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The antioxidant edaravone attenuates ER-stress-mediated cardiac apoptosis and dysfunction in rats with autoimmune myocarditis

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Abstract

Experimental autoimmune myocarditis (EAM) is mediated by myocardial infiltration by myosin-specific T-cells secreting inflammatory cytokines. In this study, rat models of EAM were prepared by injection with porcine cardiac myosin. One week after immunization, edaravone was administered intraperitoneally at 3 or 10 mg/kg/day to rats for 2 weeks. Cardiac function was measured by haemodynamic and echocardiographic studies and TUNEL assay was performed. Left ventricular (LV) expression of NADPH oxidase sub-units ($p47^{phox}$ and $p67^{phox}$), pro-inflammatory cytokines (TNF-α), endoplasmic reticulum (ER) stress signalling proteins (GRP78, caspase-12 and GADD153) and mitogen-activated protein kinase (MAPK) family proteins (phospho-p38 MAPK and phospho-JNK) were measured by western blotting. Edaravone improved LV function in a dose-dependent manner. Central venous pressure was significantly low and LV ejection fraction and fractional shortening was significantly high in edaravone groups compared with those in the vehicle group. In addition, edaravone treatment down-regulated LV expressions of $p47^{phox}$, TNF-α, GADD153, phospho-p38 MAPK and phospho-JNK. Furthermore, the LV expressions of $p67^{phox}$, GRP78, caspase-12 and TUNEL-positive cells of rats with EAM treated with edaravone were significantly low compared with those of the vehicle group. These findings suggest that edaravone ameliorated the progression of EAM by inhibiting oxidative and ER stress and, subsequently, cardiac apoptosis.

Keywords: *Edaravone, oxidative stress, endoplasmic reticulum stress, apoptosis, experimental autoimmune myocarditis*

Abbreviations: *BCA, bicinchoninic acid; CHOPC/EBP, homologous protein; CVP, central venous pressure; ±dP/dt, rate of intra-ventricular pressure rise and decline; EAM, experimental autoimmune myocarditis; EF, ejection fraction; FS, fractional shortening; GADD153, growth arrest and DNA damage-inducible gene 153; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GRP78, glucose regulated protein 78; HRP, horseradish peroxidase; HSP, heat shock proteins; IL-1β, interleukin-1 beta; JNK, c-Jun NH2-terminal protein kinase; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; LVEDP, left ventricular end-diastolic pressure; LVP, left ventricular pressure; MBP, mean blood pressure; NADPH, nicotinamide adenine dinucleotide phosphate; p38 MAPK, p38 mitogen-activated protein kinase; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substance; TNF-α, tumour necrosis factor-α; TUNEL, terminal deoxynucleotidyl transferase nick end labelling.*

Introduction

Myocarditis is an inflammation of the cardiac muscle and is caused by various factors such as infection, autoimmunity, medicines, viruses and bacilli [1]. It is a disease with a variable clinical presentation, ranging from asymptomatic to a fatal outcome. Experimental autoimmune myocarditis (EAM) is similar to giant cell myocarditis [2]. Giant cell myocarditis is more

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likely to progress to dilated cardiomyopathy than lymphocytic myocarditis. It is a rare disease of unknown aetiology characterized by the presence of multinucleated giant cells in the myocardium. EAM frequently precedes the development of dilated cardiomyopathy [3]. Human myocarditis can be classified into lymphocytic myocarditis and giant cell myocarditis according to the histopathologic findings. It is well understood that myocarditis is a kind of organ-destructive autoimmune disease mediated by T-cell immunity. EAM, the clinical and pathological features of which are quite different from those of viral infection, was provoked in Lewis rats by immunization with cardiac myosin fragments [4]. EAM leads to the release of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals. Oxidative stress plays an important role in the pathogenesis and development of cardiovascular disease, including hypertension, diabetes, arteriosclerosis, cardiac infarction and heart failure. Oxidative stress on cardiac myocytes is caused by an increase in ROS formation and a decrease in the antioxidative potential. An increase in oxidative stress is recognized to play an important role in genesis of endothelial dysfunction and recent studies implicate NADPH oxidase as a major source of ROS involved in this abnormality [5]. NADPH oxidase is the only source whose primary function is ROS generation and it appears to be especially well suited for involvement in redox signalling. ROS and oxidative stress play a central role in apoptosis and endoplasmic reticulum (ER) stress [6,7]. ER stress triggers complex adaptive or pro-apoptotic signalling defined as the unfolded protein response, which is involved in several pathophysiological processes [8]. At least three apoptotic pathways are known to be involved in this apoptotic event. The first is transcriptional activation of the gene for C/EBP homologous protein (CHOP). The second is activation of the cJUN NH2-terminal kinase (JNK) pathway, which is mediated by formation of the 'inositol requiring 1 (Ire1)-TNF receptor-associated factor 2 (TRAF2)-apoptosis signal-regulating kinase 1 (ASK1)' complex. The third is activation of ER-associated caspase-12. Caspase-12 is activated by ER stress, but apparently not by death-receptor-mediated or mitochondria-targeted apoptotic signals [9].

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel free-radical scavenger. It acts as an antioxidant by trapping hydroxyl radicals, quenching active oxygen and so on. It has been used in patients with acute brain infarction [10]. Edaravone has been shown to prevent brain oedema after ischemia and reperfusion injury in animal models [11–16]. Moreover, it has been shown that edaravone has preventive effects on myocardial injury following ischemia and reperfusion in patients with myocardial infarction [17]. Recently, Fukudome et al. [18] reported that edaravone attenuates the severity of hyperglycaemia and insulitis in a multiple low-dose streptozotocin model of type 1 diabetes and the effect of edaravone might be due to an inhibition of oxidative stress in islet β cells. In addition, edaravone prevents anthracycline-induced myocardial cell death [19]. Moreover, edaravone protects against hypoxia/ischemia-induced ER dysfunction [20]. Nevertheless, it remains to be determined whether edaravone protects against apoptosis in autoimmune myocarditis.

The present study was undertaken to assess whether edaravone attenuates myocardial apoptosis and decreases ER stress of EAM in rats. In addition, we examined the effect of edaravone on cardiac function in the same model.

Materials and methods

Animals and protocol

Male Lewis rats (8 weeks old) were purchased from Charles River, Japan (Yokohama, Kanagawa, Japan). EAM was induced by immunization with purified pig cardiac myosin as previously described [21]. The morbidity of EAM was 100% in the immunized rats using our protocol [21]. Seven days after myosin immunization the surviving rats were treated with edaravone by intraperitoneal administration for 2 weeks. We designated three groups: Group V, saline ($n = 8$); Group E3, 3 mg/kg edaravone ($n = 8$); and Group E10, 10 mg/kg edaravone. Age-matched Lewis rats were used as normal controls as indicated (Group N, $n = 5$). Edaravone was kindly supplied by Mitsubishi Tanabe Pharma (Tokyo, Japan). On the 21st day, haemodynamic parameters were measured under halothane anaesthesia and blood samples were then obtained from the inferior vena cava. Then, the heart was immediately excised. The heart was separated and weighed and then divided into three parts. The mid-ventricle was fixed in 10% formalin for histopathological examination. The apical and basal ventricles were immediately frozen in liquid nitrogen, and stored at –80°C. Throughout the studies, all animals were treated in accordance with the guidelines for animal experimentation of our institute.

Haemodynamic and echocardiographic studies

Rats were anaesthetized with 2% halothane in O_2 during the surgical procedures to measure the haemodynamic parameters and then this concentration was reduced to 0.5% to minimize the anaesthetic effect on haemodynamic parameters. Mean blood pressure, central venous pressure, peak left ventricular (LV) pressure (LVP), left ventricular end-diastolic pressure (LVEDP) and the rate of intraventricular pressure rise and decline ($\pm$dP/dt) were measured in accordance with a procedure described previously [22]. Two-dimensional echocardiographic studies

were performed under 0.5% halothane using an echocardiographic machine equipped with a 7.5-MHz transducer (SDD-5500; Aloka, Tokyo, Japan). M-mode tracings were recorded from the epicardial surface of the right ventricle and the short-axis view of the left ventricle was recorded to measure the LV dimension in diastole (LVDd) and the LV dimension in systole (LVDs). LV fractional shortening (FS) and ejection fraction (EF) were calculated as diastolic dimension minus systolic dimension divided by diastolic dimension, expressed as a percentage. The study was performed in a blinded manner.

Terminal deoxynucleotidyl transferase nick end labelling (TUNEL) assay

Frozen LV tissues embedded in OCT compound were cut into 4-μM-thick sections and fixed in 4% paraformaldehyde (pH 7.4) at room temperature. TUNEL assay was performed as specified in the *in situ* apoptosis detection kit (Takara Bio. Inc.; Shiga, Japan) using paraffin sections. For each animal, four sections were scored for apoptotic nuclei. Only nuclei that were clearly located in cardiac myocytes were considered.

Western blot analysis

Proteins were extracted from freshly frozen heart. Myocardial tissue was homogenized in a lysis buffer containing 50 mmol/L Tris HCl (pH 7.4), 200 mmol/L NaCl, 20 mmol/L NaF, 1 mmol/L Na_3VO_4 and 0.01% 2-mercaptoethanol with protease inhibitors. Then, samples were incubated at 37°C for 1 h, centrifuged at 15 000 rpm for 10 min at 4°C and supernatants were stored at -80°C until use. Total protein concentration in samples was measured by bicinchoninic acid (BCA) method. For the determination of protein levels of $p47^{phox}$, $p67^{phox}$, tumour necrosis factor-α (TNF-α), glucose regulated protein 78 (GRP78), caspase-12, growth arrest and DNA damage-inducible gene 153 (GADD153), p38 mitogen-activated protein kinase (p38 MAPK), phospho-p38 MAPK, JNK, phospho-JNK, with equal amounts of protein extracts (30 μg) were separated by 10% and 12.5% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (Bio-Rad, CA) and electrophoretically transferred to nitrocellulose membranes. Membranes were blocked with 1% non-fat dry milk and 1% BSA (Sigma, St. Louis, MO) in TBS-T (20 mM/l Tris, pH 7.6, 137 mM/l NaCl and 0.05% Tween). All antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), aside from phospho-p38 MAPK, p38 MAPK, phospho-JNK and JNK (Cell Signaling Technology, Beverly, MA). After incubation with primary antibody, the bound antibody was visualized with the respective horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) and chemiluminescence developing agents (Amersham Biosciences, Buckinghamshire, UK). The level of cardiac glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was estimated in every sample. Films were scanned and band densities were quantified with densitometric analysis using Scion Image program (Epson GT-X700, Tokyo, Japan). Finally, Western blot data were normalized with cardiac GAPDH. MAPK activation was quantified by normalizing phospho-MAPK expression level with MAPK expression in the same sample.

Statistical analysis

All the values are represented as mean ± SEM. Statistical assessment of the groups was performed by one-way analysis of variance (ANOVA), followed by

Table I. Changes in haemodynamic, echocardiographic parameters in rats with EAM after treatment with edaravone.

	Group N	Group V	Group E3	Group E10
Body weight (g)	338 ± 5	204 ± 11**	248 ± 6**	250 ± 8**
Heart weight (g)	0.85 ± 0.01	1.13 ± 0.03**	1.24 ± 0.04**	1.00 ± 0.04$^{*,\#\#,\dagger\dagger}$
Heart weight/body weight ratio (g/kg)	2.53 ± 0.02	4.72 ± 0.12**	5.01 ± 0.14**	4.01 ± 0.07$^{**,\#\#,\dagger\dagger}$
Heart rate (beats/min)	379 ± 12	340 ± 23	355 ± 28	358 ± 25
Mean blood pressure (mmHg)	95 ± 4	62 ± 2**	68 ± 5**	75 ± 3**
Central venous pressure (mmHg)	0.8 ± 0.4	9.0 ± 1.1**	5.3 ± 1.4*	2.8 ± 0.5$^{\#\#}$
LVP (mmHg)	124 ± 3	90 ± 6*	86 ± 6**	87 ± 8**
LVEDP (mmHg)	21.4 ± 3.0	39.0 ± 3.4**	37.7 ± 2.6**	33.9 ± 6.0**
dP/dt max (mmHg/min)	5836 ± 305	4054 ± 1244	3450 ± 452	3746 ± 498
dP/dt min (mmHg/min)	8248 ± 648	3930 ± 674**	3326 ± 332**	3765 ± 600**
LVDd (mm)	7.32 ± 0.24	7.58 ± 0.36	6.23 ± 0.72	6.87 ± 0.42
LVDs (mm)	4.5 ± 0.21	6.4 ± 0.26**	4.7 ± 0.58$^{\#}$	4.6 ± 0.31$^{\#\#}$
FS (%)	38.4 ± 2.2	15.5 ± 3.6**	26.2 ± 1.6$^{*,\#}$	32.6 ± 1.38$^{\#\#}$
EF (%)	74.0 ± 2.5	36.2 ± 7.2**	57.5 ± 2.7$^{\#}$	66.8 ± 1.9$^{\#\#}$

Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg); LVP, left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt, rate of intra-ventricular pressure rise and decline; LVDd, left ventricular dimension in diastole; LVDs, left ventricular dimension in systole; FS, fractional shortening; EF, ejection fraction. $^{**}p < 0.01$ and $^{*}p < 0.05$ vs Group N, $^{\#\#}p < 0.01$ and $\#p < 0.05$ vs Group V, $^{\dagger\dagger}p < 0.01$ vs Group E3. Values are mean ± SEM.

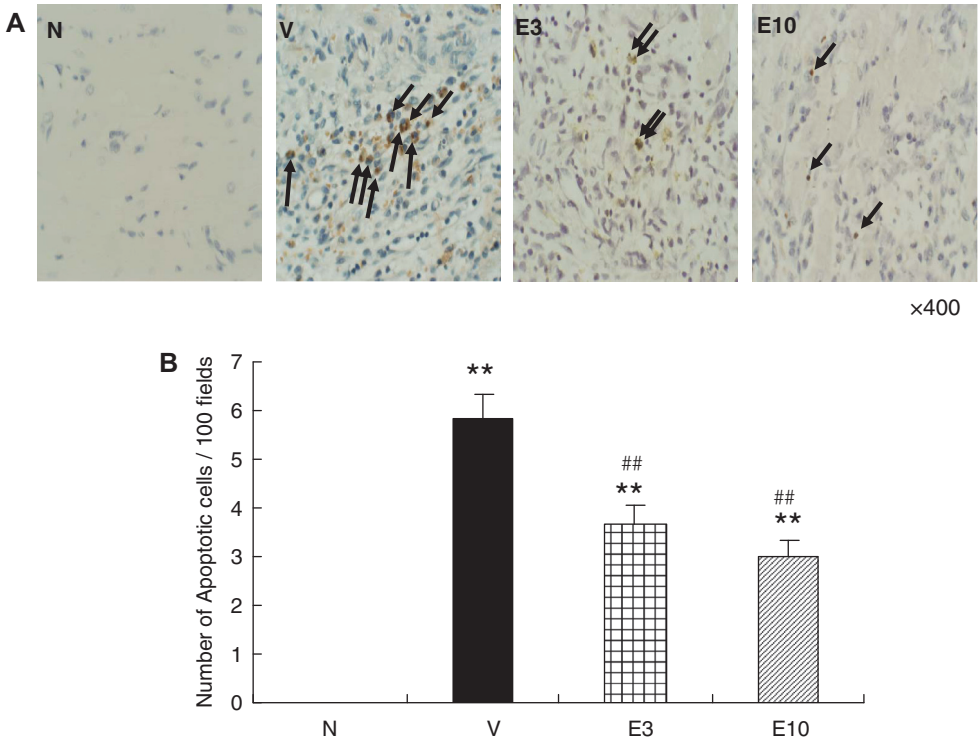

Figure 1. Detection of apoptotic myocytes using the terminal deoxynucleotidyl transferase nick end labelling (TUNEL) technique. (A) Myocardial tissue sections stained for TUNEL-positive apoptotic nuclei (indicated by arrows). Magnification ×400. (B) Quantification of TUNEL-positive apoptotic cardiac myocytes. Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg); $^{**}p < 0.01$ vs Group N, $^{\#\#}p < 0.01$ vs Group V, $^{\dagger}p < 0.05$ vs Group E3. Arrows indicate TUNEL-positive apoptotic nuclei. Values are mean ± SEM.

Tukey's test. The differences were considered significant at $p < 0.05$.

Results

Effects of edaravone on body weight and heart weight

The body weights in Group V and Groups E3 and 10 were significantly decreased compared with that in Group N (Table I). The heart weight and the heart weight-to-body weight ratio in Group V were significantly increased compared with those in Group N. Group E10 exhibited significantly reduced heart weight and heart weight-to-body weight ratio compared with those in Group V (Table I).

Effects of edaravone on haemodynamic and echocardiographic parameters

There were no significant changes in heart rate, dP/dt max and LVDd among the four groups of rats. Central venous pressure (CVP) was significantly increased in Group V compared with that in Group N. Group E10 exhibited significantly decreased CVP compared with that in Group V. Mean blood pressure and LVP in Group V were lower than those in Group N. LVEDP was significantly increased in Group V compared with that in Group N. LVDs was significantly decreased in Groups E3 and E10 compared with that in Group V. Moreover, FS and EF were significantly decreased in Group V compared with those in Group N. Furthermore, FS and EF were significantly increased in Groups E3 and E10 compared with those in Group V in a dose-dependent manner (Table I).

Effect of edaravone in myocardial apoptosis

TUNEL-positive nuclei were rare or absent in the hearts of normal rats, whereas the number of TUNEL-positive nuclei was significantly increased in Group V (Figure 1B). Treatment with edaravone significantly decreased the number of TUNEL-positive nuclei in the myocardium in a dose-dependent manner (Figure 1B). A representative microphotograph of TUNEL staining is shown in Figure 1A.

Effects of edaravone on myocardial protein expressions of p47^{phox}, p67^{phox}, TNF-α, GRP78, caspase-12, GADD153, p38 MAPK and JNK

Western blot analysis showed that the p67^{phox} protein level in cardiac tissue was significantly increased in Group V (Figures 2A and C) compared with that in Group N and the myocardial protein expression of p67^{phox} was significantly decreased in Group E10

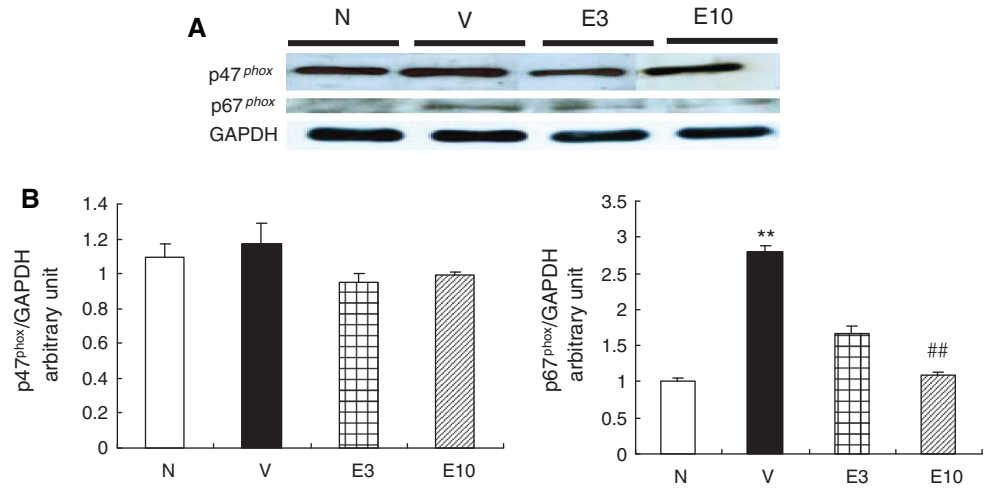

Figure 2. Myocardial expressions of p47^{phox}, p67^{phox} and GAPDH. (A) Representative western blots showing specific bands for p47^{phox}, p67^{phox} and GAPDH as an internal control. An equal amount of protein sample obtained from whole ventricular homogenate was applied in each lane. These bands are representative of three separate experiments. (B and C) Densitometric data of protein analysis. The mean density value of p47^{phox}, p67^{phox} was expressed as a ratio relative to that of GAPDH. Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg); $^{**}p < 0.01$ vs Group N, $^{\#\#}p < 0.01$ vs Group V. Values are mean ± SEM.

compared with that in Group V (Figures 2A and C). Although the effect was not significant, edaravone treatment tended to decrease the myocardial protein expressions of p47^{phox} and TNF-α (Figures 2A and B and 3A and B). The myocardial protein levels of GRP78 and caspase-12 were significantly increased in Group V compared with those in Group N (Figures 4A–C). Treatment with edaravone significantly decreased the myocardial protein expressions of GRP78 and caspase-12 compared with those in Group V in a dose-dependent manner (Figures 4A–C). GADD153 protein level in cardiac tissue was significantly increased in Group V compared with that in Group N (Figures 4A and D). Edaravone treatment tended to decrease the myocardial protein expression of GADD153 (Figures 4A and D). Myocardial protein expression of p38 MAPK was markedly increased in Group V compared with that in Group N (Figure 5B). Although the effect was not significant, edaravone treatment tended to decrease the myocardial protein expressions of p38 MAPK and JNK (Figures 5B and C).

Discussion

This is the first observation that edaravone, a free-radical scavenger, causes decreased cardiac apoptosis in EAM model rats and that the cardioprotection of edaravone may be due not only to the suppression of ER stress but also to a decrease in oxidative stress. We investigated the effect of edaravone on oxidative stress and ER stress in this EAM model. Several studies have described that oxidative stress is involved in the pathogenesis and development of cardiac diseases including heart failure, atherosclerosis and hypertension [23,24].

Edaravone was proven to have an inhibitory effect on a water-soluble and a lipid-soluble peroxyl radical-induced peroxidation system and to have sufficient accessibility to tissue, including heart, so that it can effectively scavenge ROS in heart [14]. Edaravone

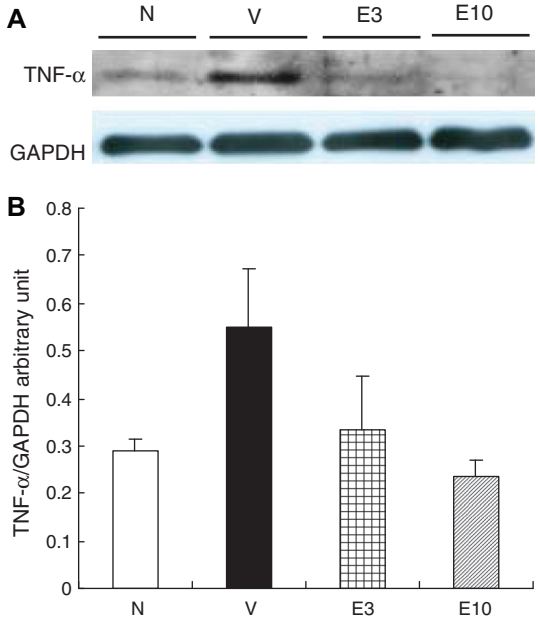

Figure 3. Myocardial expressions of tumour necrosis factor-α (TNF-α) and GAPDH. (A) Representative western blots showing specific bands for TNF-α and GAPDH as an internal control. An equal amount of protein sample obtained from whole ventricular homogenate was applied in each lane. These bands are representative of three separate experiments. (B) Densitometric data of protein analysis. The mean density value of TNF-α was expressed as a ratio relative to that of GAPDH. Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg). Values are mean ± SEM.

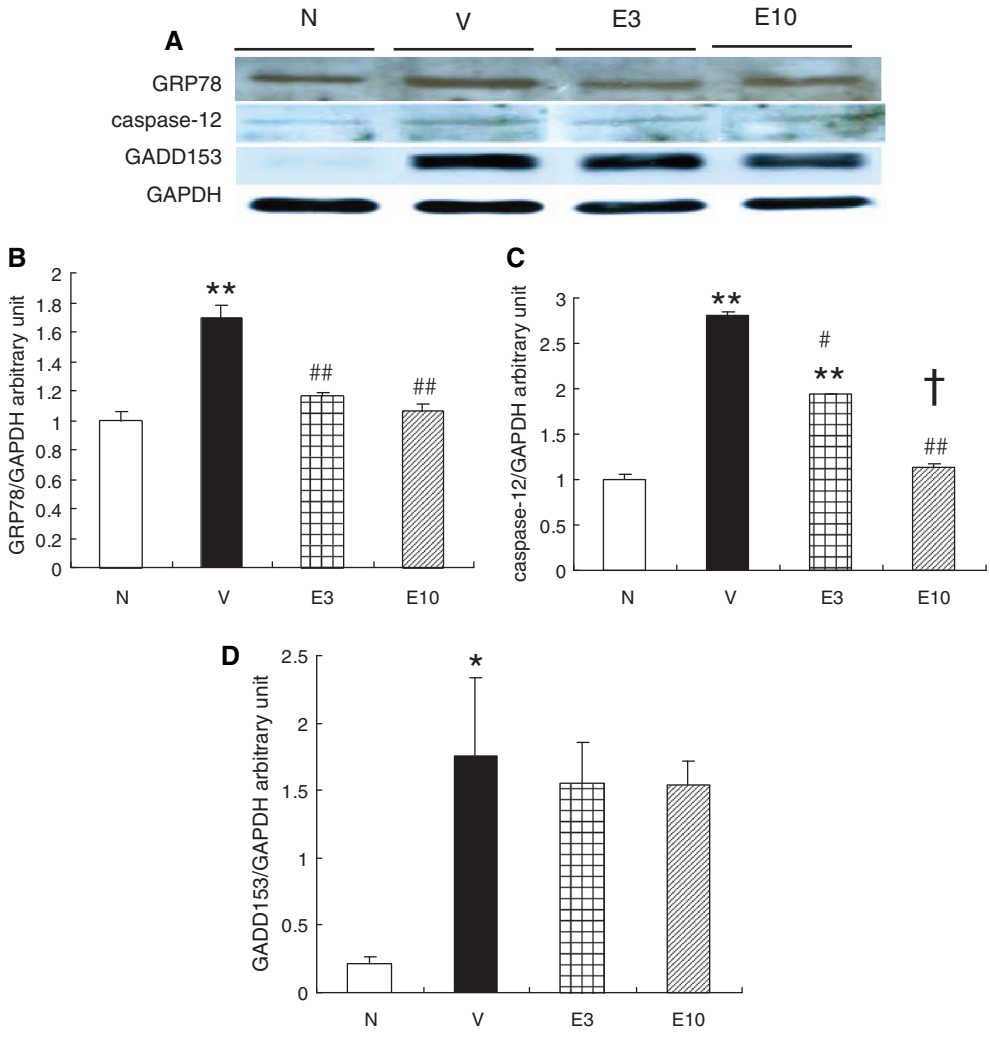

Figure 4 Myocardial expressions of glucose regulated protein 78 (GRP78), caspase-12, growth arrest and DNA damage-inducible gene 153 (GADD153) and GAPDH. (A) Representative western blots showing specific bands for GRP78, caspase-12, GADD153 and GAPDH as an internal control. An equal amount of protein sample obtained from whole ventricular homogenate was applied in each lane. These bands are representative of three separate experiments. (B–D) Densitometric data of protein analysis. The mean density value of GRP78, caspase-12 and GADD153 was expressed as a ratio relative to that of GAPDH. Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg); $^{**}p < 0.01$ and $^{*}p < 0.05$ vs Group N, $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ vs Group V, $^{\dagger}p < 0.05$ vs Group E3. Values are mean ± SEM.

has already been used in Japan as an antioxidant in the treatment of patients with ischemic brain damage [25].

In the present study, edaravone treatment suppressed cardiac apoptosis in EAM. TUNEL has been used successfully for detection of DNA degradation in paraffin-embedded tissue sections [26]. A TUNEL staining study showed that the number of apoptotic cells was reduced by edaravone treatment. Amemiya et al. [27] reported that edaravone ameliorated the apoptosis of cells in an ischemia model.

Beneficial haemodynamic effects of edaravone were shown in a dose-dependent manner in our study. Edaravone treatment significantly decreased CVP and improved FS and EF. Our results are consistent with those of other reports [28,29].

In addition, we demonstrated that edaravone effectively attenuated oxidative stress. An increase in myocardial oxidative stress was previously shown to be involved in the pathophysiology of EAM [30]. Oxidative stress may result in increased oxidative damage and can be caused either by an over-production of free radicals and ROS or by an impairment of the endogenous antioxidant defence system [31]. Excessive oxidative stress can damage many biological molecules, protein and DNA [32]. NADPH oxidase is an important source of increased cardiac ROS. The NADPH oxidase complex is a cluster of proteins that promote donation of an electron from NADPH to molecular oxygen to produce superoxide. Activation of electron transfer from NADPH to molecular oxygen requires recruitment of the cytosolic oxidase subunits $p47^{phox}$ and $p67^{phox}$ [33,34]. We have found that edaravone attenuated electron transfer from NADPH to molecular oxygen. In several studies, edaravone

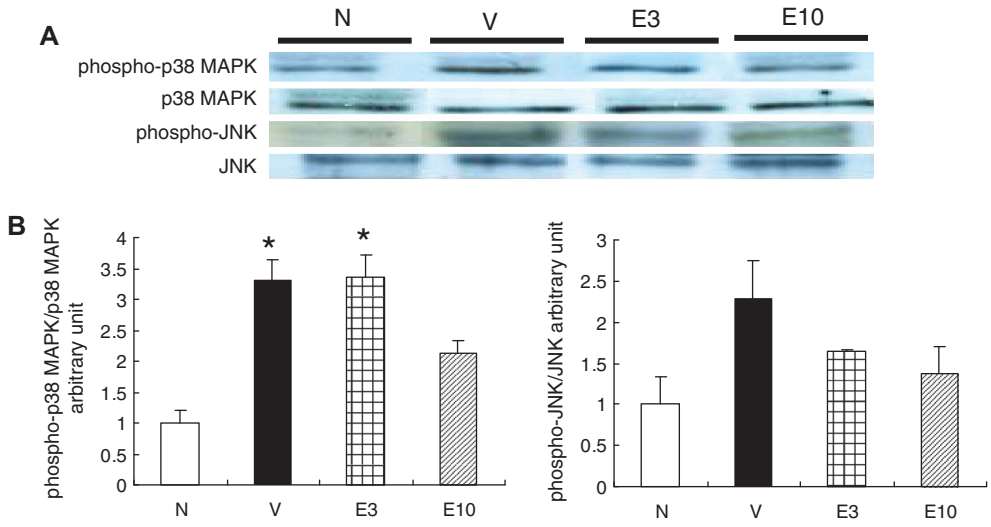

Figure 5. Myocardial expressions of phospho-p38 MAPK, phospho-JNK, p38 MAPK and JNK. (A) Representative western blots showing specific bands for phospho-p38 MAPK, phospho-JNK, p38 MAPK and JNK as an internal control. An equal amount of protein sample obtained from whole ventricular homogenate was applied in each lane. These bands are representative of three separate experiments. (B and C) Densitometric data of protein analysis. The mean density value of phospho-p38 MAPK and phospho-JNK was expressed as a ratio relative to that of p38 MAPK and JNK. Group N, age-matched normal rats; Group V, rats with EAM treated with vehicle; Group E3, rats with EAM treated with edaravone (3 mg/kg); Group E10, rats with EAM treated with edaravone (10 mg/kg); $^{*}p < 0.05$ vs Group N. Values are mean ± SEM.

decreased malondialdehyde contents and myocardial carbonyl contents [28,29]. Our result shows that edaravone could reduce oxidative stress, which might be another protective mechanism of its anti-oxidative effects. Increased oxidative stress also evokes many intracellular events including inflammation, myocyte apoptosis and ER stress [5,6,35].

Cytokines play important roles in the pathogenesis of myocarditis. Cardiac myosin-specific T-cells are activated by antigen presentation from resident dendritic cells after entering the myocardium. The activated T-cells secrete various cytokines which activate inflammatory cells in the myocardium [4]. The cytokine TNF-α is classified as a pro-inflammatory cytokine and is expressed in cardiac tissue during the acute phase of EAM and its levels were reported to be up-regulated on day 21 of EAM [36]. Yang et al. [37] reported that edaravone attenuated the elevated mRNA levels of pro-inflammatory cytokine TNF-α in rat lungs after acute pancreatitis. In this study, we found that edaravone treatment tended to decrease the expression of TNF-α in EAM rats.

ER stress refers to the accumulation of misfolded or unfolded proteins within the lumen of the ER in response to some insult [38]. ER stress also plays an essential role in viral damage of myocardium. GRP78 has been widely used as a marker for ER stress and caspase-12 mediated apoptosis was found to be a specific apoptotic pathway of ER. CHOP is also known as GADD153, although it is induced by ER stress more than by growth arrest or DNA damage [39]. EAM caused significant elevations in GRP78 and caspase-12, while edaravone reduced these ER stresses. Qi et al. [20] reported that edaravone protects against ER dysfunction in hypoxia/ischemia animal models. We consider that edaravone has another protective mechanism against ER stress via suppression of oxidative stress and directly inhibits ER stress. Excessive ER stress and oxidative stress can trigger the activation of multiple signalling pathways such as phosphorylation of p38 MAPK and JNK, which are closely associated with cell death [40,41]. Recent study has demonstrated that the p38 MAPK and JNK pathways are activated in an EAM animal model [42].

In this study, we found that oxidative stress resulted in increased activation of p38 MAPK. On the other hand, a slight increase in the activation of JNK was observed in the vehicle group compared with that in the normal group, but this increase was not significant. Edaravone slightly reduced these MAPKs, but these decreases were not significant. In another model, we reported that edaravone treatment significantly decreases the expression of JNK [43]. In the present study, our results suggested that edaravone did not reduce the expressions of GADD153, p38 MAPK and JNK but did reduce the expressions of GRP78 and caspase-12 significantly. Therefore, we consider that edaravone has an effect on the ER stress signalling pathway rather than the MAPK signalling pathway.

In conclusion, edaravone protects against EAM in rats and the cardioprotective effects of edaravone may be attributed to the suppression of apoptotic events associated with antioxidant properties. However, the exact mechanism and signalling pathway involved in

the protective role of edaravone in EAM need to be studied.

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