

## Original Article

# Effects of edaravone on serum TNF- $\alpha$ , IL-8 levels and neural function in rats with cerebral infarction

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Received January 13, 2020; Accepted March 18, 2020; Epub June 15, 2020; Published June 30, 2020

**Abstract:** Objective: This study aims to analyze the effects of edaravone on cerebral infarction in rats in terms of neural function, serum TNF- $\alpha$  and IL-8 levels. Methods: Sixty healthy SD rats were selected to establish models of cerebral infarction and then divided into two groups: the conventional group (n=30) was treated with normal saline and the edaravone group (n=30) was given the edaravone treatment. The levels of serum TNF- $\alpha$ , IL-8, plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator (t-PA) and neural function were compared between the two groups. Results: (1) Serum TNF- $\alpha$  and IL-8 levels in the edaravone group were lower than those in the conventional group ( $P < 0.05$ ). (2) In the edaravone group, neurological function scores on the 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, and 14<sup>th</sup> day were lower than those in the conventional group ( $P < 0.05$ ); (3) PAI-1 levels in the edaravone group were significantly lower than those in the conventional group, and t-PA levels were significantly higher than those in the conventional group ( $P < 0.05$ ) (4) The cerebral infarction size at 14 days after treatment was ( $6.40 \pm 1.03$ ) mm<sup>2</sup> in the edaravone group which accounted for (2.34  $\pm$  0.84)%, and (12.78  $\pm$  1.16) mm<sup>2</sup> in the conventional group which accounted for (6.22  $\pm$  1.05)% ( $P < 0.05$ ). Conclusion: Edaravone treatment can effectively improve serum TNF- $\alpha$  and IL-8 levels as well as the neural function of cerebral infarction rats, suggesting that edaravone has a good therapeutic value for cerebral infarction.

**Keywords:** Rats, cerebral infarction, edaravone, serum TNF- $\alpha$ , IL-8, neural function

## Introduction

Cerebral infarction is a type of cerebrovascular disease with a very high incidence. In recent years, with the enhancement of people's awareness of stressful events in life and the increase of population aging, the incidence of cerebral infarction has gradually increased, cerebral infarction seriously threatens patients' physical health and safety [1, 2]. Although the treatment effect and survival rate of cerebral infarction have been significantly improved with the development of medical technology, the surviving patients will suffer from varying degrees of dysfunction [3].

After the occurrence of cerebral infarction, there will be a variety of physiological and pathological reactions, including energy metabolism disorder in brain tissues, resulting in the excessive formation of oxygen free radicals [4]. Neural therapy is an important clinical tool for

patients with cerebral infarction. Neuroprotective treatment could reduce pathological cell damage and effectively inhibit the formation of neuronal apoptosis [5]. As a free radical scavenger, edaravone is widely used in the treatment of cerebral infarction. It can directly remove substances such as hydroxyl free radicals and reactive oxygen species (ROS), activate antioxidant enzymes, provide electrons of free radicals, and provide the receptors for intracellular ions [6, 7]. Jiang MY et al. [8] found that in the treatment of acute cerebral infarction, edaravone can play a good role in antioxidative stress, and simultaneously extend the treatment window for blood flow recovery of ischemic peripheral tissues, thus offering more time for cerebral infarction treatment.

The current clinical studies mostly focus on patients with cerebral infarction, and animal experiments were rare. This study specifically explores the therapeutic effect of edaravone on

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rats with cerebral infarction. The rats were modeled and divided into groups to assess the effect of treatment on the serum TNF- $\alpha$  and IL-8 levels as well as the neural function, in order to further analyze the mechanism of action of edaravone.

### Materials and methods

#### *Model sourcing*

Sixty healthy SD rats, weighing between 250 g and 300 g, were selected and the rats were maintained in the clear cages exposed to broad-spectrum visible light. The rats were fed food and water, with the humidity at 60%-80% and a temperature of 18-25°C. Rats were housed and managed strictly in accordance with the regulations of the Animal Protection Association. Every procedure was approved by the Animal Care and Use Committee of Jingzhou Central Hospital.

#### *Modelling and treatment*

All rats were established with the focal cerebral ischemia model by the middle cerebral artery occlusion method. Five% chloral hydrate was injected into the peritoneal cavity of the rats for anesthesia.

A small opening was made with ophthalmic scissors at the internal carotid artery "stump", a fishing line (0.23 mm diameter) was guided from the external carotid artery into the internal carotid artery, and stopped when there was a sense of resistance, which is the middle cerebral artery. At the beginning, the suture and the external carotid artery "stump" were ligated, and the neck incision was sutured. All rats were successfully modeled and randomly divided into two groups: edaravone group and conventional group, with 30 rats in each group.

The conventional group was injected with normal saline (3 mg/kg) via the tail vein once daily for 5 days; the edaravone group was injected with edaravone (3 mg/kg) through the tail vein (cat. No. H20050280; Nanjing Xiansheng Dongyuan Pharmaceutical Co., Ltd.), once daily for 5 days.

#### *Outcome measurements*

Serum indicators: The serum TNF- $\alpha$  and IL-8 levels of the two groups were measured before

and after 14 days of treatment by sandwich ELISA. All operations were performed strictly according to the kit instructions.

Neural function: Modified Neurological Severity Score (mNSS) [9] method was used to evaluate neural function before treatment and at 1 day, 3 days, 7 days, and 14 days after treatment. The scale includes 7 items: neurobehavioral score, animal behavioral assessment, neurological severity scores (NSS), beam balance test (BBT), elevated body swing test (EBST), step-down passive avoidance test (SDPAT), and water maze test (WMT); scoring 0-18 points. A higher score indicates worse neural function.

Inflammatory indicators: Plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (t-PA) were measured before and 14 days after treatment with ELISA (Shanghai Enzyme Biotechnology Co., Ltd.).

Three ml of blood was collected via the tail vein before and after treatment. After sitting for half an hour, the blood was centrifuged at a speed of 3200 r/min for 5 min. The supernatant was collected and stored at -80°C.

Cerebral infarction size: After 14 days of treatment, anaesthesia was performed by intraperitoneal injection of chloral hydrate. After decapitation, the brain was removed and rinsed with normal saline, and frozen at -20°C for 10 min. The brains were sliced along the coronal plane with 2 mm thickness. The sections of tissue were stained with 2% TTC solution and observed after 10 minutes. Normal brain tissue presented a red color, and infarcted brain tissue showed a pale color. The cerebral infarction size was calculated with a pathological image analyzer.

#### *Statistical analysis*

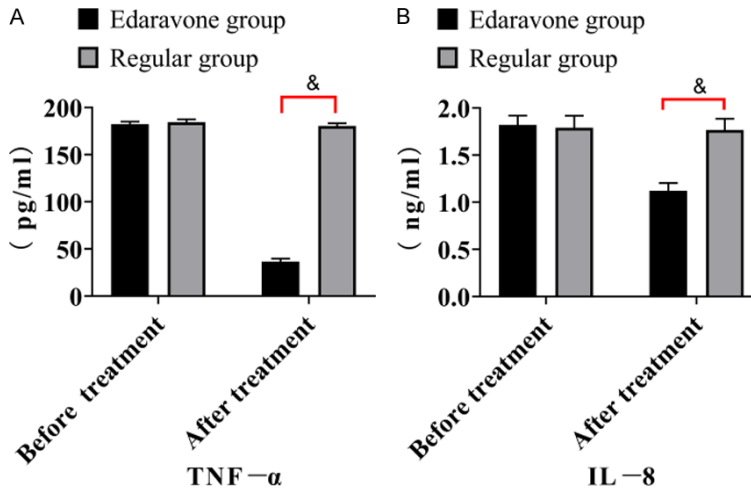
SPSS 22.0 was used for statistical analysis. Measurement data were expressed as mean  $\pm$  standard deviation. Intra-group comparison was performed with paired t test; Enumeration data were expressed as [n (%)], and enumeration data between groups were compared with the  $X^2$  test. Intra-group and inter-group comparisons at different time points were performed with analysis of variance (ANOVA), F test.  $P < 0.05$  indicated statistical significance.

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**Table 1.** Comparison of serum TNF- $\alpha$  and IL-8 levels between the edaravone group and the conventional group ( $\bar{x} \pm s$ )

Group	TNF- $\alpha$ (pg/ml)		IL-8 (ng/ml)	
	Before treatment	After treatment	Before treatment	After treatment
Edaravone group (n=30)	184.26 $\pm$ 20.28	38.75 $\pm$ 8.92*	1.89 $\pm$ 0.19	1.18 $\pm$ 0.14*
Conventional group (n=30)	186.54 $\pm$ 21.34	182.47 $\pm$ 20.79*	1.88 $\pm$ 0.16	1.85 $\pm$ 0.15
T	0.424	34.796	0.221	17.885
P	0.673	0.000	0.826	0.000

Note: Compared with that before treatment in the group, \* $P < 0.05$ .



**Figure 1.** Serum TNF- $\alpha$  and IL-8 levels of the Edaravone group and the Conventional group. Comparison of pre-treatment TNF- $\alpha$  levels in the conventional group with the conventional group,  $P > 0.05$ ; Post-treatment TNF- $\alpha$  levels compared with the conventional group,  $^{\&}P < 0.05$ . The IL-8 level before treatment in the Edaravone group was compared with the conventional group,  $P > 0.05$ ; the IL-8 level after treatment was compared with the conventional group,  $^{\&}P < 0.05$ .

### Results

#### Comparison of serum indicators between the two groups

After treatment, serum levels of TNF- $\alpha$  and IL-8 in the edaravone group were reduced and were significantly lower than those in the conventional group ( $P < 0.05$ ) (Table 1; Figure 1).

#### Comparison of neural function between the two groups

After treatment, the neural function of the conventional group at all time points was not statistically different from those before treatment ( $P > 0.05$ ). The neural scores of the edaravone group were decreased on the 1<sup>st</sup>, 3<sup>rd</sup>, 7<sup>th</sup>, and 14<sup>th</sup> days after treatment ( $P < 0.05$ ) (Table 2; Figure 2).

#### Comparison of inflammatory indicators between the two groups

Before treatment, PAI-1 and t-PA levels in the edaravone group were not significantly different from those in the conventional group ( $P > 0.05$ ). After treatment, the edaravone group showed decreased PAI-1 levels and increased t-PA levels ( $P < 0.05$ ). The PAI-1 level in the edaravone group was significantly lower than that in the conventional group, and the t-PA level was significantly higher than that in the conventional group ( $P < 0.05$ ) (Table 3; Figure 3).

#### Comparison of cerebral infarction size between the two groups

The cerebral infarction size at 14 days after treatment in the edaravone group was (6.40  $\pm$  1.03) mm<sup>2</sup>, which was significantly smaller than that of (12.78  $\pm$  1.16) mm<sup>2</sup> in the conventional group ( $P < 0.05$ ). The infarct size ratio at 4 days after treatment was (2.34  $\pm$  0.84)% in the edaravone group, which was significantly smaller than that of (6.22  $\pm$  1.05)% in the conventional group ( $P < 0.05$ ) (Table 4; Figure 4).

### Discussion

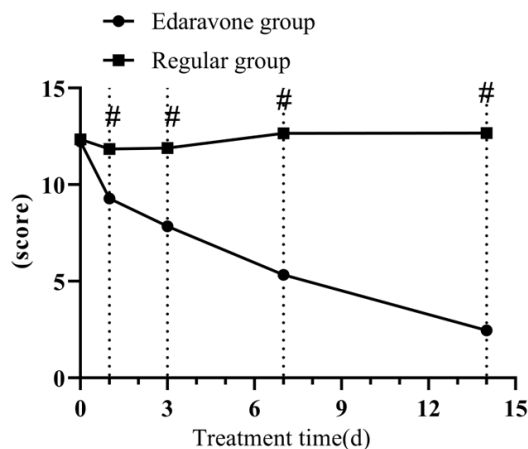
Cerebral infarction is caused by cerebral vascular blockage, impaired cerebral perfusion, hypoxia and ischemia, eventually leading to neuronal damage [10]. The treatment of acute phase after the onset of cerebral infarction is the key factor to improve the prognosis. Intravenous thrombolysis is a commonly used treatment in the acute phase. However, there is

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**Table 2.** Comparison of neural function between the edaravone group and the conventional group ( $\bar{x} \pm s$ )

Group	Cases	Before treatment	1 day after treatment	3 days after treatment	7 days after treatment	14 days after treatment
Edaravone group	30	12.23 $\pm$ 2.61	9.28 $\pm$ 2.42*	7.84 $\pm$ 2.19*.&	5.33 $\pm$ 2.04*.&.#	2.46 $\pm$ 1.09*.&.#.a
Conventional group	30	12.35 $\pm$ 2.66	11.85 $\pm$ 2.47	11.89 $\pm$ 2.31	12.66 $\pm$ 2.11	12.67 $\pm$ 2.50
T		0.176	4.071	6.969	12.679	20.505
P		0.861	0.000	0.000	0.000	0.000

Note: Compared with that before treatment in the group, \* $P < 0.05$ ; compared with 1 day after treatment in the group, & $P < 0.05$ ; compared with 3 days after treatment in the group, # $P < 0.05$ ; compared with 7 days after treatment in the group, a $P < 0.05$ .



**Figure 2.** Neural functions of the Edaravone group and the Conventional group. Neural function of the edaravone group compared with the conventional group before treatment,  $P > 0.05$ ; Edaravone group vs conventional group at 1 day after treatment, \* $P < 0.05$ ; Edaravone group vs conventional group at 3 days after treatment, # $P < 0.05$ ; Edaravone group vs conventional group at 7 days after treatment, # $P < 0.05$ ; Edaravone group vs conventional group at 14 days after treatment, # $P < 0.05$ .

a strict time window for thrombolytic therapy, generally within 6 hours after the onset of the insult [11]. However, the proportion of patients who can successfully receive thrombolytic therapy during this period is relatively small, and the majority of patients need to receive other effective treatments [12].

Clinical practice has found that a large proportion of patients with cerebral infarction could be maintained well with neuroprotective treatment in acute attacks. Edaravone is a widely used neuroprotective agent, which can scavenge free radicals, inhibit lipid peroxidation and reduce hydroxyl radicals [13, 14]. Edaravone treatment can reduce the degree of damage to vascular endothelial cells and nerve

cells, inhibit neuronal apoptosis, thus improve nerve function [15]. In this study, by modeling cerebral infarction in SD rats, it was found that serum TNF- $\alpha$  and IL-8 levels in the edaravone group were significantly lower than those in the conventional group ( $P < 0.05$ ).

Naderi Y et al. [16] found that after cerebral infarction, local hypoxia and ischemia will occur in brain tissue, inflammatory cells will aggregate, and inflammatory factors will be released in large quantities. TNF- $\alpha$  and IL-8 are indicators of early inflammation, which increase rapidly and significantly in the acute phase of cerebral infarction. Zhang Q et al. [17] showed that the number of monocytes expressing IL-8 mRNA was significantly increased in patients with cerebral ischemia, and the plasma IL-8 level was positively correlated with the plasma IL-8 mRNA level, indicating that IL-8 is a maker of leukocyte aggregation in response to the ischemic site. This study showed that the edaravone could regulate the level of inflammation in rats with cerebral infarction. The reason may be that the neuronal damage can be alleviated by reducing the inflammatory response in the brain lesion area in the acute stage of focal cerebral ischemia, thus improving cerebral infarction [18].

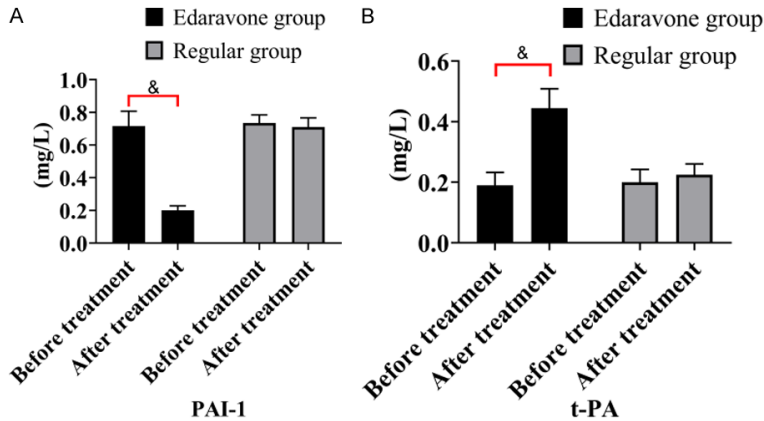
In this study, the neurological scores of the edaravone group were significantly lower than those of the conventional group at 1 day, 3 days, 7 days, and 14 days after treatment ( $P < 0.05$ ), demonstrating that edaravone can significantly improve the neural function of rats with cerebral infarction. Edaravone is an effective neuroprotective agent, which plays a neuroprotective role through its anti-oxidative ability and effectively inhibits the cascade of arachidonic acid [19, 20].

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**Table 3.** Comparison of inflammatory indicators between the edaravone group and the conventional group ( $\bar{x} \pm s$ , mg/L)

Group	PAI-1		t-PA	
	Before treatment	After treatment	Before treatment	After treatment
Edaravone group (n=30)	0.78 $\pm$ 0.10	0.22 $\pm$ 0.03*	0.22 $\pm$ 0.12	0.49 $\pm$ 0.16*
Conventional group (n=30)	0.77 $\pm$ 0.11	0.75 $\pm$ 0.08	0.23 $\pm$ 0.13	0.25 $\pm$ 0.14
T	0.368	33.976	0.310	6.183
P	0.714	0.000	0.758	0.000

Note: Compared with that before treatment in the group, \* $P < 0.05$ .



**Figure 3.** PAI-1 and t-PA levels of the edaravone group and the conventional group. Before treatment, the PAI-1 levels had no significant difference,  $P > 0.05$ ; after treatment, the PAI-1 level in the edaravone group was lower as compared with the conventional group,  $^{\&}P < 0.05$ ; before treatment, the t-PA level had no significant difference,  $P > 0.05$ ; after treatment, the t-PA level in the edaravone group was higher as compared with the conventional group,  $^{\&}P < 0.05$ .

**Table 4.** Comparison of cerebral infarction size between the edaravone group and the conventional group ( $\bar{x} \pm s$ , %)

Group	Cases	Cerebral infarction size	Infarct volume ratio
Edaravone group	30	6.40 $\pm$ 1.03*	2.34 $\pm$ 0.84*
Conventional group	30	12.78 $\pm$ 1.16	6.22 $\pm$ 1.05
T		22.526	15.805
P		0.000	0.000

Note: Compared with the conventional group, \* $P < 0.05$ .

Zhang Y et al. [21] found that edaravone could not only relieve symptoms, but also effectively reduce neurological deficits and improve neural function in the treatment of patients with cerebral infarction. In this study, the PAI-1 level in the edaravone group was significantly lower than that in the conventional group, and the t-PA level was significantly higher than that in the conventional group ( $P < 0.05$ ), suggesting that the edaravone could effectively improve PAI-1 and t-PA levels. Zong L et al. have shown

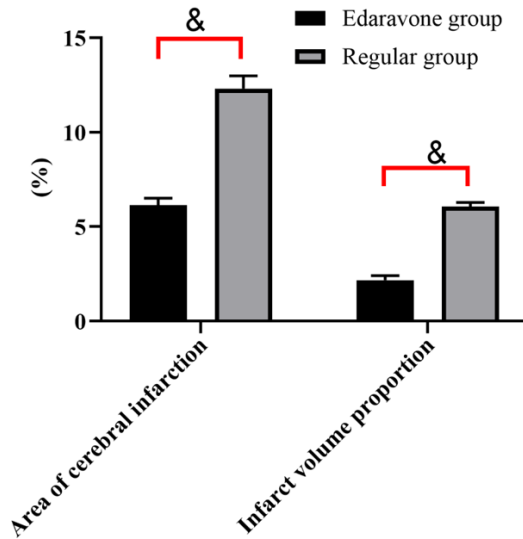
[22] that edaravone can effectively inhibit the expression of PAI-1 and t-PA, reduce cerebral infarction size or volume, and improve the prognosis of cerebral infarction.

In this study, the size of cerebral infarction in the edaravone group was smaller than that in the conventional group, and the proportion of infarct volume was smaller than that in the conventional group at 14 days after treatment ( $P < 0.05$ ). The underlying mechanism may be that edaravone can scavenge free radicals and reduce the induced tissue damage, inhibit neuronal apoptosis, reduce the brain tissue edema, and finally reduce the volume of cerebral infarction [23]. In addition, Yoshida H et al. [24] demonstrated that edaravone can increase the expression of nerve growth factor and promote the growth of astrocytes. A study by Akaiwa K et al. [25] showed that edaravone can reduce the mortality

of patients with cerebral infarction in the acute phase, reduce the volume of cortical infarction, and significantly improve the neural function.

In summary, the treatment of cerebral infarction with edaravone can reduce the levels of TNF- $\alpha$  and IL-8, improve the neural function of rats, and effectively reduce cerebral infarction size. However, this study only compared edaravone treatment with conventional treatment, and only a few indicators were observed, so the

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**Figure 4.** Cerebral infarct size and infarct volume ratio of the edaravone group and the conventional group. Cerebral infarction area,  $^{\&}P < 0.05$ ; Infarct volume ratio,  $^{\&}P < 0.05$ .

results obtained are not sufficiently representative. In future studies, more extensive, deeper and more comprehensive studies should be conducted to further explore the underlying mechanism of edaravone in the treatment of cerebral infarction.

### Disclosure of conflict of interest

None.

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