

Edaravone protects against tissue damage in the lung and kidney induced by myoneuropathic metabolic syndrome

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PURPOSE: Free radicals have been implicated in myoneuropathic metabolic syndrome (MNMS), which not only damages muscles but also the kidneys and lungs. It was recently shown that the free radical scavenger edaravone suppressed reperfusion injury in rat extremities. The present study evaluated whether edaravone also protects against MNMS-induced tissue damage in the lungs and kidneys after reperfusion injury of rat extremities.

METHODS: Ten male Lewis rats (mean [\pm SD] weight 508 \pm 33 g) were divided into two groups. The MNMS models were created by clamping the bilateral common femoral arteries for 5 h, followed by declamping. In another group, 3.0 mg/kg of edaravone was injected into the peritoneal cavity before clamping the bilateral common femoral arteries. Five hours after starting reperfusion, the kidneys and

Free radicals cause lower extremity reperfusion injury and myoneuropathic metabolic syndrome (MNMS), which damages not only muscles but also the kidneys and lungs (1). Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, C₁₀H₁₀N₂O, molecular weight = 174.2; Radicut, Mitsubishi Tanabe Pharma Co, Japan) was the first free radical scavenger introduced for the treatment of acute cerebral infarction (2). We previously suggested that edaravone also suppresses reperfusion injury in rat extremities (3,4).

In the present study, we focused on other organs – the kidneys and lungs. We evaluated whether edaravone can protect against MNMS-induced tissue damage in the kidneys and lungs after reperfusion injury of rat extremities.

METHODS

MNMS model

All animal procedures complied with the Hyogo College of Medicine Animal Research Committee criteria (No B-09-010). Ten male Lewis rats (mean [\pm SD] weight 508 \pm 33 g) were divided into two groups, and treated with either edaravone (3.0 mg/kg; edaravone group) or the same volume of saline (control group).

MNMS was induced by clamping the bilateral common femoral arteries for 5 h, followed by removal of the clamp to

lungs were harvested from each rat for histological study (n=10). Kidney damage was expressed as the number of infiltrating cells in the glomeruli. Lung damage was expressed as the percentage area of the alveolar wall thickness with cellular infiltration, using computerized densitometry.

RESULTS: Kidneys in the edaravone group showed less cellular infiltration than in the control group (62.2 \pm 2.4 cells versus 75.8 \pm 3.6 cells per glomerulus, respectively; P=0.002). Lungs in the edaravone group also showed a significantly lower percentage of damaged lung tissue area than in the control group (20.5 \pm 1.5% versus 63.6 \pm 2.8%, respectively; P<0.001).

CONCLUSION: The results suggest that the free radical scavenger edaravone might protect against kidney and lung damage induced by MNMS after reperfusion injury of rat extremities.

Key Words: Edaravone; Free radical; Myoneuropathic metabolic syndrome (MNMS)

allow reperfusion, as previously described (3,4). Complete general anesthesia was induced before MNMS by intraperitoneal administration of 30 mg/kg of sodium pentobarbital (Nembutal; Abbott Laboratories, USA). At the same time, 3.0 mg/kg of edaravone or the same volume of saline was injected into the peritoneal cavity preoperatively. Under the microscope (OME-J&N J73507R, Olympus, Japan) at original magnification \times 30, both common femoral arteries were clamped for 5 h after ligating both the deep femoral arteries and epigastric arteries. The rat lower extremity reperfusion injuries were subsequently induced by removing the clamps from the bilateral common femoral arteries after 5 h.

Histological study

The kidneys and lungs were harvested bilaterally for histological study. Tissue sections (5 μ m thick) of the kidney and lung were taken and immediately stained with hematoxylin and eosin.

Kidney damage was expressed as the number of infiltrating cells in the glomeruli. Each number of cells was counted in 10 glomeruli at original magnification \times 400, in three different fields. The mean number of viable cells in each section was calculated and expressed per area (cells per glomerulus).

Lung damage was expressed as the percentage area of the alveolar wall thickness with cell infiltration using computerized

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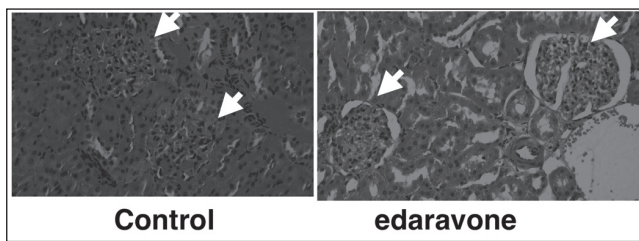


Figure 1) Representative kidney sections of the control group and edaravone group (hematoxylin and eosin stain, original magnification $\times 200$). Arrows indicate glomeruli

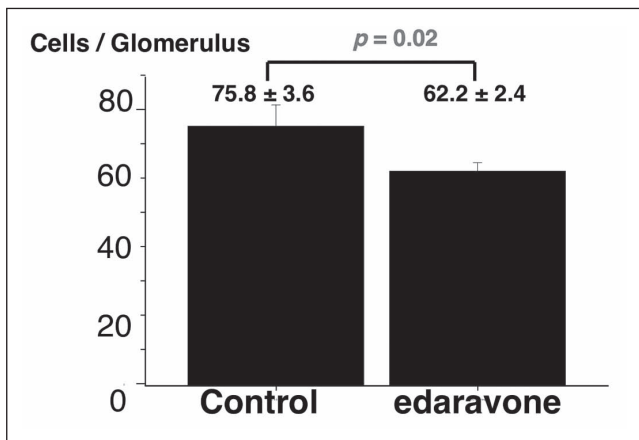


Figure 2) Comparison of the number of infiltrating cells per glomerulus in the control group and edaravone group. Results are expressed as mean \pm SEM

densitometry (Image J, version 1.37, National Institutes of Health, USA; <http://rsb.info.nih.gov/ij>). On Image J densitometry, positive areas were defined as a number of dots between 125 and 250 over.

Statistical analysis

All results are expressed as mean \pm SEM. The results were statistically analyzed using unpaired *t* tests (Stat View J-4.5 for Macintosh, Abacus Concept, USA). $P < 0.05$ was considered to be statistically significant.

RESULTS

There were no operative deaths in either group.

Kidney damage

Representative kidney sections after reperfusion injury of rat lower extremities are shown in Figure 1. In the control group, there was extensive cellular infiltration of the glomeruli. However, in the edaravone group, there was less cellular infiltration in the glomeruli.

A statistical comparison of kidney cellular infiltration is shown in Figure 2. In the edaravone group, cellular infiltration was significantly lower than that in the control group (62.2 ± 2.4 cells per glomerulus versus 75.8 ± 3.6 cells per glomerulus, respectively; $P = 0.02$).

Lung damage

The lungs from the saline-treated control group had extensive swelling. In contrast, the lungs from the edaravone group were filled with air, similar to normal lungs.

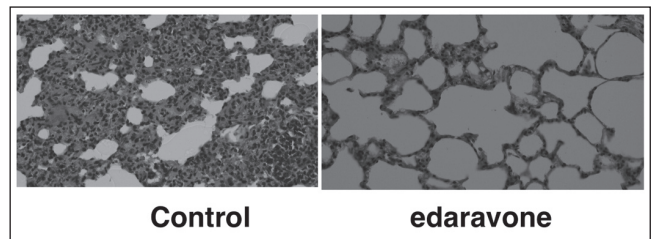


Figure 3) Representative lung sections of the control group and edaravone group (hematoxylin and eosin stain, original magnification $\times 200$)

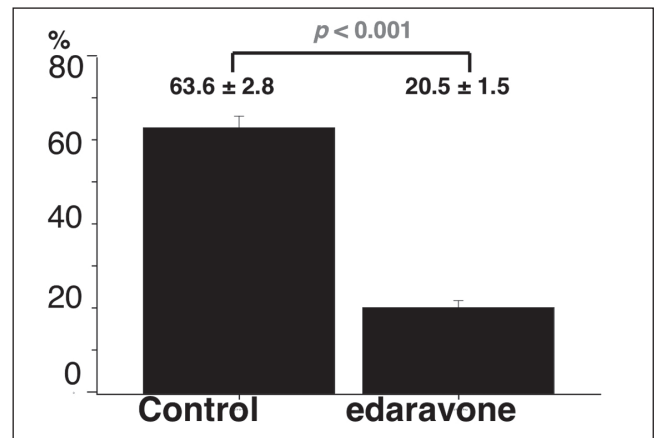


Figure 4) Comparison of percentage area of the alveolar wall thickness (damaged area of lung tissue) in the control group and edaravone group. Results are expressed as mean \pm SEM

Representative lung sections after reperfusion injury are shown in Figure 3. In the control group, there was extensive cellular infiltration in the alveolar wall and a small amount in the lung cavity. The alveolar wall was very thick in the control group. In contrast, in the edaravone group there was less cellular infiltration and the lung cavity appeared nearly normal. Furthermore, the alveolar walls were very thin.

The percentage of the alveolar wall thickness (lung damage) is shown in Figure 4. In the edaravone group, the mean percentage of alveolar wall thickness was significantly lower than that in the control group ($20.5 \pm 1.5\%$ versus $63.6 \pm 2.8\%$, respectively; $P < 0.001$).

DISCUSSION

Free radicals are responsible for MNMS induced by lower extremity reperfusion injury (1). In our previous studies (3,4), we showed that edaravone suppressed reperfusion injury in a rat model. In another *in vivo* rat study (5), we showed that edaravone also suppressed endothelial cell injury, resulting in postoperative intimal hyperplasia of the vein graft.

In the present study, we evaluated the effect of edaravone on tissue damage in the kidneys and lungs. We found that both kidneys and lungs in the edaravone group showed significantly less cellular infiltration after reperfusion injury. This may offer a clinical advantage because edaravone is effective not only in muscles, but it also protects against tissue damage in kidneys and lungs caused by lower extremity ischemia. Therefore, results of the present study suggest that edaravone has a protective effect against tissue damage resulting from MNMS after reperfusion injury in rat extremities.

The *in vitro* mechanism of edaravone as a peroxy radical scavenger was first reported in 1996 (6). The mechanism of edaravone after acute cerebral infarction is believed to involve the scavenging effect of peroxy free radicals (7). Moreover, edaravone reduced mitochondrial damage in homogenate made from ischemic incubated rat brain (8). Mitochondria are a rich source of free radicals; however, they also contain a free radical scavenger (superoxide dismutase). Our recent immunohistological study (4) showed that edaravone can maintain high levels of mitochondrial activity after reperfusion injury in rat lower extremities. Recently, using an electron microscope, we observed well-characterized mitochondrial swelling in the lower extremities of rats after reperfusion injury (unpublished data). Therefore, we believe that the mechanism of edaravone is protection from mitochondrial reperfusion damage to the lower extremities, which may lead to MNMS in the lungs and kidneys.

As the next step in the investigation of the clinical application of edaravone, we are identifying the types of cells infiltrating the kidneys and lungs. We suspect that macrophages and monocytes might be infiltrating the kidneys and lungs

because these cells have chemoattractant activity. However, ED-1 staining, which is specific for rat macrophages and monocytes, demonstrated only a few cells (unpublished data). Thus, more studies are needed to determine the precise effects of edaravone before it is used clinically.

CONCLUSION

We provide evidence that the free radical scavenger edaravone might protect against tissue damage induced by MNMS in the lungs and kidneys after reperfusion injury in rat lower extremities.

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