Basic Medical Research

Effects of edaravone on hypoxia-induced lethality in male Swiss albino mice

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ABSTRACT

BACKGROUND Edaravone has recently been used to treat acute cerebral infarction. This study aimed to evaluate the protective effects of edaravone against hypoxia-induced lethality and oxidative stress in mice using 3 experimental models of hypoxia: asphyxia, circulatory, and hemic hypoxia.

METHODS 60 Swiss albino mice from the animal facility of Mazandaran University of Medical Sciences, Iran, were randomly housed in groups of 10 during January–March 2020 and received edaravone for 4 consecutive days. After inducing hypoxia, oxidative stress, lipid peroxidation (LPO), and glutathione (GSH) content were assessed.

RESULTS The findings showed significant protective effects of edaravone in all hypoxia models, with the strongest effects in asphyxia and circulatory hypoxia, showing a dose-dependent response. It prolonged survival time at 2.5 mg/kg by 26.08 (0.79) min (p = 0.031) In addition, edaravone significantly inhibited hypoxia-induced oxidative stress (LPO and GSH oxidation) in all 3 hypoxia models.

CONCLUSIONS Edaravone exhibits an excellent protective effect against different models of hypoxia by decreasing oxidative stress in brain tissue. In addition, the results showed dose-dependent effects of edaravone in the asphyxia and circulatory hypoxia models. Antioxidant activity might be a proposed mechanism for the anti-hypoxic activity of this drug.

KEYWORDS antihypoxia, asphyxia hypoxia, oxidative stress

Organ hypoxia results from an imbalance between oxygen supply and demand, mainly occurring in ischemia and heart disease, with severe effects on different tissues, especially the brain. As the brain consumes high levels of oxygen, 20–25% of the body's oxygen is highly susceptible to hypoxia.[1] Edaravone, a potent free-radical scavenger, has shown protective effects against cerebral ischemia-reperfusion injury (CI/RI) in experimental models.[2] Notably, it can inhibit lipoxygenase activation and peroxidation of phosphatidylcholine liposomal membranes *in vitro*,[3] with clinical studies confirming its efficacy against ischemic brain attacks by showing significant improvements in functional outcomes in human studies.[3,4] Moreover, systemic administration attenuated malondialdehyde (MDA) levels, reduced superoxide dismutase activity, and suppressed retinal dysfunction after retinal ischemia or reperfusion in rats.[5]

In addition to its role in cerebral infarction, edaravone has demonstrated protective effects under various hypoxia-related conditions. Clinical studies

link its use before myocardial reperfusion to smaller enzymatic infarcts and improved clinical outcomes in patients with acute myocardial infarction.[6] Furthermore, edaravone has been explored for the treatment of acute lung injury (ALI), and studies have indicated that its distinct properties offer a novel approach for treating various lung injuries, including ALI.[7] It also helps prevent lung damage from hepatic ischemia-reperfusion by attenuating oxidative stress in the reperfused liver and subsequent lung injury.[8] This study aimed to investigate the anti-hypoxic effect of edaravone, its possible mechanism of action in cerebrovascular diseases, and its broad implications in hypoxia-induced organ dysfunction.

METHODS

Ethics approval and consent

All experimental procedures followed the National Institutes of Health guidelines of laboratory animal care and use and complied with the animal research: reporting of *in vivo* experiments guidelines. The Research Ethics Committee of Mazandaran University of Medical Sciences approved the experimental protocol (No: IR.MAZUMS.REC.93.976).

Experimental animals and diet

Male Swiss albino mice (20 [2] g) were randomly housed in groups of 10 in polypropylene cages under controlled conditions (25 ± 1°C, 45–55% humidity, 12-h light/dark cycle) at the animal house of Mazandaran University of Medical Sciences, Sari, Iran. Animals had *ad libitum* access to standard pellets and water. The study was conducted from January to March 2020. The mice were obtained from the animal facility of Mazandaran University of Medical Sciences.

Hypoxia models

Three experimental hypoxic models were used in this study, with each model involving 60 mice divided into 10 groups of six. In the asphyxia-hypoxia model, mice were placed individually in a 300 ml airtight glass container coated with parafilm. The mice received single intraperitoneal (i.p.) injections of 2.5 and 5 mg/kg edaravone for 4 consecutive days. On the 4th day, edaravone or phenytoin (50 mg/kg) was injected 30 min before exposure to hypoxia. The circulatory hypoxia model was administered an i.p. injection of sodium fluoride (NaF) (150 mg/kg), whereas the hemic hypoxia models received sodium nitrite ($NaNO_2$) (360 mg/kg). On the fourth day, 30 min after the final edaravone administration, mice were subjected to hypoxia. Both models received intraperitoneal injections of edaravone at doses of 2.5, 5, or 10 mg/kg for 4 consecutive days, with the final dose administered 30 min prior to hypoxia induction. All the models were recorded for mortality latency. The negative and positive control groups received normal saline and propranolol, respectively.[9]

Oxidative stress assessment

Sixty mice were divided into ten groups of six and exposed to hypoxia using the three methods described earlier. After the cutoff time, they were anesthetized with ketamine (80 mg/kg) and xylazine (5 mg/kg). Brain tissue was separated, minced, and homogenized using a handheld glass homogenizer. Tissue homogenates were centrifuged at 3,000 × *g* for 10 min at 4°C; moreover, the supernatant was used to assess oxidative stress markers, including lipid peroxidation (LPO) and glutathione (GSH). The amount of MDA was assessed by measuring the absorbance at 532 nm using an enzyme-linked immunosorbent assay reader (ELX800; Biotek, USA), with tetramethoxypropane as the standard. MDA content was expressed as nmol/mg protein. GSH content was determined using 5,5'-Dithiobis (2-nitrobenzoic acid) as an indicator, read at 412 nm on a spectrophotometer (CE2501; CECIL, France), and expressed as µM.[10]

Statistical analysis

The Kolmogorov–Smirnov test was used to assess whether the continuous variables were normal. The results are presented as mean (standard error of the mean) or median (interquartile range). Qualitative data are expressed as frequencies (percentages). Analysis of variance was performed, followed by Duncan's multiple range test to determine the differences in means. Statistical significance was set at $p<0.05$. Statistical analyses were performed using GraphPad Prism version 6 (GraphPad Software Inc., USA).

RESULTS

The effects of edaravone on asphyxia hypoxia are shown in Figure 1a. Edaravone prolonged the survival time in a dose-dependent manner, with both 2.5 and

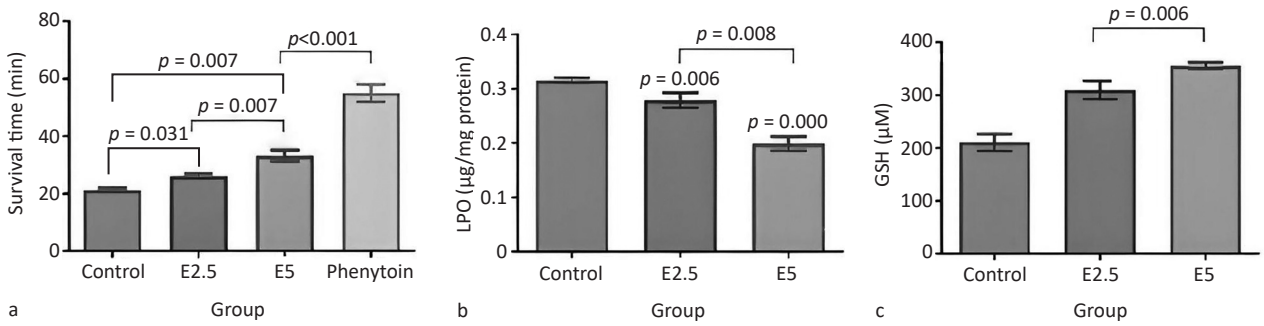

Figure 1. Dose-dependent protective effects of edaravone at 2.5 mg/kg (E2.5) and 5 mg/kg (E5) against asphyxia hypoxia–induced oxidative damage in mice. (a) Antihypoxic activities of E2.5 and E5 compared with control and phenytoin groups in asphyxia hypoxia; (b) effects of E2.5 and E5 on hypoxia-induced LPO in brain tissue; (c) effects of E2.5 and E5 on hypoxia-induced GSH oxidation in brain tissue. Data were expressed as mean (SEM) (n = 6). GSH=glutathione; LPO=lipid peroxidation; SEM=standard error of the mean

Table 1. Antihypoxic activities of ederavone in hemic and circulatory hypoxia in mice

	Dose (mg/kg)					
	Control (normal saline)	Edaravone			Propranolol	
		2.5	5	10	20	30
Hemic hypoxia, mean (SEM)						
Hypoxia (min)	**8.01 (0.29)**	-	9.57 (0.50)	**13.14 (0.82)†**	**16.44 (0.62)†**	-
MDA (µg/mg protein)	**0.273 (0.003)**	-	0.274 (0.008)	**0.200 (0.006)†**	-	-
GSH (µM)	**211.3 (6.90)**	-	**304.3 (9.25)†**	**303.8 (3.55)†**	-	-
Circulatory hypoxia, mean (SEM)						
Hypoxia (min)	**9.78 (0.41)**	12.05 (0.55)	**8.30 (2.70)†**	-	12.76 (0.40)	16.16 (0.30)
MDA	**0.222 (0.007)**	0.214 (0.006)	**0.177 (0.007)†**	-	-	-
GSH	**183.0 (7.83)**	**229.3 (6.98)***	**239.3 (8.79)†**	-	-	-

MDA=malondialdehyde; GSH=glutathione; SEM=standard error of the mean
*$p<0.001$; †$p<0.0001$

5 mg/kg producing statistically significant effects compared with the control group.

As shown in Figure 1b, MDA levels, used as an index of lipid LPO, were significantly reduced by edaravone treatment. This reduction was evident at both doses, with a more pronounced effect at 5 mg/kg.

Figure 1c demonstrates that GSH levels in brain tissue were significantly increased by edaravone administration. The elevation was observed at both doses, indicating protection of the antioxidant defense system, with higher efficacy at 5 mg/kg.

DISCUSSION

Edaravone demonstrated statistically significant antihypoxic activity in experimental mouse models of hypoxia, with effectiveness increasing in a dose-dependent manner. Higher doses resulted in significantly enhanced protective effects. A previous study confirmed the role of edaravone in CI/RI.[11] Research has also highlighted the close association between oxidative metabolism and cholinergic function, particularly regarding the effects of $NaNO_2$ on brain metabolism. $NaNO_2$ induces chemical hypoxia by converting hemoglobin to methemoglobin, thereby reducing the oxygen-carrying capacity of blood. The toxic dose was administered 30 min after phenytoin therapy, following documentation of the interval between $NaNO_2$ injection and respiratory cessation in mice. Edaravone showed moderate protective activity in a rat model of hemic hypoxia.

Research indicates that NaF induces circulatory hypoxia, increases blood histamine content, and reduces oxygen-carrying capacity. In the present study, edaravone (5 mg/kg) was highly effective in protecting against these effects, partly due to its

antioxidant properties. Assessment of oxidative stress markers in brain tissue across all models showed that edaravone significantly reduced oxidative stress compared with the control group. Hypoxia-induced reactive oxygen species (ROS) can oxidize various cellular macromolecules including proteins, DNA, RNA, and lipids through LPO, leading to neuronal dysfunction and cell death.[12,13] LPO, one of the most critical indicators of oxidative stress, can exert harmful effects on cells and tissues and can increase free radical production via chain reactions, ultimately disrupting cell membranes.

This study confirmed that edaravone effectively inhibits LPO in brain tissue following hypoxic exposure. Previous studies have demonstrated edaravone's ability to prevent LPO and nitric oxide production in the neonatal rat brain following hypoxic-ischemic injury, thereby protecting against neuronal damage.[14,15] Another study found that prophylactic edaravone administration mitigated transient hypoxic-ischemic brain injury by reducing oxidative stress.[16] Hypoxia initiates a cascade of cellular homeostatic disruptions, including excessive ROS generation, increased LPO, and mitochondrial dysfunction, which collectively trigger apoptosis. Edaravone mitigated these effects by scavenging free radicals, reducing oxidative stress, and preserving mitochondrial integrity. Studies have shown that edaravone markedly decreases ROS production, inhibits LPO under hypoxic conditions,[17] and suppresses the activation of apoptosis-related markers.[18]

Under cerebral hypoxia, phenytoin significantly protected neurons in the hippocampus and dentate gyrus compared with saline treatment. These protective effects can be attributed to four key mechanisms. First, phenytoin reduces the cerebral metabolic rate of oxygen consumption by 40–60%, decreases cerebral lactate production, and increases cerebral levels of glucose, glycogen, and phosphocreatine, thereby enhancing tolerance to brain ischemia. Second, during total brain ischemia, phenytoin increases cerebral blood flow through vasodilation. Third, since cerebral ischemia is associated with increased extracellular potassium levels, phenytoin inhibits this increase, helping to reduce brain edema following cerebral ischemia. Additionally, phenytoin stabilizes neuronal membranes and prevents increases in intracellular sodium typically observed under cerebral hypoxic conditions by regulating the Na^+/K^+-ATPase system. This membrane-stabilizing action of phenytoin may reduce some of the morphological and biochemical changes observed following cerebral hypoxia. Furthermore, phenytoin blocks Ca^{2+} channels and decreases intracellular Ca^{2+} levels, which in turn inhibits free fatty acid release, protecting the cell membrane.[19,20]

Catecholamines, particularly norepinephrine, are released into the bloodstream when mammals are exposed to hypoxia or other stressors, thereby increasing cardiac sensitivity to oxygen deficiency. Propranolol inhibits the ventilatory response to norepinephrine under hypoxic conditions[21] and helps protect the fine ultrastructure of muscle tissue against hypoxia-induced damage. Additionally, propranolol preserves mitochondrial function by maintaining near-normal mitochondrial oxidative phosphorylation and Ca^{2+} accumulation following hypoxic perfusion. Previous studies have confirmed its protective effects against hypoxia and ischemia.[22]

GSH, the primary antioxidant in cellular systems, directly scavenges free radicals.[23] Previous studies have indicated that hypobaric hypoxia reduces oxidized GSH levels, likely because of the inhibition of GSH synthesis and its amplified use for detoxifying hypoxia-induced free radicals.[24] Here, decreased GSH levels were linked to the aggravation of oxidative stress and brain injury. However, edaravone treatment significantly restored GSH levels in the brain tissue after hypoxia.

This study investigated the potential antihypoxic effects of edaravone, a promising antioxidant with neuroprotective properties, using multiple established hypoxia models. Hypoxia, characterized by insufficient oxygen supply to tissues, plays a critical role in various ischemic conditions and neurodegenerative disorders, including stroke, Alzheimer's disease, and Parkinson's disease.[25] This study is particularly valuable for exploring how edaravone mitigates cellular damage under low-oxygen conditions.[26] By incorporating three distinct hypoxia models—asphyxia, circulatory impairment, and hemic hypoxia—this study enhances its relevance and applicability to diverse pathophysiological scenarios encountered in clinical settings. This comprehensive approach strengthens the findings and provides insights into potential therapeutic strategies for managing oxidative stress-related injuries.

Despite the promising results of experimental models, some limitations remained. The molecular mechanisms underlying the protective effects of

edaravone against hypoxia require further elucidation. As most supporting evidence derives from preclinical studies, factors such as optimal dosing, timing of administration, and patient variability may limit the direct translation of these findings to clinical settings. Comprehensive clinical trials are needed to fully establish the efficacy and safety of edaravone in managing hypoxia-induced cellular damage.

In conclusion, edaravone exhibited significant protective effects across different hypoxia models and markedly reduced oxidative stress in brain tissue. The findings revealed dose-dependent effects of edaravone in asphyxia and circulatory hypoxia models. The antioxidant activity appears to be a primary mechanism underlying the antihypoxic effects of this drug.

Conflict of Interest

The authors affirm no conflict of interest in this study.

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