Published in final edited form as:

Cent Nerv Syst Agents Med Chem. 2011 June 1; 11(2): 81–97.

Central Nervous System Agents for Ischemic Stroke: Neuroprotection Mechanisms

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Abstract

Stroke is the third leading cause of mortality and disability in the United States. Ischemic stroke constitutes 85% of all stroke cases. However, no effective treatment has been found to prevent damage to the brain in such cases except tissue plasminogen activator with narrow therapeutic window, and there is an unmet need to develop therapeutics for neuroprotection from ischemic stroke. Studies have shown that mechanisms including apoptosis, necrosis, inflammation, immune modulation, and oxidative stress and mediators such as excitatory amino acids, nitric oxide, inflammatory mediators, neurotransmitters, reactive oxygen species, and withdrawal of trophic factors may lead to the development of the ischemic cascade. Hence, it is essential to develop neuroprotective agents targeting either the mechanisms or the mediators leading to development of ischemic stroke. This review focuses on central nervous system agents targeting these biochemical pathways and mediators of ischemic stroke, mainly those that counteract apoptosis, inflammation, and oxidation, and well as glutamate inhibitors which have been shown to provide neuroprotection in experimental animals. All these agents have been shown to improve neurological outcome after ischemic insult in experimental animals *in vivo*, organotypic brain slice/acute slice *ex vivo*, and cell cultures *in vitro* and may therefore aid in preventing long-term morbidity and mortality associated with ischemic stroke.

Keywords

cerebral ischemia; neuroprotection; central nervous system agents; inflammation; apoptosis; oxidation; excitotoxicity

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1. INTRODUCTION

1.1 ISCHEMIC STROKE

Stroke occurs due to reduced perfusion to a brain region, resulting in death or permanent neurological deficits including hemiplegia, numbness, loss of sensory and vibratory sensation, balance problems, ptosis, decreased reflexes, visual field defects, apraxia, and aphasia due to neuronal damage of pathways of the central nervous system (CNS), including the brain stem or cerebellum. Ischemic stroke accounts for 85% of all strokes [1]. Cerebral ischemia in patients may be produced by thrombosis or embolism due to atherosclerosis from large or small vessels, embolism of cardiac origin, systemic hypoperfusion, occlusion of small blood vessels, venous thrombosis, or undetermined causes leading to reduced perfusion (No. 5 in Trial of Org 10172 in Acute Stroke Treatment, or TOAST, classification). Based on the location of the symptoms, ischemic stroke is classified as total anterior circulation infarct, partial anterior circulation infarct, lacunar infarct, or posterior circulation infarct. Computed Tomography scan and magnetic resonance imaging, has been used to detect cerebral ischemia.

1.2 PATHOGENESIS AND NEUROPROTECTION

Reduced perfusion of the brain initiates the ischemic cascade, leading to development of a reversible ischemic penumbra surrounding an irreversible area of infarction (Fig. 1). At a microscopic level, the reduced blood flow results in failure of the mitochondrial electron transport chain and oxidative phosphorylation, producing ATP depletion and failure of the Na-K-ATPase pump and leading to increased neuronal sodium and calcium influx. The resulting anoxic depolarization leads to release of excitatory neurotransmitters such as glutamate, causing neuronal toxicity [2]. Damage is also caused by reactive oxygen species (ROS), free radicals, arachidonic acid, nitric oxide, and cytokines generated in this process, leading to inflammation and further microcirculatory compromise. Activation of the immune system and apoptosis are also responsible for the pathogenesis. These pathological events do not necessarily occur sequentially and may instead follow a variable spatial and temporal course [2]. In addition, they are interlinked, triggering each other in a positive feedback loop that culminates in neuronal destruction [3].

Necrotic cell death is responsible for core infarction where hypoxia is most severe, leading to severe energy depletion and cellular collapse. In the penumbra, which is not yet irreversibly damaged and is potentially salvageable, the hypoxia is less severe due to collateral blood flow, and cells undergo apoptotic cascade [4]. Hence, neuronal protection in the ischemic penumbra and prevention of neurological deficits is the main goal of therapy with neuroprotective agents targeting the downstream events or mediators of the cascade that causes ischemic stroke [5]. Inhibition of the molecular mechanisms underlying the ischemic cascade has been recognized as an important target for the treatment of ischemic stroke.

1.3 MODELS FOR THE STUDY OF ISCHEMIC STROKE

In vivo, global, multifocal, and focal ischemia mechanisms are induced. The MCAO (middle cerebral artery occlusion) animal model of cerebral ischemia is widely utilized in stroke research. *Ex vivo* brain slice models are also utilized as models for stroke. They replicate many aspects of the *in vivo* context of ischemic stroke and preserve the tissue architecture of original brain areas. Slices are obtained from a variety of brain areas including hippocampus, striatum, cortex, and cerebellum, as well as the spinal cord. Most CA1 pyramidal neurons subjected to oxygen/glucose deprivation (OGD) injury undergo caspase-dependent, apoptotic-like neuronal death, and the CA1 damage in this model closely resembles the injury pattern found in transient global forebrain ischemia *in vivo*. *In vitro*,

neuronal cell models of ischemic stroke mainly contain primary cortical neuron (PCN) [6-8], primary cerebellar neuron [9], primary hippocampal neuron [10]. In order to mimic ischemic conditions, the cultured neurons are exposed to insults such as OGD, H_2O_2 , and NMDA [6-8].

2. CENTRAL NERVOUS AGENTS AND NEUROPROTECTION MECHANISMS

Until now the only Food and Drug Administration - approved agent in clinical use to treat stroke is tissue plasminogen activator (tPA). It is currently used in less than 5% of cases [11] because of its narrow therapeutic window [12] of 3-4 hours. Also it carries the risk of intracerebral hemorrhage [13] and greater mortality [14]. Catheter-based intrarterial thrombolysis can be used to treat patients who cannot receive intravenous therapy [15]. Endovascular therapy can also be considered in patients with large-vessel occlusion [16]. Although many drugs have a single unique mechanism of action, most have overlapping actions affecting various pathways or mediators. Ischemic neuronal death mainly occurs due to one or more mechanisms. After the induction of experimental stroke, histological assessment is performed to assess the extent of neuronal damage. Infarction volume is quantified with the help of staining with TTC, H&E, or silver staining [17]. As to ischemic injured cells from organotypic/acute hippocampal slice culture and neuronal culture, the two most common measures to assess neuronal death and survival are propidium iodide uptake and lactate dehydrogenase (LDH) release. Lucigenin assay detects ROS production, and oxidative stress formation is evaluated using the oxidative fluorescent dye dihydroethidine. Neurological assessment makes use of a wide variety of sensorimotor and behavioral tests including the rotarod test [18], Morris water maze test [17], and forelimb asymmetry and postural reflex tests [19]. Given that OGD treatment in organotypic/acute hippocampal slice cultures mimics ischemic conditions, ischemic injury exposure to OGD, together with other insults (*e.g.* H_2O_2 and NMDA), are the models most widely adopted to study ischemic stroke pathology and test various pharmacological compounds [20].

2.1 ANTIOXIDANT AGENTS AND RADICAL SCAVENGERS

Oxidative stress through formation of ROS plays a vital role in mediation of neuronal damage during cerebral ischemia. ROS causes membrane phospholipid disruption and results in ischemic cell injury [21]. Though it remains a difficult task for stroke researchers, it would be extremely useful to be able to measure superoxide, hydroxyl, and nitric oxide (NO) radicals to establish the causative role of oxygen radicals in ischemic brain injury. The role of oxygen radicals in cerebral ischemia and molecular genetic approaches using transgenic and knockout mutant mice to dissect out the molecular and cellular mechanisms involving oxygen radicals in ischemic brain damage has been discussed previously. Methods generally employed for measuring levels of oxygen free radicals in brain tissue after ischemia are indirect, including lipid and protein peroxidation and DNA damage [22,23]. Nitric oxide relaxes vascular smooth muscle and increases cerebral blood flow. It also has beneficial effects through limiting aggregation of platelets and adhesivity of white blood cells, both of which impede microvascular flow during stroke. In addition, infusing either L-arginine, the eNOS (nitric oxide synthase) substrate, or nitric oxide donors increases vascular nitric oxide production and improves blood flow. As a consequence of improved flow in the ischemic penumbra, electrical activity is restored. The mechanism of cerebral ischemia and reperfusion injury caused by oxygen radicals is shown in Fig. 2.

Several oxygen radical species, including $O_2^{\cdot-}$, $HO_2^{\cdot}$, H_2O_2 , and $\cdot OH$, are formed following the initial reduction of oxygen:

In Equation 3, SOD catalyzes this reaction at physiological pH at an extremely fast, constant rate ($2 \times 10^9 \text{ L} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$), forming H_2O_2 that is then detoxified to H_2O and O_2 by catalase in mammalian cells or in the brain by GSHPx at the expense of reduced glutathione (Equation 4).

The oxidized glutathione can be recycled to reduced glutathione by glutathione reductase in the presence of nicotinamide-adenine dinucleotide phosphate.

Hydroxyl radicals are extremely active oxidants, known to initiate lipid peroxidation and to cause protein oxidation and DNA damage in cells (Fig. 2). Superoxide radicals, on the other hand, are much less reactive but have a longer half-life and can form hydroxyl radicals through a Haber-Weiss reaction (see Equation 5). It is generally believed that this reaction (Equation 5) proceeds rapidly in the presence of trace metal iron (Fenton reaction). Another pathway for forming $\cdot\text{OH}$ is through the reaction of $\text{O}_2^{\cdot-}$ and $\text{NO}\cdot$ (a gas radical that is constantly being formed in the brain by neuronal, endothelial, and glial NOS). The product of this reaction is ONOO^- .

This reaction (Equation 6) is extremely rapid, and the constant rate of ONOO^- formation ($6.7 \times 10^9 \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$) is diffusion-limited. The reaction of ONOO^- and H^+ forms ONOOH (Equation 7). At physiological pH, ONOO^- degrades instantly to $\cdot\text{OH}$ and nitrogen dioxide ($\text{NO}_2\cdot$) (see Equation 8). ONOO^- , a stronger pro-oxidant, can react with SOD to form a powerful nitrating agent, resulting in the nitration of tyrosines of cellular proteins and initiation of cellular dysfunction and death (Fig. 2). However, the role of ONOO^- in ischemic brain injury has only been investigated recently. On the other hand, contradictory reports have surfaced with regard to the role of $\text{NO}\cdot$ in ischemic neuronal injury, primarily because of its dual role as a vasodilator (i.e., it increases cerebral blood flow) and as a free radical that can injure neuronal cells. Other studies have suggested that the redox state of $\text{NO}\cdot$ may determine its role as either neuronal protector or injurious mediator after activation of *N*-methyl-D-aspartate receptors. It is of interest to note that downregulation of CuZn-SOD activity in PC12 cells by exposure to antisense oligonucleotides leads to apoptotic cell death via the $\text{NO}\cdot$ to ONOO^- pathway.

This neurotoxicity can be attenuated by antioxidants and inhibitors of lipid peroxidation. AM 36 is an arylalkylpiperazine [1-(2-(4-chlorophenyl)-2-hydroxyethyl)-4-(3,5-bis-(1, 1-dimethylethyl)-4-hydroxyphenyl) methylpiperazine]. It functions as an antioxidant and inhibitor of lipid peroxidation and has been shown to provide neuroprotection by acting as a Na^+ channel blocker in experimental stroke. Large amounts of energy are required to maintain the Na^+/K^+ gradient across the cell membranes of excitable tissues. Blocking Na^+ channels can reduce the energy expenditure in the compromised tissues and prevent further damage. Hence the use of AM-36 can ameliorate damage of ischemic tissue. Studies have shown that it provides neuroprotection *in vivo* in experimental animals and reduces striatal damage. Rotarod performance test, sensory hemineglect, and neurological deficit scores also improved 72 hours after stroke [18].

It has been shown that large quantities of iron are released during ischemia [24]. Free iron crosses the damaged blood-brain barrier (BBB) and is taken up by cells in a transferrin-independent manner [25]. This induces oxidative stress by formation of ROS, triggering the ischemic cascade. Tempol mimics the effects of superoxide dismutase, a major antioxidant enzyme in the cell system. It reduces the infarct volume induced by iron in experimental animals. It also has the capacity to oxidize Fe^{2+} ions that may lead to ischemic damage via generation of hydroxyl radicals. Twenty-four hours before induction of MCAO, male Sprague-Dawley rats were given intraperitoneal (IP) injection of iron dextran at a dose of 0.5 g/kg body weight, which caused significantly larger brain infarcts. Pretreatment with tempol significantly reduced the infarct size. Thus it was shown that tempol can reverse the neurotoxicity caused by iron in the rat permanent MCAO model [26]. Edema formation was seen in slices of rat brain subjected to OGD. Tempol, administered with other neuroprotective agents including AP5/CNQX and CsA after OGD exposure, provided neuroprotection by preventing edema formation [27].

Ebselen (2-phenyl-1, 2-benzisoselenazol-3[2H]-one) is a seleno-organic compound currently undergoing clinical trials for the treatment of ischemic stroke [28]. Its action as a

neuroprotectant, similar to glutathione peroxidase, is via cyclic reduction and oxidation reactions. Ebselen acts through the selenium core to remove hydroperoxides and the lipoperoxides. It has been shown that NMDA receptors are involved in the pathogenesis of various neurological disorders. Reducing agents potentiate the physiological responses in neurons mediated by NMDA receptors. Conversely, oxidants can lower NMDA receptor function from baseline levels. Ebselen is believed to oxidize the NMDA receptor via the NR1 redox modulatory site. The potentiation of dithiothreitol, a reducing agent, was reversed by ebselen [29], which acts as an oxidant without scavenging free radicals. In addition, because ebselen interacts with cysteine residues, leading to thiol oxidation, its putative role in interaction with redox-sensitive NMDA receptor is being investigated. Ebselen has a wide range of substrate specificity [30]. Cortical cultures from E16 Sprague-Dawley rats were used in tissue cultures to demonstrate these effects [31].

Ginsenoside Rd (GSRd) is one of the main active components in *Panax Ginseng*. It is highly lipophilic and can diffuse the BBB easily in an energy-deficient environment. GSRd, administered in different concentrations during exposure of hippocampal cultures to OGD, reduced OGD-induced apoptotic cell death. Its antioxidative actions reduced intracellular ROS and malondialdehyde production, as shown by DCFH-DA assay. It increased glutathione content and also enhanced the antioxidant activity of the enzymes catalase, superoxide dismutase, and glutathione peroxidase. Mitochondrial membrane potential is an important predictor of neuronal survival. GSRd stabilized membrane depolarization produced by OGD exposure, as shown by fluorescence intensity of Rh 123 [10].

Carvedilol, a widely used antihypertensive agent, has also been shown to inhibit lipid peroxidation and to scavenge free radicals in experimental animals. Free radicals lead to excitotoxicity and are neurotoxic to the ischemic brain areas either following or enhancing glutamate release [32]. Carvedilol has been shown to inhibit the release of human neutrophil-generated superoxide and superoxide radicals [33]. In vitro, primary cultures of rat cerebellar neurons with free radical generating system were used. Carvedilol inhibits the DHF-Fe⁺³/ADP free radical-generating system and Fe⁺²/vitamin C-catalyzed lipid peroxidation. In vivo, CA1 hippocampal neurons were protected against oxygen free radicals, suggesting carvedilol's potential as a therapeutic agent in ischemic stroke [9].

IAC (bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidiny)-decandiol) is a low-molecular-weight compound that can easily cross the BBB. It is a unique radical scavenger, capable of migrating intracellularly and extracellularly and scavenging most of biological free radicals including peroxy, superoxide, and peroxynitrite [34]. Along with other molecular pathological events, the ischemic cascade is associated with increased superoxide anion, hydroxyl radical, hydrogenperoxide, nitric oxide, and peroxynitrite radical during ischemia and reperfusion. Pretreatment of animals with IAC prevents neuronal loss and malondialdehyde formation in the CA1 area of the hippocampus in a dose-dependent manner. In addition, posttreatment with IAC after bilateral common carotid artery occlusion (BCCO) provided protection against neuronal loss in hippocampus in a dose-dependent manner. Thus IAC may prove to be a useful tool in protecting against neuronal damage in ischemic stroke pathogenesis [35].

4-phenyl-1-(4-phenylbutyl) piperidine (PPBP) is a potent σ_1 -receptor ligand. σ -receptor ligands inhibit activation of nitric oxide synthase induced by glutamate [36] in *in vitro* models and may reduce intracellular increase in Ca⁺ induced by NMDA [37], which may in turn influence Ca⁺-dependent NOS activation. Nitric oxide plays a vital role in mediation of ischemic brain injury [38]. The inducible isoform (iNOS) as well as that derived from neurons (nNOS) both play roles in the excitotoxic cascade, inducing brain injury [38]. PPBP reduces infarct volume in cortex and striatum in the MCAO animal model. However PPBP

does not reduce infarct size when administered to NOS-knockout mice, indicating that the neuroprotection provided by PPBP is via attenuation of nNOS activity and ischemia-evoked NO production activity and ischemia-evoked NO production [39].

Bromocriptine is a dopamine D₂ receptor agonist that acts *in vitro* as a free radical scavenger and, when present at high concentrations, also an inhibitor of lipid peroxidation in rat brain homogenates [40]. It attenuates the reduction of copper/zinc superoxide dismutase and manganese superoxide dismutase in ischemic neurons in experimental animals. Thus it raises antioxidant levels in ischemic neurons and helps in neuroprotection [41].

2.2 ANTI-APOPTOTIC AGENTS

Apoptosis is involved in the pathogenesis of ischemic stroke. Increased expression of Fas and other mediators through the extrinsic caspase-dependent pathway has been implicated in focal ischemia. In addition, studies show that in ischemic penumbra there is increased expression of caspase-1, -3, -8, -9 and cleaved caspase-8 [42]. Activation of caspase-1 and -3 occurs about 2 hours after ischemia and reperfusion [43].

Melatonin (N-acetyl-5-methoxytryptamine), secreted by the pineal gland, is an amphiphilic molecule with anti-apoptotic effects and also acts as a free-radical scavenger and antioxidant [44]. Melatonin exhibited antioxidant properties in a mouse brain slice model of stroke [20]. Melatonin was also shown to maintain the phosphorylated form of Akt in a mouse model of MCAO [45]. It also activates the PI3K/Akt survival pathway [46] and JNK pathway [47], and inhibits caspase-3 and caspase-1 activation in *in vivo* and *in vitro* experiments [6]. MCAO focal cerebral ischemia was induced on C57BL/6J mice. PCNs were exposed to OGD and incubated with melatonin. Melatonin administration diminished the damage caused by cerebral ischemia and cell death by OGD exposure, as well as reduced cytochrome c (cyto. c) release and caspase-3 activation [6]. Melatonin stabilized the mitochondrial membrane potential, as shown by punctate Rh 123 staining [6].

Humanin is a 24-amino-acid peptide providing neuroprotection against damage caused by Alzheimer's disease-related protein; it is isolated from the brains of patients with that disease [48]. Humanin significantly improves neurological deficits, and posttreatment reduces infarct volume in cortex and striatum in ischemia/reperfusion injury [49]. Gly¹⁴-Humanin attenuates neuronal apoptosis in cortex and lowers levels of cleaved PARP in the ischemic hemisphere, as shown by a decrease in TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling)-positive nuclei. Gly¹⁴-Humanin can cross the intact BBB probably via erythropoietin receptors in endothelial cells. It inhibits ERK activation, which may lead to reduction of some proapoptotic transcriptional gene products such as c-fos. However, it has no effect on JNK and p38 activation [50].

The energy imbalance in neuronal ischemia results in depletion of ATP, triggering the ischemic cascade that may lead to irreversible cell injury. Nicotinamide provides protection against energy loss and decreased production of ATP by preventing the depletion of nicotinamide adenine dinucleotide [51], significantly reducing sensory and motor behavioral deficits induced by ischemia. There was also improvement in weight gain in Nicotinamide-treated rats. In addition, Nicotinamide treatment also reduced infarct volume as shown by TTC stain. Nicotinamide may be neuroprotective via various mechanisms. It has been shown to protect against necrosis- and/or apoptosis-induced injury [51] and may also be protective against reperfusion injury [52].

Erythropoietin (EPO) is a hormone that regulates the production of red blood cells [53], effects that are mediated through the EPO receptor. Rat hippocampus and frontal cortex have high levels of EPO receptor. The ischemic injury to CA1 neurons of the hippocampus

was attenuated by EPO in a dose- and time-dependent manner when rhEPO (recombinant human EPO) was administered intracerebroventricularly (ICV) after the onset of reperfusion. This was shown by H&E staining for live neurons and DNA polymerase I-mediated biotin-dATP translation assay. Phosphorylation of Akt was increased when rhEPO was administered after ischemia. In addition, phosphorylation of GSK-3 β and Ser9 was increased by rhEPO administration, as shown by immunohistochemistry and western blotting. BDNF is neuroprotective for CA1 neurons of the hippocampus [54] even when administered several hours after ischemia. Western blot analysis showed increased BDNF following global ischemia when rhEPO was administered [55].

Acetaminophen is a widely used over-the-counter analgesic and antipyretic that has been found to be neuroprotective in experimental animals. It prevents mitochondrial dysfunction by reducing swelling and preserving morphology, thus reducing apoptosis after ischemia-reperfusion in experimental animals. Acetaminophen acts through the intrinsic mitochondrial pathway. Expression of antiapoptotic Bcl-2 is increased in neurons and endothelial brain cells [56]. It also attenuates apoptosis via inhibition of NF- κ B [57] and reduces superoxide anion generation and lipid peroxidation [58,59].

The flavonoid baicalein, extracted from the roots of *Scutellaria baicalensis*, has been shown to affect the PI3K/Akt and PTEN signal pathways. The PI3K/Akt pathway promotes neuroprotection via phosphorylating pro-apoptotic proteins including GSK3, Bcl-2/Bcl-xL-associated death protein, caspase-9, and forkhead transcription factor like 1. Conversely, PTEN, the phosphatase and tensin homolog deleted on chromosome 10, negatively influences the PI3K/Akt pathway and induces cell death. Baicalein was studied by administering it before and after the onset of ischemia in experimental rats. Its impact was also studied *in vitro* in an OGD/R model in PCNs, which showed increased cell viability and reduced ROS levels. The neuronal cultures showed the upregulation of phospho-Akt. BAD, the downstream molecule of Akt was reduced by baicalein treatment, and the anti-apoptotic molecule Bcl-2 was induced. Baicalein reduced the immunoreactivity of cleaved caspase-3 in the cortical penumbra area. The cortical, subcortical, and total infarction volume in transient MCAO was significantly reduced. Neurological scoring indicated improvement in neurological impairment [60].

Transplantation of human embryonic neural stem cell (NSC) in the cortical peri-infarct area induced neuroprotection according to neurological and histological assessment of the effect of ischemic stroke in experimental animals [61]. The neurological outcomes as evaluated by neurological severity score showed improvement after transplantation. The Bcl-2/Bax ratio, which determines resistance against apoptosis, was also increased in the penumbra at 7 days compared to control. However, very little change in the number of Bax-positive cells was seen at 14 and 28 days. Additionally, NSCs can induce neurotrophic factors [62] and anti-inflammatory cytokines [63]. In another experiment, the intravenous (IV) administration of rodent forebrain-derived neural stem cells into rats subjected to MCAO improved behavior and reduced infarct volume. These neural stem cells showed anti-apoptotic effects, inducing heat shock protein and downregulating caspase-3. In addition, inflammatory mediators including cyclooxygenase-2 (COX-2) and IL-1 beta showed reduced expression [64]. The neuroprotective role of bone marrow-derived mononuclear cells was also investigated in primary neuronal cell cultures exposed to injury to OGD, hydrogen peroxide, and hypoxia. As shown by MTT, caspase-3 activation, and TUNEL staining cell death assays, it was found that the mononuclear cells reduced cell death in the cultures. Various trophic factors including interleukin-10, vascular endothelial growth factor, insulin-like growth factor-1, and stromal cell-derived growth factor were also found in the supernatant [65].

Aminoguanidine is an inhibitor of diamine oxidase and inducible NOS. Of the three known forms, neuronal NOS and inducible NOS are neurotoxic, either by forming ROS or by inhibiting the mitochondrial complexes. NOS is also capable of activation of calpain and caspase-3. The cytotoxic effects of calpain are due to the destruction of the cytoskeletal proteins MAP-2 and spectrin. The latter can be degraded by both calpain and caspase-3. Aminoguanidine in experimental animals has been proven to reduce calpain and caspase-3 activation. Casein zymography showed reduced levels of μ -calpain and m-calpain in the penumbra of aminoguanidine-treated animals. Levels of calpastatin, an endogenous calpain inhibitor, were markedly elevated in the core as shown by western blot analysis. Caspase levels were attenuated in core as well as penumbra, reflected by an increase in levels of MAP-2 and spectrin in those areas. Histological analysis by TUNEL staining showed reduced numbers of apoptotic cells in the penumbra. Aminoguanidine also improves neurological function and reduces the area of infarction [66].

4-HBA is one of the major active phenolic constituents of a traditional Chinese medicinal herb, *Gastrodia elata* Blume. It has various protective effects in CNS including antioxidant, sedative, and anxiolytic [67]. It can also inhibit DNA degradation, thus improving memory and learning. Its role in stroke may involve antioxidant activity related to genes PDI and I-Cys Prx [68]. The neuroprotective effect of 4-HBA is also associated with its anti-apoptotic action. It enhances expression of Bcl-2 and inhibits the apoptotic cascade including caspase-3 and reduces the number of TUNEL-positive cells. 4-HBA also reduces cortical and subcortical infarct volume and neurological deficits [69].

2.3 ANTI-INFLAMMATORY AGENTS

Inflammation is important contributor to neurological deterioration in cerebral ischemic stroke [70]. Because inflammation occurs over a long period of time after the onset of stroke, anti-inflammatory agents have a relatively wide therapeutic window.

Melanocortins, the endogenous peptides of adrenocorticotropin/melanocyte-stimulating hormone, may be neuroprotective through the activation of CNS melanocortin MC₄ receptors. This was shown in a rat model of ischemic brain injury by up-regulation of mRNA expression of melanocortin MC₄ receptors in the uninjured striatum [71]. MC₄ receptors are widely distributed in brain areas such as the striatum and hippocampus [72]. Striatum plays an important role in learning and memory and also in sensory-motor orientation. In experimental animals the melanocortin analog NDP- α -MSH improved memory, learning, and sensory-motor orientation of limb use with a broad therapeutic window. Histological damage to striatum was also reduced, as evidenced by decreased neuronal death, reduced demyelination of white matter, increased cell density in ischemic areas, and decreased phagocytic activity. In addition, melanocortins make some anti-inflammatory contribution by modulating cytokine interleukin-10 in the ischemic cascade of stroke [72,73]. Hence, melanocortins should be investigated for their neuroprotective effects against development of pathogenesis in ischemic stroke [74].

Over several days, inflammation progresses to exacerbate the neuronal injury caused by ischemia, worsening neurological outcomes. Interferon- β (IFN- β) is a cytokine with anti-inflammatory properties. IFN- β reduces the massive infiltration of leukocytes in the ischemic area, especially neutrophils, but to a lesser extent monocytes. Stimulation of cytokines IL-8 and IL-10 may be responsible for the recruitment of neutrophils in the ischemic area. This action may be mediated by downregulation of the expression of adhesion molecules like vascular CAM-1 and intercellular CAM-1 [75]. IFN- β reduces ischemic lesion size in cortex and caudate putamen. In addition, IFN- β maintains the integrity of the BBB in cortex and striatum [76].

6-mercaptopurine, a purine metabolite, is widely used as an immunosuppressant in various neoplastic diseases. It may be useful as an agent to improve cerebral infarct in ischemic stroke. Ischemic stroke triggers mechanisms including oxidative stress via upregulation of pro-inflammatory cytokines such as IL-1 β and TNF- α . Cytokines, chemokines, and pro-inflammatory enzymes are upregulated after ischemic stroke. 6-mercaptopurine decreases the mRNA levels of IL-1, IL-6, and TNF- α . 6-mercaptopurine improves functional outcomes in experimental rats and reduces infarct volume and tissue edema after stroke in rats [19].

2.4 ANTIEXCITOTOXIC AGENTS

Reduced blood supply results in depolarization of cells with a release of glutamate and other excitatory amino acids. This in turn releases intracellular calcium and increases formation of oxygen free radicals due to activation of postsynaptic glutamate receptors, eventually resulting in neuronal damage [77].

Suramin is a well-known anthelmintic and anti-neoplastic agent [78]. Stimulation of P_{2X} ionotropic ligand-gated ion channel purinocentors leads to increased intracellular calcium levels and excitotoxic neuronal damage. Antagonism of P_{2X} purinocentors is responsible for the neuroprotective effects of suramin in experimental animals, where it significantly reduced infarct and edema volume. At higher concentrations it also blocks metabotropic P_{2Y} receptors and NMDA receptors and confers neuroprotection. Thus the neuroprotective effects of suramin are dose-dependent and independent of its vasoactive mechanisms [79].

YM-202074 (N-cyclohexyl-6-[80]-N-methylthiazolo[3,2-a]benzimidazole-2-carboxamide), a mGluR₁ antagonist, leads to positive modulation of NMDA receptors, which implies that antagonism of the mGluR₁ receptor can be neuroprotective through mediating NMDA receptor inhibition. It has been shown that neuroprotective mechanism of mGluR₁ antagonists also involves release of GABA and stimulation of GABA receptors. The neurotoxic effect of mGluR₁ is also mediated through calpain activation [81] and stimulation of the receptors by glutamate-induced Ca⁺² entry [82]. Cerebellar granule cells were prepared from Wistar rat pups, cerebellum membrane fractions were prepared from male Wistar rats, and microdialysis study and transient MCAO model were implemented in Sprague-Dawley rats. Infarct volume was reduced in both cerebral hemisphere and cortex in a dose-dependent manner. This neuroprotection was seen beginning at administration until 2 hours after ischemia, and improvement in neurological deficits were noted 1 week after ischemia. Thus it has been shown that stimulation of mGluR₁ receptors plays an important role in mediation of ischemia, and that its antagonism with YM202074 can be neuroprotective [80].

Mefenamic acid (MFA) is a fenamate widely used as an anti-inflammatory, analgesic, and antipyretic. MFA is believed to be neuroprotective via multiple target action indirectly inhibiting glutamate. This includes cyclooxygenase inhibition, potentiation of neuronal GABA_A receptors [83], potassium channel enhancement [84], and calcium-activated nonselective cation channel inhibition. Hippocampal cell cultures were obtained from 19-day embryonic rat brain. MFA was administered to the cells at glutamate exposure. MFA reduced the extent of cell death due to glutamate exposure in a dose-dependent manner. Multiple IV injections of MFA reduced brain injury and edema volume when given before MCAO. Though infarct volume was reduced, penumbra volume was not. Thus MFA can be neuroprotective in ischemic stroke [85].

Nortriptyline is an Food and Drug Administration - approved antidepressant that can penetrate the BBB and is well-tolerated by patients. It is found to inhibit the mitochondrial permeability transition. It prevents the loss of mitochondrial membrane potential and

mitochondrial dysfunction by protecting against Ca^{+2} overload. It also reduces the translocation of postischemic apoptogenic factors including cyto. c, smac/Diablo, and apoptosis-inducing factor from mitochondria to cytosol, improved neurological score, and reduced infarct volume. In vitro OGD was induced in PCNs and treated with nortriptyline. It reduced cell death according to lactate dehydrogenase assay and also preserved normal cell morphology. Nortriptyline inhibited the release of cyto. c, smac/Diablo, and apoptosis-inducing factor from mitochondria and activation of caspase 3, along with preventing the dissipation of the mitochondrial membrane potential in live PCN. Thus nortriptyline is neuroprotective in both cellular and animal models of cerebral ischemia [7].

Memantine (1-amino-3,5-dimethyladamantane) is an uncompetitive N-methyl-D-aspartate antagonist. It attenuates glutamate excitotoxicity and provides neuroprotection. Preischemic administration of memantine reduces glutamate levels in cortex, striatum, and hippocampus and reduces glutamine synthetase activity in striatum and hippocampus. It also increases $\text{Na}^{+}\text{-K}^{+}\text{ATPase}$ activity and glutathione content in the ischemic region. However, it has no effect on the energy metabolite [86].

Valdecoxib is a COX-2 inhibitor used as an analgesic and antipyretic. Excitatory amino acids induce the expression of COX-2 in neurons [87], and expression is increased in the penumbral cortices. Valdecoxib inhibits PGE_2 , which exerts its neurotoxic effects by activating EP1 receptors, G protein-coupled receptors that increase the intracellular calcium levels by inhibiting intracellular cAMP levels [88].

Felbamate is an antiepileptic agent with GABA agonist and glutamate antagonist action. The preischemic and post ischemic felbamate provided neuroprotection in cortex hippocampus, thalamus, and striatum at 7 and 28 days. However, post ischemic felbamate was more effective. No significant behavioral differences in performance on the water maze test were noted between treated and untreated animals. Thus felbamate could be useful as a neuroprotective agent against ischemic stroke due to its postischemic extended window of protection [17].

Diazepam is a benzodiazepine widely used to treat anxiety, insomnia, and seizures. It is neuroprotective in models of ischemic stroke by delaying neuronal death, thus expanding the therapeutic time window. This could be beneficial as it can give more time to treat the patients. However it does not provide permanent neuroprotection. It is believed to act via activation of GABA_A receptors [89] and/or by reducing the hyperthermic effect typical in global ischemia [90]. It stabilizes the field potential amplitude and delays death of CA1 neurons following global ischemia [91]. Administered in the OGD edema model of hippocampal slice culture, diazepam reduced water content under basal conditions, but edema formation was not affected [92].

Resveratrol (trans-3, 5, 4"-trihydroxystibene) is a polyphenol extracted from red wine that protects against excitotoxicity in PCNs by inducing the enzyme heme oxygenase 1. Heme oxygenase 1 converts hemoglobin and other heme-containing proteins to bilirubin/biliverdin and prevents damage by free radicals [93]. PCNs with different concentrations of resveratrol were harvested, and the protein HO1 was analyzed by western blot. Neurotoxicity was not seen until a dose of $75\text{ }\mu\text{M}$ was reached. In a mouse model of MCAO, the area of infarction was reduced by resveratrol pretreatment [94]. Resveratrol also provides neuroprotection by mimicking ischemic preconditioning. In hippocampal organotypic slice cultures treated with different concentrations of resveratrol and subjected to ischemia, it provided neuroprotection to the CA1 region of the hippocampus [95].

NBQX (2, 3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[f]quinoxaline-7-sulfonamide), an AMPA (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate/kainite) receptor

antagonist, prevents the depolarization induced by extracellular glutamate during ischemia, thus preventing neuronal calcium entry and cell death. Administered to experimental rats subjected to MCAO, NBQX reduced the infarct volume significantly. Heat shock proteins were not induced, suggesting the absence of neuronal injury. However, renal failure and uremia were seen in experimental animals, which precludes the clinical use of NBQX [96]. Furthermore, despite its inability to alter ischemic Ca^{2+} and Na^{+} transients, NBQX was significantly neuroprotective in organotypic hippocampal slice cultures exposed to OGD by either the short or long protocol [97]. OGD induction in organotypic hippocampal slice cultures [98,99] elicits region-selective death of pyramidal neurons in the CA1 region, similar to that observed *in vivo* [100].

2.5 HORMONAL THERAPY

It has been established that the risk of stroke is lower in premenopausal women than in men, but the risk increases after menopause. Experiments have shown that female rats sustain less cortical and striatal infarct than corresponding age-matched males. This impact was considered to be due to estrogen, as the decrease in infarct size was prevented in ovariectomized animals, which have lower levels of estrogen but not of progesterone [101]. The putative neuroprotective effects of estrogen are due to antioxidant, antiexcitotoxic, antiapoptotic, and vascular mechanisms. Neuroprotection has been seen with estrogen preconditioning in organotypic hippocampal cultures. The slice cultures were pretreated with estradiol-17 β for various durations prior to ischemia by OGD. Results from propidium iodide fluorescence staining of the hippocampal CA1 region demonstrated ischemic preconditioning induced significant neurosurvival [102].

Progesterone is shown to have membrane-stabilizing properties [103]; it also reduces inflammatory mediators and attenuates the disruption of the BBB [104]. Migration of newborn neural cells to ischemic areas is potentiated by progesterone [105], which has also been shown to improve neurological outcome and reduce infarct volume and brain edema in aged rats [106].

2.6 MULTI-ACTION AGENT

PAN-811, also known as 3-aminopyridine-2-carboxaldehyde thiosemicarbazone or Triapine, is a multifunctional cytoprotective agent that effectively reduces neurodegeneration after ischemia. Though $[\text{Ca}^{+}]$ plays an important role in the pathogenesis of the necrotic core, this mechanism can be blocked by PAN-811, since it chelates calcium as efficiently as EDTA. ROS may be the dominant influence in the pathogenesis of the hypoxic penumbra. PAN 811 reduces ROS via free radical scavenging, as shown with dihydrorhodamine 123. The increased intensity of dihydrorhodamine 123 in the hypoxic area was reduced by PAN-811 to normal levels. PAN-811 also blocks necrosis as well as apoptosis. Levels of the antiapoptotic proteins Bcl-2 and Bcl-xL are preserved by PAN-811, though Bax signal was not suppressed. This was shown by decreased appearance of DNA smear and the ladder pattern of DNA fragmentation analysis. PAN-811 reduces infarct volume in rat model of MCAO [107].

Telmisartan is an angiotensin II type 1 receptor (AT_1R) blocker with a unique capacity for neuroprotection: it acts as an agonist to peroxisome proliferator-activated receptor gamma. AT_1R blocker treatment may also be neuroprotective through mechanisms of angiogenesis and vascular remodeling [108]. Blocking AT_1R can also be neuroprotective, as it has been shown that AT_1R signaling leads not only to increased expression of phospholipases A_2 , C, and D but also increases cytosolic Ca^{+2} levels and stimulates PKC-MAPK, Jak-STAT, and PKC-NF- κB pathways. These signals lead to increased neuronal expression of cytoplasmic phospholipase A_2 (cPLA $_2$), which in turn leads to neurotoxicity through release of

arachidonic acid and ROS into the cytosol. cPLA₂ also potentiates glutamate's effect on NMDA receptors [109]. AT₁R signaling and the mechanism of cPLA₂ induction overlap; thus telmisartan prevents damage by downregulation of cPLA₂ expression. Different dosages of telmisartan were given to different groups of animals; pretreatment reduced total infarct volume and cortical infarct volume but made little difference in the striatal infarct volume. Neurological deficits were improved and levels of cPLA₂ expression were reduced in peri-infarct cortical regions [110].

Minocycline is a broad-spectrum, second-generation tetracycline antibiotic. It provides neuroprotection through different mechanisms of action depending on the injury paradigm of the experiment. In models of stroke it has been reported to protect neurons directly via anti-inflammatory and anti-apoptotic actions without affecting astrocytes. It inhibits microglial activation and prevents the release of cytokines via phosphorylation of p38 and translocation of 5-lipoxygenase [111,112]. In addition, minocycline also plays an anti-apoptotic role by upregulating Bcl-2 expression and reducing caspase-3 and caspase-7 activity in neurons. Minocycline's neuroprotective role is dose-dependent and is seen only with low doses, reducing both cerebral infarct and ischemia in the peri-infarct area [113]. In addition, the administration of minocycline in organotypic hippocampal slices significantly reduces neural injury from OGD induction [114]. However less neuroprotection was seen when the BV2 cell line of microglia was added to organotypic hippocampal cultures that were subjected to OGD after pretreatment with minocycline, also a monocyte inhibitor [115].

G-CSF is a cytokine from the family of growth factors that causes stimulation, proliferation, and maturation of the neutrophilic granulocytic lineage. The presence of G-CSF receptors facilitates the transfer of G-CSF through intact BBB via receptor-mediated endocytosis. Mortality rate and infarction volume were significantly reduced in experimental animals treated with G-CSF. Subcutaneous injections increase mobilization of CD34⁺ in the ischemic areas, which in turn can reconstruct the neural circuitry restore neural function by stimulating the parenchymal cells to release trophic factors. G-CSF also increases the number of BDNF (brain-derived neurotrophic factor) positive cells and fibronectin, which may also contribute to axonal regeneration. G-CSF also plays an antiapoptotic role by upregulating STAT3, Pstat3, and Bcl-2 [116], drives neuronal stem cell differentiation, and enhances structural repair and function. G-CSF encourages angiogenesis via the VEGF pathway and increased mobilization of myelomonocytic cells [117]. It also inhibits inflammatory mediators including TNF α , iNOS and IL-1 β , IL-6, and IL-8. This evidence suggests that G-CSF has a role in neuroprotection via different mechanisms; alone or in combination with other agents, it may prove beneficial in neuroprotection against stroke [118].

2.6 COMBINATION AGENTS

In treating stroke, the window of efficacy is less than 3 hours if only thrombolytic therapy is used. In addition, there is the risk of systemic and intra-cerebral hemorrhage [13]. Thus, the combination of a neuroprotective agent and a thrombolytic is investigated. Topiramate is a lipophilic compound that readily crosses the BBB and protects neurons in the ischemic penumbra but not the core. Its mechanism of action includes Na⁺ channel antagonizing, GABA_A agonizing, and inhibiting the activation of kainite-subtype glutamate receptors [119]. After autogenous embolus to the MCA, combination therapy of intra-arterial urokinase plus topiramate in rats produced severe hemisphere infarction. The neurological score was improved, and combination therapy further augmented neuroprotection by reducing neuronal damage compared to treatment with a single agent. Hence it is postulated that the thrombolytic may facilitate entry of the neuroprotective agent into the ischemic core, and this combination therapy may helping reduce neuronal damage [120].

Ethyl pyruvate, an aliphatic ester of pyruvic acid and aspirin, a nonsteroidal anti-inflammatory drug provides neuroprotection with a wide therapeutic window when co-administered post ischemia [121]. The time window was extended to 9 hours post ischemia when administered in combination. The number of microglia was reduced by combination treatment, as was the release of TNF- α , IL-1 β , COX-2, and NF- κ B. Infarction volume was attenuated, with improvement in motor and other neurological deficits. The combination of drugs was IV administered to the experimental animals. PCNs were treated with NMDA, and mixed cortical cell cultures were subjected to OGD.

Velcade is a selective proteasome inhibitor that also induces endothelial NOS. Combined with low-dose tissue plasminogen activator, it provides significant neuroprotection in aged rats. Experimental animals subjected to embolic MCAO were administered a combination of velcade and tPA after occlusion, which reduced lesion volume and neurological deficits. Cerebrovascular and BBB integrity are compromised in aged individuals [122]. Treatment with velcade alone or in combination with tPA maintains integrity by reducing the plasma protein fibrin/fibrinogen extravasation into the parenchyma after stroke. Matrix metalloproteinase-9, which damages BBB integrity by degrading collagen Type IV [123], is significantly reduced by combination treatment. In addition, combination treatment increases eNOS activity, which both increases cerebral blood flow after ischemia and is neuroprotective. Thus potent neuroprotection in the event of ischemic stroke can be achieved by the synergistic combination treatment of velcade and tPA [124].

A combination of caspase inhibitors such as z-DVED.FMK and MK-801 provides neuroprotection and reduces ischemic injury by 60%, which is the maximal protection possible in the ischemia model. Apoptosis occurs in the peri-infarct zone [125] in mild ischemia a few minutes to hours after the occlusion, whereas excitotoxicity is a very early event mediating cellular necrosis [125,126]. Both these mechanisms can be controlled by the use of this combination of agents. MK-801 was injected before MCA occlusion, and Z-DEVD.FMK was administered before and after MCA occlusion [43]. This combination lowered the infarct volume and reduced neurological deficits. Subthreshold doses of MK-801 prolonged the therapeutic window of caspase inhibitors. When exposed to OGD and treated with MK-801, organotypic hippocampal slice cultures showed significantly less neuronal damage and a rise in $[Ca^{+2}]_i$ [97].

Eliprodil and recombinant tissue-plasminogen activator (rt-PA) confer neuroprotection, greatly reducing infarct volume and improving histological outcomes when given in combination. Combined therapy of eliprodil and rt-PA show neuroprotection in a rat thromboembolic stroke model [127]. Eliprodil is a piperidine-1-ethanol derivative and NMDA antagonist at the polyamine site, which protects against damage by glutamate in cortical and hippocampal cell cultures [128]. It also provides neuroprotection in rat, cat, and mouse models after permanent or reversible focal ischemia [129]. The protective effect of rt-PA is due to its fibrinolytic action, which lyses the clot and can salvage the tissue [127].

3. DISCUSSION AND FUTURE PERSPECTIVES

Many therapeutic regimens are explored for the treatment and protection for ischemic stroke. Because of the multifactorial mechanisms involved in stroke, no single agent provides complete neuroprotection, multiple agents targeting one or more pathways of the ischemic cascade can potentially reduce the size of ischemic penumbra in the therapeutic time window, will be beneficial. To extend the therapeutic time window and enhance the preventing effects of ischemic damage, the neuroprotection strategy hence involves the development of combination therapy with neuroprotective agent including velcade administered in addition to tPA, eliprodil in addition to rt-PA, ethyl pyruvate in addition to

aspirin, and urokinase in addition to topiramate, as well as caspase inhibitors in addition to MK 801 (Fig. 1). Another therapeutic strategy is to discover a pleiotropic agent including PAN-811, telmisartan, and minocycline, as well as melatonin with multiple effects to attenuate the neurotoxicity and provide neuroprotection (Fig. 1).

Though many other CNS agents with wider therapeutic windows have been investigated as effective therapies for stroke, it is essential to determine the optimal duration of treatment and route of administration of these drugs for maximum efficacy. The route of administration and duration of treatment (tables 1A-1G) are influenced by various factors. Given the CNS agents may counteract different mechanisms of ischemic stroke mainly including apoptosis, oxidation, and inflammation, as well as excitotoxic (Fig. 1), the action mechanism of individual drug is the important factor in determining when the drug should be given. For instance, free radical scavengers can be given later than other agents, as reperfusion is a delayed phenomenon which occurs hours or days after stroke. Conversely the anti-excitotoxic agents should be given early, as excitotoxic injury in rodents takes about 4 hours to evolve after stroke; up to 48 hours after stroke. Hence administration of neuroprotective agents should be initiated during this window. Excitatory amino acids remain elevated for 6 days in some patients. Because cerebral autoregulation is deranged for 3-4 days after the onset of stroke, these patients should receive prolonged administration. Another factor in determining the route of administration is the pharmacological properties of the drug; side effects and safety should also be taken into consideration [130].

So far robust neuroprotection has been seen by some of the neuroprotective agents in animal studies of ischemic stroke which has a tremendous potential to be translated in the clinical trials. However some of these agents have not proven effective in humans [131], which may be due to poor choice of animal models, the agent and its time of administration, or the molecular mechanism targeted. Preclinical and clinical trials should be designed more appropriately [2].

Silver nanoparticles have innate antiplatelet [132] and antibacterial properties both *in vitro* as well as *ex vivo*. They have been shown to inhibit fibrin polymerization at the lesion site, preventing thrombus formation—and opening up new possibilities for research into the prevention/treatment of ischemic stroke [133]. The neuroprotective effects of glucose transporters (GLUTs) have also been investigated as having potential in treatment of ischemic stroke. In brain GLUT3 are found in neurons, and GLUT1 are present in the microvascular endothelial cells of the BBB and glia. Upregulation of GLUTs occurs during ischemic stroke as a protective mechanism during excitotoxicity, mitochondrial dysfunction, glucose deprivation, and hypoxia [134]. MicroRNAs are a small, endogenous group of noncoding RNA 5-25 nucleotides long that inhibit or degrade their target mRNAs to downregulate gene expression. Following focal cerebral ischemia, the miRNA transcriptome is modified without affecting the expression of miRNA machinery, implicating miRNA in the pathological cascade of events including BBB disruption (miR-15a) and caspase-mediated cell death signaling (miR-497) [135]. Certain microRNAs may be upregulated following experimental stroke, while others may be downregulated. Hence targeting drugs on these small molecules may be neuroprotective. In addition to playing a role in pathophysiologic conditions like tumor progression/regression, cholesterol and glucose homeostasis, etc., miRNA are highly expressed in the brain and have also been detected in the blood of rats subjected to MCAO. Hence, blood microRNA levels can also be used as biomarkers for cerebral ischemic stroke [136].

Biomarkers have recently begun to be used in clinical diagnosis of ischemic stroke, especially in the acute phase [137]. However, a specific biomarker database of all receptors that trigger or affect pathological processes in ischemic stroke has not yet been constructed.

It is still very difficult to identify specific biomarkers for a particular neuroreceptors, since the mechanism underlying each receptor's involvement in the disease's development is not yet well understood. However target-based drug design and discovery for treatment of ischemic stroke could be a good starting point.

We hope this review provides a useful current perspective on treatments for and protection against ischemic stroke, a summary of promising CNS agents, and a discussion of other therapeutic regimens.

Acknowledgments

We thank Dr. Ning Lin for discussion. This work was supported by grants from the National Institutes of Health/ National Institute of Neurological Disorders and Stroke (X.W.) and the Muscular Dystrophy Association (to X.W.).

ABBREVIATIONS

AT₁R	angiotensin II type 1 receptor
BBB	blood brain barrier
BCCO	bilateral common carotid artery occlusion
BDNF	brain-derived neurotrophic factor
COX-2	cyclooxygenase-2
CNS	central nervous system
cPLA₂	cytoplasmic phospholipase A ₂
cyto. c	cytochrome c
EPO	Erythropoietin
GLUT	glucose transporters
ICV	intracerebroventricular
IFN-β	Interferon-β
IP	intraperitoneal
IV	intravenous
LDH	lactate dehydrogenase
MCAO	middle cerebral artery occlusion
MFA	Mefenamic acid
NO	nitric oxide
NOS	nitric oxide synthase
NSC	neural stem cell
OGD	oxygen-glucose deprivation
PCN	primary cortical neuron
PPBP	4-phenyl-1-(4-phenylbutyl) piperidine
Rh 123	Rhodamine 123
ROS	reactive oxygen species
rt-PA	recombinant tissue-plasminogen activator

SC	subcutaneous
tPA	tissue plasminogen activator
TTC	2,3,4 triphenyl tetrazolium chloride
TUNEL	terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeli

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Fig. (1). Summary of CNS agents for ischemic stroke with neuroprotective mechanisms
Ischemic stroke is mediated by various mechanisms including oxidative stress through formation of free radicals and reactive oxygen species, apoptosis, inflammation, excitotoxicity by glutamate and other extracellular aminoacids, overlapping each other, forming a reversible ischemic penumbra and an irreversible infarct core. Agents acting on these mechanisms may prove to be neuroprotective in ischemic stroke.

Fig. (2). Cerebral ischemia and reperfusion injury by oxygen radicals

Fe²⁺, ferrous iron. Cu⁺: cuprous ion. COX: cyclooxygenase. NOS: nitric oxide synthase. XO: xanthine oxidase. SOD: superoxide dismutase.

Table 1A

Anti-oxidant agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
AM 36	MCAO-transient focal ischemia	6 mg/kg/IP	180 mins post ischemia	Male hooded wistar rats [18].
Tempol	MCAO	1 mM/IP	3 days before occlusion	Male sprague dawley rats [26].
	Brain slice	-	During OGD exposure	Male Long-Evans rats, 7-10 weeks [27].
Ebselen	Cortical cultures	10 – 30 μ M	Pretreatment and post treatment	E16 Sprague Dawley rats [13].
Ginsenoside R	Primary hippocampal cultures	0.1 - 10 μ M	During OGD exposure	E18 Sprague Dawley rats [10].
Carvedilol	MCAO global ischemic stroke	1 mg/kg/sc	30 mins before and 4 hours after ischemia	Mongolian gerbil [9].
	Primary cerebellar neuron cultures	0.1 - 10 μ M	20 mins before exposure to free radical system	Sprague Dawley rats P8 pups [9].
IAC	BCCO transient global ischemia	0.3 ml/IP	1 hour before, 1 and 6 hours after occlusion	Mongolian gerbils [35].
PPBP	MCAO transient focal ischemia	1 μ mol/kg/IV	30 mins before ischemia	Male wister rats [39].
Bromocriptine	BCCO	0.3–3 mg/kg/IP	30 mins before occlusion	Mongolian gerbil [41].

Table 1B

Anti-apoptotic agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
Melatonin	MCAO focal cerebral ischemia	10 mg/kg/IP	1 hour before or 30 mins after ischemia	C57BL/6 mice [6].
	Organotypic brain slice cultures	100 μ M	NMDA insult	Mice [18].
	PCNs	10 μ M	2 - 3 hours OGD exposure	C57BL/6 mice [6].
Humanin	MCAO transient focal ischemia	0.1 μ g Intraventricularly 1 μ g/IP	30 mins before and 0, 2, 4, 6 hours after ischemia 1 hour before ischemia	CD 1 mice [50].
Nicotinamide	MCAO transient focal ischemia	500 mg/kg/IP	At the time of reperfusion	Male wistar rats [52].
Erythropoietin	Four vessel occlusion global cerebral ischemia	50 U/ICV	20 mins after ischemia	Male Sprague[55]. Dawley rats
Acetaminophen	BCCO global ischemia	15 mg/kg/IV	Intraischemic	Male Sprague dawley rats [59].
Baicalein	MCAO	20 mg/kg/IP	30 mins before and 2, 4 hours after ischemia	Sprague Dawley rats [60].
Human NSC cell	MCAO	Intracortically	24 hours after ischemia	Sprague Dawley [61].
Aminoguanidine	MCAO	100 mg/kg/IP	5 mins before ischemia	Sprague Dawley [66].
4-HBA	MCAO	25-50 mg/kg/IP	30 mins before occlusion	Male Sprague dawley rats [69].

Table 1C

Anti-inflammatory agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
Melanocortins	MCAO focal cerebral ischemia	IP	Every 24 hours 3-9 hours after ischemia	Male wistar rats [74].
IFN- β	MCAO transient ischemia	500,000 U(8 μ g)/sc	Once daily until 7 days after reperfusion	Male fisher rats [76].
6-mercaptopurine	MCAO	2 mg/kg/IP	30 mins after occlusion	Sprague-Dawley rats [19].

Table 1D

Anti-excitotoxic agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
Suramin	MCAO	100 mg/kg/IV	30 mins before occlusion	Sprague dawley rats [79].
YM-202074	MCAO transient ischemia	20 mg/kg/hour 5 mg/kg/hour/IV	20 mg/kg/hour for 0.5 hour followed by 5 mg/kg/hour for 23.5 hours after occlusion	Sprague dawley rats [80].
Mefenamic acid	MCAO focal transient	20 mg/kg/IV	1 hour before occlusion	Male wistar rats [85].
Nortryptiline	MCAO transient focal ischemi PCNs	2 mg/kg/IP	30 mins before and 12 hours after ischemia Pretreatment	C57BL/6 mice [7].
Memantine	MCAO transient focal ischemia	20 mg/kg/IP	30 mins before occlusion	Sprague dawley rats [86,88].
Valdecobix	MCAO transient focal ischemia	1 mg/kg/Orally	15 mins before occlusion and 1.5 and 3 hours after reperfusion	Rats [88].
Felbamate	BCCO	300 mg/kg/IP	30 mins before occlusion	Mongolian gerbils [17].
Diazepam	BCCO	10 mg/kg/IP	30-90 mins after occlusion	Gerbils [91].
Resveratrol	MCAO	20 mg/kg/Orally	Pretreatment	Mice [94].
	Hippocampal organotypic slice cultures			Rats [95].
	PCNs			E17 C57BL/6 mice [93].
NBQX	MCAO	40, 60 or 100 mg/kg/ IV	15, 45 or 90 mins post occlusion	Sprague-Dawley [96].
	Hippocampal organotypic slice cultures			Rats [97].

Table 1E

Hormonal agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
Estrogen	MCAO	Acute: 1 mg/kg/IV Chronic: 25 or 100 µg	Acute: 7-10 days before occlusion Chronic: 30 mins before occlusion	Wistar rats [101].
	Hippocampal organotypic slice cultures	0.5, 1, 5 nM	48 hours before OGD exposure	Female Sprague Dawley rats P9-11 pups [102].
Progesterone	MCAO	8 mg/kg/IP 8 mg/kg additional doses/IV	1 hour post occlusion Additional doses at 6, 24, and 48 hours post occlusion	Sprague-Dawley rats [106].

Table 1F

Multi-action agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
PAN-811	Neuronal cultures	2 or 5 μ M	Pretreatment 24 hours	E17 Sprague Dawley rats [107].
	MCAO	50 mg/per rat/ICV	1 hour after arterial occlusion	Rats [107].
Telmisartan	MCAO	0.25, 0.5 or 1 mg/kg /gavage using a cannula	Pretreatment for 7 days	SpragueDawleyrats [110].
Minocycline	MCAO	20 mg/kg/IV	Single bolus dose 60 mins after reperfusio	Sprague Dawley rats [113].
	Organotypic hippocampal slices	30 μ M	Together with OGD or normoxia treatment	Rats [114].
G-CSF	MCAO	10 μ g/kg/sc	5 days after ischemia	Rats [118].

Table 1G

Combined agents in ischemic stroke

Agent	Model	Dose/route	Time of administration	Species
Urokinase and topiramate	Embolic MCAO	2500 U Urokinase and 20 mg/kg Topiramate/IP	2 hours after embolisation	Male Wistar rats [120].
Ethyl pyruvate and aspirin	MCAO	5mg/kg Ethyl pyruvate and 5 mg/kg aspirin/IV	1 hour after the occlusion	Sprague Dawley rats [121].