EDARAVONE AND NRF2-INDUCERS AS NEUROPROTECTIVE AGENTS IN HUMAN ASTROCYTES EXPOSED TO HYPOXIA/REOXYGENATION

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Abstract Optimization of neuronal function and survival is an important goal in the treatment of cerebrovascular diseases in order to avoid or improve devastating long-term sequelae. Nerve growth factor (NGF) is essential for neuronal growth and survival in the central nervous system (CNS). Vascular endothelial growth factor (VEGF) is a potent mitogen specific for endothelial cells and a stimulator of neovascularization. VEGF also enhances vascular permeability, which may promote the development of brain edema during cerebral ischemia. These molecules affect the outcome of ischemia/reperfusion injury in the CNS. Edaravone, a brain-penetrant, free radical scavenger, is known to ameliorate postischemic neuronal dysfunction. Transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2), a master regulator of antioxidant responses, plays an important role in the coordinated expressions of stress-inducible genes. Astrocytes express various genes involved in the regulation of neuronal functions, and the regulation of astrocyte gene expressions may be a potential therapeutic target in brain injury. This review aims to appraise the effects of radical scavenger edaravone and a natural Nrf2-inducer as neuroprotective agents in human astrocytes, particularly under an experimental model for hypoxia/reoxygenation.

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1. Introduction

Thrombolytic therapy with tissue plasminogen activator (tPA) is highly effective for ischemic stroke, by securing reperfusion in the nervous tissue with marginally compromised blood supply. Although the therapeutic time window is currently limited within 3 h after the onset, recent studies suggest that the therapy can be initiated within up to 4.5 h after the onset[1-3]. However, reperfusion may be associated with a burst of free radicals, which cause oxidative damage to lipids, proteins and nucleic acids. Oxidative damage to mitochondrial membranes triggers the release of cytochrome C and caspase 9, which leads to the activation of caspase 3, the main executioner of cell apoptosis.

Edaravone, a brain-penetrant, free radical scavenger, is known to ameliorate postischemic neuronal dysfunction in patients with acute ischemic stroke[4-6]. It is expected to be particularly effective in controlling reperfusion injury through

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its antioxidative property. In our previous study, edaravone was also found to regulate the expression of vascular endothelial growth factor (VEGF) in astrocytes[7]; and this fact may explain part of deterrent effect of edaravone on brain edema development since VEGF enhances vascular permeability.

One of the major functions of astrocytes is to maintain homeostasis in the central nervous system (CNS) through regulating the coordinated expression of various genes. Thus regulation of astrocyte gene expression may be expected to be a potential therapeutic target in stroke, and this review is intended to address such a therapeutic paradigm employing neuroprotective agents.

2. Edaravone, as a neuroprotective agent

2.1. Development of edaravone

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one (Fig. 1, upper left), MCI-186, RADICUT®, molecular weight 174.20, Mitsubishi Tanabe Pharma, Osaka, Japan) was developed for the treatment of acute cerebral infarction[6,8], and has been clinically available since June 2001 in Japan[9-12]. Edaravone is amphipathic and, thereby, brain-penetrant (permeable to the blood-brain barrier, BBB).

2.2. Edaravone as a free radical scavenger

Edaravone chemically interacts with a variety of reactive oxygen species (ROS) including hydroxyl radical ($\bullet$OH)[13,14], peroxyl radical (LOO$\bullet$)[8,13,15], alkoxyl radical (LO$\bullet$)[16], peroxynitrite (ONOO$^-$)[8] and singlet oxygen ($^1O_2$)[17], to which it donates electrons and then is transformed into the stable compound, 2-oxo-3-(phenylhydrazono)-butanoic acid[6,8]. Although the high concentration (1 or 3 mmol/L) of edaravone slightly scavenges superoxide anion (O_2^-)[18], the lower concentration ($\leq$100 μmol/L) of edaravone has no effect on superoxide anion[13,19]. Edaravone also quenches hydrogen peroxide

(H_2O_2) in cellular (neutrophil) and cell-free (xanthin-xanthin oxidase) systems[19]. Edaravone suppresses ROS generation and reduces brain edema in a rat ischemic stroke model[20]. It also represses delayed neuronal death, induced by ROS, in the hippocampus and cerebral cortex following ischemia in the rat[21].

2.3. Pharmacokinetics of edaravone
2.3.1. Permeability to BBB

Edaravone is demonstrated to have molecular property for its effectiveness in the brain with the ability to permeate to BBB. In an animal study, the ratio of the concentrations of edaravone in plasma and cerebral spinal fluid (CSF) is reported to range from 0.50 to 0.65, indicating that the BBB permeability of edaravone is around 60%[22].

2.3.2. Metabolism and excretion of edaravone

Plasma concentration of edaravone reaches a maximum level of 17.6 μmol/L after a single intravenous infusion in a dose of 1.5 mg/kg over 40 min, and after an intravenous infusion of 2.0 mg/kg over 3 h, the concentration reaches a maximum level of 7.0 μmol/L[23]. The plasma concentration reaches a maximum level of 9.3-10.4 μmol/L after 7 consecutive intravenous infusions, each over 40 min, in a dose of 1.0 mg/kg per day[23]. The pharmacokinetics in elderly subjects, over 65 years old, shows a pattern similar to that in younger subjects, indicating a maximum level of 6.0±0.6 μmol/L after 4 intravenous infusions, each over 30 min, in a dose of 0.5 mg/kg per 12 h[24].

In healthy volunteers (5 men), 87±4% of edaravone administered by infusion is metabolized and excreted into urine within 24 h[23]. Although the urinary excretion of edaravone is slow ranging 0.6-1.0% in 24 h, sulfate or glucuronide conjugation enhances the urinary excretion: 5.6-13.2% and 68.6-83.2% in 24 h, respectively[23].

After a single intravenous administration of ^{14}C-edaravone (2 mg/kg) to rats, the disappearance of radioactivity from blood vessel is delayed and the radioactivity in blood vessel is detected even after 8 days[25]. After repeated administration of ^{14}C-edaravone in a dose of 2 mg/kg/day for 21 days, the radioactivity in blood vessel is detected even after 30 days[26]. In the brain, no region-specific distribution of edaravone is observed and most of the radioactivity consists of non-metabolized edaravone, whereas most of the radioactivity in the kidney consists of the sulfated form[25].

After a single intravenous administration of ^{14}C-edaravone (2 mg/kg) to male rats, $92.0 \pm 1.7\%$ of the radioactivity is excreted into urine within 72 h, whereas $4.6 \pm 1.2\%$ into bile[25]. The glucuronide conjugate constitutes $69.6 \pm 3.1\%$ of the total biliary excretion, and most of it may enter the entero-hepatic circulation and finally excreted from the kidney: $67.3 \pm 4.5\%$ of the administered dose is excreted into urine[25].

3. Edaravone in brain injury

3.1. Effects of edaravone in experimental ischemia and/or reperfusion models

In vitro studies have demonstrated that edaravone inhibits lipid peroxidation and vascular endothelial cell injury[27]. In rat brain ischemia models, edaravone inhibited brain edema[20,28,29], tissue injury[13,30,31], inflammatory responses[32], lipid free radical formation[33] and delayed neuronal death[21]. In human umbilical vein endothelial cells, edaravone increases endothelial nitric oxide synthase (eNOS) with the inhibition of low-density lipoprotein (LDL) oxidation[34]. In mice with transient brain ischemia, edaravone suppresses the early accumulation of lipid peroxidation products and oxidative DNA damage, and eliminate a sequence of inflammatory responses resulting in the reduction of inducible NOS[35]. In rat ischemia/reperfusion models, edaravone is demonstrated to reduce intracellular free Ca^{2+} concentration,

raise superoxide dismutase (SOD) activity, and decrease mitochondria membrane damage[36]; and prevents the dissociation of the neurovascular unit (integration of astrocyte endfeet and microvascular basement membrane)[37]. Edaravone is known to suppress the expression of VEGF in a rat ischemia/reperfusion model[38]. We confirmed the suppression, by edaravone, of the hypoxia-induced VEGF expression in cultured human astrocytes[7].

3.2. Efficacy of edaravone in patients with cerebrovascular diseases

As for the clinical effectiveness of edaravone, several trials have confirmed neuroprotective effects of edaravone in patients with acute cerebral infarction[4-6]. Several studies demonstrated the efficacy of edaravone in delaying evolution of cerebral infarcts and edema[39], reducing the generation of ROS[40], oxidized LDL, cytosolic protein S-100B and Mn-SOD[41]. Improvement of functional outcome in patients with acute ischemic stroke, including lacunar infarction[10], cardioembolic stroke[11] and aneurysmal subarachnoid hemorrhage[12], is also reported[9].

3.3. Edaravone in traumatic brain injury

In patients with traumatic brain injury, edaravone is reported to suppress the blood levels of alkoxyl radicals that contribute to lipid peroxidation[16]. Edaravone is also demonstrated to inhibit production of free radicals in a rat model for traumatic brain injury, and this is associated with the protection of neuronal stem cells that have the potential to differentiate into neurons and glia around the injured area[42].

3.4. Edaravone in diabetic neuropathy

In the development of diabetic neuropathy, oxidative stress is implicated as a final common pathway. Edaravone is demonstrated to inhibit the augmented angiotensin II-induced contraction in endothelium intact aortic spinal preparations

isolated from thoracic aorta in diabetic rats[43]. Edaravone also shows improving effects on nerve conduction velocity, nociception, lipid peroxidation status, and anti-oxidant enzymes (SOD and catalase) in a rat model of diabetic neuropathy[44].

3.5. Side effects of edaravone

During the phase I study in healthy volunteers, an increase of serum total bilirubin (0.2 mg/kg dose) and a decrease of platelet count (0.2 mg/kg dose) were observed; however, they were recovered 1 w after the infusion. No other problems were observed, and edaravone was regarded to have a good tolerability in healthy volunteers[23].

Adverse reactions to edaravone, including renal and hepatic disorders, have been reported after its launch in June 2001, and renal disorders have been the most frequent and sometimes serious. In a study reviewed 207 Japanese patients who were treated with edaravone for acute stroke and developed renal disorder, no particular factor other than edaravone administration was found as a possible cause for the renal disorders in 17 patients (8.2%), whereas factors other than edaravone were associated with renal disorders in the remaining 190 patients[45]. The frequency of renal dysfunction as a complication of edaravone treatment is estimated to be 0.04% (207/530000) since edaravone was given to 530000 patients in Japan during the same period of analysis, June 2001 to September 2005[45].

In the 207 patients with renal disorders, the overall recovery rate of renal function was 43%; risk factors for the nonrecovery of renal function were the complication of severe infection and the implication of blood purification, and risk factors for death were advanced age (≥ 80 years) and the complication of severe infection[46].

The precise role(s) of edaravone in the pathogenesis of renal disorders remain(s) unclear. Early detection of renal disorders and control of infection are critical during edaravone treatment. Also, it has been pointed out that care should be taken with the clinical use of edaravone when pterin derivatives stay in the body[47].

4. Therapeutic potential of Nrf2-inducers

Carnosic acid (Fig. 1, upper right), a component of rosemary (*Rosmarinus officinalis* L.), induces a transcription factor Nrf2 (nuclear factor-erythroid 2-related factor 2), a master regulator of antioxidant response, by inhibiting Keap1 (Kelch-like ECH-associated protein 1)[48-50]. Keap1 is a bifunctional protein that serves as an Nrf2-specific adaptor for the Cullin3 ubiquitin ligase complex[51,52] and a sensor for oxidative and/or electrophilic stresses[53]. Keap1 has many reactive cysteine residues that have the potential to sense various electrophiles[54].

Nrf2 plays an important role in the coordinated expression of many phase 2 detoxifiying enzymes such as glutathione S-transferases, heme oxygenase-1 and NAD(P)H quinone oxidoreductase[55,56] and is considered as an indicator and modulator of oxidative stress in neurodegeneration[57]. Nrf2 expression in astrocytes, by overexpression, prevents neuronal death in a mouse model of Parkinson's disease[58].

The Nrf2 pathway is also activated by other electrophilic compounds including natural products such as sulforaphane[59] and curcumin[60] (Fig. 1, bottom left and right). The mitogen-activated protein kinases (MAPK) are activated by phase 2 gene inducers and involved in survival response leading to the transcriptional activation of defense genes mediated by ARE/EpRE (antioxidant or electrophile response element)[61]. A marked nuclear accumulation of Nrf2 protein is reported in cultures of HK-2 human renal proximal tubular epithelial cells or T84 human intestinal epithelial cells exposed to hypoxia[62]. Some Nrf2-inducers may be a potential modulator of the expression of

neuroprotective genes in astrocytes and useful as therapeutic agents.

5. Astrocytes as a therapeutic target in ischemic brain injury

5.1. Regulation of VEGF in astrocytes

Astrocytes play integral roles in maintaining homeostasis and regulating responses to various stresses in the CNS[63,64]. Hypoxia upregulates many genes in astrocytes, including glycolytic enzymes and angiogenic growth factors as *VEGF*[65,66]. VEGF, a potent mitogen specific for endothelial cells and a stimulator of neovascularization, is also known to enhance vascular permeability[67-70], which may be involved in the development of brain edema during cerebral ischemia[71]. A neutralizing antibody against VEGF is reported to reduce infarct volume in a rat 2-vein occlusion model[72]. Edaravone may protect ischemic brain tissue from the development of vasogenic edema, in part, through the suppression of the enhanced *VEGF* transcription in astrocytes[7].

5.2. Regulation of nerve growth factor (NGF) in astrocytes

Neurotrophins including NGF are essential for neuronal growth and subsequent survival[73]. Astrocytes continue to produce NGF[74-76], and its circulating and brain levels undergo significant variations after exposure to stressful events[77].

Figure 1 Chemical structures of edaravone and Nrf2-inducers.

For instance, transient focal cerebral ischemia enhances the expression of NGF in reactive astrocytes particularly in the peri-infarct penumbra[78]. In our previous study, platelet-activating factor (PAF), a proinflammatory phospholipid[79], was found to enhance the NGF expression in human astrocytes[80]. Since PAF is generated in high levels in ischemic brain tissue[81,82], it may play a dual role, by promoting inflammation and protecting neuronal cells, in ischemic brain injury. Also, carnosic acid is demonstrated to upregulate the NGF expression in T98G human glioblastoma cells[83]. NGF exerts protective effects on cultured neurons against a variety of deleterious factors[84-86].

Signaling pathways through MAPK[87,88] (including extracellular signal-regulated kinase (ERK)[89]) and phosphatidylinositol 3-kinase (PI3K)[90] are known to mediate NGF expression in astrocytes. Ethanol extracts of an edible mushroom, *Hericium erinaceus* (Yamabushitake), are reported to promote NGF expressions for mRNA and protein, *via* c-Jun N-terminal kinase (JNK) signaling, in 1321N1 human astrocytoma cells[91]. Also, JNK pathway is likely to mediate the astrocyte NGF expression in response to edaravone[92] or carnosic acid (Yoshida et al., unpublished data).

5.3. Perspectives

The benefit of edaravone as a therapeutic measure against ischemic stroke is almost established both from the viewpoints of immediate prognostic assessment and from functional outcome of the patients in chronic stage. Thrombolysis with tPA is now an essential therapeutic strategy in the treatment of acute cerebral infarction, and edaravone is suggested to be useful for the extension of the therapeutic time window for thrombolytic therapy[6,35,93]. The combination of neuroprotective agents, such as edaravone or carnosic acid, with tPA may be considered as a current standard
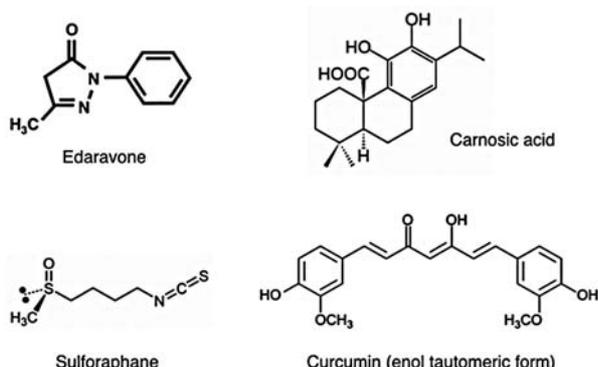
in the treatment of acute cerebral infarction; and this is expected to expand the indication of thrombolytic therapy beyond 3 h after stroke.

Acknowledgments

References

1) Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317-29.

2) Lees KR. Stroke: success for extending acute treatment. Lancet Neurol 2009;8:2-4.

3) Wahlgren N, Ahmed N, Dávalos A, et al. Thrombolysis with alteplase 3-4.5 h after acute ischaemic stroke (SITS-ISTR): an observational study. Lancet 2008;372:1303-9.

4) Higashi Y. Edaravone for the treatment of acute cerebral infarction: role of endothelium-derived nitric oxide and oxidative stress. Expert Opin Pharmacother 2009;10:323-31.

5) Kitagawa Y. Edaravone in acute ischemic stroke. Intern Med 2006;45:225-6.

6) Yoshida H, Yanai H, Namiki Y, Fukatu-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.

7) Ishikawa A, Yoshida H, Metoki N, et al. Edaravone inhibits the expression of vascular endothelial growth factor in human astrocytes exposed to hypoxia. Neurosci Res 2007;59:406-12.

8) Watanabe T, Tanaka M, Watanabe K, Takamatsu Y, Tobe A. Research and development of the free radical scavenger edaravone as a neuroprotectant. Yakugaku Zasshi 2004;124:99-111. (in Japanese with English abstract, figures and tables)

9) Edaravone Acute Brain Infarction Study Group (Chair: Otomo, E). Effect of a novel free radical scavenger, edaravone (MCI-186), on acute brain infarction. Randomized, placebo-controlled, double-blind study at multicenters. Cerebrovasc Dis 2003;15:222-9.

10) Mishina M, Komaba Y, Kobayashi S, et al. Efficacy of edaravone, a free radical scavenger, for the treatment of acute lacunar infarction. Neurol Med Chir (Tokyo) 2005;45:344-8.

11) Inatomi Y, Takita T, Yonehara T, et al. Efficacy of edaravone in cardioembolic stroke. Intern Med 2006;45:253-7.

12) Munakata A, Ohkuma H, Nakano T, Shimamura N, Asano K, Naraoka M. Effect of a free radical scavenger, edaravone, in the treatment of patients with aneurysm subarachnoid hemorrhage. Neurosurgery 2009;64:423-8; discussion 428-9.

13) Watanabe T, Yuki S, Egawa M, Nishi H. Protective effects of MCI-186 on cerebral ischemia: possible involvement of free radical scavenging and antioxidant actions. J Pharmacol Exp Ther 1994;268:1597-604.

14) Watanabe T, Yuki S, Saito K, Sato S, Sugimoto J, Ohori Y. Pharmacological studies of MCI-186, a new drug for acute stroke. Jpn Pharmacol Ther 1997;25:S-1691–8. (in Japanese with English abstract, figures and tables)

15) Watanabe K, Morinaka Y, Iseki K, Watanabe T, Yuki S, Nishi H. Structure-activity relationship of 3-methyl-1-phenyl-2-pyrazolin-5-one (edaravone). Redox Rep 2003;8:151-5.

16) Dohi K, Satoh K, Mihara Y, et al. Alkoxyl radical-scavenging activity of edaravone in patients with traumatic brain injury. J Neurotrauma 2006;23:1591-9.

17) Sommani P, Arai T, Yamashita K, et al. Effects of edaravone on singlet oxygen released from activated human neutrophils. J Pharmcol Sci 2007;103:117-20; erratum 252.

18）Wu T-W, Zeng L-H, Wu J, Fung K-P. MCI-186: further histochemical and biochemical evidence of neuroprotection. Life Sci 2000;67:2387-92.

19）Mikawa K, Akamatsu H, Nshina K, Obara H, Niwa Y. Effects of edaravone on human neutrophil function. Acta Anaesthesiol Scand 2005;49:385-9.

20）Nishi H, Watanabe T, Sakurai H, Yuki S, Ishibashi A. Effect of MCI-186 on brain edema in rats. Stroke 1989;20:1236-40.

21）Yamamoto T, Yuki S, Watanabe T, Mitsuka M, Saito KI, Kogure K. Delayed neuronal death prevented by inhibition of increased hydroxyl radical formation in a transient cerebral ischemia. Brain Res 1997;762:240-2.

22）Takamatsu Y, Watanabe T. Studies on the concentration of 3-methy-1-phenyl-2-pyrazolin-5-one（MCI-136）in dog plasma and cerebral spinal fluid. Jpn Pharmacol Ther 1997;25: S-1793–7. （in Japanese with English abstract, figures and tables）

23）Shibata H, Arai S, Izawa M, et al. Phase I clinical study of MCI-186（edaravone, 3-methyl-1- phenyl-2-pyrazolin-5-one）in healthy volunteers: safety and pharmacokinetics of single and multiple administrations. Jpn J Clin Pharmacol Ther 1998;29:863-76. （in Japanese with English abstract）

24）Yokota S, Kumagai Y, Uchiumi M, et al. A pharmacokinetic study of MCI-186, a novel drug for cerebrovascular disease in elderly and young healthy subjects. Jpn J Clin Pharmacol Ther 1997;28:693-702. （in Japanese with English abstract）

25）Komatsu T, Nakai H, Masaki K, Obata R, Nakai K, Iida S. Pharmacokinetic studies of 3-methyl-1-phenyl-2-pyrazolin-5-one（MCI-186）in rats（1）: blood and plasma levels, distribution, metabolism and excretion after a single intravenous administration. Jpn Pharmacol Ther 1996;11:463-80. （in Japanese with English abstract, figures and tables）

26）Komatsu T, Nakai H, Masaki K, Obata R, Nakai K, Iida S. Pharmacokinetic studies of 3-methyl-1-phenyl-2-pyrazolin-5-one（MCI-186）in rats（2）: blood and plasma levels, distribution, metabolism, excretion and accumulation during and after repeated intravenous administration. Jpn Pharmacol Ther 1996;11:481-91. （in Japanese with English abstract, figures and tables）

27）Watanabe T, Morita I, Nishi H, Murota S. Preventive effect of MCI-186 on 15-HPETE induced vascular endothelial cell injury in vitro. Prostaglandins Leukot Essent Fatty Acids 1988; 33:81-7.

28）Abe K, Yuki S, Kogure K. Strong attenuation of ischemic and postischemic brain edema in rats by a novel free radical scavenger. Stroke 1988;19:480-5.

29）Watanabe T, Egawa M. Effects of an antistroke agent MCl-186 on cerebral arachidonate cascade. J Pharmacol Exp Ther 1994;271:1624-9.

30）Mizuno A, Umemura K, Nakashima M. Inhibitory effect of MCI-186, a free radical scavenger, on cerebral ischemia following rat middle cerebral artery occlusion. Gen Pharmacol 1998;30:575-8.

31）Kawai H, Nakai H, Suga M, Yuki S, Watanabe T, Saito KI. Effects of a novel free radical scavenger, MCl-186, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. J Pharmacol Exp Ther 1997;281:921-7.

32）Shichinohe H, Kuroda S, Yasuda H, et al. Neuroprotective effects of the free radical scavenger Edaravone（MCI-186）in mice permanent focal brain ischemia. Brain Res 2004;1029:200-6.

33）Noor JI, Ueda Y, Ikeda T, Ikenoue T. Edaravone inhibits lipid peroxidation in neonatal hypoxic-ischemic rats: an in vivo microdialysis study. Neurosci Lett 2007;414:5-9.

34）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.

35）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.

36）Wu T, Ding XS, Wang W, Wu J. MCI-186 （3-methyl-1-phenyl-2-pyrazolin-5-one）attenuated

simulated ischemia/reperfusion injury in cultured rat hippocampal cells. Biol Pharm Bull 2006; 29:1613-7.

37) Yamashita T, Kamiya T, Deguchi K, et al. Dissociation and protection of the neurovascular unit after thrombolysis and reperfusion in ischemic rat brain. J Cereb Blood Flow Metab 2009;29:715-25.

38) Otani H, Togashi H, Jesmin S, et al. Temporal effects of edaravone, a free radical scavenger, on transient ischemia-induced neuronal dysfunction in the rat hippocampus. Eur J Pharmacol 2005; 512:129-37.

39) Toyoda K, Fujii K, Kamouchi M, et al. Free radical scavenger, edaravone, in stroke with internal carotid artery occlusion. J Neurol Sci 2004;221:11-7.

40) Aizawa H, Makita Y, Sumitomo K, et al. Edaravone diminishes free radicals from circulating neutrophils in patients with ischemic brain attack. Intern Med 2006;45:1-4.

41) Uno M, Kitazato KT, Suzue A, et al. Inhibition of brain damage by edaravone, a free radical scavenger, can be monitored by plasma biomarkers that detect oxidative and astrocyte damage in patients with acute cerebral infarction. Free Radic Biol Med 2005;39:1109-16.

42) Itoh T, Satou T, Nishida S, Tsubaki M, Hashimoto S, Ito H. The novel free radical scavenger, edaravone, increases neural stem cell number around the area of damage following rat traumatic brain injury. Neurotox Res 2009;16:378-89.

43) Saini AK, Patel RJ, Sharma SS, Kumar HSA. Edaravone attenuates hydroxyl radical stress and augmented angiotensin II response in diabetic rats. Pharmocol Res 2006;54:6-10.

44) Saini AK, Kumar HSA, Sharma SS. Preventive and curative effect of edaravone on nerve functions and oxidative stress in experimental diabetic neuropathy. Eur J Pharmacol 2007;568:164-72.

45) 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.

46) Hishida A. Determinants for the prognosis of acute renal disorders that developed during or after treatment with edaravone. Clin Exp Nephrol 2009;13:118-22.

47) Arai T, Nonogawa M, Makino K, et al. The radical scavenger edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) reacts with a pterin derivative and produces a cytotoxic substance that induces intracellular reactive oxygen species generation and cell death. J Pharmacol Exp Ther 2008;324: 529-38.

48) Satoh T, Izumi M, Inukai Y, et al. Carnosic acid protects neuronal HT22 Cells through activation of the antioxidant-responsive element in free carboxylic acid- and catechol hydroxyl moieties-dependent manners. Neurosci Lett 2008;434:260-5.

49) Satoh T, Kosaka K, Itoh K, et al. Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. J Neurochem 2008;104:1116-31.

50) Takahashi T, Tabuchi T, Tamaki Y, Kosaka K, Takikawa Y, Satoh T. Carnosic acid and carnosol inhibit adipocyte differentiation in mouse 3T3-L1 cells through induction of phase2 enzymes and activation of glutathione metabolism. Biochem Biophys Res Commun 2009;382:549-54.

51) Itoh K, Wakabayashi N, Katoh Y, et al. Keap1 represses nuclear activation of antioxidant responsive elements by Nrf2 through binding to the amino-terminal Neh2 domain. Genes Dev 1999;13:76-86.

52) Kobayashi A, Kang MI, Okawa H, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. Mol Cell Biol 2004;24:7130-9.

53) Kobayashi A, Kang MI, Watai Y, et al. Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol Cell Biol 2006;26:221-9.

54) Kobayashi M, Li L, Iwamoto N, et al. The antioxidant defense system Keap1-Nrf2 comprises a multiple sensing mechanism for responding to a wide range of chemical compounds. Mol Cell Biol 2009;29:493-502.

55) Hayes JD, McMahon M. *NRF2* and *KEAP1* mutations: permanent activation of an adaptive response in cancer. Trends Biochem Sci 2009;34: 176-88.

56) Motohashi H, Yamamoto M. Nrf2-Keap1 defines a physiologically important stress response mechanism. Trends Mol Med 2004:10:549-57.

57) Johnson JA, Johnson DA, Kraft, AD, et al. The Nrf2-ARE pathway: an indicator and modulator of oxidative stress in neurodegeneration. Ann N Y Acad Sci 2008;1147:61-9.

58) Chen PC, Vargas MR, Pani AK, et al. Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: critical role of astrocytes. Proc Natl Acad Sci U S A 2009;106:2933-8.

59) Morimitsu Y, Nakagawa Y, Hayashi K, et al. A sulforaphane analogue that potently activates the Nrf2-dependent detoxification pathway. J Biol Chem 2002;277:3456-63.

60) Lipton SA. Pathologically activated therapeutics for neuroprotection. Nat Rev Neurosci 2007;8: 803-8.

61) Kong A-NT, Yu R, Hebbar V, et al. Signal transduction events elicited by cancer prevention compounds. Mutat Res Fund Mol M 2001;480-1:231-41.

62) Leonard MO, Kieran NE, Howell K, et al. Reoxygenation-specific activation of the antioxidant transcription factor Nrf2 mediates cytoprotective gene expression in ischemia-reperfusion injury. FASEB J 2006;20:2624-6.

63) Liberto CM, Albrecht PJ, Herx LM, Yong VW, Levison SW. Pro-regenerative properties of cytokine-activated astrocytes. J Neurochem 2004;89:1092-100.

64) Tsacopoulos M, Magistretti PJ. Metabolic coupling between glia and neurons. J Neurosci 1996;16:877-85.

65) Mense SM, Sengupta A, Zhou M, et al. Gene expression profiling reveals the profound upregulation of hypoxia-responsive genes in primary human astrocytes. Physiol Genomics 2006;25:435-49.

66) Yoshida H, Imaizumi T, Tanji K, et al. Platelet-activating factor enhances the expression of vascular endothelial growth factor in normal human astrocytes. Brain Res 2002:944:65-72.

67) Bates DO, Curry FE. Vascular endothelial growth factor increases hydraulic conductivity of isolated perfused microvessels. Am J Physiol Heart Circ Physiol 1996;271:2520-8.

68) Hippenstiel S, Krull M, Ikemann A, Risau W, Clauss, M, Suttorp N. VEGF induces hyperpermeability by a direct action on endothelial cells. Am J Physiol 1998;274:678-84.

69) Mayhan WG. VEGF increases permeability of the blood-brain barrier via a nitric oxide synthase/cGMP-dependent pathway. Am J Physiol 1999; 276:1148-53.

70) Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497-504.

71) Josko J, Knefel K. The role of vascular endothelial growth factor in cerebral oedema formation. Folia Neuropathol 2003;41:161-6.

72) Kimura R, Nakase H, Tamaki R, Sakaki T. Vascular endothelial growth factor antagonist reduces brain edema formation and venous infarction. Stroke 2005;36:1259-63.

73) Lyskissas MG, Batistatou AK, Charalabopoulos KA, Beris A. The role of neurotrophins in axonal growth, guidance, and regeneration. Curr Neurovasc Res 2007;4:143-51.

74) Oderfeld-Nowak B, Bacia A. Expression of astroglial nerve growth factor in damaged brain. Acta Neurobiol Exp (Wars) 1994;54:73-80.

75) Otten U, März P, Heese K, Hoch C, Kunz D, Rose-John S. Cytokines and neurotrophins interact in normal and diseased states. Ann N Y Acad Sci 2000;917:322-30.

76) Villoslada P, Genain CP. Role of nerve growth factor and other trophic factors in brain inflammation. Prog Brain Res 2004;146:403-14.

77) Aloe A, Alleva E, Fiore M. Stress and nerve growth factor. Finding in animal models and humans. Pharmacol Biochem Behav 2002;73:159-

66.

78) Lee T-H, Kato H, Chen S-T, Kogure K, Itoyama Y. Expression of nerve growth factor and trkA after transient focal cerebral ischemia in rats. Stroke 1998;29:1687-97.

79) Stanimirovic D, Satoh K. Inflammatory mediators of cerebral endothelium: a role in ischemic brain inflammation. Brain Pathol 2000;10:113-26.

80) Yoshida H, Imaizumi T, Tanji K, et al. Platelet-activating factor enhances the expression of nerve growth factor in normal human astrocytes under hypoxia. Mol Brain Res 2005;133:95-101.

81) Braque P, Spinnewyn B, Demerle C, et al. The role of platelet-activating factor in cerebral ischemia and related disorders. Ann N Y Acad Sci 1989; 559:296-312.

82) Lindsberg PJ, Yue T-L, Frericks KU, Hallenbeck JM, Feuerstein G. Evidence for platelet-activating factor as a novel mediator in experimental stroke in rabbits. Stroke 1990;21:1452-7.

83) Kosaka K, Yokoi T. Carnosic acid, a component of rosemary (*Rosmarinus officinalis* L.), promotes synthesis of nerve growth factor in T98G human glioblastoma cells. Biol Pharm Bull 2003;26:1620-2.

84) Brown A, Ricci MJ, Weaver LC. NGF message and protein distribution in the injured rat spinal cord. Exp Neurol 2004;188:115-27.

85) Cheng B, Mattson MP. NGF and bFGF protect rat hippocampal and human cortical neurons against hypoglycemic damage by stabilizing calcium homeostasis. Neuron 1991;7:1031-41.

86) Zhang Y, Tatsuno T, Carney JM, Mattson MP. Basic FGF, NGF, and IGFs protect hippocampal and cortical neurons against iron-induced degeneration. J Cereb Blood Flow Metab 1993;13:378-88.

87) Galve-Roperh I, Haro A, Díaz-Laviada I. Induction of nerve growth factor synthesis by sphingomyelinase and ceramide in primary astrocyte cultures. Mol Brain Res 1997;52:90-7.

88) Zaheer A, Yorek MA, Lim R. Effects of glia maturation factor overexpression in primary astrocytes on MAP kinase activation, transcription factor activation, and neurotrophin secretion. Neurochem Res 2001;26:1293-9.

89) Park MH, Lee SM, Lee JW, et al. ERK-mediated production of neurotrophic factors by astrocytes promotes neuronal stem cell differentiation by erythropoietin. Biochem Biophys Res Commun 2006;339:1021-8.

90) Takuma K, Yoshida T, Lee E, et al. CV-2619 protects cultured astrocytes against reperfusion injury via nerve growth factor production. Eur J Pharmacol 2000;406:333-9.

91) Mori K, Obara Y, Hirota M, et al. Nerve growth factor-inducing activity of Hericium erinaceus in 1321N1 human astrocytoma cells. Biol Pharmacol Bull 2008;31:1727-32.

92) Yoshida H, Metoki N, Ishikawa A, et al. Edaravone improves the expression of nerve growth factor in human astrocytes subjected to hypoxia/reoxygenation. Neurosci Res 2010;66:284-9.

93) Zhang W, Sato K, Hayashi T, et al. Extension of ischemic therapeutic time window by a free radical scavenger, edaravone, reperfused with tPA in rat brain. Neurol Res 2004;26:342-8.