Biomedical Letters
2016 | Volume 2 | Issue 2|Pages 72-81

REVIEW ARTICLE OPEN ACCESS

Neuroprotection and Acute Ischemic Stroke: The Clinical Quest

Abdoulaye Idriss Ali, Yi Jing Guo*

Department of Neurology, Southeast University affiliated Zhong Da Hospital, No. 87 Dingjiaqiao, Nanjing 210009, Jiangsu Province, China.

Abstract
Stroke is one of the leading causes of disability and mortality affecting millions worldwide with an ever-increasing prevalence in developing countries. The psychological and physical burdens, decreased quality of life and the considerable cost generated, can only emphasis the need for additional, better and more effective management options. The concept of neuroprotection is a mean to achieve better outcome through exploiting the complex pathophysiological changes deriving from stroke, and have been and continue to be the focus of numerous therapeutic strategies. Several compounds targeting various ischemic stroke mechanisms from excitotoxicity, apoptosis, and oxidative stress to inflammation have been explored with the majority yielding disappointing results in clinical phase. Yet, few have proven their worth and others remain promising showing that neuroprotection is still a viable option. In this review, potential targets for neuroprotection and the corresponding targeting treatments are discussed, additionally; a summary of clinically explored compounds, their mechanisms and the outcome is provided.
Keywords: Neuroprotection, stroke, apoptosis, inflammation, excitotoxicity, oxidative stress.

Received July 26, 2016; **Revised** September 4, 2016; **Accepted** September 24, 2016
***Correspondence:** Yi Jing Guo **Email** janegyj@aliyun.com, **Contact**: +86 18651657263

***To cite this manuscript*:** Abdoulaye IA, Guo YJ. Neuroprotection and Acute Ischemic Stroke: The Clinical Quest. Biomed Lett 2016; 2(2):72-81

Introduction

Stroke, as one of the leading causes of death and disability [1], can be traced back to the 2nd millennium BC [2] when Hippocrates first mentioned it as a phenomenon of sudden paralysis associated with ischemia. He initially referred to it as Apoplexy, from the Greek word meaning "struck down with violence" [3]. Acute ischemic stroke or cerebral ischemia refers to a sudden brain cells death due to inadequate blood supply. This acts as a precursor for a devastation cascade of pathophysiological events starting by an energy metabolism failure, membrane depolarization, inhibition of protein synthesis, Ca^{2+} influx, overstimulation and glutamate release, cytoskeleton and membranes damage, microglial-activated inflammation and lysosomal membrane rupture inducing cell death [4-6]. These events combined act synergistically with an amplifying effect than their own [7].

Although the prevalence and cost of stroke exponentially increased throughout the ages, currently the only approved treatment is tissue plasminogen activator (rtPA), which, is not suitable to a larger number of patients due to its limitations: a shorted time window and increased risk of subsequent hemorrhage [8-10].

New approaches for ischemic stroke management have been extensively studied with some directly aimed at the pathophysiological changes induced or occurring seconds to minutes after stroke. Neuroprotection, one of those explored complementary alternatives, has been studied, with the earlier clinical trials dating back to the 1980s [11], and has yielded various degree of success [12]. Neuroprotection is basically defined as the process aiming to limit or reduce the volume of an infarct, and salvage surrounding vulnerable cells by inhibition of intracellular calcium increase, and or activation of free radical reactions and cell death [13]. The final goal is to prevent or slow disease progression as well as secondary injuries through stopping or at least slowing neuronal loss. The main targets for neuroprotective therapy are oxidative stress, mitochondrial dysfunction, apoptosis, autophagy, excitotoxicity, and inflammatory changes [8]. These pathophysiological changes, sometimes referred to as the ischemic cascade (**Figure 1**) [14] , are subsequent to stroke and are responsible for the death of most ischemic neurons.

Several potential pharmacological agents with direct or indirect effects on above ischemic stroke-related changes have been studied for their neurotrotective potential and underwent several clinical trials in hope of a future implementation as anti-ischemic agents. This review aims to examine the pathophysiological mechanisms of interest for neuroprotection and

provides an overview of clinically tested neuroprotective treatments regardless of the outcome.

1. Ischemic mechanisms targeted by Neuroprotection

1.1 Excitotoxicity

Excitotoxicity is a process defined by excess stimulation of nerve cells due to a release of toxic amounts of neurotransmitters such as glutamate into the extracellular space [15], resulting eventually in cell death by apoptosis [16-18]. This is a result of a pathological excess of glutamate, inducing an over activation of glutamate receptors (*N*-Methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor), an intracellular shift of high levels of calcium and neuronal depolarization [19-21]. Higher intracellular calcium levels have dramatic effects on neuronal cells. Excess of calcium serves as an activator for several enzymes, such as phospholipases, proteases and calpain [22], with a devastating effect on cellular structure and deoxyribonucleic acid (DNA). Another immediate effect of higher calcium levels in the cytosol is unlocking the transition membranes of mitochondria, which acts as a loophole thus increasing permeability, causing swell and release of reactive oxygen species [21]. Additionally, all this changes affect the production of adenosine triphosphate (ATP) [23] leading to a loss of ion gradients and a stop of glutamate uptake, further damaging the activation of glutamate receptors, due to buildup [24]. Therefore glutamate excitotoxicity is an important aspect of stroke pathology and a precursor to the initiation of necrosis, apoptosis, and autophagy. Several methods have been explored in the aim of improving the outcome by directly influencing the excitotoxicity process. The general idea is to achieve an inhibition of glutamate release and or block the action of glutamate receptors in the brain. Various drugs (**Table 1**) underwent clinical trials with many unsatisfactory results.

1.2 Calcium channel blockers

This subgroup is comprised of drugs such as *Nimodipine*, a dihydropyridine calcium channel blocker originally developed for hypertension treatment; now mainly used to prevent cerebral vasospasm and associated ischemia. It has been proposed as a neuroprotectant due to its action on voltage dependent calcium channels. Although promising at first, around 28 trials assessing its effects on ischemic stroke patients have shown that there was no difference in death rates or clear improvement of disabilities seen on patients that were given Nimodipine compared to the control group [25].

1.3 NMDA antagonists

NMDA antagonists, mainly comprised of anesthetics possessing an inhibiting or antagonizing effect on the action of NMDA receptor (NMDAR), have been explored for treatment of excitotoxicity due to the ionotropic nature of NMDAR. They are classified into four categories, with competitive (bind to and block the binding site of glutamate) and noncompetitive (inhibit NMDAR by binding to allosteric sites) antagonists being subject of our focus. Among NMDA antagonists, *Magnesium*, a noncompetitive antagonist and voltage-gated calcium channel blocker [26]. Magnesium has been investigated as a potential anti-excitotoxicity treatment and has shown promises particularly upon immediate administration after surgical occlusion of middle cerebral artery (MCA) in rat stroke model [27]. Two main studies assessing the efficacy of magnesium have been conducted (the Intravenous Magnesium Efficacy in Stroke trial (IMAGES), being a multicenter randomized controlled trial (RCT) with administration of high dose of magnesium within 12h of onset of stroke [28]; and the FAST-MAG, a multicenter randomized double-blind placebo-controlled trial [29], with administration of magnesium within 2h of onset at phase III trial [30]. Unfortunately, both studies showed no significant benefit in outcome, whether death or disability in comparison to the control group [28, 30]. Another competitive antagonist, *Selfotel* [31], has shown, in experimental studies, to significantly reduce the size of infarct [32] if given within 5min of MCA occlusion [33]. It has proven to be effective and safe in human at a phase II study with a significant improvement of the outcome assessed by an independent Barthel index score on day 90 compared to control [34]. However, the phase III trials, two double-blinded randomized placebo-controlled parallel-design trials involving a total of 567 patients with within 6 h after stroke onset administration, were abandoned due to high mortality and no therapeutic benefit in primary outcome [35].

1.4 AMPA antagonists

A competitive AMPA receptor (AMPAR) antagonist *YM872*, known as *Zonampanel*, a quinoxalinedione derived drug, has also been investigated for its neuroprotective properties [36]. It has been proven that YM872 can reduce infarct volume and improve symptoms on rat embolic stroke model [37]. The

Table 1: Summary of clinically explored neuroprotective agents in ischemic stroke: mechanisms and outcome.

Compounds	Mechanisms	Outcome
Nimodipine	Ca^{2+} channel blocker.	No benefit observed
Magnesium	Anti-excitotoxic, non-competitive NMDA antagonist (voltage-gated Ca^{2+} channels).	No outcome improvement seen
Zonampanel	AMPA receptor blocker.	Early termination due to side effects
Nicardipine	Ca^{2+} channel blocker.	No benefit observed
Flunarizine	Ca^{2+} channel blocker.	No benefit observed
Selfotel	Competitive NMDA antagonist.	No benefit observed, increased mortality
Aptiganel	Non-competitive NMDA antagonist.	No benefit observed
Clomethiazole	GABA agonist.	No benefit observed
ZK-200775	AMPA antagonist.	Worsen outcome
Fosphenytoin	Na^{+} channel blocker.	No benefit observed
BMS-204352	K^{+} channel opener.	No benefit observed
Eliprodil	NMDA blocker (polyamine site).	Abandoned (interferes with neuronal survival)
Gavestinel	Glycine site antagonist.	No benefit observed
Lubeluzole	Blockage of ion channel and NO inhibitor.	No benefit observed
Enlimomab	Anti-ICAM-1 antibody (leucocyte adhesion inhibitor).	Not effective, may worsen outcome
Ramecemide	Non-competitive NMDA antagonist.	No outcome improvement seen
Trafermin	Growth factor.	No benefit observed
G-CSF	Anti-excitotoxicity, anti-inflammatory, anti-apoptosis, angiogenesis and neurogenesis enhancer.	No benefit observed
Cerebrolysin	Enhancement of neurogenesis, stabilization of cell integrity, apoptotic cells reduction	Possible benefit
Erythropoietin	Anti-excitotoxicity, anti-inflammatory, anti-apoptosis, angiogenesis and neurogenesis enhancer.	No improvement of functional outcome observed
Fingolimod	Immunomodulatory agent, regulation of myelination and microglial activation, proliferation and migration of neural precursor cells, anti-apoptotic and anti-inflammatory.	Further clinical investigation needed
Ginsenoside-Rd	Ca2+ channel antagonist.	Improves outcome
Glyceryl trinitrate	Reduction of lesion size, increase blood flow and induction of transient ROS production.	Ongoing
Minocycline	Anti-inflammatory, anti-apoptotic, antioxidant and reduction of microglial activation and MMP-9.	NeuMAST (phase Ⅳ stopped due to futility); ongoing further investigation
Repinotan	$5\text{-}HT_{1A}$ receptor agonist.	No benefit observed
Hypothermia	Reduction of cerebral metabolism, anti-inflammatory, antioxidant, prevention of apoptosis, reduction of BBB disruption and edema.	Improves outcome
Cyclosporin A	Anti-excitotoxicity and anti-inflammatory.	Further clinical investigation needed
Citicoline	Stimulation/restoration of Na^{+}/K^{+} ATPase activity; prevention of neuronal ATP loss and of PLA2 activation; induction of glutathione reductase activity; increase in the Bcl-2 expression; up regulation of SIRT Ⅰ protein; down regulation of procaspase and caspase expression.	Improves outcome
Resveratrol	Activation of SIRT1, AMPA-activated kinase and nuclear factorNrf2.	Further clinical investigation needed
Ebselen	Antioxidant (free radical scavenger).	Ongoing
Tirilazad	Inhibition of lipid peroxidation (free radical scavenger).	No benefit observed
Albumin	Promotion of Collateral circulation, reduction of swelling.	No clinical benefit, safety concerns
Statin	HMG-CoA reductase inhibition (antioxidant).	Ongoing
Edaravone	Antioxidant (ROS scavenger).	Improves size of ischemic stroke lesions and neurological deficits in small vessel occlusion within 1year
NXY-059	Antioxidant.	Mixed results
Uric acid	Antioxidant (ROS scavenger).	Ongoing
Dextrorphan	Non-competitive NMDA antagonist.	No benefit observed

NBP	Anti-oxidation, anti-inflammation, promotion of neurogenesis, improvement of mitochondrial functions, inhibition of platelet aggregation.	Improves outcome
HUK	Activation of bradykinin B1 and B2 receptors, anti-inflammation, anti-apoptosis, promoting angiogenesis, cerebral perfusion and neurogenesis.	Improves outcome

Note: GABA=Gamma-Aminobutyric acid; ICAM-1=Intercellular Adhesion Molecule 1; MMP-9=Matrix metallopeptidase 9; 5-HT_{1A}=5-Hydroxytryptamine 1A; BBB=Blood-brain barrier; NO=Nitric Oxide; ATP=Adenosine triphosphate; PLA2=Phospholipase A2; Bcl-2 =B-cell lymphoma 2; SIRT Ⅰ=NAD-dependent deacetylase sirtuin-1; Nrf2=Erythroid-derived 2; HMG-CoA=3-Hydroxy-3-methylglutaryl-coenzyme A.

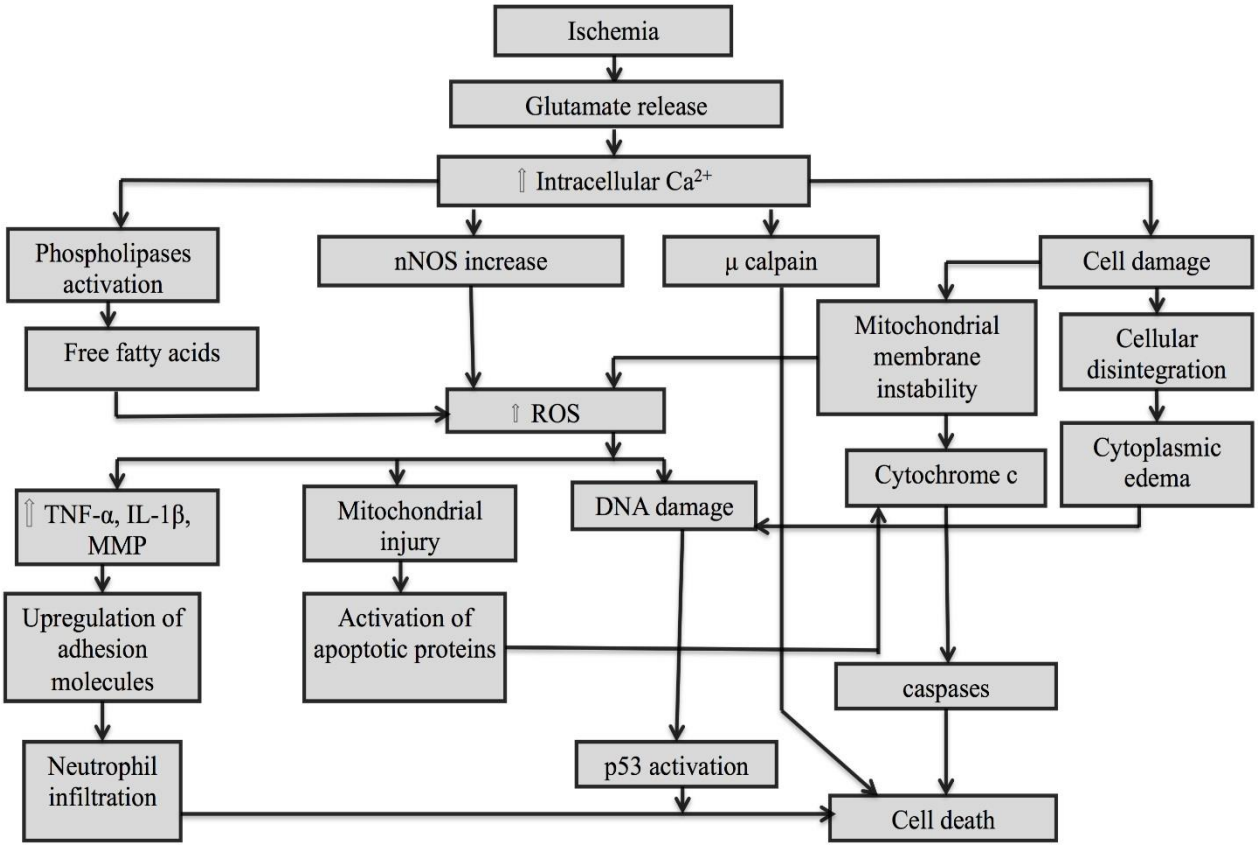

Figure 1: Pathophysiological changes during ischemic stroke. nNOS=Neuronal nitric oxide synthase; ROS=Reactive oxygen species; TNF-α=Tumor necrosis factor-α; IL-β =Interleukin-β; MMP=Matrix metalloproteinase.

mechanism is believed to be through the reduction of cortical tissue loss and cerebral edema [38]. However, clinical trials were early terminated due to severe side effects such as hallucinations, catatonia, and agitation [39].

2. Apoptosis

For a long time, post stroke cell death has been considered exclusively a result of necrosis defined by damaging events culminating in plasma membrane disruption and release of intracellular content [40]. This event occurs within the ischemic penumbra [41].

Nonetheless, further evidence has shown that apoptosis, a form of programmed cell death, plays a prominent role after ischemic brain injury in animal models [42, 43]. This phenomenon is essentially defined by the apoptotic cascades involving a myriad of changes among which: an increase of intracellular calcium; an increased expression of proteins such as prostate apoptosis response-4, which promotes

mitochondrial dysfunction and stops the anti-apoptotic process; mitochondrial membrane depolarization; and release of cytochrome c, with activation of caspases and nuclear DNA as ultimate result [44]. Recently, the aim of achieving neuroprotection through countering the effects of apoptosis has obtained a great deal of attention [45]. Several anti-apoptotic compounds have been successfully tested in experimental models with significant reduction of infarct, inhibition of caspase, blockage of pro-apoptotic gene expression and stimulation of anti-apoptotic gene expression. However, experimental results have not always been concordant with clinical ones. Among clinically evaluated formulas, erythropoietin (EPO) and cerebrolysin have been tested for possible implementation for post stroke treatment.

2.1 Erythropoietin (EPO)

Besides its well-know function which is red blood cell production through stimulation of erythropoiesis, controversial studies have shown EPO to possess a range of actions including stimulation of angiogenesis, promotion of cell survival via activation of EPO receptors, resulting in an anti-apoptotic effect on ischemic tissues [46, 47]. It's effect on apoptotic and anti-apoptotic gene is believed to be through an increased expression of apoptosis-inhibitor genes XIAP and c-IAP2, demonstrated in cortical cells pre-incubated with recombinant human erythropoietin (rhEPO) [48]. Furthermore, an increase in B-cell lymphoma extra-large (bcl-xL) expression in the hippocampal CA1 region of ischemia was shown after rhEPO administration [49]. Although pilot trial demonstrated the safety and benefit of EPO in stroke patients [50], those findings were not further validated by a larger study [51]. Moreover, a larger study (AXIS-2) of 328 stroke patients receiving either granulocyte-colony stimulating factor (G-CSF) or placebo within 9 h after stroke onset found no differences regarding the primary endpoint and clinical outcome at day 90 [9]. This typically outlined the problems associated with translation from experimental to clinical.

2.2 Cerebrolysin

Cerebrolysin is a mixture of purified porcine brain-derived peptides of low molecular weight neuropeptides and free aminoacids [52], including (not limited to) brain-derived neurotrophic factor, glial cell line-derived neurotrophic factor, nerve growth factor, and ciliary neurotrophic factor [53]. It has demonstrated neuroprotective properties and effectiveness against excitotoxicity, by inhibiting free radical formation, microglial activation, neuro-inflammation, calpain activation/apoptosis, and exhibited neurotrophic activity [54-57]. Previous studies came to the conclusion that cerebrolysin significantly improved functional outcome [58] but did not reduce infarct size, this is believed to be due to an augmentation of proliferation, migration, and surviving of neuroblasts [59]; moreover, a decrease in the pulsatility index (PI) of the right middle cerebral artery was observed on day 30, 60, and 90 in one of those studies [60]. All these findings are suggestive of a clinical usage for cerebrolysin in acute ischemic stroke although further investigation is required.

3. Inflammation

The first reported inflammation of the central nervous system dated as early as 1900s, with further findings implicating inflammatory mediators in the modulatory process of neurogenesis. Any form of insult to the brain whether is it hypoxia, ischemia or infection, has profound consequences and elicits a characteristic inflammation response. Brain inflammation has been seen as a secondary injury mechanism following stroke [61, 62] and is a result of several factors, such as reactive oxygen species or necrotic cells; with the key players and mediators being microglia, astrocytes and peripheral macrophages. Those factors lead to microglial activation, resulting in more cytokine generation and adhesion molecules induction within the cerebral blood vessels [63, 64]. Adhesion molecules in turn induce adhesion of circulating leukocytes causing micro-vascular occlusion and infiltration of immune cells into the brain parenchyma. Activated inflammatory cells then elaborate a variety of cytotoxic molecules such as an array of pro- and anti-inflammatory cytokines and chemokines, which have double-edged effects. On one hand providing immune protection to the brain and on the other hand initiating a regenerative loop of inflammation leading to disruption of the blood-brain barrier [65] and neuronal death [66]. During the years and following successful experimental studies, few clinical trials have been carried out to assess the value of some anti-inflammatory strategies. Although several of these trials resulted in a failure due to unforeseen side effects or inefficacy, few still hold great promises.

3.1 Targeted temperature management (TTM)

TTM's aim is to improve outcomes after an ischemic period or stroke through body temperature

management for a specific duration [67]. It was believed that hypothermia acts as neuro-protectant by slowing cellular metabolism and decreasing the brain's oxygen demand [68-71]. Later on, several studies conducted have shown that hypothermia affects pathways that extend beyond those limits. The mechanisms underlying the neuroprotective effects of hypothermia are found to be throughout a decrease in: cerebral metabolism (decrease 6-10% per °C below 37 °C), mitochondrial injury and dysfunction, reperfusion injury, ion pump dysfunction, influx of calcium into cell, neuro-excitotoxicity, cell membrane leakage, formation of cytotoxic edema, intracellular acidosis, production of free radicals, apoptosis, calpain-mediated proteolysis, DNA injury, vascular permeability, permeability of the blood-brain barrier, coagulation activation, formation of micro-thrombi, immune response, neuro-inflammation, and an improved tolerance for ischemia [72].

However, using a single factor to explain the neuroprotective effect of hypothermia is proven to be quite challenging due to the fact that hypothermia affects several pathways of the ischemic cascade ranging from excitotoxicity, apoptosis, inflammation, free radical production, blood flow and intracranial pressure [73, 74]. On the immunological count, hypothermia's main effect is not only through activation of microglia and reduction of neutrophils in ischemic areas [75]; but also reduction of reactive oxygen species (ROS) [76], adhesion molecules, macrophage inflammatory protein-3 α, pro-inflammatory cytokines, tumor necrosis factor alpha (TNF- α), interleukin 6 (IL-6), interleukin 10 (IL-10) [77], reactive nitrogen species, and most importantly nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). Additionally, it has been shown to affect the mitogen-activated protein kinase, an important enzyme system in inflammation [78]. All this evidences are suggestive of the important future role that targeted temperature management might play in post stroke therapy.

3.2 Minocycline

Minocycline, a broad–spectrum bacteriostatic antibiotic, member of the tetracycline family, has been found to possess several neuroprotective properties ranging from anti-inflammation [79], anti-apoptosis, blood-brain barrier (BBB) protection, reduction of microglial activation [80, 81], Matrix Metalloproteinase (MMP)-9 reduction [82], to nitric oxide (NO) production. The anti-inflammatory functions of minocycline are believed to be by inhibition several cellular targets, including caspase-3 [83], caspase-1 [84], cyclo-oxygenase-2, inducible nitric oxide synthase [85], p38 mitogen-activated protein kinase [81], TNF- α, and blockade of NF-κB. Whereas its BBB integrity protection comes from its inhibition of hypoxia-inducible factor 1-alpha (HIF-1α)-mediated cellular responses through the NAD-dependent deacetylase sirtuin-3 (SIRT-3)/ prolyl hydroxylase domain-containing protein 2 (PHD-2) pathway [86]. However, further clinical studies for a possible therapeutic use against ischemic stroke are still much needed.

4. Oxidative stress

Oxidative stress in stroke translate by an increased production of reactive oxygen species and other free radicals; with phospholipase A2 and arachidonic acid playing a major role in this process. During ischemia, glutamate release and increase intracellular Ca^{2+} activate cytosolic Ca^{2+}-dependent PLA2 [87] leading to hydrolysis of phospholipids and free fatty acids release [88]. On the other hand, intracellular free arachidonic acid accumulation promotes the formation of oxygen reactive species, which amplifies oxidative damage [88]. The final result is a direct neuron cell death or other events such as mitochondrial dysfunction, glial cell activation, protein misfolding [89, 90], disruption of gene expression and cellular signaling [91], and proteasomal malfunction [92]; with several of those mechanism leading to apoptosis. It has been shown that oxidative stress is a byproduct of excitotoxicity, cellular respiration inhibition, and inflammation [93]. Although the neutralization of oxidative stress presents an interesting potential therapeutic option for stroke, the discovering of viable compounds has proven quite challenging.

4.1 Citicoline

Also known as cytidine diphosphate-choline (CDP-Choline), is a two molecules compound: cytidine and choline, and an intermediary in the generation of phosphatidylcholine from choline. It has been shown, in experimental studies, to be multi-targeted and effective against several disorders of the central nervous system [94-96]; with both molecules capable of passing through the blood-brain barrier [97]. In those of stroke, Citicoline has been shown to have a larger time window with multistage effects [98, 99]. Similar findings have been found in clinical studies with a clear functional outcome improvement. The antioxidant effect of citicoline is believed to be through preventing or attenuating the activation of

phospholipase A2 in membrane and mitochondria [100], stabilizing cell membranes, decreasing arachidonic acid [101] and extra free fatty acids after reperfusion. Additionally, it has shown to modulate synthesis of glutathione through the choline-S-adenosyl-L-methionine pathway [102]. Thus citicoline represents an extra in stroke treatment.

4.2 Resveratrol

Resveratrol is a type of natural phenol and a phytoalexin produced by plants as a response to bacterial, fungal infection or injury [103]. Originally known for its antioxidant properties and availability in grapes, blueberries, mulberries skin [104]; resveratrol has been shown to improve outcomes in acute central nervous system injuries [105]. However, through which exact mechanism it exerts that effect still remains elusive [106, 107]. It is believed to be by activation of a nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase, which improves survival [108, 109]. Additionally, it also activates AMP-activated kinase [110] and nuclear factor (erythroid derived 2)-like 2(Nrf2) [111]. Extensive studies on resveratrol effects have suggested that it can be a determinant option for future stroke management. Although further clinical studies for better understanding and quantification of its effects are essentials.

5. Multi-targeting options

On the quest for new therapeutic options for stroke, many compounds have shown a multi-targeted and multi-purposed effect on the pathophysiological changes deriving from ischemia; among which two compounds: N-Butylphthalide (NBP) and Human Urinary Kallidinogenase, already approved and implemented for clinical usage in China.

5.1 N-Butylphthalide (NBP)

One of the constituents in celery oil has shown remarkable multi-targeted neuroprotective properties. It demonstrated anti-platelet aggregation and anti-thrombotic effects, which are believe to be through increasing cyclic adenosine monophosphate (cAMP) levels in platelets and inhibiting serotonin release [112]. Extensive studies have also concluded that NBP possesses anti-apoptotic effect by increasing levels of Bcl-2 and HIF-1 α, and decreasing caspase-3 expression [113]. Additionally, it exhibited protective properties against mitochondrial damage through improvement of Na^+/K^+-ATPase and Ca^{2+}-ATPase in mitochondria, and reduction of cytochrome c release [114]. Furthermore, NBP has shown antioxidant effects after long term administration in experimental models. The basic mechanism is through increasing malondialdehyde (MDA) levels [115] and reducing hydrogen peroxide (H_2O_2)-dependent ROS accumulation [116]. All those findings indicated it's efficiency against stroke. After extensive clinical trials, NBP has already been approved and used in China for 12 years with satisfactory results.

5.2 Human Urinary Kallidinogenase (HUK)

Tissue kallikrein, a component of the kallikrein/kinin system (KKS), has been shown to possess protective effects against ischemic stroke in clinical and experimental studies. Human urinary kallidinogenase, a KKS regulator and kallikrein producer, exhibited anti-inflammatory, anti-apoptotic, angiogenesis and neurogenesis promoting effects [117], and has been approved for stroke treatment in China. Several studies demonstrated that HUK ameliorates functional deficits [118, 119], promotes angiogenensis and improves cerebral-blood flow [120]. The basic mechanism is through up-regulating vascular endothelial growth factor and apelin/APJ pathway [121] and activating the bradykinin B1 and B2 receptors [122]. Additionally, HUK have been shown to improve cognition and decrease Aβ1-40 and Aβ1-42 serum levels [123]. Furthermore, tissue kallikrein pre-treatment have been shown to alleviate glutamate-induced oxidative stress through inhibition of neuronal nitric oxide synthase activity and activation of extracellular signal-regulated kinase 1 (ERK1) and NF-κB; resulting in enhancement of brain derived neurotrophic factor mRNA and anti-apoptotic gene Bcl-2 protein [124].

Summary

The quest for new therapeutic options for the management of ischemic stroke has been an ever captivating. However, due to the intricacy of its pathology, developing new neuroprotective drugs have proven to be an even greater challenge. On one hand, an ever-increasing number of failed clinical translations, emphasizing the complexity of stroke and the need of higher quality controlled studies; on the other hand, the dual effect of many post stroke pathophysiological activities which, can be beneficial or harmful depending on the concentration, timing, location and circumstances of occurrence. Nevertheless, positive results, with few compounds successfully implemented for clinical use, have shown us that although the process of discovering

new effective neuroprotective options might be long and sometimes disappointing, neuroprotection is still a viable and one of the best options for future treatments.

References

[1] Martynov MY, Gusev EI. Current Knowledge on the Neuroprotective and Neuroregenerative Properties of Citicoline in Acute Ischemic Stroke. J Exp Pharmacol. 2015;7:17-28.

[2] Ashrafian H. Familial Stroke 2700 Years Ago. Stroke. 2010;41:e187-e.

[3] Thompson JE. The Evolution of Surgery for the Treatment and Prevention of Stroke: The Willis Lecture. Stroke. 1996;27:1427-34.

[4] Villa RF, Gorini A, Ferrari F, Hoyer S. Energy Metabolism of Cerebral Mitochondria During Aging, Ischemia and Post-Ischemic Recovery Assessed by Functional Proteomics of Enzymes. Neurochem Int. 2013;63:765-81.

[5] Moskowitz MA, Lo EH, Iadecola C. The Science of Stroke: Mechanisms in Search of Treatments. Neuron. 2010;67:181-98.

[6] Hossmann KA. Pathophysiology and Therapy of Experimental Stroke. Cell Mol Neurobiol. 2006;26:1057-83.

[7] Zadori D, Klivenyi P, Szalardy L, Fulop F, Toldi J, Vecsei L. Mitochondrial Disturbances, Excitotoxicity, Neuroinflammation and Kynurenines: Novel Therapeutic Strategies for Neurodegenerative Disorders. J Neurol Sci. 2012;322:187-91.

[8] Majid A. Neuroprotection in Stroke: Past, Present, and Future. ISRN Neurol. 2014;2014:515716.

[9] Minnerup J, Sutherland BA, Buchan AM, Kleinschnitz C. Neuroprotection for Stroke: Current Status and Future Perspectives. Int J Mol Sci. 2012;13:11753-72.

[10] Donnan GA, Davis SM, Parsons MW, Ma H, Dewey HM, Howells DW. How to Make Better Use of Thrombolytic Therapy in Acute Ischemic Stroke. Nat Rev Neurol. 2011;7:400-9.

[11] Shuaib A, Hussain MS. The Past and Future of Neuroprotection in Cerebral Ischaemic Stroke. Eur Neurol. 2008;59:4-14.

[12] Fisher M. New Approaches to Neuroprotective Drug Development. Stroke. 2011;42:S24-7.

[13] Wahlgren NG, Ahmed N. Neuroprotection in Cerebral Ischaemia: Facts and Fancies--the Need for New Approaches. Cerebrovasc Dis. 2004;17 Suppl 1:153-66.

[14] Deb P, Sharma S, Hassan KM. Pathophysiologic Mechanisms of Acute Ischemic Stroke: An Overview with Emphasis on Therapeutic Significance Beyond Thrombolysis. Pathophysiology. 2010;17:197-218.

[15] Jablonska A, Lukomska B. Stroke Induced Brain Changes: Implications for Stem Cell Transplantation. Acta Neurobiol Exp (Wars). 2011;71:74-85.

[16] Yang DD, Kuan CY, Whitmarsh AJ, Rincon M, Zheng TS, Davis RJ, et al. Absence of Excitotoxicity-Induced Apoptosis in the Hippocampus of Mice Lacking the Jnk3 Gene. Nature. 1997;389:865-70.

[17] Ankarcrona M, Dypbukt JM, Bonfoco E, Zhivotovsky B, Orrenius S, Lipton SA, et al. Glutamate-Induced Neuronal Death: A Succession of Necrosis or Apoptosis Depending on Mitochondrial Function. Neuron. 1995;15:961-73.

[18] Dutta R, Trapp BD. Mechanisms of Neuronal Dysfunction and Degeneration in Multiple Sclerosis. Prog Neurobiol. 2011;93:1-12.

[19] Jaiswal MK, Zech WD, Goos M, Leutbecher C, Ferri A, Zippelius A, et al. Impairment of Mitochondrial Calcium Handling in a Mtsod1 Cell Culture Model of Motoneuron Disease. BMC Neurosci. 2009;10:64.

[20] Manev H, Favaron M, Guidotti A, Costa E. Delayed Increase of Ca2+ Influx Elicited by Glutamate: Role in Neuronal Death. Mol Pharmacol. 1989;36:106-12.

[21] Kaur H, Prakash A, Medhi B. Drug Therapy in Stroke: From Preclinical to Clinical Studies. Pharmacology. 2013;92:324-34.

[22] White BC, Sullivan JM, DeGracia DJ, O'Neil BJ, Neumar RW, Grossman LI, et al. Brain Ischemia and Reperfusion: Molecular Mechanisms of Neuronal Injury. J Neurol Sci. 2000;179:1-33.

[23] Stavrovskaya IG, Kristal BS. The Powerhouse Takes Control of the Cell: Is the Mitochondrial Permeability Transition a Viable Therapeutic Target against Neuronal Dysfunction and Death? Free Radic Biol Med. 2005;38:687-97.

[24] Kristian T, Siesjo BK. Calcium-Related Damage in Ischemia. Life Sci. 1996;59:357-67.

[25] Zhang J, Yang J, Zhang C, Jiang X, Zhou H, Liu M. Calcium Antagonists for Acute Ischemic Stroke. Cochrane Database Syst Rev. 2012:CD001928.

[26] Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A. Magnesium Gates Glutamate-Activated Channels in Mouse Central Neurones. Nature. 1984;307:462-5.

[27] Izumi Y, Roussel S, Pinard E, Seylaz J. Reduction of Infarct Volume by Magnesium after Middle Cerebral Artery Occlusion in Rats. J Cereb Blood Flow Metab. 1991;11:1025-30.

[28] Muir KW, Lees KR, Ford I, Davis S, Intravenous Magnesium Efficacy in Stroke Study I. Magnesium for Acute Stroke (Intravenous Magnesium Efficacy in Stroke Trial): Randomised Controlled Trial. Lancet. 2004;363:439-45.

[29] Saver JL, Kidwell C, Eckstein M, Starkman S, Investigators F-MPT. Prehospital Neuroprotective Therapy for Acute Stroke: Results of the Field Administration of Stroke Therapy-Magnesium (Fast-Mag) Pilot Trial. Stroke. 2004;35:e106-8.

[30] Grupke S, Hall J, Dobbs M, Bix GJ, Fraser JF. Understanding History, and Not Repeating It. Neuroprotection for Acute Ischemic Stroke: From Review to Preview. Clin Neurol Neurosurg. 2015;129:1-9.

[31] Lehmann J, Hutchison AJ, McPherson SE, Mondadori C, Schmutz M, Sinton CM, et al. Cgs 19755, a Selective and Competitive N-Methyl-D-Aspartate-Type Excitatory Amino Acid Receptor Antagonist. J Pharmacol Exp Ther. 1988;246:65-75.

[32] Miyabe M, Kirsch JR, Nishikawa T, Koehler RC, Traystman RJ. Comparative Analysis of Brain Protection by N-Methyl-D-Aspartate Receptor Antagonists after Transient Focal Ischemia in Cats. Crit Care Med. 1997;25:1037-43.

[33] Simon R, Shiraishi K. N-Methyl-D-Aspartate Antagonist Reduces Stroke Size and Regional Glucose Metabolism. Ann Neurol. 1990;27:606-11.

[34] Grotta J, Clark W, Coull B, Pettigrew LC, Mackay B, Goldstein LB, et al. Safety and Tolerability of the Glutamate Antagonist Cgs 19755 (Selfotel) in Patients with Acute Ischemic Stroke. Results of a Phase Iia Randomized Trial. Stroke. 1995;26:602-5.

[35] Davis SM, Lees KR, Albers GW, Diener HC, Markabi S, Karlsson G, et al. Selfotel in Acute Ischemic Stroke : Possible Neurotoxic Effects of an Nmda Antagonist. Stroke. 2000;31:347-54.

[36] Jain KK. The Handbook of Neuroprotection. 1. ed. Totowa, NJ: Springer Science+Business Media, LLC; 2011.

[37] Suzuki M, Sasamata M, Miyata K. Neuroprotective Effects of Ym872 Coadministered with T-Pa in a Rat Embolic Stroke Model. Brain Res. 2003;959:169-72.

[38] Furukawa T, Hoshino S, Kobayashi S, Asakura T, Takahashi M, Atsumi T, et al. The Glutamate Ampa Receptor Antagonist, Ym872, Attenuates Cortical Tissue Loss, Regional Cerebral Edema, and Neurological Motor Deficits after Experimental Brain Injury in Rats. J Neurotrauma. 2003;20:269-78.

[39] Farooqui AA. Neurochemical Aspects of Neurotraumatic and Neurodegenerative Diseases. New York, NY: Springer Science+Business Media, LLC; 2010.

[40] Garcia JH, Yoshida Y, Chen H, Li Y, Zhang ZG, Lian J, et al. Progression from Ischemic Injury to Infarct Following Middle Cerebral Artery Occlusion in the Rat. Am J Pathol. 1993;142:623-35.

[41] Broughton BR, Reutens DC, Sobey CG. Apoptotic Mechanisms after Cerebral Ischemia. Stroke. 2009;40:e331-9.

[42] Li Y, Chopp M, Jiang N, Yao F, Zaloga C. Temporal Profile of in Situ DNA Fragmentation after Transient Middle Cerebral Artery Occlusion in the Rat. J Cereb Blood Flow Metab. 1995;15:389-97.

[43] Li Y, Chopp M, Jiang N, Zhang ZG, Zaloga C. Induction of DNA Fragmentation after 10 to 120 Minutes of Focal Cerebral Ischemia in Rats. Stroke. 1995;26:1252-7; discussion 7-8.
[44] Mattson MP, Culmsee C, Yu ZF. Apoptotic and Antiapoptotic Mechanisms in Stroke. Cell Tissue Res. 2000;301:173-87.
[45] MacManus JP, Buchan AM. Apoptosis after Experimental Stroke: Fact or Fashion? J Neurotrauma. 2000;17:899-914.
[46] Elliott S, Sinclair AM. The Effect of Erythropoietin on Normal and Neoplastic Cells. Biologics. 2012;6:163-89.
[47] Chong ZZ, Kang JQ, Maiese K. Erythropoietin Is a Novel Vascular Protectant through Activation of Akt1 and Mitochondrial Modulation of Cysteine Proteases. Circulation. 2002;106:2973-9.
[48] Digicaylioglu M. Erythropoietin in Stroke: Quo Vadis. Expert Opin Biol Ther. 2010;10:937-49.
[49] Wen TC, Sadamoto Y, Tanaka J, Zhu PX, Nakata K, Ma YJ, et al. Erythropoietin Protects Neurons against Chemical Hypoxia and Cerebral Ischemic Injury by up-Regulating Bcl-Xl Expression. J Neurosci Res. 2002;67:795-803.
[50] Ehrenreich H, Hasselblatt M, Dembowski C, Cepek L, Lewczuk P, Stiefel M, et al. Erythropoietin Therapy for Acute Stroke Is Both Safe and Beneficial. Mol Med. 2002;8:495-505.
[51] Ehrenreich H, Weissenborn K, Prange H, Schneider D, Weimar C, Wartenberg K, et al. Recombinant Human Erythropoietin in the Treatment of Acute Ischemic Stroke. Stroke. 2009;40:e647-56.
[52] Heiss WD, Brainin M, Bornstein NM, Tuomilehto J, Hong Z, Cerebrolysin Acute Stroke Treatment in Asia I. Cerebrolysin in Patients with Acute Ischemic Stroke in Asia: Results of a Double-Blind, Placebo-Controlled Randomized Trial. Stroke. 2012;43:630-6.
[53] Menon PK, Muresanu DF, Sharma A, Mossler H, Sharma HS. Cerebrolysin, a Mixture of Neurotrophic Factors Induces Marked Neuroprotection in Spinal Cord Injury Following Intoxication of Engineered Nanoparticles from Metals. CNS Neurol Disord Drug Targets. 2012;11:40-9.
[54] Hartbauer M, Hutter-Paier B, Skofitsch G, Windisch M. Antiapoptotic Effects of the Peptidergic Drug Cerebrolysin on Primary Cultures of Embryonic Chick Cortical Neurons. J Neural Transm (Vienna). 2001;108:459-73.
[55] Zhang L, Chopp M, Meier DH, Winter S, Wang L, Szalad A, et al. Sonic Hedgehog Signaling Pathway Mediates Cerebrolysin-Improved Neurological Function after Stroke. Stroke. 2013;44:1965-72.
[56] Gutmann B, Hutter-Paier B, Skofitsch G, Windisch M, Gmeinbauer R. In Vitro Models of Brain Ischemia: The Peptidergic Drug Cerebrolysin Protects Cultured Chick Cortical Neurons from Cell Death. Neurotox Res. 2002;4:59-65.
[57] Masliah E, Diez-Tejedor E. The Pharmacology of Neurotrophic Treatment with Cerebrolysin: Brain Protection and Repair to Counteract Pathologies of Acute and Chronic Neurological Disorders. Drugs Today (Barc). 2012;48 Suppl A:3-24.
[58] Muresanu DF, Heiss WD, Hoemberg V, Bajenaru O, Popescu CD, Vester JC, et al. Cerebrolysin and Recovery after Stroke (Cars): A Randomized, Placebo-Controlled, Double-Blind, Multicenter Trial. Stroke. 2016;47:151-9.
[59] Zhang C, Chopp M, Cui Y, Wang L, Zhang R, Zhang L, et al. Cerebrolysin Enhances Neurogenesis in the Ischemic Brain and Improves Functional Outcome after Stroke. J Neurosci Res. 2010;88:3275-81.
[60] Amiri-Nikpour MR, Nazarbaghi S, Ahmadi-Salmasi B, Mokari T, Tahamtan U, Rezaei Y. Cerebrolysin Effects on Neurological Outcomes and Cerebral Blood Flow in Acute Ischemic Stroke. Neuropsychiatr Dis Treat. 2014;10:2299-306.
[61] Chamorro A, Hallenbeck J. The Harms and Benefits of Inflammatory and Immune Responses in Vascular Disease. Stroke. 2006;37:291-3.
[62] Barone FC, Feuerstein GZ. Inflammatory Mediators and Stroke: New Opportunities for Novel Therapeutics. J Cereb Blood Flow Metab. 1999;19:819-34.
[63] Becker KJ. Inflammation and Acute Stroke. Curr Opin Neurol. 1998;11:45-9.
[64] Stanimirovic DB, Wong J, Shapiro A, Durkin JP. Increase in Surface Expression of Icam-1, Vcam-1 and E-Selectin in Human Cerebromicrovascular Endothelial Cells Subjected to Ischemia-Like Insults. Acta Neurochir Suppl. 1997;70:12-6.
[65] Danton GH, Dietrich WD. Inflammatory Mechanisms after Ischemia and Stroke. J Neuropathol Exp Neurol. 2003;62:127-36.
[66] Basu A, Lazovic J, Krady JK, Mauger DT, Rothstein RP, Smith MB, et al. Interleukin-1 and the Interleukin-1 Type 1 Receptor Are Essential for the Progressive Neurodegeneration That Ensues Subsequent to a Mild Hypoxic/Ischemic Injury. J Cereb Blood Flow Metab. 2005;25:17-29.
[67] Peberdy MA, Callaway CW, Neumar RW, Geocadin RG, Zimmerman JL, Donnino M, et al. Part 9: Post-Cardiac Arrest Care: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. Circulation. 2010;122:S768-86.
[68] Polderman KH. Application of Therapeutic Hypothermia in the Icu: Opportunities and Pitfalls of a Promising Treatment Modality. Part 1: Indications and Evidence. Intensive Care Med. 2004;30:556-75.
[69] Milde LN. Clinical Use of Mild Hypothermia for Brain Protection: A Dream Revisited. J Neurosurg Anesthesiol. 1992;4:211-5.
[70] Ehrlich MP, McCullough JN, Zhang N, Weisz DJ, Juvonen T, Bodian CA, et al. Effect of Hypothermia on Cerebral Blood Flow and Metabolism in the Pig. Ann Thorac Surg. 2002;73:191-7.
[71] Erecinska M, Thoresen M, Silver IA. Effects of Hypothermia on Energy Metabolism in Mammalian Central Nervous System. J Cereb Blood Flow Metab. 2003;23:513-30.
[72] Polderman KH. Mechanisms of Action, Physiological Effects, and Complications of Hypothermia. Crit Care Med. 2009;37:S186-202.
[73] Busto R, Dietrich WD, Globus MY, Ginsberg MD. Postischemic Moderate Hypothermia Inhibits Ca1 Hippocampal Ischemic Neuronal Injury. Neurosci Lett. 1989;101:299-304.
[74] Baldwin WA, Kirsch JR, Hurn PD, Toung WS, Traystman RJ. Hypothermic Cerebral Reperfusion and Recovery from Ischemia. Am J Physiol. 1991;261:H774-81.
[75] Wang GJ, Deng HY, Maier CM, Sun GH, Yenari MA. Mild Hypothermia Reduces Icam-1 Expression, Neutrophil Infiltration and Microglia/Monocyte Accumulation Following Experimental Stroke. Neuroscience. 2002;114:1081-90.
[76] Perrone S, Szabo M, Bellieni CV, Longini M, Bango M, Kelen D, et al. Whole Body Hypothermia and Oxidative Stress in Babies with Hypoxic-Ischemic Brain Injury. Pediatr Neurol. 2010;43:236-40.
[77] Meybohm P, Gruenewald M, Zacharowski KD, Albrecht M, Lucius R, Fosel N, et al. Mild Hypothermia Alone or in Combination with Anesthetic Post-Conditioning Reduces Expression of Inflammatory Cytokines in the Cerebral Cortex of Pigs after Cardiopulmonary Resuscitation. Crit Care. 2010;14:R21.
[78] Choi JS, Park J, Suk K, Moon C, Park YK, Han HS. Mild Hypothermia Attenuates Intercellular Adhesion Molecule-1 Induction Via Activation of Extracellular Signal-Regulated Kinase-1/2 in a Focal Cerebral Ischemia Model. Stroke Res Treat. 2011;2011:846716.
[79] Maier K, Merkler D, Gerber J, Taheri N, Kuhnert AV, Williams SK, et al. Multiple Neuroprotective Mechanisms of Minocycline in Autoimmune Cns Inflammation. Neurobiol Dis. 2007;25:514-25.
[80] Tikka TM, Vartiainen NE, Goldsteins G, Oja SS, Andersen PM, Marklund SL, et al. Minocycline Prevents Neurotoxicity Induced by Cerebrospinal Fluid from Patients with Motor Neurone Disease. Brain. 2002;125:722-31.
[81] Tikka TM, Koistinaho JE. Minocycline Provides Neuroprotection against N-Methyl-D-Aspartate Neurotoxicity by Inhibiting Microglia. J Immunol. 2001;166:7527-33.
[82] Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting Leukocyte Mmps and Transmigration: Minocycline as a Potential Therapy for Multiple Sclerosis. Brain. 2002;125:1297-308.
[83] Wang X, Zhu S, Drozda M, Zhang W, Stavrovskaya IG, Cattaneo E, et al. Minocycline Inhibits Caspase-Independent and -Dependent

Mitochondrial Cell Death Pathways in Models of Huntington's Disease. Proc Natl Acad Sci U S A. 2003;100:10483-7.
[84] Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, et al. Minocycline Inhibits Caspase-1 and Caspase-3 Expression and Delays Mortality in a Transgenic Mouse Model of Huntington Disease. Nat Med. 2000;6:797-801.
[85] Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, Koistinaho J. A Tetracycline Derivative, Minocycline, Reduces Inflammation and Protects against Focal Cerebral Ischemia with a Wide Therapeutic Window. Proc Natl Acad Sci U S A. 1999;96:13496-500.
[86] Yang F, Zhou L, Wang D, Wang Z, Huang QY. Minocycline Ameliorates Hypoxia-Induced Blood–Brain Barrier Damage by Inhibition of Hif-1α through Sirt-3/Phd-2 Degradation Pathway. Neuroscience. 2015;304:250-9.
[87] Kim DK, Rordorf G, Nemenoff RA, Koroshetz WJ, Bonventre JV. Glutamate Stably Enhances the Activity of Two Cytosolic Forms of Phospholipase A2 in Brain Cortical Cultures. Biochem J. 1995;310 (Pt 1):83-90.
[88] Katsuki H, Okuda S. Arachidonic Acid as a Neurotoxic and Neurotrophic Substance. Prog Neurobiol. 1995;46:607-36.
[89] Seidl SE, Potashkin JA. The Promise of Neuroprotective Agents in Parkinson's Disease. Front Neurol. 2011;2:68.
[90] Dunnett SB, Bjorklund A. Prospects for New Restorative and Neuroprotective Treatments in Parkinson's Disease. Nature. 1999;399:A32-9.
[91] Chan PH. Reactive Oxygen Radicals in Signaling and Damage in the Ischemic Brain. J Cereb Blood Flow Metab. 2001;21:2-14.
[92] Liu T, Bitan G. Modulating Self-Assembly of Amyloidogenic Proteins as a Therapeutic Approach for Neurodegenerative Diseases: Strategies and Mechanisms. ChemMedChem. 2012;7:359-74.
[93] Pradeep H, Diya JB, Shashikumar S, Rajanikant GK. Oxidative Stress--Assassin Behind the Ischemic Stroke. Folia Neuropathol. 2012;50:219-30.
[94] Secades JJ, Alvarez-Sabin J, Rubio F, Lozano R, Davalos A, Castillo J, et al. Citicoline in Intracerebral Haemorrhage: A Double-Blind, Randomized, Placebo-Controlled, Multi-Centre Pilot Study. Cerebrovasc Dis. 2006;21:380-5.
[95] Cho HJ, Kim YJ. Efficacy and Safety of Oral Citicoline in Acute Ischemic Stroke: Drug Surveillance Study in 4,191 Cases. Methods Find Exp Clin Pharmacol. 2009;31:171-6.
[96] Alvarez-Sabin J, Ortega G, Jacas C, Santamarina E, Maisterra O, Ribo M, et al. Long-Term Treatment with Citicoline May Improve Poststroke Vascular Cognitive Impairment. Cerebrovasc Dis. 2013;35:146-54.
[97] IL GC, Wurtman RJ. Enhancement by Cytidine of Membrane Phospholipid Synthesis. J Neurochem. 1992;59:338-43.
[98] Schabitz WR, Weber J, Takano K, Sandage BW, Locke KW, Fisher M. The Effects of Prolonged Treatment with Citicoline in Temporary Experimental Focal Ischemia. J Neurol Sci. 1996;138:21-5.
[99] Hurtado O, Pradillo JM, Fernandez-Lopez D, Morales JR, Sobrino T, Castillo J, et al. Delayed Post-Ischemic Administration of Cdp-Choline Increases Eaat2 Association to Lipid Rafts and Affords Neuroprotection in Exp Stroke. Neurobiol Dis. 2008;29:123-31.
[100] Adibhatla RM, Hatcher JF, Dempsey RJ. Citicoline: Neuroprotective Mechanisms in Cerebral Ischemia. J Neurochem. 2002;80:12-23.
[101] Rao AM, Hatcher JF, Dempsey RJ. Cdp-Choline: Neuroprotection in Transient Forebrain Ischemia of Gerbils. J Neurosci Res. 1999;58:697-705.
[102] De La Cruz JP, Villalobos MA, Cuerda MA, Guerrero A, Gonzalez-Correa JA, Sanchez De La Cuesta F. Effects of S-Adenosyl-L-Methionine on Lipid Peroxidation and Glutathione Levels in Rat Brain Slices Exposed to Reoxygenation after Oxygen-Glucose Deprivation. Neurosci Lett. 2002;318:103-7.
[103] Fremont L. Biological Effects of Resveratrol. Life Sci. 2000;66:663-73.
[104] Jasinski M, Jasinska L, Ogrodowczyk M. Resveratrol in Prostate Diseases - a Short Review. Cent European J Urol. 2013;66:144-9.
[105] Girbovan C, Morin L, Plamondon H. Repeated Resveratrol Administration Confers Lasting Protection against Neuronal Damage but Induces Dose-Related Alterations of Behavioral Impairments after Global Ischemia. Behav Pharmacol. 2012;23:1-13.
[106] Morris-Blanco KC, Cohan CH, Neumann JT, Sick TJ, Perez-Pinzon MA. Protein Kinase C Epsilon Regulates Mitochondrial Pools of Nampt and Nad Following Resveratrol and Ischemic Preconditioning in the Rat Cortex. J Cereb Blood Flow Metab. 2014;34:1024-32.
[107] Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, et al. Resveratrol Ameliorates Aging-Related Metabolic Phenotypes by Inhibiting Camp Phosphodiesterases. Cell. 2012;148:421-33.
[108] Yang Y, Duan W, Li Y, Yan J, Yi W, Liang Z, et al. New Role of Silent Information Regulator 1 in Cerebral Ischemia. Neurobiol Aging. 2013;34:2879-88.
[109] Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, et al. Sirtuin Activators Mimic Caloric Restriction and Delay Ageing in Metazoans. Nature. 2004;430:686-9.
[110] Dasgupta B, Milbrandt J. Resveratrol Stimulates Amp Kinase Activity in Neurons. Proc Natl Acad Sci U S A. 2007;104:7217-22.
[111] Ungvari Z, Bagi Z, Feher A, Recchia FA, Sonntag WE, Pearson K, et al. Resveratrol Confers Endothelial Protection Via Activation of the Antioxidant Transcription Factor Nrf2. Am J Physiol Heart Circ Physiol. 2010;299:H18-24.
[112] Peng Y, Zeng X, Feng Y, Wang X. Antiplatelet and Antithrombotic Activity of L-3-N-Butylphthalide in Rats. J Cardiovasc Pharmacol. 2004;43:876-81.
[113] Yang WH, Li L, Huang RX, Pei Z, Liao SJ, Zeng JS. Hypoxia Inducible Factor-1alpha Mediates Protection of Dl-3-N-Butylphthalide in Brain Microvascular Endothelial Cells against Oxygen Glucose Deprivation-Induced Injury. Neural Regeneration Research. 2012;7:948-54.
[114] He WF, Zhou WS, Hu ZP. Chinese Herbal Extract Dl-3n-Butylphthalide a Commonly Used Drug for the Treatment of Ischemic Stroke as a Novel Therapeutic Approach to Treat Neurodegenerative Diseases. Neural Regeneration Research. 2011;6:2773-8.
[115] Zhao W, Luo C, Wang J, Gong J, Li B, Gong Y, et al. 3-N-Butylphthalide Improves Neuronal Morphology after Chronic Cerebral Ischemia. Neural Regen Res. 2014;9:719-26.
[116] Sun B, Feng MJ, Tian XY, Lu XW, Zhang YY, Ke XJ, et al. Dl-3-N-Butylphthalide Protects Rat Bone Marrow Stem Cells against Hydrogen Peroxide-Induced Cell Death through Antioxidation and Activation of Pi3k-Akt Pathway. Neuroscience Letters. 2012;516:247-52.
[117] Chao J, Chao L. Experimental Therapy with Tissue Kallikrein against Cerebral Ischemia. Front Biosci. 2006;11:1323-7.
[118] Zhang C, Tao W, Liu M, Wang D. Efficacy and Safety of Human Urinary Kallidinogenase Injection for Acute Ischemic Stroke: A Systematic Review. J Evid Based Med. 2012;5:31-9.
[119] Li C, Zha OG, He QY, Wu YZ, Wang TS, Teng JF. Study on the Clinical Efficacy of Human Urinary Kalllikrein in the Treatment of Acute Cerebral Infarction According to Toast Classification. Pak J Pharm Sci. 2015;28:1505-10.
[120] Miao J, Deng F, Zhang Y, Xie HY, Feng JC. Exogenous Human Urinary Kallidinogenase Increases Cerebral Blood Flow in Patients with Acute Ischemic Stroke. Neurosci (Riyadh). 2016;21:126-30.
[121] Li J, Chen Y, Zhang X, Zhang B, Zhang M, Xu Y. Human Urinary Kallidinogenase Improves Outcome of Stroke Patients by Shortening Mean Transit Time of Perfusion Magnetic Resonance Imaging. J Stroke Cerebrovasc Dis. 2015;24:1730-7.
[122] Han L, Li J, Chen Y, Zhang M, Qian L, Chen Y, et al. Human Urinary Kallidinogenase Promotes Angiogenesis and Cerebral Perfusion in Experimental Stroke. PLoS One. 2015;10:e0134543.
[123] Zhao L, Zhao Y, Wan Q, Zhang H. Urinary Kallidinogenase for the Treatment of Cerebral Arterial Stenosis. Drug Des Devel Ther. 2015;9:5595-600.
[124] Liu L, Zhang R, Liu K, Zhou H, Tang Y, Su J, et al. Tissue Kallikrein Alleviates Glutamate-Induced Neurotoxicity by Activating Erk1. Journal of Neuroscience Research. 2009;87:3576-90.