

## Edaravone, a Free Radical Scavenger, can Effect on the Inflammatory Biomarkers in Acute Ischemic Stroke Patients

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### Abstract

**Background:** This study was aimed to explore whether the free radical scavenger, edaravone, can reduce the inflammatory response providing better outcome along with not only conventional therapy but also recombinant tissue plasminogen activator (rt-PA) thrombolytic therapy.

**Results:** Acute ischemic stroke patients were enrolled (n=64, average 73.3 year-old) for measuring interleukin-6 (IL-6), tumor necrosis factor (TNF $\alpha$ ) and malondialdehyde-modified LDL (MDA-LDL) as inflammatory biomarkers at 3 time points: before medication, 5 hrs and 24hrs following medication. All patients were classified into four groups depending on the medication: conventional therapy without edaravone (n=15), conventional therapy with edaravone (n=34), rt-PA thrombolytic therapy without edaravone (n=4) and rt-PA thrombolytic therapy with edaravone (n=11). Neurological alteration was assessed by NIH Stroke Scale on admission and at 1 month. As the results, the inflammatory markers were reduced in the edaravone treated group, although it was not significant. While, IL-6 was significantly increased at 24 hrs in the thrombolytic group than the conventional group. Patients treated with rt-PA and edaravone exhibited better outcome than only rt-PA treated patients, although there was no difference of the recanalization rate.

**Conclusion:** Edaravone may be able to decrease the amplified inflammatory response following brain infarction, providing better outcome.

**Keywords:** Inflammatory biomarkers; Brain infarction; Acute therapy; Edaravone

### Introduction

The inflammatory response is exacerbated at the acute phase of brain infarction. The amplified inflammation might relate to not only the worsening of ischemic lesion but also the poor outcome [1,2]. Especially, acute thrombolytic therapy by intravenous administration of recombinant tissue plasminogen activator (rt-PA) can cause the increase of inflammation [3,4]. Therefore, the reduction of inflammatory response following ischemia might be critical for the acute treatment of brain infarction. Up to the present, several studies have been reported the effect of statins regarding the reduction of inflammatory response [5,6]. Meanwhile, edaravone, a free radical scavenger, has been used for treatment of acute ischemic stroke patients in Japan since 2001. The effect of edaravone has been reported to reduce oxidative stress [7] and protecting neurovascular unit [8,9], resulting in clinically better outcome [10]. Herein, this study was aimed to investigate how edaravone could reduce the inflammatory response in the acute brain infarction in the clinical situation.

### Methods

#### Patients

Following the approval of the ethical committee of Research Institute for Brain and Blood Vessels –Akita (ref.#09-10), acute

ischemic stroke patients admitted to the hospital within 24 hrs after the onset were consecutively asked to participate in this study between July 2009 and December 2010. Consented patients were enrolled in this study (n=64, 73.3  $\pm$  12.2 y.o.). All patients were clinically diagnosed by magnetic resonance imaging (MRI: Sigma 1.5T, GE healthcare, Tokyo, Japan) using diffusion weighted images (DWI, TR: 5800 sec, TE: 76.2 sec) and T2 weighted images (T2WI, TR: 3600 sec, TE: 96 sec) on admission. Magnetic resonance angiography (MRA) images were constructed by the 3-dimensional time-of-flight method and rendered using the maximum intensity projection method. Then, these patients were classified into cardiogenic embolism (n=33), atherothrombotic infarction (n=20) and lacunar infarction (n=11) following the clinical examinations and records. According to the acute medication, patients were classified into the group of conventional therapy (n=49) and the group of thrombolytic therapy (n=15). For the conventional therapy, anti-platelet medicines such as clopidogrel (75 mg once a day) and cilostazol (100mg twice a day) or anti-coagulation medicine such as warfarin (optimal dose was set by PT-INR 2-3) were adopted to patients following the guideline [11]. For the thrombolytic therapy, rt-PA (alteplase) was intravenously administered within 3 hrs after stroke onset (0.6 mg/kg intravenous drip infusion taking 1hr following 10% bolus injection). For the neuroprotective therapy, edaravone (Radicut®, Mitsubishi Tanabe Pharma Corp. Osaka, Japan) was administered with intravenous drip infusion (30 mg twice a day). The decision of the treatment in each patient was performed following the standard guidelines [12]. Because edaravone has toxicity for kidney function, edaravone was administered to a patient unless who showed kidney dysfunction

(Blood Urea Nitrogen > 30 mg/dl or Serum Creatinine >1.5 mg/dl). Therefore, in each medication group, we could classify all patients into four subgroups: 1) conventional therapy without edaravone, 2) conventional therapy with edaravone, 3) thrombolytic therapy without edaravone and 4) thrombolytic therapy with edaravone. Neurological deficits were measured by National Institute of Health Stroke Scale (NIHSS), and the alteration was assessed by comparing that of on admission and at 1 month later. Recanalization of the occluded artery by rt-PA administration was assessed on the findings of MRA performed on the second day. Hemorrhagic change of the ischemic lesion was assessed by the observations of follow-up computed brain tomography (Aquilion One, Toshiba Medical Systems, Tochigi, Japan). Clinical risk factors were collected from the patient's record. Criteria of risks are as following: hypertension (>140 mmHg of the systolic BP or >90 mmHg of the diastolic BP, or currently prescribed anti-hypertensive drugs), diabetes mellitus (spontaneous blood sugar level >200 mg/dL or currently prescribed anti-diabetic medication), hyperlipidemia (>220 mg/dL serum total cholesterol or >150 mg/dL triglyceride, or currently prescribed anti-dyslipidemic medication), alcohol drinking (>180 ml of sake per day) and smoking. Details of patients' background were presented in Table 1. Blood sampling for measuring the concentration of interleukin-6 (IL-6), tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and malondialdehyde-modified low-density lipoprotein (MDA-LDL), which stands for oxidized LDL, were performed at 3 time points: before medication, 5 hrs and 24 hrs following medication started. Briefly describing the laboratory methods, serum amount of IL-6 and MDA-LDL was measured by enzyme-linked immunosorbent assay (ELISA) method, and serum amount of TNF $\alpha$  was measured by chemiluminescent enzyme immunoassay (CLEIA) method. All blood examination for inflammatory biomarkers was performed by the external laboratory (SRL Inc. Tokyo, Japan).

## Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD). Because the number of patients in the thrombolytic therapy without edaravone group was very small (n=4), we could not adopt data into the statistical analysis. Clinical backgrounds were compared between the edaravone treated group and the no edaravone group by Pearson's  $\chi^2$  test. A comparison of mean value of the NIHSS and the alteration of NIHSS between that of on admission and at 1 month was performed with factorial ANOVA post-hoc test, Scheffe method among three subgroups. The amount of serum IL-6 was compared between the patients of all thrombolytic therapy and the patients of conventional therapy at the different time points by a nonparametric Mann-Whitney's U test. All statistical analysis was performed by JMP9 software (SAS Institute Inc., Cary, NC).

## Results

Clinical backgrounds of all patients were shown in Table 1. Age was significantly younger in the edaravone treated group compared with the no edaravone group. There was no significant difference in the percentage of risk factors between the edaravone treated and the no edaravone groups. The distribution of stroke subtypes was not different between two groups.

Both the conventional and the thrombolytic therapy groups were subdivided into two groups according to whether edaravone was

administered or not (Table 2). The thrombolytic therapy without edaravone group showed relatively higher age compared with other groups. NIHSS on admission was significantly increased in the thrombolytic therapy with edaravone group compared with the conventional groups ( $p < 0.001$ ). The thrombolytic therapy without edaravone group showed higher point of NIHSS at 1 month compared with other groups. Moreover, the reduction of NIHSS at one month was significantly higher in the thrombolytic therapy with edaravone group compared with the conventional group with and without edaravone ( $p = 0.014$  and  $0.003$ , respectively). The recanalization rate was slightly better in the thrombolytic therapy with edaravone compared with the thrombolytic therapy without edaravone group. The rate of the hemorrhagic transformation of the ischemic lesion was the same between the thrombolytic therapy with and without edaravone (4/4 cases and 10/11 cases, respectively). The hematoma at the cortical area was observed in 2 of 4 patients in the thrombolytic therapy without edaravone, while no patients exhibited the cortical hematoma in the thrombolytic therapy with edaravone group.

Edaravone	Used	None
n	45	19
m/f	31/14	13-Jun
Age	69.2 $\pm$ 11.2	78.2 $\pm$ 10.8 ‡
Subtype (%)		
Embolic	20 (44.4)	5 (26.3)
Atherothrombotic	16 (35.6)	11 (57.9)
Lacunar	9 (20.0)	3 (15.8)
rt-PA used (%)	11 (24.4)	4 (21.1)
Risks (%)		
Hypertension	31 (68.9)	16 (84.2)
Hyperlipidemia	11 (24.4)	8 (42.1)
Diabetes Mellitus	9 (20.0)	5 (26.3)
AF	12 (26.7)	5 (26.3)
CKD	2 (4.4)	2 (10.5)
Alcohol	12 (26.7)	3 (15.8)
Smoking	9 (20.0)	3 (15.8)
Past stroke history	9 (20.0)	8 (42.1)

**Table 1:** Background of all cases.

m/f: male female ratio; age: average  $\pm$  standard deviation; embolic: embolic stroke; atherothrombotic: atherothrombotic infarction, lacunar: lacunar infarction, rt-PA: recombinant tissue plasminogen activator, Af: Atrial Fibrillation and CKD: Chronic Kidney Disease. ‡:  $p < 0.01$ .

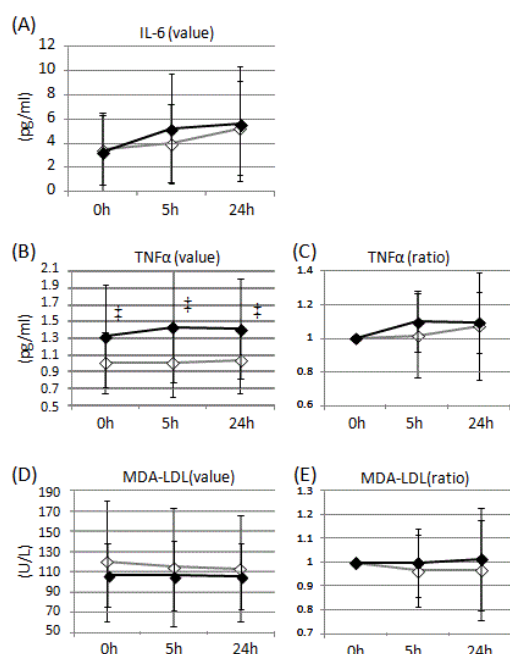
Therapy	Conventional		Thrombolytic	
Edaravone	(-)	(+)	(-)	(+)
n	15	34	4	11
Age	77.7 ± 9.8	70.8 ± 11.6	80.3 ± 15.5	64.3 ± 8.5
NIHSS				
On admission	5.4 ± 6.5	6.6 ± 5.9	19.8 ± 9.9	16.0 ± 5.6 ‡
At 1 month	4.4 ± 6.0	3.4 ± 4.4	18.0 ± 19.2	7.5 ± 5.5
ΔNIHSS	1.0 ± 5.9	3.3 ± 3.6	1.8 ± 17.3	8.5 ± 4.4 **
Recanalization (%)	n.a.	n.a.	2 (50.0)	8 (72.7)

**Table 2:** Neurological alteration after each treatment

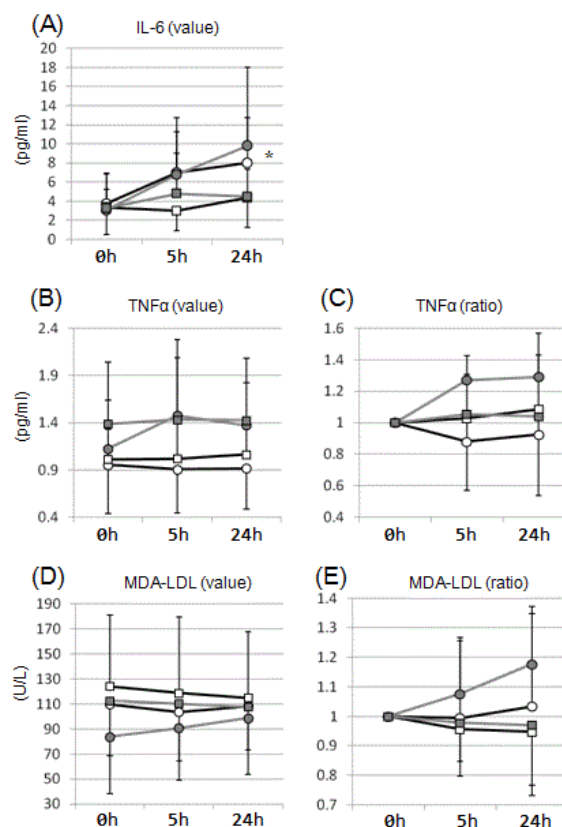
NIHSS: National Institute of Health Stroke Scale,

ΔNIHSS=(NIHSS on admission) – (NIHSS at 1 month). ‡: p<0.001, compared with the conventional groups, and \*\*: p=0.01, compared with the conventional groups.

### Alteration of inflammatory markers



**Figure 1:** The alteration of inflammatory biomarkers (A: IL-6, B and C: TNFα, D and E: MDA-LDL) at different time points regarding with or without edaravone treatment.



**Figure 2**

**Figure 2:** The alteration of inflammatory biomarkers (A: IL-6, B and C: TNFα, D and E: MDA-LDL) at different time points regarding 4 different therapy groups.

The results of inflammatory markers were shown in Figures 1 and 2. The edaravone treated group showed a slight reduction of IL-6 and

TNF $\alpha$  at 5 hrs following onset (Figure 1A and 1C). MDA-LDL was continuously decreased at 5 hrs and 24 hrs following onset (Figure 1E). The value of TNF $\alpha$  was significantly higher in the no edaravone group compared with the edaravone treated group (Figure 1B:  $p=0.001$ ). As shown in Figure 2A, although the level of IL-6 was already increased in the patients with thrombolytic therapy at 5 hrs after medication started, the increase was not observed in the conventional therapy groups. At the time point of 24 hrs, the average amount of IL-6 was significantly increased in the thrombolytic groups compared with the conventional groups (Figure 2A:  $p=0.008$ ,  $9.02 \pm 5.57$  and  $4.44 \pm 3.10$ , respectively).

The measured amount of TNF $\alpha$  presented a widely dispersed among four groups, suggesting the influence of stroke subtypes (Figure 2B). Thus far, the ratio of TNF $\alpha$  was referred for assessing the effect of medication. The conventional therapy did not alter the level of TNF $\alpha$  at both 5 hrs and 24 hrs regardless of edaravone administration. While the rt-PA administration amplified the amount of TNF $\alpha$ , the edaravone concurrent administration could decrease the TNF $\alpha$  level (Figure 2C).

As shown in Figure 2D, the measured amount of MDA-LDL was scattered among individual patients, however, in the calculation of MDA-LDL ratio, the thrombolytic therapy without edaravone group showed a continuous increase compared with other groups (Figure 2E).

Each line graph indicates the measured value of inflammatory biomarkers (A, B and D) and the ratio against first time point (C and E). White diamond and black diamond show the edaravone administered group and the no edaravone group, respectively. (A) The edaravone administered group indicates slightly lower amount of IL-6 compared with the no edaravone group at 5 hrs. (B) The amount of TNF $\alpha$  is significantly higher in the no edaravone group compared with the edaravone administered group throughout the periods. (C) TNF $\alpha$  ratio is also decreased at 5 hrs in the edaravone administered group. (E) MDA-LDL ratio is slightly decreased in the edaravone administered group compared with the no edaravone group at both 5 hrs and 24 hrs.  $\ddagger$ :  $p<0.01$ .

Each line graph indicates the measured value of inflammatory biomarkers (A, B and D) and the ratio against first time point (C and E). Gray circle, white circle, gray square and white square show the thrombolytic therapy without edaravone, the thrombolytic therapy with edaravone, the conventional therapy without edaravone and the conventional therapy with edaravone, respectively. (A) The thrombolytic groups indicate significantly higher amount of IL-6 compared with the conventional groups at 24 hrs regardless of edaravone administration. \*:  $p<0.05$ , between the conventional groups and the thrombolytic groups. (C) Although the conventional therapy groups show no increase of TNF $\alpha$  ratio, the thrombolytic therapy without edaravone group shows the increase of TNF $\alpha$  ratio and the thrombolytic therapy with edaravone shows the decrease of TNF $\alpha$  ratio at both 5 hrs and 24 hrs. (E) Only the thrombolytic therapy without edaravone group shows a continuous increase of MDA-LDL compared with other groups.

## Discussion

In this study, we revealed that a free radical scavenger, edaravone, might be able to reduce the inflammatory response at the acute phase of brain infarction. Moreover, our data showed that the possibility in

which the amplified inflammation by the rt-PA intravenous administration could be also decreased by edaravone.

Actually, the inflammation can be exacerbated by the ischemic cellular damage in the brain lesion. This inflammatory response at acute phase might not only influence on the expansion of ischemic lesion but also lead to the worse outcome [1,2]. Therefore, it can be said that the reduction of inflammation is a critical issue as the acute brain infarction therapy.

Statins have been interested in its effects in which the inflammatory response following ischemia can be reduced [5,6]. However, considering the effective dose and the time to achieve effective concentration, the statin administration has been still under debate regarding acute therapy of brain infarction [13,14]. Meanwhile, in Japan, edaravone has been officially available for clinical use for the treatment of acute brain infarction [10]. Edaravone was reported to decrease oxidative stress resulting in the reduction of inflammation [7,15]. Our previous study reported that the inflammatory response was restrained by edaravone administration in the acute phase of ischemic brain infarction, and the extent of gliosis was reduced at the chronic phase [16].

In this study, inflammatory markers of IL-6 and TNF $\alpha$  were revealed to be increased at the acute phase of brain infarction. Moreover, edaravone was able to decrease this amplification at 5 hrs after medication started, although it was not significant. While, the prominent increase of IL-6 by rt-PA administration could not be reduced by edaravone. Moreover, the increase of MDA-LDL, which stands for the amplified oxidative stress, was observed only in the rt-PA treated group. This oxidative stress might be also reduced by edaravone. The rt-PA expresses the thrombolytic efficacy by means of converting plasminogen to plasmin which could expedite the degradation of fibrin clot. However at the same time, plasmin has been reported to activate MMP3, resulting in the increase of the expression of MMP9 [17]. MMP9 has been reported to disrupt the basal membrane of the endothelial cells, causing decomposition of the blood brain barrier (BBB) [18,19]. Moreover, MMP9 was reported to increase the expression of cytokines such as granulocyte culture-stimulating factor, and promote the activation of inflammatory cells [20]. Furthermore, previous reports suggested that rt-PA could directly raise inflammation by activating astrocytes and microglia [21,22]. According to the clinical studies, the better outcome following thrombolytic therapy might be related to the improvement of inflammatory response [23,24]. Thus far, although rt-PA is useful agent for thrombolytic therapy, it has a problematic issue in which inflammatory response will be exaggerated. Recently, experimental studies have revealed that edaravone could decrease the activation of MMP9 by restricting the expression of nuclear factor (NF $\kappa$ B) in the endothelial cells [25], and protect BBB in the ischemia-reperfusion injury [8,26]. Actually, our observation showed that hemorrhagic formation was not increased in the thrombolytic therapy with edaravone group. Rather, edaravone might be able to restrict hematoma formation. Moreover, edaravone has been reported to increase the rate of recanalization with rt-PA administration [27]. Oxidative stress induced by ischemia will cause the activation of plasminogen activator inhibitor-1 (PAI-1), resulting in the development of thrombosis [28]. Tumor necrosis factor (TNF $\alpha$ ) has been reported to stimulate inflammatory cells for inducing various cytokines such as IL-1, platelet-activating factor (PAF) and PAI-1 [29]. It could be said that edaravone might be able to protect brain tissue



against not only ischemic stress but also inflammatory response which was amplified by the recanalized blood flow by rt-PA.

This study was a prospective observational and not a randomized control study. Therefore, it is undeniable that patients who had not been administered edaravone might have some bias. Actually, edaravone was not adopted if a patient had kidney dysfunction. Moreover, the number of thrombolytic therapy without edaravone group was only 4, and it was not able to perform the statistical analysis on this group. Therefore, since the number of patients in this study is also quite small, these findings have to be examined by a randomized control study with large number of cases. However, edaravone decreased the inflammatory response introduced by rt-PA and provide better recovery of neurological deficit. Therefore, it might be said that edaravone should be introduced at the same time as much as possible in case of acute brain infarction.

Meanwhile, according to the report from the pharmaceutical company (Mitsubishi Tanabe Pharma Corp.), edaravone should not be administered to the elderly patients who have complications of chronic renal dysfunction or dehydration. If edaravone is prescribed for two weeks complying with the informed document, the required clauses for avoiding side effects should be obeyed. Meanwhile, if we precisely looked at the report, such side effects were observed around 4th day after edaravone administrated. Nevertheless, we will propose that it might be acceptable to administrate edaravone at least only one time for reducing the inflammatory response following brain ischemia.

## Conclusion

Even though inflammatory response was amplified following brain infarction, edaravone may be able to present the anti-inflammatory effects, providing better outcome. Further investigation will be intended to confirm our results.

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