Treatment with ALK Inhibitor. For the experiments based on i.v. administration of EML4-ALK/3T3 cells, the cells (2×10^5) were injected into the tail vein of 4-week-old *nu/nu* mice (Clea Japan). An inhibitor specific for the tyrosine kinase activity of ALK [example 3-39 in the patent application: Garcia-Echeverria C, *et al.*, inventors; Novartis AG, Novartis Pharma GmbH, IRM LLC, applicants (24 Feb 2005). 2,4-Pyrimidinediamines useful in the treatment of neoplastic disease and in inflammatory and immune system disorders. PCT WO 2005016894] was synthesized by Astellas Pharma and was orally administered each day at a dose of 10 mg/kg to the injected mice or to *EML4-ALK*-transgenic mice. Sequential examination of lung tumors was performed with an X-ray CT apparatus for experimental animals (LCT-100; Aloka).

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ORIGINAL ARTICLE

Preventive effects of edaravone, a free radical scavenger, on lipopolysaccharide-induced lung injury in mice

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TAJIMA S, SODA M, BANDO M, ENOMOTO M, YAMASAWA H, OHNO S, TAKADA T, SUZUKI E, GEJYO F, SUGIYAMA Y. *Respirology* 2008; **13**: 646–653
Background and objective: Reactive oxygen species (ROS) play an important role in the pathogenesis of acute lung injury (ALI) and pulmonary fibrosis. It was hypothesized that edaravone, a free radical scavenger, would be able to attenuate LPS-induced lung injury in mice by decreasing oxidative stress.
Methods: For the *in vivo* experiments, lung injury was induced in female BALB/c mice by the intranasal instillation of LPS. Edaravone was given by intraperitoneal administration 1 h before the LPS challenge. For the *in vitro* experiments, MH-S cells (murine alveolar macrophage cell line) were exposed to edaravone, followed by stimulation with LPS.
Results: In the LPS-induced ALI mouse model, the administration of edaravone attenuated cellular infiltration into and the concentrations of albumin, IL-6, tumour necrosis factor-α, keratinocyte-derived chemokine and macrophage inflammatory protein-2 in BAL fluid. In addition, the *in vitro* studies showed that the elevated IL-6 secretion from MH-S cells in response to LPS was significantly attenuated by co-incubation with edaravone.
Conclusions: In an experimental murine model, a free radical scavenger may prevent ALI via repression of pro-inflammatory cytokine production by lung macrophages.

Key words: acute lung injury, edaravone, free radical scavenger, LPS, pro-inflammatory cytokine, reactive oxygen species.

INTRODUCTION

Acute lung injury (ALI) and its severest form, acute ARDS, are frequent complications in critically ill patients and cause significant morbidity and mortality.[1] An initiating event (sepsis, shock, trauma, multiple transfusions, pancreatitis, etc.) leads to the activation of an acute inflammatory response on a systemic level. One of the earliest manifestations of ALI/ARDS is the activation of the pulmonary endothelium and macrophages (alveolar and interstitial), the upregulation of adhesion molecules and the production of cytokines and chemokines that induce a massive sequestration of neutrophils within the pulmonary microvasculature. These cells transmigrate across the endothelium and epithelium into the alveolar space and then release a variety of cytotoxic and pro-inflammatory compounds, including proteolytic enzymes, reactive oxygen species (ROS) and nitrogen species, cationic proteins, lipid mediators and additional inflammatory cytokines.[2] These activities perpetuate a vicious cycle by recruiting additional inflammatory cells that in turn produce more cytotoxic mediators, ultimately leading to the occurrence of profound injury of the alveolo-capillary membrane and respiratory failure. However, aside from the use of

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activated protein C in a subset of ALI/ARDS patients with sepsis, specific therapies are lacking, and the cascade of events leading to ALI and ARDS, once initiated, is much less amenable to specific treatment modalities.[3] New preventive and/or therapeutic measures against ALI/ARDS are eagerly awaited.

Edaravone (3-methyl-1-phenyl-2-pyrazoline-5-one) is a potent free radical scavenger and has the antioxidant ability to inhibit lipid peroxidation.[4] It is postulated that edaravone administration might improve tissue damage induced by ROS. The protective effects of edaravone on both hemispheric embolization and transient cerebral ischaemia have already been used clinically to treat acute brain infarction in Japan.[5–7] Studies have demonstrated that edaravone prevents endotoxin-induced liver injury in rats via the suppression of pro-inflammatory cytokine production.[8,9] Ito *et al.* showed that edaravone ameliorated the lung injury induced by intestinal ischaemia/reperfusion. It decreased the neutrophil infiltration, the lipid membrane peroxidation and the expression of IL-6 mRNA in the lungs, resulting in a reduction in mortality.[10]

The purpose of this study was to investigate the anti-inflammatory effects of edaravone in LPS-induced lung injury in mice. It was hypothesized that attenuation of lipid peroxidation and/or inhibition of pro-inflammatory cytokine and chemokine activation may lead to inhibition of airway inflammation.

METHODS

Mice, cells and reagents

All mice received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH publication 8523, revised 1985; http://www.nyu.edu/uawc/Forms/Guide-excerpts.pdf). The study protocol was approved by the institutional Ethics Committee. Female BALB/c mice, 6–8 weeks of age, were obtained from Japan SLC (Tochigi, Japan) and housed in the animal facility. The MH-S (murine alveolar macrophage) cells were obtained from the American Type Culture Collection (Rockville, MD, USA). LPS from *Escherichia coli* was purchased from Sigma (St Louis, MO, USA). Edaravone was obtained from Mitsubishi Pharma Corporation (Tokyo, Japan).

LPS-induced acute lung injury model

After BALB/c mice were anaesthetized by the intraperitoneal administration of 0.01 mL/g of 10% pentobarbital sodium solution (Abbott Laboratories, North Chicago, IL, USA), 100 μg/kg LPS in 60 μL saline was administered intranasally as previously described.[11] Edaravone dissolved in saline at concentrations of 1.5, 15 or 150 mg/kg or the same volume of saline was administered by a single intraperitoneal injection 1 h before LPS injection. BAL fluid (BALF) and serum were sampled 6 and 24 h after LPS treatment.

Sampling of BALF and serum

Blood samples were obtained from the right atrium of the anaesthetized mice at each time point. After centrifugation at 3000 *g* for 10 min at 4°C, the serum was frozen and stored at –80°C until assayed. BAL was performed four times through a tracheal cannula with 0.7 mL saline. In each mouse examined, approximately 2.5 mL (90%) of BALF was recovered. A 100-μL aliquot was used for the total cell count, and the remainder was immediately centrifuged at 1000 *g* for 10 min. The total cell number was counted using a haemocytometre, and cell differentiation was determined for more than 500 cells on cytocentrifuge slides with Wright–Giemsa staining. The supernatant of BALF was stored at –80°C until use.

Treatment of LPS-stimulated cells with edaravone

For the *in vitro* experiments, MH-S cells were cultured in tissue flasks incubated in 100% humidity and 5% CO_2 at 37°C in RPMI 1640 medium (Sigma, St Louis, MO) supplemented with 10% heat-inactivated fetal bovine serum (GIBCO BRL, Grand Island, NY, USA) and penicillin-streptomycin (50 mg/mL, GIBCO BRL) at 1×10^6 cells/mL. MH-S cells were then plated onto six-well, flat-bottom tissue culture plates (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) at a density of 3×10^5 cells/well in RPMI 1640 medium. The medium was changed every 2 days until the cells became confluent, At this point, the cultures were washed three times with 2 mL PBS, followed by the addition of 2 mL fresh serum-free media (RPMI 1640). Cells were then treated with various concentrations of edaravone (0–100 μmol/L) for 1 h followed by incubation with 1 μg/mL of LPS or medium alone. After 3, 6 and 24 h of LPS challenge, cell-free culture supernatants were collected and stored at –80°C until assay.

Assays for cytokines and lipid hydroperoxide

The levels of pro-inflammatory cytokines, IL-6 and tumour necrosis factor-α (TNF-α) and CXC chemokines, macrophage inflammatory protein-2 (MIP-2) and keratinocyte-derived chemokine (KC), were measured in BALF, serum, and culture supernatant. The levels of IL-6 and TNF-α were measured by a sandwich ELISA kit (Biosource International, Camarillo, CA, USA). MIP-2 was measured by the Mouse MIP-2 ELISA kit (R&D Systems, Minneapolis, MN, USA). KC was measured by the Mouse KC sandwich EIA kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan). The concentrations of lipid hydroperoxide (LPO) in serum and BALF were measured as an indicator of oxidative stress using the Lipid Hydroperoxide Assay kit (Cayman Chemical, Ann Arbor, MI, USA). The albumin concentration was determined in the cell-free BALF supernatant by dye-binding assay (Bio-Rad protein assay; Bio-Rad, Richmond, CA).

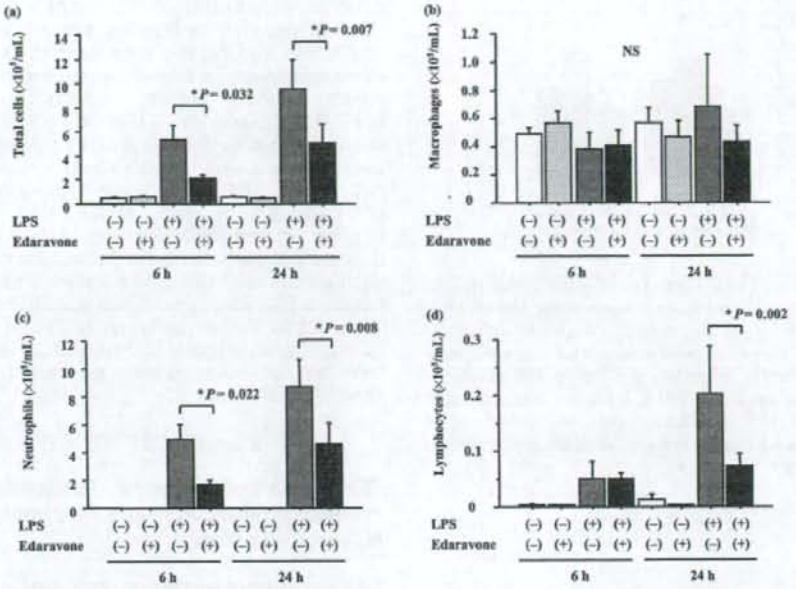

Figure 1 Effects of edaravone on the cell counts in BAL fluid (BALF) in a LPS-induced acute lung injury (ALI) mouse model (□, control group; ▨, edaravone group; ■, LPS group; ■, LPS + edaravone group). To induce ALI, LPS was instilled intranasally 1 h after the intraperitoneal administration of 150 mg/kg edaravone. The number of total cells (a) and neutrophils (c) in BALF in the LPS + edaravone group at 6 and 24 h after the LPS challenge was significantly decreased in comparison to the LPS group. At 24 h after the LPS challenge, edaravone caused a significant reduction in the number of lymphocytes (d); however, no difference was seen in the number of macrophages (b). Data are presented as mean ± SEM ($n = 6$ in each group). *$P < 0.05$ in comparison to LPS group. NS, not significant.

Statistical analysis

Data were expressed as means ± SEM. For multiple comparisons, one-way analysis of variance was used with the Fisher protected least-significant differences method used as the *post hoc* test. Differences between two variables were assessed with the Mann–Whitney *U*-test. A *P*-value < 0.05 was considered statistically significant.

RESULTS

Effects of edaravone pretreatment on BALF inflammatory cells

Pretreatment with 150 mg/kg of edaravone caused a significant reduction in inflammatory cell infiltration in BALF.

The number of total cells in BALF in the edaravone-treated mice at 6 and 24 h after the LPS challenge was significantly decreased in comparison to that in untreated mice (Fig. 1a). Similarly, the pre-administration of edaravone caused a significant reduction in the neutrophils in BALF at 6 and 24 h after the LPS challenge (Fig. 1c). At 24 h after the LPS challenge, the pre-administration of edaravone caused a significant reduction in the lymphocytes in BALF (Fig. 1d); however, no difference was seen in the BALF macrophages (Fig. 1b). Low-dose (1.5 or 15 mg/kg) edaravone did not prevent LPS-induced ALI (data not shown).

Effects of edaravone pretreatment on albumin levels in BALF

The preventive effects of edaravone on lung permeability were assessed by measurement of the albumin content in the BALF of the LPS-induced ALI model. The albumin level in BALF was significantly increased in mice given LPS, but pretreatment with 150 mg/kg edaravone significantly reduced BALF albumin levels at 6 and 24 h after LPS instillation (Fig. 2).

Effects of edaravone pretreatment on pro-inflammatory cytokine and LPO production in BALF and serum

Pro-inflammatory cytokines IL-6 and TNF-α are secreted by activated alveolar macrophages in

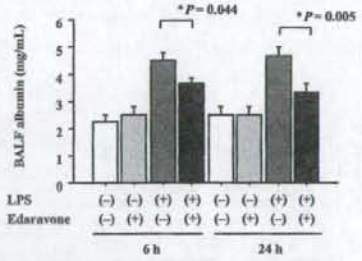

Figure 2 Effects of edaravone on albumin levels in BAL fluid (BALF) in a LPS-induced acute lung injury (ALI) mouse model (□, control group; ▨, edaravone group; ■, LPS group; ■, LPS + edaravone group). The single administration of 150 mg/kg edaravone 1 h before LPS challenge significantly reduced the BALF levels of albumin at 6 and 24 h after LPS instillation. Data are presented as mean ± SEM ($n = 6$ in each group). *$P < 0.05$ in comparison to the LPS group.

patients with ALI/ARDS.[1] The BALF levels of IL-6 (Fig. 3a) and TNF-α (Fig. 3b) peaked 6 h after LPS instillation and rapidly decreased thereafter. These elevations were significantly attenuated by 150 mg/kg edaravone administration ($P < 0.05$). The BALF levels of KC (Fig. 3c) and MIP-2 (Fig. 3d), which are chemotaxins for neutrophils similar to human IL-8, also peaked at 6 h, and the elevations were significantly attenuated by edaravone pre-administration ($P < 0.05$). Serum levels of IL-6 (Fig. 4a) and KC (Fig. 4c) showed similar profiles to the BALF levels of IL-6 and KC. They peaked at 6 h and the elevation was significantly attenuated by edaravone before administration ($P < 0.05$). No significant differences were observed in the serum levels of TNF-α (Fig. 4b) or MIP-2 (Fig. 4d). The serum and BALF levels of LPO were not decreased in mice pretreated with edaravone (data not shown).

Effects of edaravone on pro-inflammatory cytokine production from LPS-stimulated MH-S cells *in vitro*

MH-S cells were pretreated with various concentrations of edaravone (0~100 μmol/L) for 1 h and then

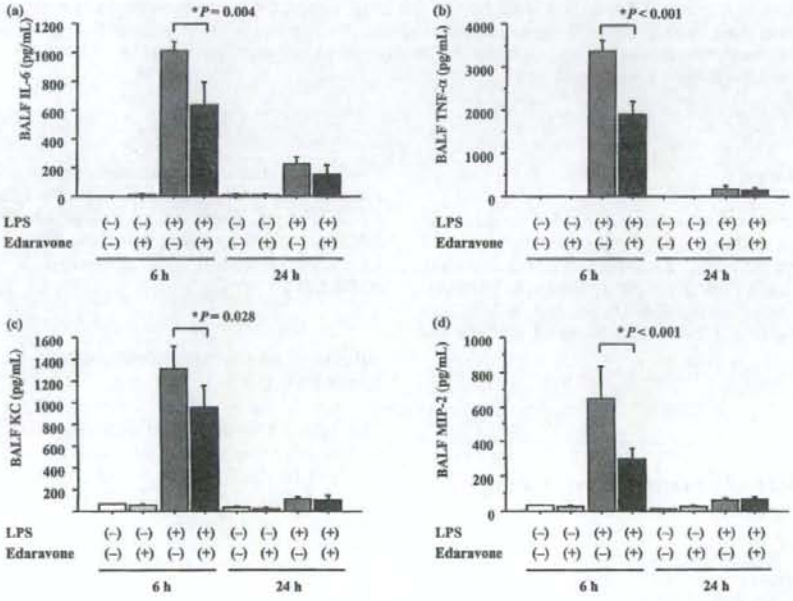

Figure 3 Effects of edaravone on pro-inflammatory cytokine production in BAL fluid (BALF) in a lioppolysaccharide (LPS)-induced acute lung injury (ALI) mouse model (□, control group; ▨, edaravone group; ■, LPS group; ■, LPS + edaravone group) To induce ALI, LPS was instilled intranasally 1 h after the intraperitoneal administration of 150 mg/kg edaravone. BALF levels of IL-6 (a), tumour necrosis factor (TNF)-α (b), keratinocyte-derived chemokine (KC) (c) and macrophage inflammatory protein-2 (MIP-2) (d) were all significantly attenuated in the LPS + edaravone group in comparison to the LPS group at 6 h after the LPS challenge. Data are presented as mean ± SEM ($n = 6$ in each group). *$P < 0.05$ in comparison to the LPS group.

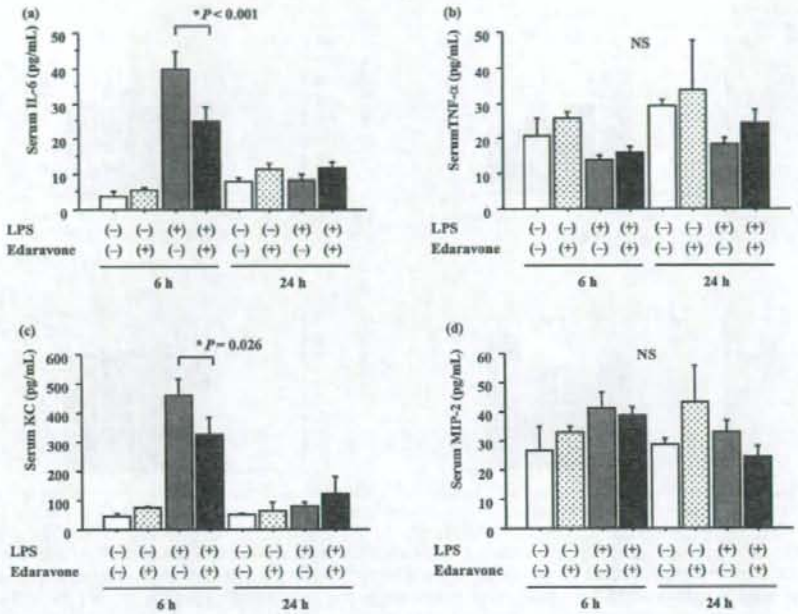

Figure 4 Effects of edaravone on pro-inflammatory cytokine production in sera in a LPS-induced acute lung injury (ALI) mouse model (□, control group; ▨, edaravone group; ■, LPS group; ■, LPS + edaravone group). To induce ALI, LPS was instilled intranasally 1 h after the intraperitoneal administration of 150 mg/kg edaravone. Although serum levels of IL-6 (a) and keratinocyte-derived chemokine (KC) (c) were significantly attenuated in the LPS + edaravone group in comparison to the LPS group, no significant differences were observed in the serum levels of tumour necrosis factor (TNF)-α (b) and macrophage inflammatory protein-2 (MIP-2) (d) among the four groups 6 h after the LPS challenge. Data are presented as mean ± SEM ($n = 6$ in each group). *$P < 0.05$ in comparison to the LPS group. NS, not significant.

were left unstimulated or stimulated with 1 μg/mL LPS for 24 h. The concentrations of IL-6, TNF-α, KC and MIP-2 in the culture supernatant were measured by ELISA. Edaravone dose-dependently inhibited IL-6 production by LPS-stimulated MH-S cells (Fig. 5a). Levels of TNF-α, KC and MIP-2 in the culture supernatant were similar in all treatment groups (Fig. 5b–d). The levels of IL-6, TNF-α, KC and MIP-2 in the culture supernatant at 3 and 6 h after LPS exposure were not attenuated by any dose of edaravone (data not shown). Furthermore, similar experiments were conducted using murine alveolar macrophages, but not cell lines, harvested by BALF, *in vitro* to determine the specific mechanics, but there was no significant difference compared with MH-S cells (data not shown).

DISCUSSION

The present study demonstrated that in the LPS-induced ALI model, edaravone attenuated cellular infiltration and concentrations of albumin, IL-6, TNF-α, KC and MIP-2 in BALF. *In vitro* studies demonstrated that elevated IL-6 secretion from MH-S cells in response to LPS was significantly attenuated by edaravone. These findings suggested that edaravone could prevent ALI via repression of cytokine release from macrophages.

Neutrophils are key players in the pathogenesis of ALI, releasing lipid, enzyme mediators and oxygen radicals.[12,13] Accumulation of neutrophils within the lung in the setting of LPS-induced stimulation probably depends on the coordinated expression of pro-inflammatory cytokines, adhesion molecules and the establishment of chemotactic gradients via the local generation of chemotactic factors. TNF-α, an early pro-inflammatory cytokine, is believed to trigger the activation of other pro-inflammatory cytokines, such as IL-6 and IL-8.[14] A sufficient concentration of edaravone for the inhibition of IL-6 from MH-S cells ranged from 10–100 μmol/L. At 5 min after a single intravenous administration of edaravone (2 mg/kg/day: roughly equivalent to the daily human dose) to male rats, edaravone levels in plasma and lung tissue were about 1 and 0.5 μmol/L, respectively.[15] To inhibit pro-inflammatory cytokine production by LPS, concentrations of edaravone 10–100 times higher than the daily human dose might be necessary. The levels of TNF-α, KC and MIP-2 in the

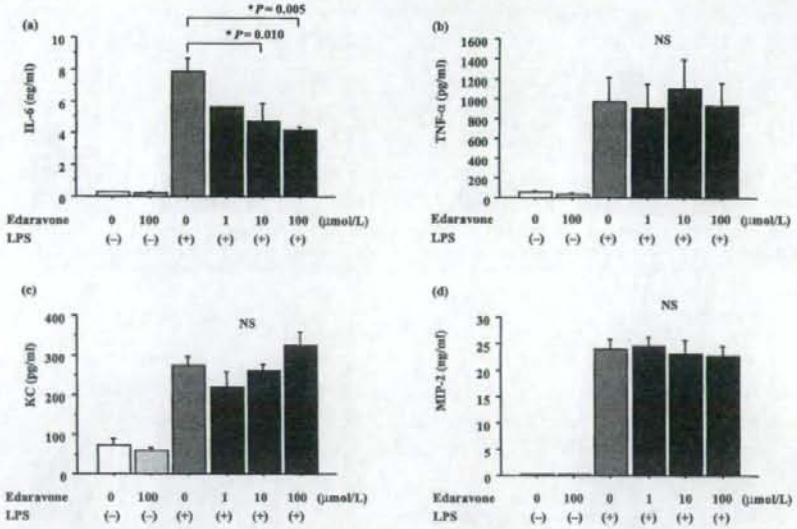

Figure 5 Effects of edaravone on pro-inflammatory cytokine production from LPS-stimulated MH-S cells *in vitro* (□, control group; ▨, edaravone group; ■, LPS group; ■, LPS + edaravone group). MH-S cells pretreated with various concentrations of edaravone (0–100 μmol/L) for 1 h were left unstimulated or stimulated with 1 μg/mL of LPS for 24 h. Treatment with edaravone significantly inhibited IL-6 production by LPS-stimulated MH-S cells, dose-dependently (a). No differences were found in tumour necrosis factor (TNF)-α, keratinocyte-derived chemokine (KC), or macrophage inflammatory protein-2 (MIP-2) levels in culture supernatants (b–d). The mean pro-inflammatory cytokine concentrations ± SEM are shown for the cumulative data from each of three samples in three independent experiments. *$P < 0.05$ in comparison to the LPS group. NS, not significant.

culture supernatant of MH-S cells were not affected by treatment with edaravone 3, 6 or 24 h after LPS exposure. Although the reasons for the differences in the effective concentration of edaravone between *in vivo* and *in vitro* studies are not known, other cells such as neutrophils, epithelial cells, endothelial cells, dendritic cells or lymphocytes might cooperate with monocyte-macrophages at different concentrations of edaravone.

The main adverse effects of edaravone are renal failure and liver dysfunction.[15] No significant effect on heart rate or blood pressure at the dose of 450 mg/kg of edaravone was reported.[16] No adverse effects of a single administration of 150 mg/kg/day of edaravone were observed in the present study. The usual daily dose of edaravone in humans for the treatment of cerebral infarction is approximately 1.5 mg/kg, and the medication is continued for 14 days.[4–7] In the present study, LPS-induced ALI could not be prevented by a low dose (1.5–15 mg/kg/day) of edaravone. The dose of edavarone used, 150 mg/kg, was roughly 100 times the daily human dose administered as a single injection in this study. Anzai *et al.* have shown the radioprotective effect of edaravone on whole body X-ray irradiation in C3H mice.[16] An increase in the survival rate required 450 mg/kg intraperitoneally of edavarone given 30 min prior to the irradiation.[16] High-dose pulse therapy with edaravone might be needed for the prevention of lung injury induced by LPS; as it is needed to prevent injury induced by irradiation.

Edaravone is a potent-free radical scavenger and has the antioxidant ability to inhibit lipid peroxidation.[4] The amount of LPO in BALF and serum is an indicator of oxidative stress.[17] The results of the present study, however, demonstrated that the serum and BALF levels of LPO did not decrease in edaravone-treated mice. Kono *et al.* have shown that the increases in 4-hydroxynonenal (HNE)-modified protein, a cytotoxic lipid peroxidation product, were blunted in the liver by edaravone.[8] Tsuji *et al.* have shown that the elevated levels of serum malondialdehyde (MDA), which is one of the most frequently used indicators of lipid peroxidation, were inhibited significantly by edaravone administration.[9] In the present study, no inhibition of ROS production by edaravne was shown. The reasons for the discrepancy between these studies are not known, but might relate to the differences in drug delivery or transition to the liver or lung. Investigation of the indicators of lipid peroxidation without LPO, such as HNE and MDA, could determine the mechanics of anti-oxidants.

Nuclear factor-kappa B (NF-κB) is one of the crucial transcription factors required for the maximal transcription of a wide array of pro-inflammatory molecules including TNF-α and other mediators. In endotoxaemia, NF-κB freed from IκB (inhibitor κB) translocates into the nucleus, where it enhances the transcription of cytokines such as TNF-α and IL-6.[18] The pivotal role of the activation of NF-κB in inflammation during ALI has been well

elucidated in previous studies.[12,19,20] Although the mechanism of the inhibitory effects of edaravone in the inflammatory response is incompletely understood, several studies of the relationship between NF-κB and edaravone have been published.[9,21] In the pathway of activation of NF-κB, ROS are regarded as second messengers.[22,23] It has been reported that several anti-oxidants block the activation of NF-κB through the inhibition of ROS *in vitro*.[24–26] Kokura *et al.* have shown that pretreatment with edaravone scavenged the ROS and inhibited the activation of NF-κB.[21] Furthermore, Tsuji *et al.* have shown that edaravone prevented endotoxin-induced liver injury after partial hepatectomy not only by attenuating oxidative damage, but also by reducing the production of inflammatory cytokines, cytokine-induced neutrophil chemoattractant and inducible nitric oxide synthase, in part through the inhibition of NF-κB activation.[9] Although NF-κB was not examined, in the present study, it is possible that edaravone might affect the activation of NF-κB, causing the decrease of pro-inflammatory cytokine production in this LPS-induced ALI model.

In summary, the results of the present study indicated that edaravone could inhibit LPS-induced lung injury via repression of pro-inflammatory cytokine production. Clinical studies in patients with ALI/ARDS will be necessary to determine the appropriate dose, route of administration and duration of treatment.

ACKNOWLEDGEMENT

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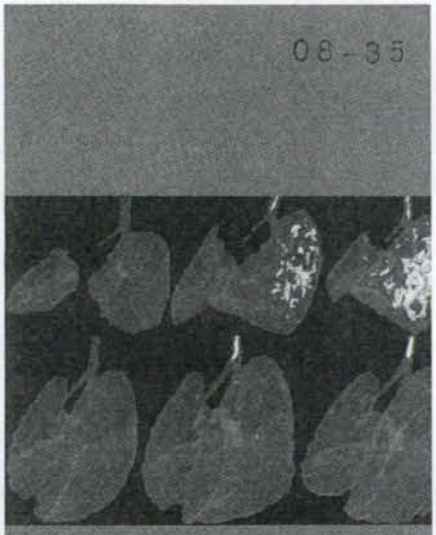

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Effects of edaravone, a free-radical scavenger, on bleomycin-induced lung injury in mice

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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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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

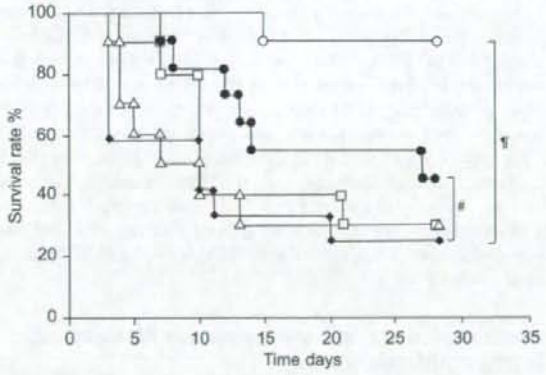

FIGURE 1. Effects of edaravone on mortality in a bleomycin (BLM)-induced lung injury mouse model. The survival rates of five study groups of mice are shown over a 28-day observation period (10–12 mice in each group). The four BLM + edaravone groups received single intraperitoneal infusion of edaravone 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

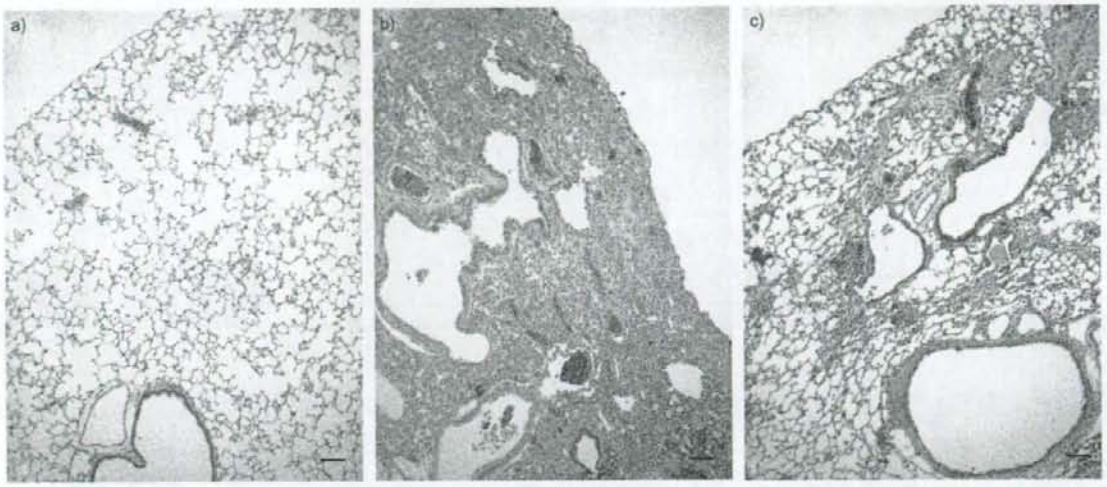

FIGURE 2. Effects of edaravone on histopathological changes. Lung tissue was obtained on day 28 after instillation of bleomycin (BLM) or saline and was stained with haematoxylin and eosin. a) Saline-group lung tissue sample showing thin interalveolar septa, a lack of inflamed cells, and normal-appearing 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.

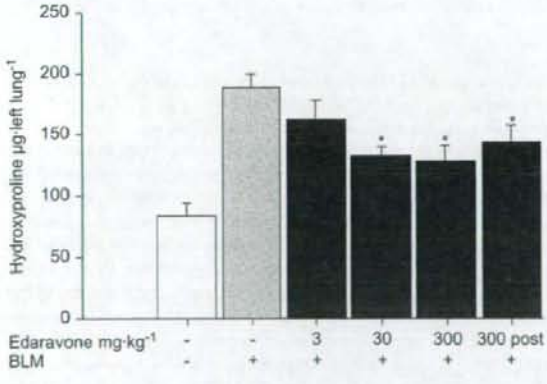

FIGURE 3. Effects of edaravone on the hydroxyproline content in the left lung in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. The hydroxyproline content was significantly increased by BLM injection. Single administration of 30 or 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly 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

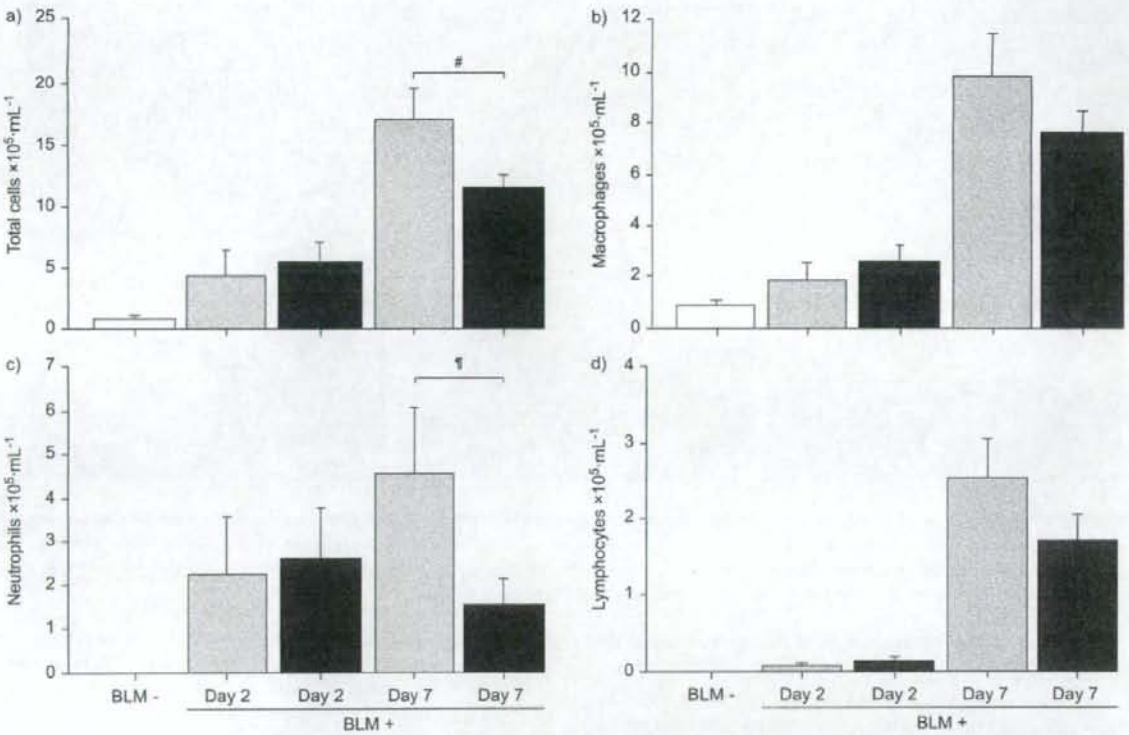

FIGURE 4. Effects of edaravone on bronchoalveolar lavage fluid (BALF) cell analysis in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Single administration of 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly reduced the number of total cells and neutrophils in BALF on 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.

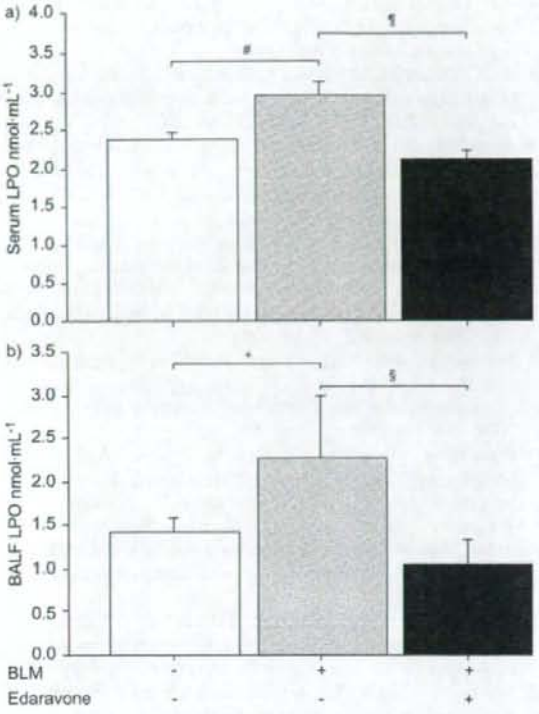

FIGURE 5. Effects of edaravone on the amount of a) lipid hydroperoxide (LPO) in serum and b) bronchoalveolar lavage fluid (BALF) in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Edaravone treatment consisted of a single administration of 300 mg·kg^{-1} 1 h before BLM instillation. a) Although 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

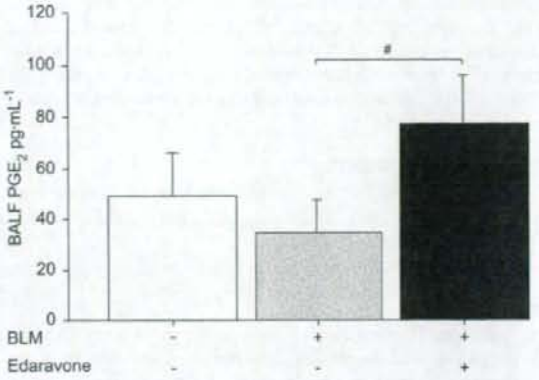

FIGURE 6. Effects of edaravone on the prostaglandin (PG)E_2 levels in bronchoalveolar lavage fluid (BALF) of a bleomycin (BLM)-induced pulmonary fibrosis mouse model. Single administration of 300 mg·kg^{-1} of edaravone 1 h before BLM instillation significantly increased PGE_2 on day 2. □: control group; ▒: 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

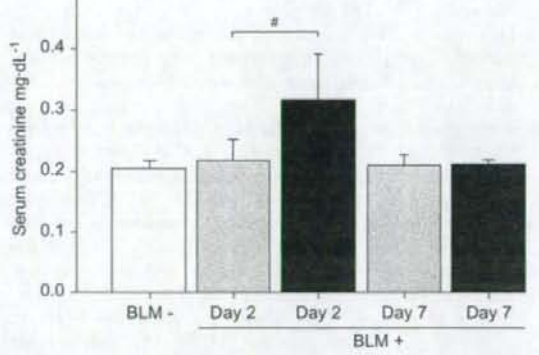

FIGURE 7. Adverse effects of edaravone on the serum creatinine levels in a bleomycin (BLM)-induced pulmonary fibrosis mouse model. The serum creatinine levels were measured by Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan). Although a temporary increase of serum creatinine levels at 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.

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Case Report

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Drug-Induced Pneumonitis Associated with Imatinib Mesylate in a Patient with Idiopathic Pulmonary Fibrosis

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Key Words
Drug-induced pneumonitis · Idiopathic pulmonary fibrosis · Imatinib mesylate

Abstract
A 74-year-old man with idiopathic pulmonary fibrosis (IPF) developed severe dyspnea on exertion after the re-administration of imatinib mesylate for chronic myeloid leukemia. Chest X-ray and CT showed ground-glass opacities in both lungs in addition to preexisting honeycombing. Discontinuation of imatinib and methylprednisolone pulse therapy followed by administration of oral prednisolone resulted in improvement in both symptoms and radiographic findings. Imatinib-induced pneumonitis was diagnosed based on the clinical course and findings. Only five previous reports of imatinib-induced pneumonitis have been published in the literature, and this is the first case reported in a patient with IPF.

Introduction

Imatinib mesylate (Gleevec, formerly STI571) belongs to a group of drugs successfully used in the molecular targeting of several cancers. Imatinib is a selective inhibitor of BCR-ABL tyrosine kinase, which has an essential role in the pathogenesis of chronic myeloid leukemia (CML). In the treatment of CML, this drug is highly effective and well tolerated; however, it has been associated with various adverse effects, e.g. superficial edema, nausea, muscle cramps and skin rashes, although such side effects are generally mild [1]. In addition, imatinib has been associated with respiratory complications, e.g. dyspnea and cough, related to pulmonary edema and pleural effusion [1]. In this report, we describe a case of imatinib-induced interstitial pneumonitis in a patient with idiopathic pulmonary fibrosis (IPF).

Case Report

In July 2001, a 74-year-old man was referred to our department for investigation of abnormal shadows on chest X-ray. He reported a 2-year history of gradually progressive dyspnea on exertion. Chest X-ray showed bilateral reticulo-nodular shadows situated predominantly in the lower lung fields (fig. 1). Chest CT revealed basal predominant reticular abnormality and honeycombing in the lower lobes (fig. 2). Serum KL-6 level was elevated (1,480 U/ml; normal <500). A diagnosis of IPF was made on the basis of the clinical diagnostic criteria established by the American Thoracic Society Consensus Statement [2]. In addition, laboratory investigations also revealed an elevated white blood cell (WBC) count (18,700/μl; normal 3,800–9,800, with 77% neutrophils, 12% lymphocytes, 8% monocytes and 1% eosinophils). At the Department of Hematolo-

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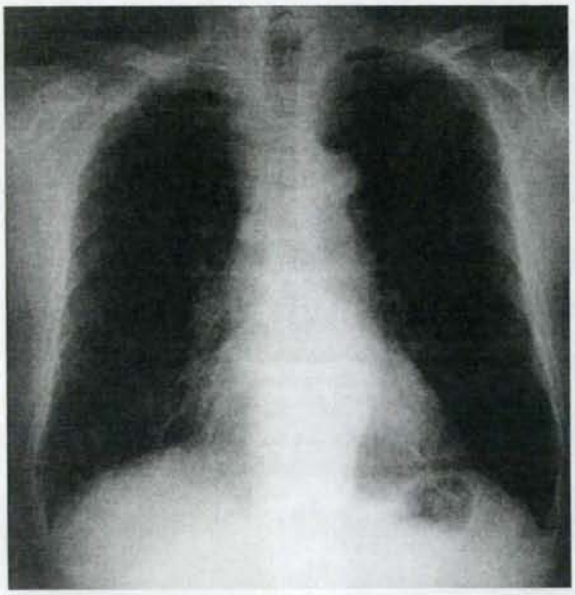

Fig. 1. Chest X-ray from July 2001. Bilateral reticulo-nodular shadows are observed predominantly in the lower lung fields.

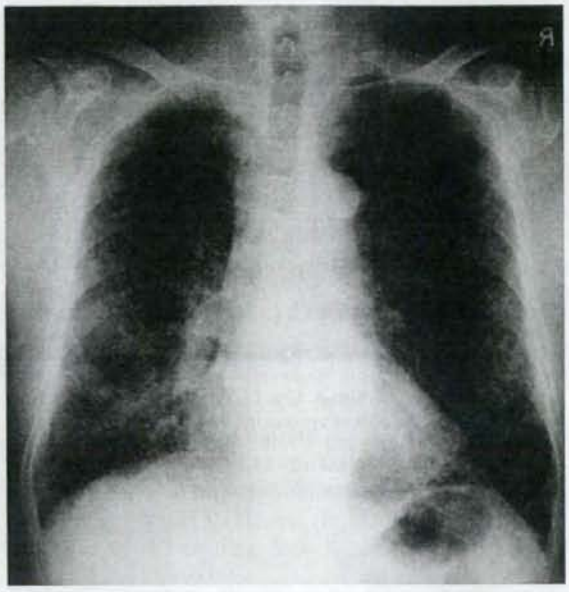

Fig. 3. Chest X-ray taken on admission (August 22, 2003). Bilateral ground-glass opacities are apparent in the middle and lower lung fields in addition to preexisting interstitial changes.

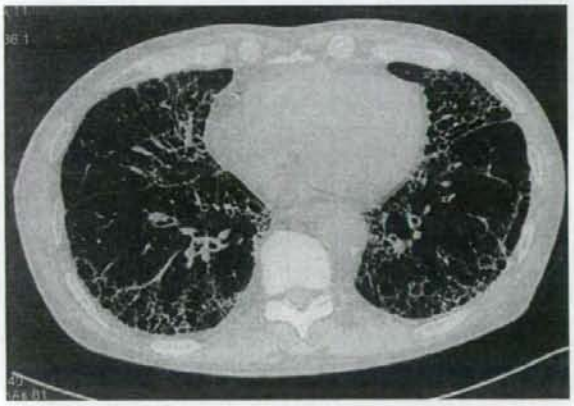

Fig. 2. Chest CT from July 2001. Bilateral reticular abnormality and honeycombing are observed in the lower lobes.

gy, the patient was diagnosed with Philadelphia-chromosome-negative, BCR-ABL-positive, chronic-phase CML based on findings of bone marrow aspiration showing myeloid hyperplasia with 0.6% blasts and positive RT-PCR findings for the major BCR-ABL fusion gene. Hydroxyurea was initiated in December 2001 and treatment was changed to imatinib (400 mg/day) in February 2002. However, in April 2002, imatinib was discontinued due to the development of facial edema and mild muscle cramps. Hydroxyurea was recommenced at this point; however, the patient decided to discontinue hydroxyurea treatment after 1 month. Although the clinical condition fortunately remained stable for 10 months, a gradual rise in WBC count (13,600/μl) occurred thereafter. On July 25, 2003, imatinib (200 mg/day) was readministered to prevent progression to accelerated/blast crisis phase. Following the readministration of imatinib, the patient gradually developed severe dry cough and progressive dyspnea on exertion. He was admitted to our department on August 22, 2003.

On physical examination, temperature was 37.2°C and respiratory rate was 24 breaths/min. Fine crackles were audible, predominantly in both posterior lower lung fields. Laboratory tests on admission showed increased C-reactive protein (4.67 mg/dl; normal <0.06) and accelerated erythrocyte sedimentation rate (43 mm/h; normal 0–10). WBC count was 9,100/μl with 79% neutrophils, 7% lymphocytes, 8% monocytes and 6% eosinophils. Serum lactate dehydrogenase was moderately increased (441 mU/ml; normal 109–216) and KL-6, surfactant protein (SP)-D and SP-A levels were also elevated (902 U/ml, 188 ng/ml; normal <110, and 138 ng/ml; normal <43.8, respectively). Total serum IgE was marginally raised (340 U/ml; normal <216). Analysis of arterial blood gases on room air revealed severe hypoxemia (PaO_2 41.2 Torr). Sputum culture was negative and no acid-fast bacilli were observed. A chest X-ray taken on admission showed bilateral ground-glass opacities in the middle and lower lung fields in addition to the pre-

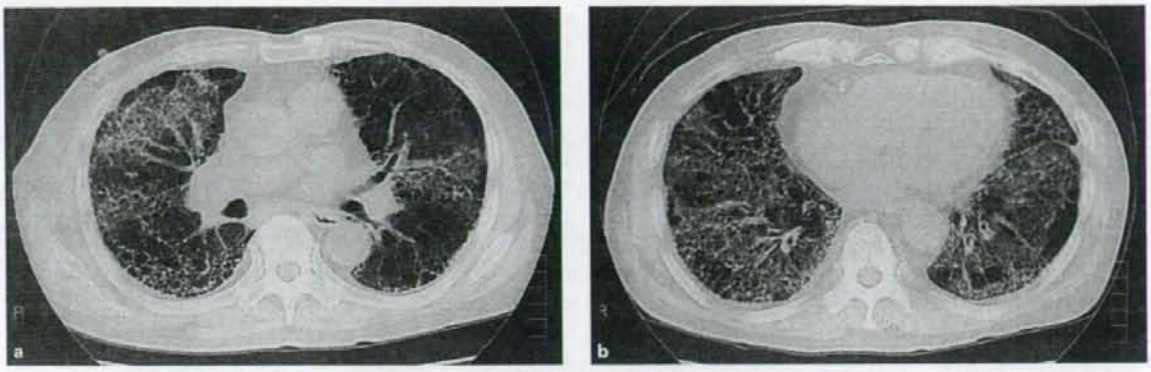

Fig. 4. Chest CT taken on admission (August 22, 2003). Diffuse bilateral ground-glass opacities are evident (**a**) and preexisting honeycombing is observed in the lower lobes (**b**).

Fig. 5. Histopathological appearance of TBLB specimens (elastica van-Gieson, original ×100). Thickened alveolar septa with modest infiltration of inflammatory cells, Masson bodies (arrow) and slight interstitial fibrosis are observed.

existing interstitial changes (fig. 3). CT revealed diffuse ground-glass opacities in both lungs (fig. 4). No pleural effusion was seen.

As these findings were highly suggestive of imatinib-induced interstitial pneumonitis, this agent was discontinued. High-dose methylprednisolone (1 g/day i.v. for 3 days) was administered, followed by oral prednisolone (60 mg/day), which was then gradually tapered. This resulted in a gradual improvement in the clinical condition. Histopathological analysis of transbronchial lung biopsy (TBLB) specimens from the right B^8 on the 35th hospital day revealed thickened alveolar septa, a few Masson bodies, and slight interstitial fibrosis. No significant infiltration of inflammatory cells was evident in the alveolar septa (fig. 5). Six weeks after this episode, chest CT showed improvement in the previously described pulmonary findings, with the exception of the preexisting honeycombing. At this point, serum levels of SP-D and SP-A had normalized, while KL-6 remained unchanged. No change in CML disease activity was noted during the course of the pulmonary complications.

Discussion

A review of the literature revealed only five case reports of imatinib-induced pneumonitis [3–7]. Among these, Bergeron et al. [3] reported a case of hypersensitivity pneumonitis related to imatinib and Yokoyama et al.