org/10.1038/bjp.1994.196

[57] Mathiasen, S.; Grotta, J. Intravenous rt-PA: a tenth anniversary reflection. J. Neurosci., 1999, 25(11), 2635-2640. DOI: http://dx.doi.org/10.1016/0022-3050(99)00071-6

[58] Karmy-Jones, T.; Nito, C.; Ueda, M.; Kato, K.; Amemiya, S.; Terasi, A.; Katayama, Y. Mild hypothermia enhances the neuroprotective effects of a selective thrombin inhibitor following transient focal ischemia in rats. Acta Neurochir. Suppl., 2003, 86, 195-198. DOI: http://dx.doi.org/10.1007/978-3-7091-0651-8-42

[59] Edgington, T. S.; Mackman, N. Astrocytes are the primary source of tissue factor in the murine central nervous system. A role for astrocytes in cerebral hemostasis. J. Clin. Invest., 1993, 92(1), 349-358. DOI: http://dx.doi.org/10.1172/JCI116573
by ethanol in vitro and by chronic in vivo ethanol ingestion. Brain Res., 1993, 602(1), 91-98. DOI: http://dx.doi.org/10.1016/0006-8993(93)00246-J

[104] Chandler, L. J.; Summers, C.; Crews, F. T. Ethanol inhibits NMDA receptor-mediated excitotoxicity in rat primary neuronal cultures. Alcohol Clin. Exp. Res., 1995, 19(4), 479-480. DOI: http://dx.doi.org/10.1111/j.1530-2729.1993.tb00726.x

[105] Strong, R.; Grotta, J. C.; Aronowski, J. Combination of low dose ethanol and caffeine protects brain from damage produced by focal ischemia in rats. Neuropharmacology, 2000, 39(5), 515-522. DOI: http://dx.doi.org/10.1016/S0028-3908(99)00156-2

[106] Martin-Schild, S.; Hallevi, H.; Shaltoni, H.; Barreto, A. D.; Gonzales, N. R.; Aronowski, J.; Savi, S. I.; Grotta, J. C. Combined neuroprotective modalities coupled with thrombolyis in acute ischemic stroke: a pilot study of caffeine and mild hypothermia. Stroke Cerebrovasc. Dis., 2009, 18(2), 86-96. DOI: http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2008.09.015

[107] Dingley, J.; Tooley, J.; Porter, H.; Thoresen, M. Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. Stroke, 2006, 37(2), 501-506. DOI: http://dx.doi.org/10.1161/01.STR.0000198867.31134.ac

[108] Luo, Y.; Ma, D.; Ieong, E.; Sanders, R. D.; Yu, B.; Hossain, M.; Maze, M. Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology, 2008, 109(5), 782-789. DOI: http://dx.doi.org/10.1097/ALN.0b013e3181895858

[109] Homi, H. M.; Yokoo, N.; Ma, D.; Warner, D. S.; Franks, N. P.; Maze, M.; Grocott, H. P. The neuroprotective effect of xenon administration during transient middle cerebral artery occlusion in mice. Anesthesiology, 2003, 99(4), 876-881. DOI: http://dx.doi.org/10.1097/00000542-200301000-00020

[110] David, H. N.; Leveille, F.; Charalvel, L.; Mackenzie, E. T.; Buisson, A.; Lemaire, M.; Abraini, J. H. Reduction of ischemic brain damage by nitrous oxide and xenon. J. Cereb. Blood Flow Metab., 2003, 23(10), 1168-1173. DOI: http://dx.doi.org/10.1097/01.WCB.0000087342.31689.18

[111] Limatola, V.; Ward, P.; Cattano, D.; Tu, J.; Giunta, F.; Maze, M.; Leveille, F.; Chazalviel, L.; MacKenzie, E. T.; Martin-Schild, S.; Hallevi, H.; Shaltoni, H.; Barreto, A. D.; Gonzales, N. R.; Aronowski, J.; Savi, S. I.; Grotta, J. C. Combined neuroprotective modalities coupled with thrombolysis in acute ischemic stroke: a pilot study of caffeine and mild hypothermia. Stroke Cerebrovasc. Dis., 2009, 18(2), 86-96. DOI: http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2008.09.015

[112] Dingley, J.; Tooley, J.; Porter, H.; Thoresen, M. Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. Stroke, 2006, 37(2), 501-506. DOI: http://dx.doi.org/10.1161/01.STR.0000198867.31134.ac

[113] Luo, Y.; Ma, D.; Ieong, E.; Sanders, R. D.; Yu, B.; Hossain, M.; Maze, M. Xenon and sevoflurane protect against brain injury in a neonatal asphyxia model. Anesthesiology, 2008, 109(5), 782-789. DOI: http://dx.doi.org/10.1097/ALN.0b013e3181895858

[114] Homi, H. M.; Yokoo, N.; Ma, D.; Warner, D. S.; Franks, N. P.; Maze, M.; Grocott, H. P. The neuroprotective effect of xenon administration during transient middle cerebral artery occlusion in mice. Anesthesiology, 2003, 99(4), 876-881. DOI: http://dx.doi.org/10.1097/00000542-200301000-00020

[115] David, H. N.; Leveille, F.; Charalvel, L.; Mackenzie, E. T.; Buisson, A.; Lemaire, M.; Abraini, J. H. Reduction of ischemic brain damage by nitrous oxide and xenon. J. Cereb. Blood Flow Metab., 2003, 23(10), 1168-1173. DOI: http://dx.doi.org/10.1097/01.WCB.0000087342.31689.18

[116] Limatola, V.; Ward, P.; Cattano, D.; Tu, J.; Giunta, F.; Maze, M.; Leveille, F.; Chazalviel, L.; MacKenzie, E. T.; Martin-Schild, S.; Hallevi, H.; Shaltoni, H.; Barreto, A. D.; Gonzales, N. R.; Aronowski, J.; Savi, S. I.; Grotta, J. C. Combined neuroprotective modalities coupled with thrombolysis in acute ischemic stroke: a pilot study of caffeine and mild hypothermia. Stroke Cerebrovasc. Dis., 2009, 18(2), 86-96. DOI: http://dx.doi.org/10.1016/j.jstrokecerebrovasdis.2008.09.015

[117] Schmidt, M.; Marx, T.; Gloggl, E.; Reinelt, H.; Schirmer, U. Xenon attenuates cerebral damage after ischemia in pigs. Anesthesiology, 2005, 102(5), 929-936. DOI: http://dx.doi.org/10.1097/00000542-200505000-00011

[118] Yamada, K.; Fujii, M. J. J.; Yuan, H.; Miki, T.; Sato, S.; Horimoto, N.; Shimizu, T.; Seino, S.; Inagaki, N. Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. Science, 2001, 292(5521), 1543-1546. DOI: http://dx.doi.org/10.1126/science.1059829

[119] Bantel, C.; Maze, M.; Trapp, S. Noble gas xenon is a novel adenosine triphosphate-sensitive potassium channel opener. Anesthesiology, 2010, 112(3), 623-630. DOI: http://dx.doi.org/10.1097/ALN.0b013e3181c8f84a

[120] Franks, N. P.; Dickinson, R.; de Sousa, S. L.; Hall, A. C.; Lieb, W. R. How does xenon produce anaesthesia? Nature, 1998, 396(6709), 324. DOI: http://dx.doi.org/10.1038/1040-463769901872-8

[121] Natale, G.; Cattano, D.; Abramo, A.; Forfori, F.; Fuceri, F.; Formi, F.; Paparelli, A.; Giunta, F. Morphological evidence that xenon protects against N-methyl-DL-aspartic acid-induced damage in the rat arcuate nucleus: a time-dependent study. Ann. N Acad. Sci., 2006, 1074, 650-658. DOI: http://dx.doi.org/10.1121/annals.1369.063

[122] Dickinson, R.; Peterson, B. K.; Banks, P.; Simillis, C.; Martin, J. C.; Valenzuela, G. A.; Maze, M.; Franks, N. P. Competitive inhibition at the glycine site of the N-methyl-D-aspartate receptor by the anesthetics xenon and isoflurane: evidence from molecular modeling and electrophysiology. Anesthesiology, 2007, 107(5), 756-767. DOI: http://dx.doi.org/10.1097/01.anes.0000287061.77674.71

[123] Petzet, C. P.; Kodirov, S.; Taschenberger, G.; Kox, W. J. Participation of the Ca(2+)-calmodulin-activated kinase II in the control of metaphase-anaphase transition in human cells. Cell Biol. Int., 2001, 25(5), 403-409. PMID: 11401327

[124] Gossens and Hachimi-Idrissi
Assessment by intracerebral brain pH, cortical blood flow, and electroencephalography. J. Neurosurg., 1987, 66(1), 109-115. DOI: http://dx.doi.org/10.3171/jns.1987.66.1.109

Bell, B. A.; Smith, M. A.; Kean, D. M.; McGhee, C. N.; MacDonald, H. L. ; Miller, J. D.; Barnett, G. H.; Tocher, J. L.; and Paulson, B. L. Brain blood measured by magnetic resonance imaging. Correlation with direct estimation and changes after mannitol and dexamethasone. Lancet, 1987, 1(8524), 66-69. DOI: http://dx.doi.org/10.1016/S0140-6736(87)91908-8

Suzuki, J. Y. T. The effect of mannitol in prolongation of permissible occlusion time of cerebral arteries: Clinical data of aneurysm surgery. Neurosurg. Rev., 1979, 1, 13-19. DOI: http://dx.doi.org/10.1007/BF01644042

Karlbe, H.; Zarow, G. J.; Weinstein, P. R. Use of mild intracerebral hypothermia versus mannitol to reduce infant size after temporary middle cerebral artery occlusion in rats. J. Neurosurg., 1995, 83(1), 93-98. DOI: http://dx.doi.org/10.3171/jns.1995.83.1.0093

Ogilvy, C. S.; Chu, D.; Kaplan, S. Mild hypothermia, hypertension, and mannitol are protective against infarction during experimental intracranial temporary vessel occlusion. Neurosurgery, 1996, 38(6), 1202-1209; discussion 1209-1210. DOI: http://dx.doi.org/10.1093/neuros/38.6.1202

Muizelaar, J. P.; Wei, E. P.; Kontos, H. A.; Becker, D. P. Mannitol decreases compensatory cerebrovascular vasodilation in response to blood viscosity changes. J. Neurosurg., 1983, 59(5), 822-828. DOI: http://dx.doi.org/10.3171/jns.1983.59.5.0822

Kotwica, Z.; Persson, L. Effect of mannitol on intracranial pressure in focal cerebral ischemia. An experimental study in a rat. Mater. Med. Pol., 1991, 23(4), 280-284. PMID: 18422334

Smith, D. S.; Rehnscrona, S.; Siejo S. K. Barbiturates as protective agents in brain ischemia and as free radical scavengers in vitro. Acta Physiol. Scand. Suppl., 1980, 492, 129-134. PMID: 6939303

Westmaier, T.; Zausinger, S.; Baethmann, A.; Steiger, H. J.; Schmid-Elsaesser, R. No additional neuroprotection provided by barbiturate-induced burst suppression under mild hypothermic conditions in rats subjected to reversible focal ischemia. J. Neurosurg. 2000, 93(5), 835-844. DOI: http://dx.doi.org/10.3171/jns.2000.93.5.0835

Geral, C.; Angelova, A.; Lesieur, S. From molecular to nanotechnology strategies for delivery of neurotrophins: emphasis on brain-derived neurotrophic factor (BDNF). Pharmaceutics, 2013, 5(1), 127-167. DOI: 10.3390/pharmaceutics5010127

Schabitz, W. R.; Schwab, S.; Spranger, M.; Hacke, W. Intraventricular brain-derived neurotrophic factor reduces infarct size after focal cerebral ischemia in rats. J Cereb Blood Flow Metab, 1997, 17(5), 500-506. PMID: 9183287

Schabitz, W. R.; Sommer, C.; Zoder, W.; Kiessling, M.; Schwabing, M.; Schwab, S. Intravenous brain-derived neurotrophic factor reduces infarct size and counterregulates Bax and Bcl-2 expression after transient focal cerebral ischemia. Stroke, 2000, 31(9), 2212-2217. PMID: 10978054

Berger, C.; Schabitz, W. R.; Wolf, M.; Mueller, H.; Sommer, C.; Schwab, S. Hypothermia and brain-derived neurotrophic factor reduce glutamate synergistically in acute stroke. Exp. Neurol., 2004, 185(2), 305-312. DOI: http://dx.doi.org/10.1016/j.expneurol.2003.10.008

Fisher, M.; Meadows, M. E.; Do; T.; Weise, J.; Trubetzkoy, V.; Charette, M.; Finklestein, S. P. Delayed treatment with intravenous basic fibroblast growth factor reduces infarct size following permanent focal cerebral ischemia in rats. J Cereb Blood Flow Metab, 1995, 15(6), 953-959. PMID: 7953356

Silasi, G.; Klahr, A. C.; Hacket, M. J.; Urrat, A. M.; Nichol, H.; Colbourne, F. Prolonged therapeutic hypothermia does not improve neurological outcome in global ischemia in rats. J Cereb Blood Flow Metab, 2012, 32(8), 1525-1534. doi: 10.1038/jcbfm.2012.38

Olson, L.; Faulkner, S.; Lundstrom, K.; Kerenyi, A.; Kelen, D.; Chandrasekaran, M.; Aden, U.; Olson, L.; Golay, X.; Lagercram, H.; Robertson, N. J.; Galter, D. Comparison of three hypothermic target temperatures for the treatment of HI HHHH ischemia: mRNA
level responses of eight genes in the piglet brain. *Transl Stroke Res.*, 2013, 4(2), 248-257. doi: 10.1007/s12975-012-0215-4

[171] Belalov, L.; Liu, Y.; Zhao, W.; Busto, R.; Ginsberg, M. D. Human albumin therapy of acute ischemic stroke: marked neuroprotective efficacy at moderate doses and with a broad therapeutic window. *Stroke*, 2001, 32(2), 553-560. DOI: http://dx.doi.org/10.1161/01.STR.32.2.553

[172] Ginsberg, M. D.; Hill, M. D.; Palesch, Y. Y.; Ryckborst, K. J.; Tamariz, D. The ALIAS Pilot Trial: a dose-escalation and safety study of albumin therapy for acute ischemic stroke: I: Physiological responses and safety results. *Stroke*, 2006, 37(8), 2100-2106. DOI: http://dx.doi.org/10.1161/01.STR.0000231388.72646.05

[173] Chen, J.; Fredrickson, V.; Ding, Y.; Cheng, H.; Wang, N.; Ling, F.; Ji, X. Enhanced neuroprotection by local intra-arterial infusion of human albumin solution and local hypothermia. *Stroke*, 2013, 44(1), 260-262. DOI: http://dx.doi.org/10.1161/STROKEAHA.112.675462

[174] Iwamoto, H. S.; Numoto, M.; Donaghy, R. M. Surgical decompression for cerebral and cerebellar infarcts. *Stroke*, 1994, 35(1), 365-370. DOI: http://dx.doi.org/10.1161/01.STR.53.365

[175] Doerfler, A.; Schwab, S.; Hoffmann, T. T.; Engelhorn, T.; Forsting, M. Combination of decompressive craniectomy and mild hypothermia ameliorates infarction volume after permanent focal ischemia in rats. *Stroke*, 2001, 32(11), 2675-2681. CrossRef DOI: http://dx.doi.org/10.1161/hs1101.098369

[176] Jieyong, B.; Zhong, W.; Shiming, Z.; Dai, Z.; Kato, Y.; Kanno, T.; Sano, H. Decompressive craniectomy and mild hypothermia reduces infarct size and counterregulates Bax and Bcl-2 expression after permanent focal ischemia in rats. *Neurosurg. Rev.*, 2006, 29(2), 167-182. DOI: http://dx.doi.org/10.1007/s10143-005-0010-8

[177] Els, T.; Oehm, E.; Voigt, S.; Kliech, J.; Hetzel, A.; Kassubeck, J. Safety and therapeutic benefit of hemicraniectomy combined with mild hypothermia in comparison with hemicraniectomy alone in patients with malignant ischemic stroke. *Cerebrovasc. Dis.*, 2006, 21(1-2), 79-85. DOI: http://dx.doi.org/10.1159/000090007

[178] Lawrence, M. S.; McLaughlin, J. R.; Sun, G. H.; Ho, D. Y.; McIntosh, L.; Kunis, D. M.; Sapolsky, R. M.; Steinberg, G. K. Herpes simplex viral vectors expressing Bcl-2 are neuroprotective when delivered after a stroke. *J. Cereb. Blood Flow Metab.*, 1997, 17(7), 740-744. DOI: http://dx.doi.org/10.1097/00004647-199707000-00003

[179] Zhao, H.; Yenari, M. A.; Sapolsky, R. M.; Steinberg, G. K. Mild postischemic hypothermia prolongs the time window for gene therapy by inhibiting cytochrome C release. *Stroke*, 2004, 35(2), 572-577. DOI: http://dx.doi.org/10.1161/01.STR.0000110787.42083.58

[180] Wadia, J. S.; Dowdy, S. F. Protein transduction technology. *Curr. Opin. Biotechnol.*, 2002, 13(1), 52-56. http://dx.doi.org/10.1016/S0958-1669(02)00284-7

[181] Beerens, A. M.; Al Hadithy, A. F.; Rots, M. G.; Haisma, H. J. Protein transduction domains and their utility in gene therapy. *Curr. Gene Ther.*, 2003, 3(5), 486-494. DOI: http://dx.doi.org/10.2174/1566523034578258

[182] Denicourt, C.; Dowdy, S. F. Protein transduction technology offers novel therapeutic approach for brain ischemia. *Trends Pharmacol. Sci.*, 2003, 24(5), 216-218. DOI: http://dx.doi.org/10.1016/S0165-6147(03)00774-9

[183] Asoh, S.; Ohsawa, I.; Mori, T.; Katsura, K.; Hiraide, T.; Katayama, Y.; Kimura, M.; Ozaki, D.; Yamagata, K.; Ohta, S. Protection against ischemic brain injury by protein therapeutics. *Proc. Natl. Acad. Sci. U. S. A.*, 2002, 99(26), 17107-17112. DOI: http://dx.doi.org/10.1073/pnas.262460299

[184] Katsura, K.; Takahashi, K.; Asoh, S.; Watanabe, M.; Sakurazawa, M.; Ohsawa, I.; Mori, T.; Igarashi, H.; Ohkubo, S.; Katayama, Y.; Ohta, S. Combination therapy with transductive anti-death FNK protein and FK506 ameliorates brain damage with focal transient ischemia in rat. *J. Neurochem.*, 2008, 106(1), 258-270. DOI: http://dx.doi.org/10.1111/j.1471-4159.2008.05360.x

[185] Sakurazawa, M.; Katsura, K.; Saito, M.; Asoh, S.; Ohta, S.; Katayama, Y. Mild hypothermia enhanced the protective effect of protein therapy with transductive anti-death FNK protein using a rat focal transient cerebral ischemia model. *Brain. Res.*, 2012, 1430, 86-92. DOI: http://dx.doi.org/10.1016/j.brainres.2011.08.041

[186] Vannucci, R. C.; Perlman, J. M. Interventions for perinatal H-I ischemic encephalopathy. *Pediatrics*, 1997, 100(6), 1004-1014.

[187] Levene, M. I.; Sands, C.; Grindulis, H.; Moore, J. R. Comparison of two methods of predicting outcome in perinatal asphyxia. *Lancet*, 1986, 1(8472), 67-69. PMID: 2867316

[188] Hobbs, C.; Thoresen, M.; Tucker, A.; Aquilina, K.; Chakkarapani, E.; Dingley, J. Xenon and hypothermia combine additively, offering long-term functional and histopathologic neuroprotection after neonatal hypoxia/ischemia. *Stroke*, 2008, 39(4), 1307-1313. doi: 10.1161/STROKEAHA.107.499822.

[189] Ikonomidou, C.; Mosinger, J. L.; Olney, J. W. Hypothermia enhances protective effect of MK-801 against hypoxic/ischemic brain damage in infant rats. *Brain Res.*, 1989, 487(1), 184-187. PMID: 2546648

[190] Alkan, T.; Kahveci, N.; Buyukyusa, L.; Korfali, E.; Ozluk, K. Neuroprotective effects of MK 801 and hypothermia used alone and in combination in hypoxic-ischemic brain injury in neonatal rats. *Arch Physiol Biochem.*, 2001, 109(2), 135-144. PMID: 11780774

[191] Xu, S. Y.; Pan, S. Y. The failure of animal models of neuroprotection in acute ischemic stroke to translate to clinical efficacy. *Med. Sci. Monit. Basic Res.*, 2013, 19, 37-45. PMID: 23353570

[192] Fisher, M.; Albers, G. W.; Donnan, G. A.; Furlan, A. J.; Grotta, J. C.; Kidwell, C. S.; Sacco, R. L.; Wechsler, L. R.; Stroke Therapy Academic Industry Roundtable, I. V. Enhancing the development and approval of acute stroke therapies: Stroke Therapy Academic Industry Roundtable. *Stroke*, 2005, 36(8), 1808-1813.DOI: http://dx.doi.org/10.1161/01.STR.0000173403.60553.27

Received: November 24, 2013  Revised: March 14, 2014  Accepted: April 22, 2014