Eur Respir J 2008; 32: 1337–1343
DOI: 10.1183/09031936.00164407

Effects of edaravone, a free-radical scavenger, on bleomycin-induced lung injury in mice

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ABSTRACT: Reactive oxygen species play an important role in the pathogenesis of acute lung injury and pulmonary fibrosis. The present authors hypothesise that edaravone, a free-radical scavenger, is able to attenuate bleomycin (BLM)-induced lung injury in mice by decreasing oxidative stress.

Lung injury was induced in female ICR mice by intratracheal instillation of 5 mg·kg^{-1} of BLM. Edaravone (300 mg·kg^{-1}) was administered by intraperitoneal administration 1 h before BLM challenge.

Edaravone significantly improved the survival rate of mice treated with BLM from 25 to 90%, reduced the number of total cells and neutrophils in bronchoalveolar lavage fluid (BALF) on day 7, and attenuated the concentrations of lipid hydroperoxide in BALF and serum on day 2. The fibrotic change in the lung on day 28 was ameliorated by edaravone, as evaluated by histological examination and measurement of hydroxyproline contents. In addition, edaravone significantly increased the prostaglandin E_2 concentration in BALF on day 2.

In summary, edaravone was shown to inhibit lung injury and fibrosis *via* the repression of lipid hydroperoxide production and the elevation of prostaglandin E_2 production in the present experimental murine system.

KEYWORDS: Bleomycin, edaravone, free-radical scavenger, lung injury, pulmonary fibrosis

Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic fibrosing interstitial pneumonia limited to the lung [1]. The aetiology of IPF is not known, and IPF remains a devastating disease with a 5-yr mortality rate of >50% [1]. Unfortunately, the pathogenesis of IPF is also incompletely understood. Although several drugs have been used or tested for IPF, there is no established treatment that definitely improves its outcome [1]. Thus, new therapies are awaited, based on new understanding of the pathogenesis of IPF. There is considerable evidence that oxygen-generated free radicals play a major role in inflammatory and immune-mediated tissue injury [2–4]. DEMEDTS *et al.* [5] have shown that acetylcysteine, a precursor of the major antioxidant glutathione, administered at a daily dose of 1,800 mg in combination with prednisone and azathioprine, preserves vital capacity and carbon monoxide diffusing capacity better in patients with IPF than the combination of prednisone and azathioprine alone. These findings suggest that an oxidant–antioxidant imbalance may contribute to the disease process in IPF.

Bleomycin (BLM), an antineoplastic agent, induces pulmonary fibrosis as an adverse effect, since the hydrolase that inactivates BLM is relatively scarce in lung tissue. The mechanism of the antineoplastic effect of BLM is that the BLM-iron complex reduces molecular oxygen to superoxide and hydroxy radicals that can then attack DNA and cause strand cleavage [6]. The role of oxygen free radicals has been supported by studies showing that the addition of superoxide dismutase, an oxygen free-radical scavenger, inhibited BLM-induced DNA breakage and cellular damage *in vitro* [7–10]. Therefore, a BLM-induced pulmonary fibrosis model in mice is a helpful tool to examine the general mechanism of fibrosis, especially the mechanism mediated by oxygen free radicals.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a potent free-radical scavenger and has the antioxidant ability to inhibit lipid peroxidation [11]. Therefore, it is speculated that edaravone administration might ameliorate the tissue damage induced by reactive oxygen species (ROS).

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Received:
December 06 2007
Accepted after revision:
July 02 2008

SUPPORT STATEMENT
This study was supported by the Health and Labour Sciences Research Grants on Diffuse Lung Diseases from the Japanese Ministry of Health, Labour and Welfare.

STATEMENT OF INTEREST
A statement of interest for this study can be found at www.erj.ersjournals.com/misc/statements.shtml

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

Edaravone has protective effects on both hemispheric embolisation and transient cerebral ischaemia, and has, therefore, been used clinically to treat acute brain infarction in Japan [12–14]. ITO *et al.* [15] have shown that edaravone ameliorated the lung injury induced by intestinal ischaemia/reperfusion. In the study by ITO *et al.* [15], edaravone decreased the neutrophil infiltration, the lipid membrane peroxidation and the expression of interleukin (IL)-6 mRNA in the lungs, resulting in a reduction in mortality. Most recently, ASAI *et al.* [16] have shown that edaravone suppressed BLM-induced acute pulmonary injury in rabbits. They reported that a 10-day intravenous edaravone administration beginning 3 days prior to intratracheal instillation of BLM significantly attenuated the acute BLM-induced lung injury and the numbers of both terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate-positive (apoptotic) and transforming growth factor-β positive cells on day 7 [16]. Although the results of ASAI *et al.* [16] support the present authors' hypothesis, it was thought that several critical points were lacking, as follows: 1) collagen accumulation at the late fibrosing stage was not evaluated; and 2) bronchoalveolar lavage (BAL) was not performed and ROS was not measured in order to evaluate inhibitory effects on the inflammatory process. Accordingly, in the present study, a BLM-induced pulmonary fibrosis model was used in mice, which is a more common animal lung fibrosis model than the rabbit model used by ASAI *et al.* [16], to investigate the ability of edaravone to: 1) inhibit pulmonary fibrosis; or 2) decrease lung inflammation and attenuate ROS.

MATERIALS AND METHODS

Mice, cells and reagents

All mice received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (Bethesda, MD, USA). The study protocol was approved by the Ethics Committee of Jichi Medical University (Tochigi, Japan). Female ICR mice, 6–8 weeks of age, were obtained from Japan SLC (Tochigi, Japan) and housed in the animal facility of Jichi Medical University. BLM was purchased from Nippon Kayaku (Tokyo, Japan). Edaravone was a gift from Mitsubishi Pharma Corporation (Tokyo, Japan). It was dissolved in a small amount of 1 N NaOH solution, the pH was adjusted to 7.0 with 1 N HCl and the concentration was adjusted to 3 $mg \cdot mL^{-1}$ in the saline solution.

BLM-induced pulmonary fibrosis model

To induce pulmonary fibrosis, ICR mice were treated with intratracheal BLM on day 0. The ICR mice were anaesthetised by the intraperitoneal administration of 0.01 $mL \cdot g^{-1}$ of 10% pentobarbital sodium solution (Abbott Laboratories, North Chicago, IL, USA), followed by intratracheal instillation of 5 $mg \cdot kg^{-1}$ body weight of BLM in 50 μL of sterile isotonic saline. The control animals received intratracheal saline only. Edaravone dissolved in saline or the same volume of saline was administered by a single intraperitoneal injection either 1 h before or 24 h after BLM injection. To ascertain the optimal dose of edaravone for the proposed experiment, mice were given edaravone at a dose of 0, 3, 30 or 300 $mg \cdot kg^{-1}$ or the same volume of saline (10–12 mice in each group). The mice were killed under anaesthesia on day 2, 7 or 28 after BLM instillation, for examination. On day 28, the left lung lobes were used for hydroxyproline assay. In the mice receiving pre-administration of 300 $mg \cdot kg^{-1}$ edaravone with BLM instillation, BAL was performed on days 2 and 7. In addition, histological examination was performed on day 28. The present authors randomly selected six or 10 mice samples from each group. Mortality calcuation, hydroxyproline assay, histological examination and BAL analysis were performed independently.

Sampling of BAL fluid and serum

Under anaesthesia, as previously described, blood samples were obtained from the right atrium at each time-point. After centrifugation at $3,000 \times g$ for 10 min at 4°C, the serum was frozen and stored at -80°C until it was assayed. BAL was performed four times through a tracheal cannula with 0.7 mL of saline. In each mouse examined, ~2.5 mL (90%) of BAL fluid (BALF) was recovered. A 100-μL aliquot was used for the total cell count, and the remainder was immediately centrifuged at $1,000 \times g$ for 10 min. The total cell count was prepared using a haemocytometer, and cell differentiation was determined for >500 cells on cytocentrifuge slides with Wright–Giemsa staining. The supernatants of BALF were stored at -80°C until used.

Morphological evaluation

Histopathological evaluation was performed on day 28 in the BLM-induced pulmonary fibrosis model. Both lungs were removed and inflated with 10% formaldehyde neutral buffer solution, and longitudinal tissue sections were stained with haematoxylin and eosin.

Assay of hydroxyproline

Hydroxyproline in the murine lung on day 28 after BLM instillation was assayed according to the commonly used

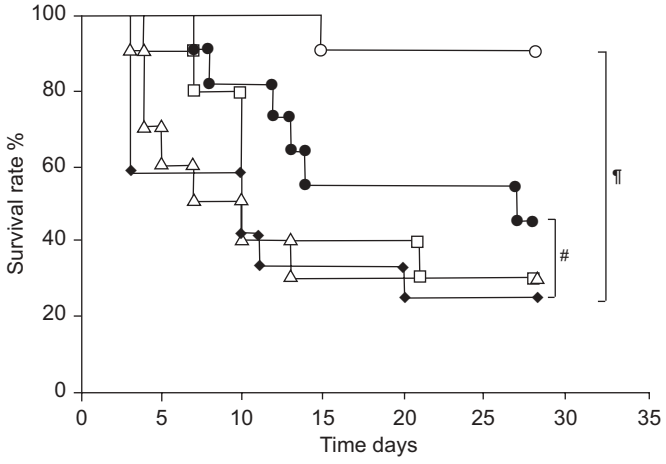

FIGURE 1. Effects of edaravone on mortality in a bleomycin (BLM)-induced lung injury mouse model. The survival rates of five study groups of mice are shown over a 28-day observation period (10–12 mice in each group). The four BLM + edaravone groups received single intraperitoneal infusion of edaravone as follows. ○: high dose of edaravone (pre-treatment, 300 $mg \cdot kg^{-1}$); □: intermediate dose of edaravone (pre-treatment, 30 $mg \cdot kg^{-1}$); △: low dose of edaravone (pre-treatment, 3 $mg \cdot kg^{-1}$) administered as a single intraperitoneal infusion 1 h before the instillation of BLM; ●: high dose of edaravone as a single intraperitoneal infusion 24 h after the instillation of BLM (treatment, 300 $mg \cdot kg^{-1}$). The survival rate of the high-dose edaravone group (pre-treatment, 300 $mg \cdot kg^{-1}$; ○) was significantly higher than the group administered intratracheal instillation of BLM (◆; $p<0.05$). The results for the control group are not shown. #: $p=0.15$; ¶: $p=0.002$.

procedure of colorimetric measurement (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Tokyo) [17, 18]. Hydroxyproline content ($\mu g \cdot lung^{-1}$) was measured in the left lung of each subject.

Assays for lipid hydroperoxide and prostaglandin E_2

The concentrations of lipid hydroperoxide (LPO) in serum and BALF were measured as an indicator of oxidative stress using a Lipid Hydroperoxide Assay kit (Cayman Chemical, Ann Arbor, MI, USA). Prostaglandin (PG)E_2 in BALF was quantified using specific immunoassays (Cayman Chemical).

Statistical analysis

Survival curves were estimated by the Kaplan–Meier method. Comparisons of all curves were carried out using the two-tailed log-rank test. Data were expressed as the mean±SEM. For multiple comparisons, ANOVA was performed followed by the Fisher's protected least-significant differences method as a *post hoc* test. Differences between two variables were assessed with the Mann–Whitney U-test. A p-value <0.05 was considered to indicate statistical significance.

RESULTS

Edaravone caused a significant reduction in the mortality of mice with BLM-induced pulmonary fibrosis

The severe lung injury caused by BLM administration was associated with high mortality. To assess the protective effects of edaravone, the compound was injected intraperitoneally in various doses at various times either before or after the BLM instillation. The survival rate of each group is shown in figure 1. In total, nine (75%) out of 12 animals died from day 3 to 20 after treatment with 5 $mg \cdot kg^{-1}$ of BLM. However, the pre-administration of 300 $mg \cdot kg^{-1}$ edaravone significantly improved the survival rate of mice treated with BLM (one out of 10 animals died, p=0.002; fig. 1). In contrast, among the mice treated with low-dose edaravone (pre-administration of 3 or 30 $mg \cdot kg^{-1}$) followed by BLM instillation, only three out of 10 mice survived in both dosage groups (fig. 1). The administration of 300 $mg \cdot kg^{-1}$ edaravone after 24 h BLM injection (post-treatment administration is the treatment group) did not improve the survival rate of mice treated with BLM (five out of 11 animals died, p=0.15; fig. 1).

Administration of edaravone ameliorated BLM-induced pulmonary fibrosis in mice

To evaluate the antifibrotic effect of edaravone, mice were treated with 5 $mg \cdot kg^{-1}$ of BLM and killed on day 28. The fibrotic change in the lung was evaluated by histological examination and measurement of hydroxyproline contents. As shown in figure 2, when 300 $mg \cdot kg^{-1}$ of edaravone was administered before BLM instillation, a significant reduction of fibrosis in the subpleural areas of the lung was observed. The hydroxyproline assay demonstrated that pre-treatment with edaravone dose-dependently reduced the total hydroxyproline contents in BLM-treated lungs (fig. 3). The post-treatment administration (treatment group) of 300 $mg \cdot kg^{-1}$ edaravone was also effective in reducing the pulmonary fibrosis caused by BLM.

Analysis of BALF cells in mice with BLM-induced pulmonary fibrosis

Following this, the cells in BALF were analysed to evaluate the effects of edaravone on the inflammatory responses induced by BLM. Edaravone (300 $mg \cdot kg^{-1}$ body weight) was administered by a single intraperitoneal injection 1 h before BLM injection. Administration of BLM elevated the number of inflammatory cells, including macrophages, lymphocytes and neutrophils, on days 2 and 7. Pre-administration of edaravone significantly reduced the number of total cells and neutrophils in BALF on day 7 ($p<0.05$; fig. 4a and c). As shown in figure 4a and c, the p-value for total cells and neutrophils in BALF between the BLM and BLM + edaravone group were significant but marginal (p=0.045 and p=0.046, respectively). Therefore, the present authors did not perform BALF cell analysis or measurement of LPO or PGE_2 without pre-treatment of 300 $mg \cdot kg^{-1}$ edaravone.

Effects of edaravone on the amount of LPO in serum and BALF in the BLM model

One of the possible reasons for the preventive effect of edaravone on BLM-induced lung injury may be its antioxidant effect. To study the antioxidant effect of edaravone, the amount of LPO in the serum and BALF was measured, which is an indicator of oxidative stress [9]. On day 2 after BLM instillation, serum LPO levels were significantly increased compared with those in the control mice (p=0.013; fig. 5a). However, pre-treatment with edaravone (300 $mg \cdot kg^{-1}$ body weight) significantly decreased the levels of LPO in serum, compared with those in the animals treated with BLM alone (p=0.001; fig. 5a). LPO production in BALF was also significantly lowered by edaravone injection on day 2 (p=0.049; fig. 5b). The serum or BALF levels of LPO in edaravone-treated mice on day 7 after BLM challenge did not differ from those in untreated mice (data not shown).

Effects of edaravone on the PGE_2 levels in BALF of the BLM model

The PGE_2 level in BALF was measured as an index of the amount of anti-inflammatory prostanoids. PGE_2 was measured by immunoassay in BLM-treated mice with or without pre-treatment of edaravone (300 $mg \cdot kg^{-1}$ body weight). As shown in figure 6, mice pre-treated with edaravone exhibited significantly greater levels of PGE_2 than mice receiving BLM alone on day 2, but this elevation of PGE_2 by edaravone rapidly decreased thereafter until day 7 (data not shown).

Adverse effects of edaravone on the serum creatin levels in a model

A temporary increase of serum creatinine levels was observed at the dose of 300 $mg \cdot kg^{-1}$ of edaravone (fig. 7). However, the creatinine elevation at day 2 after BLM instillation was normalised until day 7 (fig. 7).

DISCUSSION

The present study has shown that the anti-inflammatory effects of edaravone improved the 28-day survival in mice with acute lung injury after a BLM instillation. Edaravone could mitigate the progression of lung injury and fibrosis. It also attenuated the cellular infiltration and the concentrations of LPO in BALF. These findings suggested that edaravone could inhibit lung

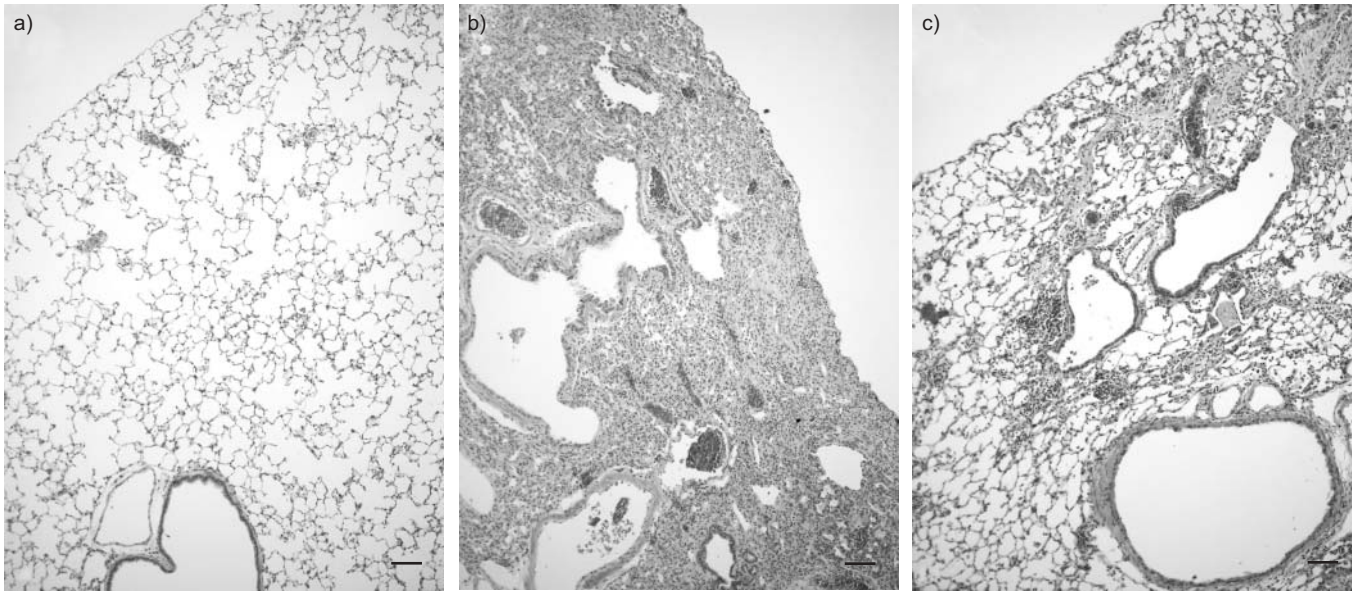

FIGURE 2. Effects of edaravone on histopathological changes. Lung tissue was obtained on day 28 after instillation of bleomycin (BLM) or saline and was stained with haematoxylin and eosin. a) Saline-group lung tissue sample showing thin interalveolar septa, a lack of inflamed cells, and normal-appearing bronchioles and alveolar ducts. b) BLM-group lung tissue sample showing alveolitis and patchy fibrosis with destruction of the alveolar structure, mainly in the subpleural regions. c) In mice pre-treated with high doses of edaravone (300 mg·kg^{-1}) these features were less severe. Scale bars=200 μm.

injury and fibrosis *via* the repression of LPO production in the current model.

In the present study, a murine BLM-induced pulmonary fibrosis model was used to examine the ability of edaravone to: 1) inhibit pulmonary fibrosis; 2) decrease lung inflammation and attenuate ROS. First, the ability of edaravone to inhibit pulmonary fibrosis was investigated using histological examination and measurement of hydroxyproline contents. It was found that a single administration of edaravone not only 1 h before but also 24 h after BLM challenge could mitigate the progression of pulmonary fibrosis on day 28 after BLM instillation.

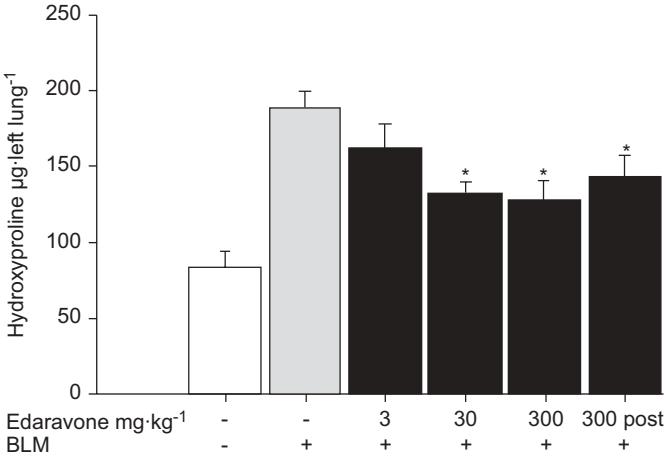

FIGURE 3. Effects of edaravone on the hydroxyproline content in the left lung in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. The hydroxyproline content was significantly increased by BLM injection. Single administration of 30 or 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly attenuated the BLM-induced increase in hydroxyproline content on day 28. In addition, a single high dose (300 mg·kg^{-1}) of edaravone by intraperitoneal infusion 24 h after the instillation of BLM also significantly decreased hydroxyproline contents. □: control group; ■: BLM group; ■: BLM + edaravone group. Data are presented as mean±SEM (six to 10 mice in each group). *: $p<0.05$ in comparison to the BLM group.

Secondly, the ability of edaravone to decrease lung inflammation and attenuate ROS was investigated. The present study demonstrated that edaravone could attenuate the concentrations of LPO (an indicator of oxidative stress) in BALF and serum on day 2. An oxidant–antioxidant imbalance may contribute to the pathogenesis of BLM-induced pulmonary fibrosis [7–10]. HAGIWARA *et al.* [9] have shown that aerosolised administration of *N*-acetylcysteine (NAC) attenuates lung fibrosis induced by BLM *via* repression of LPO production. In the present study, the number of total cells and neutrophils in BALF in edaravone-treated mice on day 7 was significantly decreased in comparison with untreated mice. These findings are consistent with previous reports [8–10]. Most of the antioxidant agents used for the treatment of BLM models have shown both antifibrosing effects and anti-inflammatory effects, *i.e.* attenuating the cellular infiltration, pro-inflammatory cytokines or chemokines in BALF [8–10]. Although pro-inflammatoy cytokines or chemokines in BALF were not measured, the current authors speculate that edaravone may have decreased the pro-inflammatory cytokine or chemokine production in the current BLM-induced lung injury model.

The present study demonstrated that a single administration of edaravone reduced the total hydroxyproline contents in BLM-treated lungs on day 28. Although numerous agents targeting diverse signalling and molecular pathways inhibited fibrosis very effectively in the BLM-induced pulmonary fibrosis model, so far none of the molecules have demonstrated clear efficacy in the treatment of IPF. One main difference between the disease and the mouse model is the inflammatory component

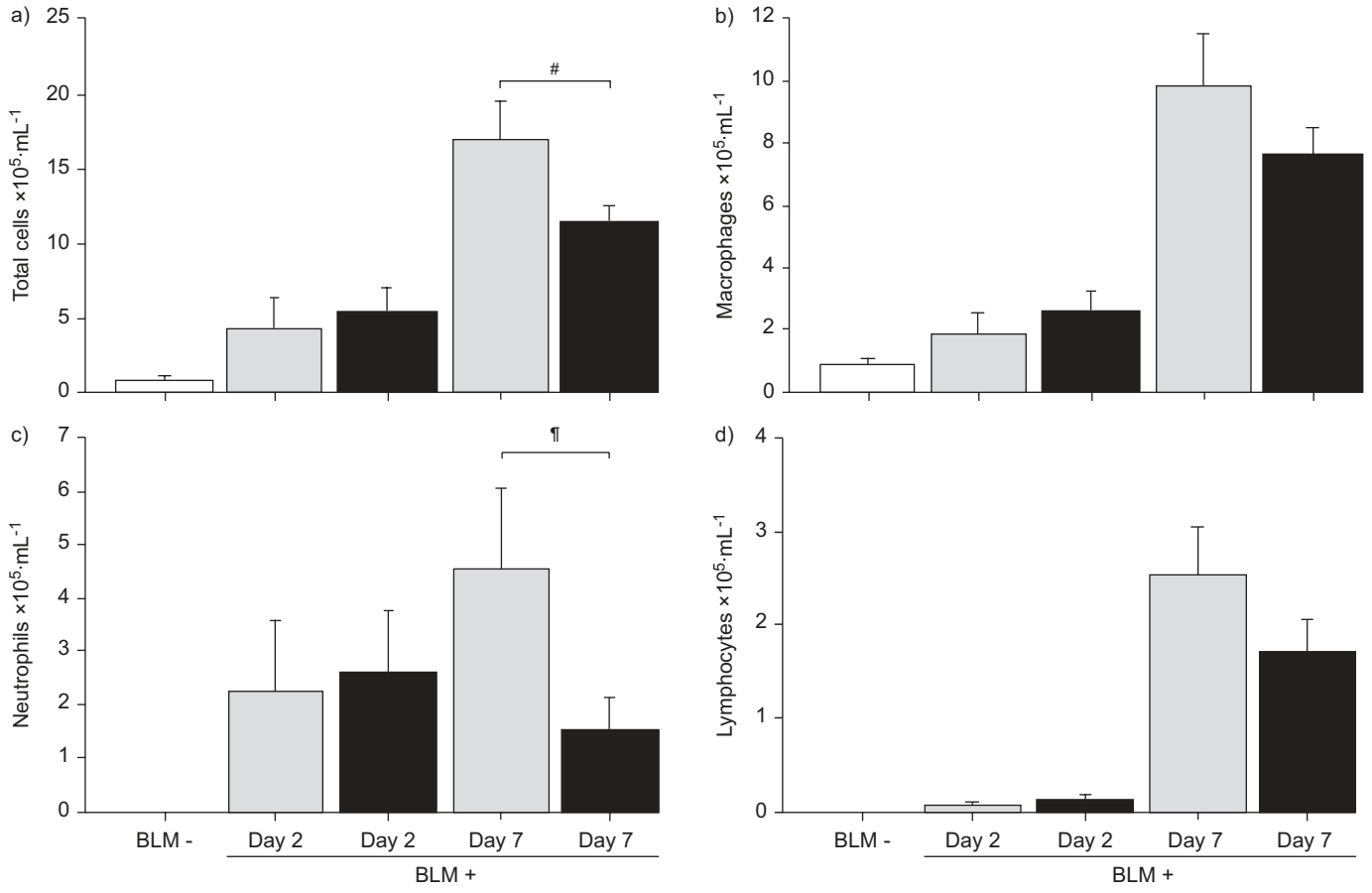

FIGURE 4. Effects of edaravone on bronchoalveolar lavage fluid (BALF) cell analysis in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Single administration of 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly reduced the number of total cells and neutrophils in BALF on day 7 ($p<0.05$; a and c). There was no change in the number of macrophages or lymphocytes in BALF on day 7 (b and d). □: control group; ■: BLM group; ■: BLM + edaravone group. Data are presented as the mean±SEM (n=6 in control and day 2 groups, n=10 in each day 7 group). #: $p=0.045$; ¶: $p=0.046$.

of early BLM-induced lung injury, which is often absent in human IPF [19]. Recently, CHAUDHARY *et al.* [20] determined the time-course of the development of inflammation and fibrosis in BLM-induced lung fibrosis. They demonstrated that in an animal model of single intratracheal injection of BLM, the ''switch'' between inflammation and fibrosis occurred on or just after day 9 [20]. Although the current authors experimented with daily intravenous or intraperitoneal injections of 60 mg·kg^{-1} edaravone from 14 days after BLM instillation, there was no beneficial effect (data not shown). HAGIWARA *et al.* [9] used NAC inhalation and obtained results similar to those in the present study. The current results suggested that edaravone might not demonstrate a therapeutic effect on chronic fibrotic lung diseases, such as IPF, but may have a preventive effect in the very accelerated phases of interstitial lung diseases, such as in acute exacerbation of IPF, acute interstitial pneumonia or drug-induced lung diseases.

WATANABE *et al.* [21] have shown that edaravone acts as: 1) a radical scavenger; 2) a stimulator of PG production; 3) an inhibitor of lipoxygenease; and 4) a protector against cell membrane damage. Thus, it was considered that arachidonic acid might be preferentially metabolised *via* the alternative cyclooxygenase (COX) pathway to prostanoids that possess anti-inflammatory and antifibrotic activity, *e.g.* PGE_2. PGE_2 is produced in large quantities by macrophages in response to pro-inflammatory molecules such as IL-1 and lipopolysaccharide [22–24] and is, therefore, also considered a pro-inflammatory mediator. In addition to its effects on inflammation, PGE_2 suppresses fibroblast proliferation [25] and reduces collagen mRNA expression [26], thereby exerting an antifibrotic activity. *In vivo*, consistent with an antifibrotic activity of PGE_2, COX2 knockout mice were found to be more susceptible to BLM-induced lung fibrosis [27]. The administration of edaravone before BLM challenge was found to produce more PGE_2 in the BALF than saline administration. EGAN *et al.* [28] have shown that the COX-PG pathway is irreversibly self-deactivated due to the natural reduction of the hydroperoxide at carbon 15 of PGG_2 to the hydroxyl on PGH_2. During this reduction, radicals, possibly hydroxyl radicals, are formed and could oxidise the enzyme [28]. Therefore, edaravone may increase both the initial rate and the total reaction prior to deactivation by partially consuming these radicals. The current authors did not examine which cells (macrophages, epithelial cells, endothelial cells or fibroblasts) contribute to PGE_2 production. Further examination will be needed to determine which cells are affected by edaravone.

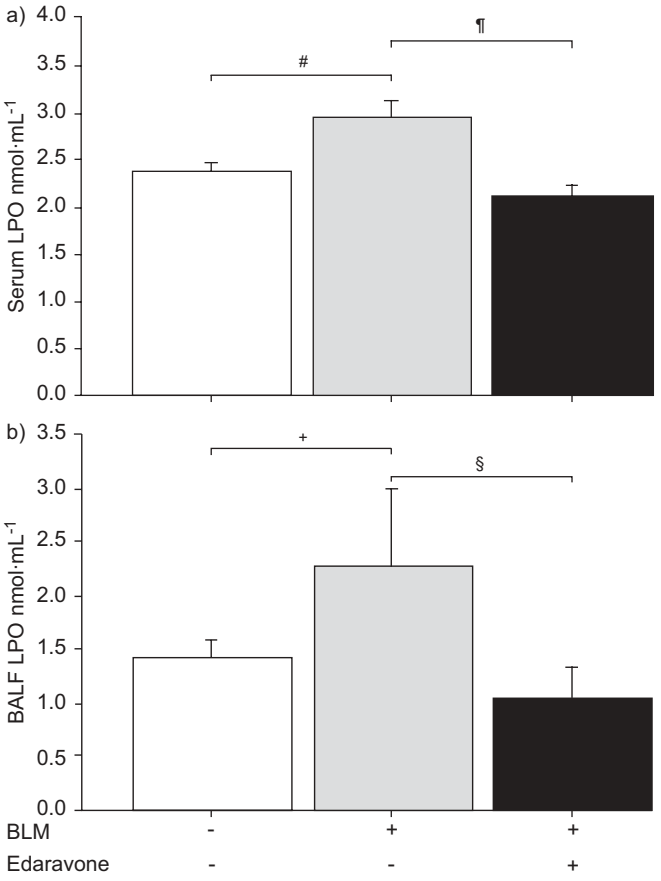

FIGURE 5. Effects of edaravone on the amount of a) lipid hydroperoxide (LPO) in serum and b) bronchoalveolar lavage fluid (BALF) in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Edaravone treatment consisted of a single administration of 300 mg·kg^{-1} 1 h before BLM instillation. a) Although on day 2 after BLM instillation serum LPO levels were significantly increased compared with the control mice, administration of edaravone significantly decreased the levels of LPO in serum. b) LPO production in BALF was also significantly lowered by edaravone injection on day 2. □: control group; ■: BLM group; ■: BLM + edaravone group. Data are presented as the mean±SEM (n=6 in each group). #: p=0.013; ¶: p=0.001; +: p=0.125; §: p=0.049.

Usually, the daily dose of edaravone is ~1.5 mg·kg^{-1}, and the treatment commences 14 days after cerebral infarction [11–14]. Although, in a previous report, no adverse effects on heart rate or blood pressure at the dose of 450 mg·kg^{-1} of edaravone were reported [29], the present authors observed a temporary increase of serum creatinine levels at the dose of 300 mg·kg^{-1} of edaravone. However, the creatinine elevation on day 2 after BLM instillation was normalised by day 7. No other adverse effects of a single daily administration of 300 mg·kg^{-1} of edaravone were observed, despite the fact that this dose was ~200 times higher than the daily dose used in humans. ANZAI *et al.* [29] have reported a radioprotective effect of edaravone against whole body X-ray irradiation in C3H mice. To increase the survival rate, the necessary dose of edaravone was 450 mg·kg^{-1} intraperitoneally, and the timing of the administration was 30 min prior to the irradiation [29]. ASAI *et al.* [16] used daily intravenous injections of 3 mg·kg^{-1} edaravone for rabbits administered 2 mg·kg^{-1} BLM. In the present study, a

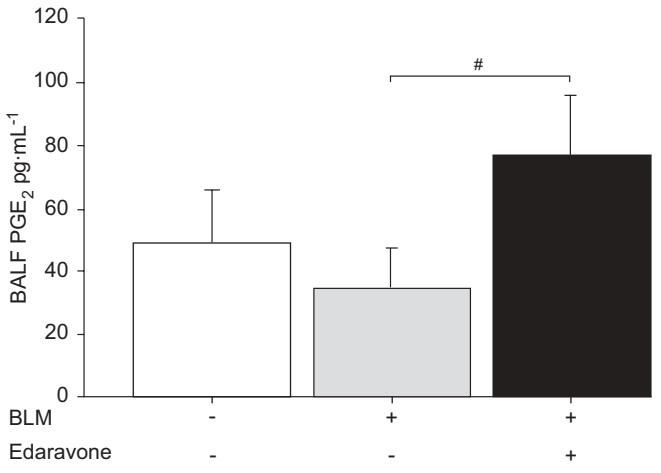

FIGURE 6. Effects of edaravone on the prostaglandin (PG)E_2 levels in bronchoalveolar lavage fluid (BALF) of a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Single administration of 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly increased PGE_2 on day 2. □: control group; ■: BLM group; ■: BLM + edaravone group. Data are presented as the mean±SEM (n=6 in each group). #: p=0.043.

high dose of edaravone was required for the treatment of lung injury in ICR mice. In addition to the dose-dependency, the efficacy of edaravone in ameliorating BLM-induced organ injury was also dependent on the administration route and the strain of mice.

In conclusion, the results of the present study suggest that edaravone could inhibit bleomycin-induced lung injury and fibrosis *via* the repression of lipid hydroperoxide production and augmentation of prostaglandin E_2 production. Additional

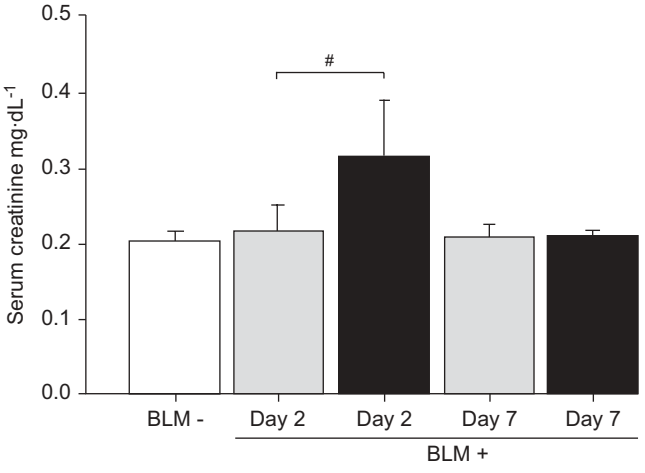

FIGURE 7. Adverse effects of edaravone on the serum creatinine levels in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. The serum creatinine levels were measured by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan). Although a temporary increase of serum creatinine levels at the dose of 300 mg·kg^{-1} of edaravone was observed on day 2 after BLM instillation, the elevation was normalised by day 7. □: control group; ■: BLM group; ■: BLM + edaravone group. Data are presented as the mean±SEM (n=6 in each group). #: p=0.044.

clinical studies on other fatal interstitial lung diseases, such as acute exacerbation of idiopathic pulmonary fibrosis, acute interstitial pneumonia associated with collagen vascular diseases or chemotherapy-related toxicity, are needed to determine the safest dose, administration route and duration times of edaravone.

ACKNOWLEDGEMENTS

The authors would like to thank T. Ikahata for (Division of Pulmonary Medicine, Dept of Medicine, Jichi Medical University) excellent assistance.

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