Review Article

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Neuroprotective approach in acute ischemic stroke: A systematic review of clinical and experimental studies

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Abstract:

Ischemic stroke is a disease with worldwide economic and social negative effects. It is a serious disease with high disability and mortality. Ionic imbalance, excitotoxicity, oxidative stress, and inflammation are induced during and after ischemic stroke. Cellular dysfunction, apoptosis, and necrosis are activated directly or indirectly mechanisms. The studies about neuroprotection in neurodegenerative diseases have increased in recent years. Data about the mechanisms of progressive molecular improvement in the brain tissue are increasing in acute ischemic stroke. Based on these data, preclinical and clinical studies on new neuroprotective treatments are being designed. An effective neuroprotective strategy can prolong the indication period of recanalization treatments in the acute stage of ischemic stroke. In addition, it can reduce neuronal necrosis and protect the brain against ischemia-related reperfusion injury. The current review has evaluated the recent clinical and experimental studies. The molecular mechanism of each of the neuroprotective strategies is also summarized. This review may help develop future strategies for combination treatment to protect the cerebral tissue from ischemia-reperfusion injury.

Keywords:

Clinical trials, ischemic stroke, neuroprotection, neuroprotective agents, preclinical studies

Introduction

Stroke is one of the major causes of mortality in the world. In addition, the risk of severe disability is higher in a patient with stroke. The rate of acute ischemic stroke (AIS) is 80% in patients with total stroke. The main cause of AIS is the occlusion of the vascular structure by thrombus. Neuronal hypoxia and cellular necrosis occur after this pathogenetic mechanism.[1] Intravenous tissue plasminogen activator is a main medical treatment option in patients with AIS. It has been approved by the Food and Drug Administration (FDA). The duration of this treatment is up to 4.5 h (limited time) after symptom. As a result, only 2%–5% of patients can be treated with intravenous thrombolytic treatment. In addition, modern endovascular treatment methods (mechanical thrombectomy, intra-arterial thrombolytic applications, etc.,) continue to develop in AIS.[2,3]

Neuroprotection has become important in recent years. Protection of neuronal tissue and prevention of degeneration are defined as "neuroprotection." It is a term used for cerebral neuronal protection for many neurological diseases such as stroke, Parkinson's disease, dementia, and traumatic/nontraumatic brain injury.[4] Positive results have been detected with many neuroprotective agents in experimental studies. However, the efficacy of neuroprotective treatments has not been confirmed by clinical studies. Many neuroprotective clinical studies have been completed and many are in progress.[5]

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Hypoxia and cerebral hypoglycemia induce a series of molecular activities in the neuronal cell membrane after cerebral vascular occlusion. There is an ion imbalance in the energy-dependent membrane channels in the neuronal cell membrane. A hypoperfused neuronal tissue named the penumbra is formed. If the vascular occlusion is not treated, progressive neuronal necrosis occurs. Glutamate overexpression occurs after cellular death. Increased glutamate triggers many chemical activities. Glutamate induces intracellular calcium (Ca) reuptake associated with α-amino-3-hydroxy-5-methyl -4-isoxazolepropionic acid receptor, voltage-dependent Ca receptor, and N-methyl-D-aspartate (NMDA) receptor. Intracellular Ca activates many signaling pathways and induces intracellular reactive oxygen species. After vascular occlusion, neuronal injury increases associated with oxidative stress, protein oxidation, and lipid peroxidation. In addition, the damage-associated molecular model demonstrated that oxidative stress-related micromolecules caused microglia activation, blood–brain barrier permeability impairment, and peripheral immune reactivation. Postischemic inflammation is the cause of secondary progression of brain injury. The severity of ischemic stroke correlates with inflammation.[6] The primary aim of neuroprotection is to prevent ischemia-reperfusion injury in the cerebral tissue. In addition, the indication period for thrombolytic treatment can be increased with neuroprotective agents.[7]

The purpose of this review is to make a general assessment of the impact of neuroprotective treatments on the neuronal tissue. The molecular mechanism of all of the cerebral neuroprotective treatment options is also described.

Neuroprotective Treatments: Clinical and Experimental Studies

Clinical and experimental studies with neuroprotective agents are popular in patients with stroke. At the first stage, experimental studies are designed. If this agent is effective in experimental studies, clinical studies are designed. Neuroprotective agents and their mechanisms in clinical and experimental studies are listed in Table 1.

Table 1: Neuroprotective agents and their mechanisms in clinical and experimental studies

Neuroprotective agents	Mechanisms for therapeutic activity
Human urinary kallidinogenase	Antioxidant activity
	Anti-inflammatory activity and immunomodulation
Anti-hyperlipidemic treatments	Antioxidant activity
	Anti-inflammatory activity and immunomodulation
	Decreased blood brain barrier dysfunction
	Increased cerebral blood flow
Edaravone	Antioxidant activity
Nerinetide	Decreased neurotoxic signaling cascades
3K3A-APC	Neuroprotection, vasculoprotection and immunomodulation
Natalizumab	Anti-inflammatory activity and immunomodulation
Vinpocetine	Antioxidant activity
	Anti-inflammatory activity and immunomodulation
	Anti-apoptotic activity
Fasudil	Decreased peripheral immune cell infiltration and oxidative stress
	Rho-kinase inhibition
Fingolimod	Sphingosine-1-phosphate receptor modulation
	Anti-inflammatory activity and immunomodulation
RIC	Decreased ischemia reperfusion injury, but the exact mechanism is unknown
Tocilizumab	Human monoclonal antibody against the IL-6 receptor
Uric acid	Decreased lipid peroxidation
	Antioxidant activity
	Increased Nrf2 protein
Magnesium	Anti-inflammatory activity
	Anti-apoptotic activity
	Increased cerebral blood flow
Therapeutic hypothermia	Antioxidant activity
	Anti-inflammatory activity and immunomodulation
	Anti-apoptotic activity
	Decreased neurotoxic signaling cascades

RIC: Remote ischemic conditioning, Nrf2: Nuclear factor E2 associated factor 2, APC: Activated protein C

Human Urinary Kallidinogenase

Human urinary kallidinogenase is a tissue kallikrein and it is extracted from urine. In addition, it is a glycoprotein that breaks down kininogen to produce kinin (kinin is a potent vasodilator).[8] Experimental animal studies about ischemic stroke and neuroprotection have demonstrated that human urinary kallidinogenase reduces infarct volume and improves neurological deficits.[9] The neuroprotective mechanism of human urinary kallidinogenase is associated with increased local vasodilation. Human urinary kallidinogenase induces the expression of toll-like receptor 4 (TLR-4). Thus, it provides neuroprotection with antioxidant and anti-inflammatory effects by using the (TLR4/nuclear factor-kappa B [NF-κb]) signaling pathway. Human urinary kallidinogenase increases the expression of transforming growth factor-beta 1. Neuroinflammation is suppressed and associated with these mechanisms.[10,11] Data from a randomized placebo-controlled and phase IIb/III clinical trial demonstrated that human urinary kallidinogenase is an effective and safe treatment within 48 h in patients with AIS.[12] Human urinary

kallidinogenase is approved by the State FDA of China in patients with mild and moderate stroke.[13]

Anti-Hyperlipidemic Treatments

Antihyperlipidemic treatments are a group of drugs used to decrease cholesterol levels. Statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The blood lipid level decreases as a result of the inhibition of HMG-CoA reductase.[14] Studies in experimental stroke models have demonstrated that statins reduce cerebral infarct volume in stroke, with or without thrombolytic treatment.[15] The efficacy of statins in ischemic brain injury is not associated with antihyperlipidemic mechanisms. Statins increase cerebral blood flow and decrease neuronal injury by up-regulating endothelial nitric oxide synthase (eNOS) in ischemic penumbra. Statins also protect against poststroke blood–brain barrier dysfunction by reducing the expression of adhesion molecules.[16,17] In addition, statins suppress NFκB-mediated gene expression and superoxide radicals; thus, it provides neuroprotection in ischemic stroke.[6]

Edaravone

Edaravone is a low-molecular-weight treatment and it crosses to blood–brain barrier.[18] The therapeutic effect of edaravone is associated with antioxidant activity and regulation of multiple signaling pathways. Studies have demonstrated that edaravone reduces delayed neuron damage, microglia-induced neurotoxicity, and long-term inflammation.[19,20] In addition, edaravone inhibits lipid oxidation and directly suppresses low-density lipoprotein oxidation.[21] Besides direct neuroprotection, it inhibits the expression of vascular endothelial growth factor and astrocyte activity. Thus, it reduces poststroke edema. Edaravone reduces the increased aquaporin-4 level after ischemic stroke. It has antiedema effect with this mechanism. It has been demonstrated that edaravone before thrombolytic treatment reduces the activity of matrix metalloproteinase-9 and prevents vascular injury and intracerebral hemorrhage.[21-24] However, reversible renal toxicity associated with edaravone has been reported in several patients.[25] Edaravone has been approved in Japan for the treatment of AIS. However, it is not accepted as the standard treatment for stroke in the general medicine.[26]

Nerinetide

Nerinetide is an intracellular peptide associated with the NMDA receptor. The NMDA receptors have an activity on nitric oxide synthase in the neuronal tissue. Nerinetide reduces intracellular neuronal Ca and nitric oxide. It has neuroprotective effects with these mechanisms.[27] Decreased infarct volume, improved functional outcomes, and lower mortality were determined in patients treated with mechanical thrombectomy and nerinetide combination. However, long-term functional outcome was similar in the placebo group and in-group treated with combination.[28]

3K3A-Activated Protein C

3K3A-Activated protein C (APC) is induced by APC protease, and it has anticoagulant and cellular signaling activities mediated by protease-activated receptor 1. 3K3A-APC activates the transmembrane G-protein, brain endothelial cells and the blood–brain barrier to protect neuronal tissue. It reduces reperfusion injury associated with multiple neuroprotective effects.[29-31] The preclinical drug safety and efficacy of 3K3A-APC were evaluated in accordance with the recommendations of the Academic Industry Round Table for the Treatment of stroke therapy academic industry roundtable.[32] It was demonstrated that this treatment has a safe pharmacokinetic profile. In experimental ischemic stroke models, 3K3A-APC treatment has been demonstrated to reduce cerebral ischemia volume.[30,33]

Natalizumab

Natalizumab is a kind of humanized antibody against the glycoprotein alpha-4 integrin. This mechanism prevents the adhesion of immune cells to the endothelium.[34] It has been reported that 300 mg of natalizumab is safe and effective in AIS. High-dose natalizumab trial was not effective and the trial of natalizumab treatment in ischemic stroke was discontinued.[35]

Vinpocetine

Vinpocetine is a synthetic ethyl-ester derivative. It is a selective phosphodiesterase type 1 (PDE1) inhibitor. Vinpocetine is a selective PDE1 inhibitor with potential neurological effects through inhibition of the voltage-gated sodium channel and reduction of neuronal Ca flux. Vinpocetine has inhibitory effects on glial and astrocyte cells during and after ischemic stroke. In this way, it has anti-inflammatory and anti-apoptotic effects.[36] Hypoxemia after ischemic stroke causes deterioration in energy metabolism. Ca increases in the extracellular area associated with neuronal necrosis. Oxidative stress and inflammation are induced. Cellular vascularity and blood–brain barrier are disrupted.[37] During ischemic stroke, activated microglia, and macrophages induce different neurotransmitters and neuroinflammation. In particular, interleukin (IL)-8, NF-κB, and tumor necrosis factor-alpha (TNF-α) are overexpressed during ischemic stroke. These inflammatory molecules play a potential role in inflammation and apoptosis.[38] The

NF-κB pathway is important in the pathogenesis of neurological disability. Endothelial cells in cerebral vascular structures are activated and associated with NF-κB pathway. Vascular obstruction and thrombosis are induced with this mechanism. Inhibition of the NF-κB pathway with vinpocetine is considered the main mechanism of neuroprotection.[39] Voltage-gated sodium channels are activated after ischemic stroke. Intracellular sodium and Ca increase. After this pathophysiological process, neuronal cellular injury, excitotoxicity, edema, acidosis, and acute neuronal dysfunction occur. Vinpocetine inhibits voltage-gated sodium channels. This leads to decreased intracellular sodium and Ca concentrations. The neuroprotective effect of vinpocetine is associated with neuronal voltage-gated Na-channel inhibition.[40] Vinpocetine induces neuronal cyclic guanosine monophosphate (cGMP) associated with inhibition of calmodulin-dependent PDE. This process improves cerebral blood flow and oxygenation.[41] Vinpocetine induces the production of adenosine triphosphate and regulates cerebral metabolism. These effects prevent ischemic stroke-induced cognitive dysfunctions. Improvement of cognitive function is mediated by neurotransmitters such as serotonin, dopamine, and noradrenaline.[36] It has been demonstrated that vinpocetine treatment decreases cerebral infarct volume after middle cerebral artery occlusion in experimental rat and mouse models.[42] However, clinical studies are required to confirm these data.

Other Neuroprotective Treatment Methods

The National Institute of Neurological Disorders and Stroke in the United States supports a Stroke Preclinical Evaluation Network for translational studies for acute neuroprotection in stroke. The main aim of the institute is to support the development of new neuroprotective agents before or after endovascular thrombectomy. The main purpose of neuroprotection is to extend the indication period for neurovascular treatments and to improve functional outcome.[6] Some neuroprotective agents continue to be investigated for this purpose.

Fasudil is a Rho-kinase inhibitor that reduces peripheral infiltration of immune cells and oxidative stress.[43] Positive results of fasudil and hydroxyfasudil in the treatment of AIS have been demonstrated in experimental studies.[44,45] The mechanisms of this treatment are to prevent immune cell infiltration. As a result of all these mechanisms, an anti-inflammatory mechanisms are induced.[43-45] A study demonstrated that treatment with fasudil within 48 h after stroke improved the outcome of AIS. In addition, fasudil is an effective and safety treatment in patients with AIS. The optimum therapeutic interval for fasudil is 48 h after stroke.[46]

In the early poststroke period, local and systemic inflammation is triggered after the deterioration of the blood-brain barrier. This mechanism plays an important role in ischemic necrosis, hemorrhagic transformation, and vasogenic edema. In addition, reperfusion injury is induced after recanalization associated with inflammation.[47] Fingolimod is a sphingosine analog associated with sphingosine-1-phosphate receptors. It was approved for the relapsing-remitting form of multiple sclerosis relapsing-remitting MS as the first oral multiple sclerosis treatment.[48] Fingolimod prevents the outflow of lymphocytes from lymph nodes.[49] The efficacy of fingolimod was investigated in patients with cerebral hemorrhage and AIS.[50,51] Long-term clinical improvement has been demonstrated after fingolimod in patients with cerebral hemorrhage. In addition, decreased perihematomal edema has been demonstrated in these groups.[50] In patients with acute cerebral artery occlusion, clinical improvement was detected with fingolimod. Decreased ischemic area and decreased microvascular permeability were reported with this treatment.[51] Fingolimod and alteplase combination treatment was compared with alteplase in AIS. The combination treatment was detected to be more effective in terms of lesion volume, hemorrhagic transformation, and clinical improvement.[52]

Remote ischemic conditioning (RIC) is reperfusion after a short cycle of focal ischemia. A preventive treatment strategy protects against more severe ischemia in distant organs.[53] RIC is usually performed with an inflated blood pressure cuffs that inhibit blood perfusion in the limbs.[54,55] The neuroprotective mechanisms of RIC are unclear. These mechanisms are explained by increased cerebral tolerance against ischemic injury.[56] Compared with traditional treatments, RIC is noninvasive, easy, and cost-effective. These results provide positive data for clinical research and practice.[57-59]

Tocilizumab is a humanized monoclonal antibody against the IL-6 receptor (IL-6R). It is an immunosuppressive drug approved by the FDA for the treatment of some autoimmune diseases such as rheumatoid arthritis.[60] TNF-a and IL-1p levels are increased after elevated IL-6 levels in ischemic stroke. However, antagonism of IL-6 has conflicting results in experimental studies. The treatment with IL-6R monoclonal antibody was associated with increased peri-infarct apoptosis, motor dysfunction, and infarct volume in a mouse model of ischemic stroke.[61] However, recent studies have demonstrated the neuroprotective effects of tocilizumab in experimental cerebral ischemia.[62,63] In a study, rats were treated with tocilizumab for 7 days in the prestroke period. It was demonstrated that tocilizumab was preventive for neuronal apoptosis in rats with cerebral ischemia.[62] Another study demonstrated that

tocilizumab within 4 h of cerebral ischemia protected against brain infarctions and brain atrophy in older male mice.[63]

The human brain has lower endogenous antioxidants compared to other organs. Therefore, the brain is more sensitive to oxidative stress.[64] Uric acid is an endogenous antioxidant derived from the metabolism of purines. Descriptive studies demonstrate that serum uric acid concentrations are associated with functional outcome with or without thrombolytic treatment in patients with acute stroke.[65,66] Serum uric acid concentration decreases after the onset of stroke symptoms.[67,68] A randomized, double-blind phase 2b/3 trial study demonstrated that combination treatment of intravenous thrombolysis and uric acid is safe in patients with stroke, but is not superior to placebo in clinical outcomes at 90-day follow-up. In addition, functional improvement is associated with uric acid treatment.[69]

Magnesium has been demonstrated to have neuroprotective effects in preclinical experimental studies. It also has anti-inflammatory and anti-apoptotic effects.[70] Magnesium induces increased cerebral blood flow in ischemic brain regions. Thus, magnesium regulates cellular metabolism. In an experimental study, magnesium sulfate was detected to be effective before intravenous thrombolytic treatment in ischemic stroke.[71] The clinical effect of magnesium could not be confirmed in patients with AIS. However, the positive effect of magnesium was demonstrated in patients with lacunar strokes.[72]

Therapeutic hypothermia is potentially advantageous over the other neuroprotective agents. Fever (body temperature >38.3°C) is detected with an incidence of 70% within 2 weeks in patients with neurological damage.[73] It activates many mechanisms during the ischemia-reperfusion process.[73,74] Therapeutic hypothermia is a neuroprotective treatment in hypoxic brain injury after cardiac arrest. It provides neurological clinical improvement after cardiac arrest.[75,76] The vagus nerve is one of the components of the autonomic nervous system. This cranial nerve contains a complex neuroendocrine-immune network associated with various cerebral physiological or pathological processes.

Promising Neuroprotective Agents in Ischemic Stroke

Neuroprotection is one of the main investigation area in ischemic stroke. There are many promising studies about this subject. Some of these studies have been completed. Some of them continue. Promising neuroprotective agents in ischemic stroke are listed in Table 2. There are many studies about protein, pharmacological agent, and microRNA for neuroprotection. Adropin is an endogenous peptide that is highly expressed in the brain tissue. Exogenous injection of this peptide has been demonstrated to decrease the permeability of the blood-brain barrier associated with eNOS phosphorylation. However, the effect of endogenous adropin on ischemic brain injury is unknown. It is hypothesized that brain adropin deficiency induces the stroke-related disability with neurovascular dysfunction. In addition, overexpression of this peptide decreases ischemic brain injury.[77] Verapamil is a treatment with L-type Ca channel blocker. In a study, intra-arterial injection of verapamil has been demonstrated to be safe and effective after mechanical thrombectomy. In addition, the efficacy of verapamil has been demonstrated to be associated with vasodilation and direct neuroprotective features.[78] MicroRNAs are important in the epigenetic field. It is mainly associated with message translation. These are the subclass of noncoding RNAs. Sequences encoding microRNAs usually originate from intronic DNA. MicroRNAs modulate stroke pathophysiology, including inflammation, blood–brain barrier dysfunction, excitotoxicity, and oxidative stress. Neuroprotective microRNAs decrease and neurotoxic microRNAs increase during the stroke process.[79,80]

Table 2: Promising neuroprotective agents in ischemic stroke

Neuroprotective agents	Mechanisms for therapeutic activity
Adropin	Decreased lipid peroxidation
	Antioxidant activity
	Increased Nrf2 protein
Verapamil	Calcium channel blockade and vasodilation
MicroRNA	Antioxidant activity
	Anti-inflammatory activity and immunomodulation
	Anti-apoptotic activity
	Decreased neurotoxic signaling cascades
Minocycline	Anti-inflammatory activity
	Anti-apoptotic activity
VNS	Antioxidant activity
	Anti-inflammatory activity

VNS: Vagus-nerve stimulation, Nrf2: Nuclear factor E2 associated factor 2

Minocycline is an antibiotic in the semi-synthetic group of tetracycline. It has neuroprotective effects. The drug is lipophilic and it crosses the blood-brain barrier with this mechanism.[81] Minocycline is increasingly recognized as a neuroprotective drug associated with its high penetration into the blood–brain barrier. In addition, it has efficacy in many neurodegenerative diseases.[82]

Vagus-nerve stimulation (VNS) stimulates the vagus nerve in the cervical area. It is an effective technique for some neurological diseases.[83] The FDA has approved the VNS devices in patients with medical refractory epilepsy and depression.[84,85] Transcutaneous cervical

vagus nerve stimulation was approved by FDA for the treatment cluster and of migraine type of headache in the year of 2017.[86] In experimental cerebral ischemia models, VNS has been reported to decrease cerebral ischemia and disability.[87] However, the data of this treatment for clinical practice are insufficient.

Conclusions

The main treatment principles after ischemic stroke are recanalization with thrombolytic treatment and/or mechanical thrombectomy. However, they are time-dependent treatments. Therefore, the minority of patients with ischemic stroke are treated with these methods. It is evident from the literature that a large number of therapeutic agents have in experimental studies for the evaluation of neuroprotective effects in AIS. Despite these studies, clinically effective neuroprotective agents could not be detected. The ability to protect the ischemic brain from injury until reperfusion and to protect the cerebral tissue from ischemia-reperfusion injury could improve the disability in patients with AIS. The investigations continue in the world to reduce stroke-related mortality and disability.

Ethics

The local Ethics Committee has approved the project, and the work that was undertaken conforms to the provisions of the Declaration of Helsinki.

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Conflicts of interest

There are no conflicts of interest.

References

1. Goenka L, Uppugunduri Satyanarayana CR, Suresh Kumar S, George M. Neuroprotective agents in acute ischemic stroke-a reality check. Biomed Pharmacother 2019;109:2539-47.
2. Reed SD, Cramer SC, Blough DK, Meyer K, Jarvik JG. Treatment with tissue plasminogen activator and inpatient mortality rates for patients with ischemic stroke treated in community hospitals. Stroke 2001;32:1832-40.
3. Saver JL, Jahan R, Levy EI, Jovin TG, Baxter B, Nogueira RG, *et al.* Solitaire flow restoration device versus the merci retriever in patients with acute ischaemic stroke (SWIFT): A randomised, parallel-group, non-inferiority trial. Lancet 2012;380:1241-9.
4. Chamorro Á, Lo EH, Renú A, van Leyen K, Lyden PD. The future of neuroprotection in stroke. J Neurol Neurosurg Psychiatry 2021;92:129-35.
5. Green AR. Pharmacological approaches to acute ischaemic stroke: Reperfusion certainly, neuroprotection possibly. Br J Pharmacol 2008;153 Suppl 1:S325-38.
6. Paul S, Candelario-Jalil E. Emerging neuroprotective strategies for the treatment of ischemic stroke: An overview of clinical and preclinical studies. Exp Neurol 2021;335:113518.
7. Chamorro Á, Dirnagl U, Urra X, Planas AM. Neuroprotection in acute stroke: Targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. Lancet Neurol 2016;15:869-81.
8. Chao J, Chao L. Kallikrein kinin in stroke, cardiovascular and renal disease. Exp Physiol 2005;90:291-8.
9. Chao J, Chao L. Experimental therapy with tissue kallikrein against cerebral ischemia. Front Biosci 2006;11:1323-7.
10. Cekanaviciute E, Fathali N, Doyle KP, Williams AM, Han J, Buckwalter MS. Astrocytic transforming growth factor-beta signaling reduces subacute neuroinflammation after stroke in mice. Glia 2014;62:1227-40.
11. Yang J, Su J, Wan F, Yang N, Jiang H, Fang M, *et al.* Tissue kallikrein protects against ischemic stroke by suppressing TLR4/NF-κB and activating Nrf2 signaling pathway in rats. Exp Ther Med 2017;14:1163-70.
12. Dong Y, Qu J, Zhang Z, Wang C, Dong Q. Human urinary kallidinogenase in treating acute ischemic stroke patients: Analyses of pooled data from a randomized double-blind placebo-controlled phase II b and phase III clinical trial. Neurol Res 2020;42:286-90.
13. Wu D, Lyu Y, Zhong P, Liu F, Liu X. Human urinary kallidinogenase promotes good recovery in ischemic stroke patients with level 3 hypertension. Brain Behav 2017;7:e00752.
14. Vaughan CJ, Gotto AM Jr., Basson CT. The evolving role of statins in the management of atherosclerosis. J Am Coll Cardiol 2000;35:1-10.
15. Sironi L, Cimino M, Guerrini U, Calvio AM, Lodetti B, Asdente M, *et al.* Treatment with statins after induction of focal ischemia in rats reduces the extent of brain damage. Arterioscler Thromb Vasc Biol 2003;23:322-7.
16. Mayanagi K, Katakam PV, Gáspár T, Domoki F, Busija DW. Acute treatment with rosuvastatin protects insulin resistant (C57BL/6J ob/ob) mice against transient cerebral ischemia. J Cereb Blood Flow Metab 2008;28:1927-35.
17. Ma M, Uekawa K, Hasegawa Y, Nakagawa T, Katayama T, Sueta D, *et al.* Pretreatment with rosuvastatin protects against focal cerebral ischemia/reperfusion injury in rats through attenuation of oxidative stress and inflammation. Brain Res 2013;1519:87-94.
18. Yoshida H, Yanai H, Namiki Y, Fukatsu-Sasaki K, Furutani N, Tada N. Neuroprotective effects of edaravone: A novel free radical scavenger in cerebrovascular injury. CNS Drug Rev 2006;12:9-20.
19. Lee BJ, Egi Y, van Leyen K, Lo EH, Arai K. Edaravone, a free radical scavenger, protects components of the neurovascular unit against oxidative stress *in vitro*. Brain Res 2010;1307:22-7.
20. Zhang N, Komine-Kobayashi M, Tanaka R, Liu M, Mizuno Y, Urabe T. Edaravone reduces early accumulation of oxidative products and sequential inflammatory responses after transient focal ischemia in mice brain. Stroke 2005;36:2220-5.
21. Yoshida H, Sasaki K, Namiki Y, Sato N, Tada N. Edaravone, a novel radical scavenger, inhibits oxidative modification of Low-Density Lipoprotein (LDL) and reverses oxidized LDL-mediated reduction in the expression of endothelial nitric oxide synthase. Atherosclerosis 2005;179:97-102.
22. Kikuchi K, Tancharoen S, Matsuda F, Biswas KK, Ito T, Morimoto Y, *et al.* Edaravone attenuates cerebral ischemic injury by suppressing aquaporin-4. Biochem Biophys Res Commun 2009;390:1121-5.
23. Nakamura T, Kuroda Y, Yamashita S, Zhang X, Miyamoto O, Tamiya T, *et al.* Edaravone attenuates brain edema and neurologic deficits in a rat model of acute intracerebral hemorrhage. Stroke 2008;39:463-9.
24. Yagi K, Kitazato KT, Uno M, Tada Y, Kinouchi T, Shimada K, *et al.* Edaravone, a free radical scavenger, inhibits MMP-9-related brain hemorrhage in rats treated with tissue plasminogen activator. Stroke 2009;40:626-31.
25. Hishida A. Clinical analysis of 207 patients who developed renal disorders during or after treatment with edaravone reported during post-marketing surveillance. Clin Exp Nephrol 2007;11:292-6.

26. Kikuchi K, Setoyama K, Kawahara KI, Nagasato T, Terashi T, Ueda K, *et al.* Edaravone, a synthetic free radical scavenger, enhances alteplase-mediated thrombolysis. Oxid Med Cell Longev 2017;2017:1-30.
27. Ballarin B, Tymianski M. Discovery and development of NA-1 for the treatment of acute ischemic stroke. Acta Pharmacol Sin 2018;39:661-8.
28. Hill MD, Goyal M, Menon BK, Nogueira RG, McTaggart RA, Demchuk AM, *et al.* Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke (ESCAPE-NA1): A multicentre, double-blind, randomised controlled trial. Lancet 2020;395:878-87.
29. Griffin JH, Zlokovic BV, Mosnier LO. Activated protein C: Biased for translation. Blood 2015;125:2898-907.
30. Wang Y, Zhao Z, Chow N, Rajput PS, Griffin JH, Lyden PD, *et al.* Activated protein C analog protects from ischemic stroke and extends the therapeutic window of tissue-type plasminogen activator in aged female mice and hypertensive rats. Stroke 2013;44:3529-36.
31. Wang Y, Zhang Z, Chow N, Davis TP, Griffin JH, Chopp M, *et al.* An activated protein C analog with reduced anticoagulant activity extends the therapeutic window of tissue plasminogen activator for ischemic stroke in rodents. Stroke 2012;43:2444-9.
32. Zlokovic BV, Griffin JH. Cytoprotective protein C pathways and implications for stroke and neurological disorders. Trends Neurosci 2011;34:198-209.
33. Lyden P, Levy H, Weymer S, Pryor K, Kramer W, Griffin JH, *et al.* Phase 1 safety, tolerability and pharmacokinetics of 3K3A-APC in healthy adult volunteers. Curr Pharm Des 2013;19:7479-85.
34. Rice GP, Hartung HP, Calabresi PA. Anti-alpha4 integrin therapy for multiple sclerosis: Mechanisms and rationale. Neurology 2005;64:1336-42.
35. Elkins J, Veltkamp R, Montaner J, Johnston SC, Singhal AB, Becker K, *et al.* Safety and efficacy of natalizumab in patients with acute ischaemic stroke (ACTION): A randomised, placebo-controlled, double-blind phase 2 trial. Lancet Neurol 2017;16:217-26.
36. Al-Kuraishy HM, Al-Gareeb AI, Naji MT, Al-Mamorry F. Role of vinpocetine in ischemic stroke and poststroke outcomes: A critical review. Brain Circ 2020;6:1-10.
37. Yang C, Hawkins KE, Doré S, Candelario-Jalil E. Neuroinflammatory mechanisms of blood-brain barrier damage in ischemic stroke. Am J Physiol Cell Physiol 2019;316:C135-53.
38. Zarruk JG, Greenhalgh AD, David S. Microglia and macrophages differ in their inflammatory profile after permanent brain ischemia. Exp Neurol 2018;301:120-32.
39. Wu LR, Liu L, Xiong XY, Zhang Q, Wang FX, Gong CX, *et al.* Vinpocetine alleviate cerebral ischemia/reperfusion injury by down-regulating TLR4/MyD88/NF-κB signaling. Oncotarget 2017;8:80315-24.
40. Svab G, Doczi J, Gerencser AA, Ambrus A, Gallyas F, Sümegi B, *et al.* The mitochondrial targets of neuroprotective drug vinpocetine on primary neuron cultures, brain capillary endothelial cells, synaptosomes, and brain mitochondria. Neurochem Res 2019;44:2435-47.
41. Zhang W, Huang Y, Li Y, Tan L, Nao J, Hu H, *et al.* Efficacy and safety of vinpocetine as part of treatment for acute cerebral infarction: A randomized, open-label, controlled, multicenter CAVIN (Chinese Assessment for Vinpocetine in Neurology) Trial. Clin Drug Investig 2016;36:697-704.
42. Bereczki D, Fekete I. Vinpocetine for acute ischaemic stroke. Cochrane Database Syst Rev 2000;2:CD000480.
43. Fukuta T, Asai T, Sato A, Namba M, Yanagida Y, Kikuchi T, *et al.* Neuroprotection against cerebral ischemia/reperfusion injury by intravenous administration of liposomal fasudil. Int J Pharm 2016;506:129-37.
44. Satoh S, Utsunomiya T, Tsurui K, Kobayashi T, Ikegaki I, Sasaki Y, *et al.* Pharmacological profile of hydroxy fasudil as a selective rho kinase inhibitor on ischemic brain damage. Life Sci 2001;69:1441-53.
45. Toshima Y, Satoh S, Ikegaki I, Asano T. A new model of cerebral microthrombosis in rats and the neuroprotective effect of a Rho-Kinase inhibitor. Stroke 2000;31:2245-50.
46. Shibuya M, Hirai S, Seto M, Satoh S, Ohtomo E, Fasudil Ischemic Stroke Study Group. Effects of fasudil in acute ischemic stroke: Results of a prospective placebo-controlled double-blind trial. J Neurol Sci 2005;238:31-9.
47. Simard JM, Kent TA, Chen M, Tarasov KV, Gerzanich V. Brain oedema in focal ischaemia: Molecular pathophysiology and theoretical implications. Lancet Neurol 2007;6:258-68.
48. Cohen JA, Chun J. Mechanisms of fingolimod's efficacy and adverse effects in multiple sclerosis. Ann Neurol 2011;69:759-77.
49. Massberg S, von Andrian UH. Fingolimod and sphingosine-1-phosphate-modifiers of lymphocyte migration. N Engl J Med 2006;355:1088-91.
50. Fu Y, Hao J, Zhang N, Ren L, Sun N, Li YJ, *et al.* Fingolimod for the treatment of intracerebral hemorrhage: A 2-arm proof-of-concept study. JAMA Neurol 2014;71:1092-101.
51. Fu Y, Zhang N, Ren L, Yan Y, Sun N, Li YJ, *et al.* Impact of an immune modulator fingolimod on acute ischemic stroke. Proc Natl Acad Sci USA 2014;111:18315-20.
52. Zhu Z, Fu Y, Tian D, Sun N, Han W, Chang G, *et al.* Combination of the immune modulator fingolimod with alteplase in acute ischemic stroke: A pilot trial. Circulation 2015;132:1104-12.
53. Przyklenk K, Bauer B, Ovize M, Kloner RA, Whittaker P. Regional ischemic 'preconditioning' protects remote virgin myocardium from subsequent sustained coronary occlusion. Circulation 1993;87:893-9.
54. Hess DC, Blauenfeldt RA, Andersen G, Hougaard KD, Hoda MN, Ding Y, *et al.* Remote ischaemic conditioning-a new paradigm of self-protection in the brain. Nat Rev Neurol 2015;11:698-710.
55. Hausenloy DJ, Barrabes JA, Bøtker HE, Davidson SM, Di Lisa F, Downey J, *et al.* Ischaemic conditioning and targeting reperfusion injury: A 30 year voyage of discovery. Basic Res Cardiol 2016;111:70.
56. Meng R, Asmaro K, Meng L, Liu Y, Ma C, Xi C, *et al.* Upper limb ischemic preconditioning prevents recurrent stroke in intracranial arterial stenosis. Neurology 2012;79:1853-61.
57. Meng R, Ding Y, Asmaro K, Brogan D, Meng L, Sui M, *et al.* Ischemic conditioning is safe and effective for Octo- and nonagenarians in stroke prevention and treatment. Neurotherapeutics 2015;12:667-77.
58. Zhao W, Zhang J, Sadowsky MG, Meng R, Ding Y, Ji X. Remote ischaemic conditioning for preventing and treating ischaemic stroke. Cochrane Database Syst Rev 2018;7:CD012503.
59. Zhao W, Li S, Ren C, Meng R, Jin K, Ji X. Remote ischemic conditioning for stroke: Clinical data, challenges, and future directions. Ann Clin Transl Neurol 2019;6:186-96.
60. Rubbert-Roth A, Furst DE, Nebesky JM, Jin A, Berber E. A review of recent advances using Tocilizumab in the treatment of rheumatic diseases. Rheumatol Ther 2018;5:21-42.
61. Yamashita T, Sawamoto K, Suzuki S, Suzuki N, Adachi K, Kawase T, *et al.* Blockade of interleukin-6 signaling aggravates ischemic cerebral damage in mice: Possible involvement of Stat3 activation in the protection of neurons. J Neurochem 2005;94:459-68.
62. Wang S, Zhou J, Kang W, Dong Z, Wang H. Tocilizumab inhibits neuronal cell apoptosis and activates STAT3 in cerebral infarction rat model. Bosn J Basic Med Sci 2016;16:145-50.
63. Hudobenko J, Chauhan A, McCullough L. Amelioration of ischemic stroke damage through inhibition of interleukin-6 signaling with Tocilizumab requires sex specific dosing. Stroke 2019;50 Suppl 1:A128-A28.
64. Amaro S, Chamorro Á. Translational stroke research of the

combination of thrombolysis and antioxidant therapy. Stroke 2011;42:1495-9.
65. Chamorro A, Obach V, Cervera A, Revilla M, Deulofeu R, Aponte JH. Prognostic significance of uric acid serum concentration in patients with acute ischemic stroke. Stroke 2002;33:1048-52.
66. Amaro S, Urra X, Gómez-Choco M, Obach V, Cervera A, Vargas M, *et al.* Uric acid levels are relevant in patients with stroke treated with thrombolysis. Stroke 2011;42:S28-32.
67. Amaro S, Soy D, Obach V, Cervera A, Planas AM, Chamorro A. A pilot study of dual treatment with recombinant tissue plasminogen activator and uric acid in acute ischemic stroke. Stroke 2007;38:2173-5.
68. Hong JM, Bang OY, Chung CS, Joo IS, Gwag BJ, Ovbiagele B. Influence of recanalization on uric acid patterns in acute ischemic stroke. Cerebrovasc Dis 2010;29:431-9.
69. Chamorro A, Amaro S, Castellanos M, Segura T, Arenillas J, Martí-Fábregas J, *et al.* Safety and efficacy of uric acid in patients with acute stroke (URICO-ICTUS): A randomised, double-blind phase 2b/3 trial. Lancet Neurol 2014;13:453-60.
70. Eren F, Öztürk Ş, Barışkaner H, Ünlü A, Uğurluoğlu C. Deneysel iskemi reperfüzyon modelinde geç trombolitik tedavi öncesi magnezyum sülfatin etkinliği. Kocatepe Tıp Dergisi 2019;20:264-74.
71. Saver JL, Starkman S, Eckstein M, Stratton SJ, Pratt FD, Hamilton S, *et al.* Prehospital use of magnesium sulfate as neuroprotection in acute stroke. N Engl J Med 2015;372:528-36.
72. Ortiz JF, Ruxmohan S, Saxena A, Morillo Cox Á, Bashir F, Tambo W, *et al.* Minocycline and magnesium as neuroprotective agents for ischemic stroke: A systematic review. Cureus 2020;12:e12339.
73. Lampe JW, Becker LB. State of the art in therapeutic hypothermia. Annu Rev Med 2011;62:79-93.
74. Zhao H, Steinberg GK, Sapolsky RM. General versus specific actions of mild-moderate hypothermia in attenuating cerebral ischemic damage. J Cereb Blood Flow Metab 2007;27:1879-94.
75. Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 2002;346:549-56.
76. Bernard SA, Gray TW, Buist MD, Jones BM, Silvester W, Gutteridge G, *et al.* Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia. N Engl J Med 2002;346:557-63.
77. Yang C, Sanz BD, DeMars KM, Butler AA, Candelario-Jalil E. Abstract WP315: Neuroprotective effects of endogenous adropin in experimental ischemic stroke. Stroke 2020;51 Suppl 1:AWP315-AWP15.
78. Fraser JF, Maniskas M, Trout A, Lukins D, Parker L, Stafford WL, *et al.* Intra-arterial verapamil post-thrombectomy is feasible, safe, and neuroprotective in stroke. J Cereb Blood Flow Metab 2017;37:3531-43.
79. Khoshnam SE, Winlow W, Farbood Y, Moghaddam HF, Farzaneh M. Emerging roles of microRNAs in ischemic stroke: As possible therapeutic agents. J Stroke 2017;19:166-87.
80. Ng GY, Lim YA, Sobey CG, Dheen T, Fann DY, Arumugam TV. Epigenetic regulation of inflammation in stroke. Ther Adv Neurol Disord 2018;11:1-30.
81. Padma Srivastava MV, Bhasin A, Bhatia R, Garg A, Gaikwad S, Prasad K, *et al.* Efficacy of minocycline in acute ischemic stroke: A single-blinded, placebo-controlled trial. Neurol India 2012;60:23-8.
82. Amiri-Nikpour MR, Nazarbaghi S, Hamdi-Holasou M, Rezaei Y. An open-label evaluator-blinded clinical study of minocycline neuroprotection in ischemic stroke: Gender-dependent effect. Acta Neurol Scand 2015;131:45-50.
83. Howland RH. Vagus nerve stimulation. Curr Behav Neurosci Rep 2014;1:64-73.
84. Conway CR, Kumar A, Xiong W, Bunker M, Aaronson ST, Rush AJ. Chronic vagus nerve stimulation significantly improves quality of life in treatment-resistant major depression. J Clin Psychiatry 2018;79:18m12178.
85. Pérez-Carbonell L, Faulkner H, Higgins S, Koutroumanidis M, Leschziner G. Vagus nerve stimulation for drug-resistant epilepsy. Pract Neurol 2020;20:189-98.
86. Schoenen J, Ambrosini A. Update on noninvasive neuromodulation for migraine treatment-vagus nerve stimulation. Prog Brain Res 2020;255:249-74.
87. Dawson J, Liu CY, Francisco GE, Cramer SC, Wolf SL, Dixit A, *et al.* Vagus nerve stimulation paired with rehabilitation for upper limb motor function after ischaemic stroke (VNS-REHAB): A randomised, blinded, pivotal, device trial. Lancet 2021;397:1545-53.