

## Beneficial Effects of the Free Radical Scavenger Edaravone (Radicut) in Neurologic Diseases

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### Abstract

Free radicals play major roles in the pathogenesis of many diseases, including neurologic diseases, making them an attractive target for therapeutic intervention. Several free radical scavengers have been developed, and some have progressed to clinical trials for the treatment of ischemic stroke. One such scavenger, edaravone is currently used to treat patients who present within 24 h of an attack. Edaravone can diffuse into many affected organs. Edaravone also exerts protective effects against brain and spinal cord injuries. Beyond its direct free radical scavenging effect, edaravone has anti-apoptotic and anti-inflammatory effects in various diseases. Here, we critically review the literature on experimental animal model and clinical studies of edaravone efficacy, and examine whether it should be considered a candidate for worldwide development. Edaravone has proven safe during 10 years of use as a free radical scavenger to treat ischemic stroke. In addition to ischemic stroke treatment, animal data suggest that edaravone may be an effective treatment option for several neurologic diseases, but additional clinical trials are necessary to verify its efficacy.

### Introduction

The World Health Organization estimates that 15 million people suffer strokes worldwide annually. More than five million die from the initial brain insult, and another five million are permanently disabled and require lifelong medical care ([www.strokecenter.org/patients/stats.htm](http://www.strokecenter.org/patients/stats.htm)). The National Institute of Neurological Disorders and Stroke (NINDS) classifies ischemic stroke types and determines incidence rates from population-based studies [1,2]. The NINDS estimates that cardioembolic stroke caused by an embolism from a cardiac source accounts for 29% of cases. Atheroembolic stroke, which is associated with narrowing of a cervicocephalic artery, makes up 16% of cases. Small vessel lacunar stroke, defined as pure motor, sensorimotor or sensory deficits with ataxic hemiparesis, results from thrombosis in one of the deep penetrating branches of the large cerebral arteries and accounts for 16% of cases [3]. Approximately 40% of ischemic strokes have an unknown cause [3]. Because oxidative stress is a major component of the ischemic stroke cascade [4], removal of free radicals may offer therapeutic benefits.

Several free radical scavengers have been assessed for their efficacy in the treatment of ischemic stroke, but few of these have shown success in studies conducted in Western countries [3]. In contrast, trials conducted in Japan have been more successful [3]. Indeed, several free radical scavengers have now been developed, and some of these (e.g., tirilazad and NXY-059) have progressed to clinical trials [5]. However, tirilazad produced inadequate therapeutic effects in patients with ischemic stroke and the trial was terminated. The Stroke-Acute

Ischemic NXY-059 Treatment (SAINT)-II trial found that NXY-059 was ineffective against ischemic stroke when administered within 4 h (mean time from onset of stroke to treatment, 3 h 46 min) after the onset of symptoms [6-8].

The free radical scavenger edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186, Radicut; Mitsubishi Tanabe Pharma Corporation, Osaka, Japan) exerts antioxidant effects by inhibiting hydroxyl radical-dependent and -independent lipid peroxidation [9,10]. This antioxidant activity, the main proposed mechanism of action, may protect against free radical-related injuries following ischemic stroke [11]. Edaravone also suppresses the increase in hydroxyl and superoxide anion radical levels in several models of ischemic stroke [12,13]. Unlike other free radical scavengers, edaravone readily crosses

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the blood-brain barrier (BBB) [3], possibly explaining its efficacy where other scavengers have failed to show any.

In addition to its free radical scavenging effects, edaravone exerts unrelated effects that may be useful in the treatment of diseases other than ischemic stroke. To the best of our knowledge, no reports have reviewed edaravone's potential use in the treatment of other neurologic diseases.

Therefore, the aims of this review are to present current research on the use of edaravone, primarily in animal models of various neurologic diseases, and to introduce a clinical study aimed at assessing the potential use of edaravone in the treatment of these diseases.

### Edaravone efficacy in ischemic stroke: Experimental studies

Edaravone exerts its antioxidant effects by quenching the hydroxyl radical and inhibiting hydroxyl radical-dependent and hydroxyl radical-independent lipid peroxidation in both a focal ischemic model and a global ischemic model [9,10,14,15]. In early studies of the antioxidant activity of edaravone, its pKa was found to be 7.0, and the rate of oxidation initiated with an azo compound was positively correlated with pH [15]. At 50  $\mu$ M, edaravone inhibited the aerobic oxidation of unilamellar soybean phosphatidylcholine liposomal membranes initiated with either a water-soluble or a lipid-soluble initiator [15]. A more recent study described the ability of edaravone to inhibit copper- and human umbilical vein endothelial cell (HUVEC)-mediated low-density lipoprotein (LDL) oxidation [14]. In rats, the mechanism of action of edaravone was found to involve an enhancement of endothelial nitric oxide synthase (eNOS) expression in HUVECs through stabilizing eNOS mRNA and reversing the inhibitory effect of oxidized LDL on eNOS expression [14]. The authors of that study speculated that the upregulation of eNOS and the decrease in LDL oxidation caused by edaravone may improve vascular blood flow, which may in turn have a protective effect in ischemic tissues. Additional studies have shown that edaravone suppresses the increases in hydroxyl radical and superoxide anion radical levels in both a focal ischemic model and a global ischemic model [10,16,17].

Many effects of edaravone have been reported in basic studies of ischemic stroke. Reactive oxygen species (ROS) and  $Ca^{2+}$  overload during ischemia/reperfusion induce cellular damage by opening the mitochondrial permeability transition pore, a non-specific pore in the inner mitochondrial membrane [18]. Notably, edaravone attenuated  $Ca^{2+}$  induced swelling of mitochondria in the rat brain [18]. In a mouse focal ischemic model, the neuroprotective effects of edaravone were mediated *via* its antioxidant actions, including suppression of lipid peroxidation and oxidant DNA damage [19]. Edaravone also suppressed inducible nitric oxide synthase (iNOS) activity, thereby exerting anti-inflammatory effects by inhibiting microglial activity and peroxynitrite production [19]. In addition, data show that edaravone protects ischemic neurons from apoptosis by suppressing the expression of Fas-associated death domain protein, death-associated protein and caspase-8 immunoreactivity in a rat middle cerebral artery occlusion (MCAO) model, a focal ischemic model [20]. Edaravone has also been shown to have an anti-apoptotic effect mediated by a decrease in the level of B-cell lymphoma 2 (Bcl-2)-associated X protein immunoreactivity and an increase in the levels of the apoptosis regulator Bcl-2 immunoreactivity in a rat MCAO model [21]. The protective effect of edaravone in hypoxic/ischemic injury has also been attributed to inhibition of response to endoplasmic reticulum (ER) stress and subsequent apoptotic signaling in a focal ischemic model

[22,23]. Using a standard ischemia model in gerbils, Jin and colleagues showed that edaravone could reduce edema and increase cerebral blood flow following ischemia [24]. They also showed that that edaravone and argatroban (a selective thrombin inhibitor and an anticoagulant agent used to treat acute noncardioembolic ischemic stroke) protect against damage to neuronal cells and increase the survival ratio ( $p < 0.05$  by Mantel-Cox test) [24], while another study revealed that edaravone can ameliorate damage when used in conjunction with argatroban [25]. Data from several animal models suggest that edaravone can suppress brain edema in hypoxic/ischemic conditions [26,27]. This effect is attributed to edaravone-mediated inhibition of vascular endothelial growth factor (VEGF) expression in astrocytes [26]. In addition to the inhibition of ROS generation, edaravone reduces the amount of ROS-induced inflammatory reactions in ischemic stroke [28]. Oxidative stress activates nuclear factor- $\kappa$ B (NF- $\kappa$ B) and several mediators of inflammation (e.g., iNOS, cytokines and cyclooxygenase-2) that are known to cause delayed damage to the ischemic area in stroke patients and models of stroke [29–36]. In cases of ischemic injury, edaravone can also reduce iNOS expression and suppress neutrophil activation and the accumulation of lipid peroxidation products and 4-hydroxy-2-nonenal (HNE)-modified proteins [19,37].

Recent discoveries indicate many new benefits of edaravone in ischemic stroke. Several lines of evidence show neuroprotection after ischemic stroke. For example, ischemic stroke is associated with enhanced expression of metalloproteinase-9 (MMP-9) and aquaporin-4 (AQP4), which cause acute edema. It is also associated with the release of high-mobility group box 1 (HMGB1) from affected tissue. These events are associated with poor clinical outcomes [3,24,38–40]. Edaravone is a low molecular weight agent that readily crosses the BBB, and its activity is not limited to the vascular compartment [41,42]. Furthermore, edaravone was reported to inhibit MMP-9-related brain hemorrhage in rats treated with recombinant tissue plasminogen activator (tPA) [40] and to attenuate cerebral ischemic injury by suppressing AQP4 expression in a focal ischemia model [27]. Moreover, edaravone rescues rats from ischemic stroke by attenuating the release of HMGB1 from neuronal cells in a focal ischemia model [38]. Taken together, these findings suggest that edaravone could be used to treat ischemic stroke by targeting and inhibiting the underlying molecular events associated with brain injury. In a tPA-treated rat MCAO model, edaravone prevented the dissociation of the neurovascular unit (e.g. neurons, glia and vascular cells), dramatically decreased hemorrhagic transformation (hemorrhages that develop inside areas of ischemia), and improved neurologic scores and survival rate [43].

### Edaravone efficacy in ischemic stroke: Clinical studies

Edaravone was the first free radical scavenger developed as a neuroprotective drug to be introduced worldwide. Since 2001, it has been used in Japan to treat many patients with ischemic stroke [44,45]. It is currently approved only in Japan. Clinical trial data show that administration of edaravone within 72 h of ischemic stroke onset significantly reduces infarct volume and provides sustained benefits over a 3-month follow-up period [46]. Administration of edaravone within 24 h of ischemic stroke onset has also been performed in patients with lacunae, large-artery atherosclerosis, and cardioembolic stroke as shown by the beneficial effects on rehabilitation [47,48]. In a retrospective study of 72 acute ischemic stroke patients, Unno et al. reported that the total dose of edaravone was associated with gains in rehabilitation [47]. Furthermore, in a randomized, controlled pilot study of 41 acute ischemic stroke patients, edaravone treatment for

up to 14 days slowed the progression of disuse muscle atrophy and improved leg locomotor function to a greater extent than shorter-term treatment (3 day treatment) [48]. Moreover, Shinohara and co-workers studied the effects of edaravone in a mixed population of 401 acute ischemic stroke patients including patients with thrombotic and lacunar stroke [25]. The study was part of a multicenter, randomized, parallel-group, open-label design comparing edaravone with sodium ozagrel (ozagrel) [25], an antiplatelet agent restricted to use in the treatment of acute noncardioembolic ischemic stroke [25]. The main conclusion was that edaravone was at least as effective as ozagrel [25]. These data showed that there was a trend toward decreased National Institutes of Health Stroke Scale scores in edaravone-treated patients. Furthermore, edaravone suppressed reduced serum MMP9 levels in 63 patients with acute ischemic stroke in another randomized study [49].

### **Edaravone efficacy in amyotrophic lateral sclerosis: Experimental studies**

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease involving selective and progressive degeneration and disappearance of motor neurons. Since Cu/Zn-dependent superoxide dismutase (SOD-1) was first identified as a contributing factor in familial ALS (FALS) in 1993, the possibility of a role of oxidative stress in the pathogenesis of FALS has been studied [50]. Oxidative lesions have been found in the nervous tissue of both sporadic ALS and FALS patients [51], and oxidative stress has been shown to contribute to the pathogenesis of ALS [52,53]. Mutant SOD1 transgenic mice also recapitulate the clinical symptoms and pathological findings of human FALS [54]. Beneficial effects of edaravone in mutant SOD1 mice in randomized experiments with a blinded design have been reported [55]. The deposition of abnormal SOD1 in the anterior horns was reduced by edaravone administration, and edaravone effectively slowed symptom progression and motor neuron degeneration [55]. Whereas FALS is well represented by transgenic mutant SOD1 mouse models, the mouse mutant 'wobbler' develops progressive motor neuron degeneration as a result of a point mutation in the Vps54 gene, and provides an animal model for sporadic ALS [56]. There is evidence of increased oxidative stress in the spinal cords of wobbler mice, and beneficial effects of edaravone in these mice have been reported [57].

### **Edaravone efficacy in amyotrophic lateral sclerosis: Clinical studies**

Yoshino and Kimura reported the results of a small-sized open trial of edaravone in 19 ALS patients [58]. Edaravone markedly reduced the concentration of 3-nitrotyrosine, which is indicative of oxidative cellular damage and is increased in sporadic ALS patients [52], in the cerebrospinal fluid of enrolled patients. In addition, edaravone reduced the rate of decline in ALS functional rating scale scores during the six-month treatment period. Thus, treatment with edaravone may delay the progression of functional motor disturbances in ALS patients [58]. More promising evidence of the beneficial effects of edaravone in human ALS patients is expected with the publication of the results of a phase III clinical trial of edaravone in ALS patients, currently underway in Japan [59].

### **Edaravone efficacy in traumatic brain injury: Experimental and clinical studies**

Like acute ischemic stroke, a large number of studies have reported that oxidative stress has a key role in the development of traumatic brain injury (TBI). Edaravone administration inhibited free radical-induced neuronal degeneration and apoptotic cell death around the

injured area, and improved cerebral dysfunction in a rat TBI model in which injury was introduced with a pneumatic controlled injury device [60]. Moreover, edaravone scavenged alkoxy radicals in a rat cryoinjury TBI model [61], and, in a different study, increased neural stem cell numbers around the area of damage following TBI in rats [62]. Edaravone was also shown to scavenge alkoxy radicals in 17 human patients with TBI [63]. However, neither neurologic function nor outcome was evaluated in this study.

### **Edaravone efficacy in other neurologic diseases: Experimental studies**

A neuroprotective effect of edaravone was observed in a Parkinson's disease (PD) rat model, both *in vitro* and *in vivo* [64]. The rat PD model was produced by administration of 6-hydroxydopamine, a neurotoxin targeting dopaminergic neurons. The authors reported reductions in the numbers of terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling-positive apoptotic cells and hydroethidine-positive cells, suggesting that edaravone may have anti-apoptotic and anti-oxidative effects [64]. Staining for ionized calcium binding adaptor molecule 1 in a PD rat model revealed that inflammation was also suppressed after edaravone administration [64]. Edaravone also protects neurons from apoptosis after cranial irradiation and protects against spatial memory retention deficits in mice [65]. In addition, edaravone has been shown to protect human neural stem cells from radiation-induced apoptosis [30].

The neuroprotective effects of edaravone extend to spinal cord damage models. Edaravone administration reduced eNOS and SOD1 levels after transient ischemia in rabbits (a rabbit MCAO model) [66]. Furthermore, edaravone reduced oxidative DNA damage, as shown by the prolonged expression of the redox effector factor Raf-1, a multifunctional enzyme involved in the DNA repair process, in the spinal cord of the same rabbit transient ischemia model [67]. Moreover, evaluation of the effect of edaravone on lipid peroxide formation downstream of the ROS production cascade, by measuring malonyldialdehyde levels in injured spinal cord homogenates, found that edaravone significantly attenuated lipid peroxide formation by >45% in the acute stage of spinal cord injury in rats [68].

Anti-inflammatory effects of edaravone have been reported in a few experimental studies. In a murine experimental multiple sclerosis model, edaravone significantly ameliorated the clinical severity, reduced infiltration of lymphocytes and lowered iNOS expression [69]. Edaravone effectively inhibited NF- $\kappa$ B activation and decreased expression of chemoattractant protein-1, vascular cell adhesion molecular-1 and matrix metalloproteinase-2, resulting in significant inhibition of macrophage infiltration into aneurysmal walls of a rat cerebral aneurysm (CA) model generated by artery ligation [70]. Edaravone also inhibited enlargement and medial degradation of CAs without influencing systemic blood pressure in this rat model [70]. These results suggest the possibility of a preventive effect of edaravone against CA rupture.

Finally, edaravone was shown to attenuate intracerebral hemorrhage-induced brain edema, neurologic deficits and oxidative injury in rats [71]. Furthermore, edaravone reduced iron- and thrombin-induced brain injury in rats [71]. A clear and selective inhibitory effect of edaravone against hydroxyl radical-induced vasoconstriction was shown in the canine basilar artery *in vitro* [72].

### **Conclusions**

The findings of recent research on the efficacy and mechanism of action of edaravone suggest tremendous potential for this scavenger

in the treatment of several neurologic diseases. However, edaravone is currently only used to treat ischemic stroke within Japan. The abnormal generation of free radicals appears to be common to the etiology and progression of multiple diseases, in particular, a variety of neurologic diseases. Therefore, edaravone may have potential therapeutic effects in patients with neurologic diseases. In addition, edaravone has been shown to have many effects beyond free radical scavenging, such as anti-apoptotic and anti-cytokine effects, in animal models of various neurologic diseases. To date, the therapeutic effects of edaravone in humans have only been reported in ALS and ischemic stroke patients. Further clinical studies are desired to extend the effects of edaravone seen in various animal models of disease to humans.

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