

Neuroprotective Effect of an Antioxidant in Ischemic Stroke: Involvement of Neuronal Death Signaling and Blood-brain Barrier Disruption

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Cerebral ischemia is a major cause of death and disability worldwide, and treatments for it are limited. A new therapeutic strategy of brain protection after acute stroke targets the neurovascular unit, such as endothelial cells, astrocytes, microglia, and neurons, instead of neurons only (**Fig. 1**)¹.

Edaravone, a novel free radical scavenger, has been used to treat acute ischemic stroke in Japan. We have previously reported the antiedema effect of the combination therapy of edaravone and mild hypothermia at 35°C following transient focal cerebral ischemia (tFCI)². However, the mechanism of the effect remains unclear. In the present study, we tested the hypothesis that this combination therapy can reduce oxidative stress and the activation of matrix metalloproteinase-9 (MMP-9), which is related to blood-brain barrier (BBB) dysfunction, and brain edema formation after tFCI.

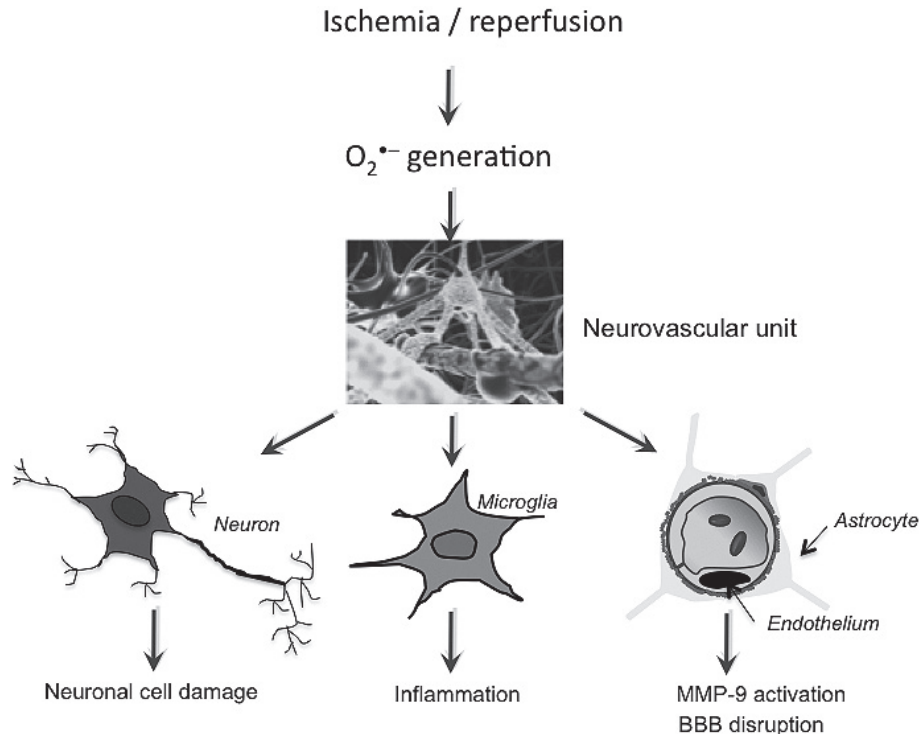


Fig. 1 Vascular unit damage by oxygen radicals after cerebral ischemia and reperfusion

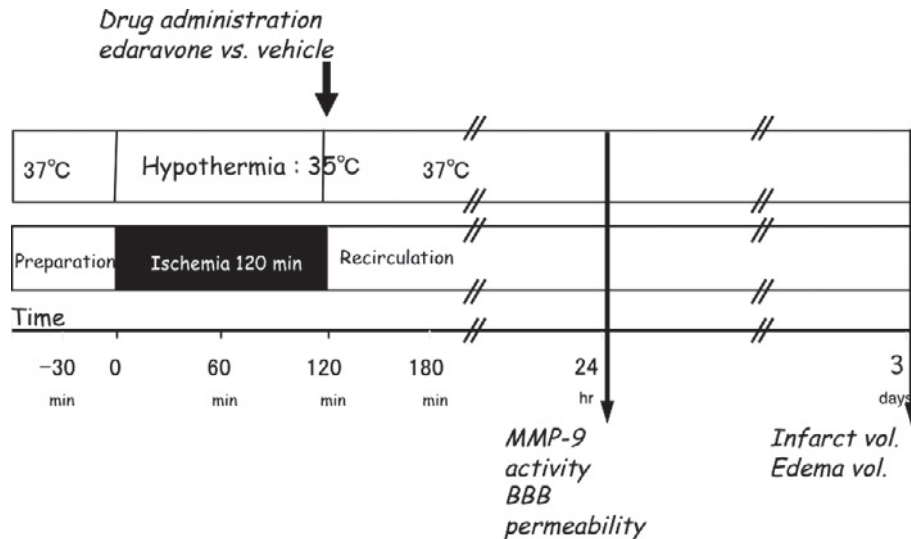


Fig. 2 Experimental procedure

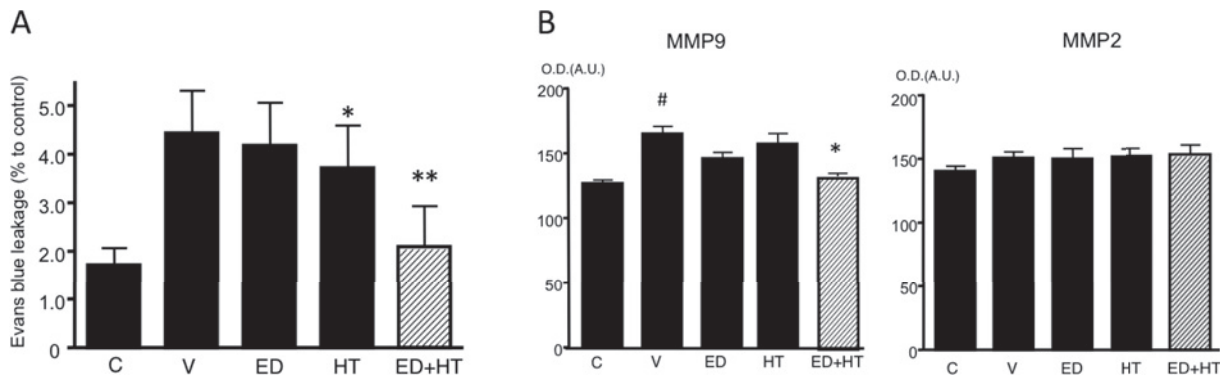


Fig. 3 A: Quantitative assay of Evans blue leakage in each group 24 hours after tMCAO. The level of Evans blue leakage was significantly decreased in the HT group and ED+HT group compared with the V group (* $p < 0.05$, ** $p < 0.01$).

C: control, V: vehicle + normothermia, HT: vehicle + hypothermia, ED: edaravone + normothermia, ED+HT: edaravone + hypothermia

B: Zymographic analysis in each group 24 hours after tMCAO.

ED+HT group significantly decreased MMP-9 activity (* $p < 0.01$) but did not change MMP-2 activity in any group. # $p < 0.01$ vs control

Sprague-Dawley rats were subjected to middle cerebral artery (MCA) occlusion for 2 hours by using an intraluminal suture technique. The rats were randomly divided into 4 groups: 1) vehicle + normothermia (control); 2) vehicle + mild hypothermia; 3) edaravone + normothermia; and 4) edaravone + mild hypothermia. Temporal muscle and rectal temperatures were maintained during ischemia at $37^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in the normothermic groups or at $35^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ in the hypothermic groups by means of a thermal regulatory system. After reperfusion, the rats were decapitated. The hemisphere of the affected side was removed at 24 hours and 3 days. **Figure 2** shows the procedures for this experiment. The activity of MMP-9 and MMP-2 was assessed with with gelatin zymography, and BBB permeability was investigated by Evans blue method. The size of the cerebral infarction or edema was evaluated with 2,3,5-triphenyltetrazolium chloride staining.

There were no statistically significant differences in the mean arterial blood pressure, pH, PaCO_2 , PaO_2 , or blood glucose levels among the experimental groups. The combined treatment with edaravone and mild hypothermia significantly reduced Evans blue leakage (**Fig. 3A**) and MMP-9 activity 24 hours after reperfusion

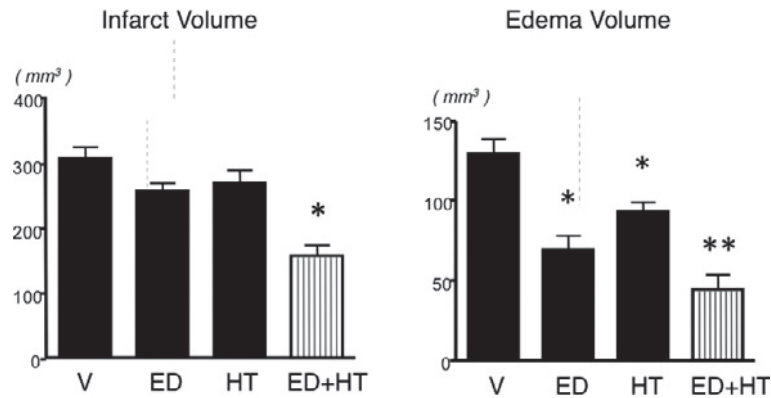


Fig. 4 Infarct and edema volume of 3 days after tMCAO in each group. Edema volume was significantly reduced in the ED group and the ED+HT group (** $p < 0.01$, * $p < 0.05$), but only in the ED+HT group, infarct volume was decreased 3 days after tMCAO (** $p < 0.01$).

(Fig. 3B) and decreased edema and infarct volume 3 days after reperfusion (Fig. 4). The MMP-2 level was not significantly different between these 4 groups (Fig. 3B). Treatment with edaravone alone or hypothermia alone reduced edema volume but not infarct volume. We also found that the oxidized hydroethidine signals were decreased to a greater extent in the combined therapy group than in the control group.

These results demonstrate that edaravone combined with mild hypothermia reduces BBB disruption by blocking MMP-9 activity and attenuates edema formation and infarction by suppressing free radical production following tFCI in rats. We suggest that this combination therapy has a potent neurovascular protective effect after acute ischemic stroke.

References

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2. Nito C, Kamiya T, Amemiya S, et al: The neuroprotective effect of a free radical scavenger and mild hypothermia following transient focal ischemia in rats. *Acta Neurochir Suppl* 2003; 86: 199-203.