MCI-186 (edaravone), a free radical scavenger, attenuates ischemia—reperfusion injury and activation of phospholipase A2 in an isolated rat lung model after 18 h of cold preservation

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Abstract

Objective: Increased microvascular permeability and extravasation of inflammatory cells are key events in ischemia—reperfusion (IR) injury. We hypothesized that edaravone, a free radical scavenger, is able to attenuate IR lung injury by decreasing oxidative stress and phospholipase A2 (PLA2) activation, which otherwise may lead to lung injury through PAF receptor (PAF-R) activation.

Methods: We used an isolated rat lung model. Five groups were defined (n = 7, each): in the sham and vehicle group, lungs were immediately washed after thoracotomy or perfused for 2 h without an ischemic period, respectively. In the ischemic groups, 10 mg/kg of MCI-186 (edaravone group), 1 mg/kg of PAF-R inhibitor (ABT-491 group) or saline (control group) were i.v. administered 20 min before harvest. Lungs were flushed with LPD solution, stored at 4°C for 18 h, and reperfused for 2 h.

Results: Compared to vehicle group, IR significantly decreased the PO2 level and increased the wet-to-dry ratio, proteins in bronchoalveolar lavage (BAL), and myeloperoxidase (MPO) activity in the control group, while edaravone treatment maintained the PO2 similar to the vehicle group and significantly reduced edema formation and neutrophil extravasation. Consistently, IR significantly increased lipid peroxidation, cytosolic-PLA2 activity mainly via alveolar macrophages, soluble-PLA2 activity, leukotriene B4, and PAF-R expression in control lungs, together with a decreased PAF acetylhydrolase (PAF-AH) activity. Edaravone significantly reduced all of these, but increased PAF-AH activity. Furthermore, pharmacological inhibition of the PAF-R attenuated IR injury resembling edaravone action.

Conclusion: Edaravone attenuates lung IR injury by suppressing oxidative damage and PLA2 activation, which otherwise partially mediates edema formation and neutrophil extravasation through PAF-R activation.

Keywords: Edaravone; Phospholipase A2; Lung; Ischemia—reperfusion

1. Introduction

Lung transplantation (LT) has become the mainstay of therapy for most end-stage lung disease [1]. Ischemia—reperfusion (IR) lung injury is a major complication of the early postoperative period after LT [2]. Life-threatening IR injury occurs in up to 20% of transplants leading to primary graft failure (PGF) with a mortality rate of up to 60% [3]. Nonspecific alveolar damage, hypoxemia, increased pulmonary vascular resistance, lung edema, and extravasation of leukocytes characterize the syndrome.

Lung transplantation activates multiple inflammatory pathways that promote PGF [2]; however, clinical and experimental studies suggest that the oxidative stress induced by reactive oxygen species (ROS) is one of the most important mediators of IR injury [4]. The endothelium appears to be one of the predominant sources of oxidants [5], although macrophages and/or marginated neutrophils also contribute to the lung oxidant burden [6]. ROS are highly reactive substances that can cause direct oxidative damage [2,4] and able to modulate many inflammatory responses including the arachidonic acid cascade [7,8].

Phospholipase A2 (PLA2) is a key enzyme that plays a crucial role in orchestrating the inflammatory response in lung injury through the synthesis of lipid mediators such as eicosanoids and platelet-activating factor (PAF) [9]. The PLA2 family is classified into three main subtypes: secretory PLA2 (sPLA2), cytosolic Ca2+-dependent PLA2 (cPLA2), and intracellular Ca2+-independent PLA2 (iPLA2) [10]. Studies on acute lung injury and IR have mainly focused on sPLA2 forms [11]; however, recent reports have highlighted the importance of the cPLA2 in the pathogenesis of these processes [12,13].

PAF, a potent lipid autacoid, mediates its effects through a G-protein-coupled membrane PAF receptor (PAF-R) [14]. PAF amplifies the inflammatory response by promoting neutrophil extravasation, increased vascular permeability,
and pulmonary edema leading to lung injury [14,15]. Several groups have reported that antagonist of PAF attenuates IR injury in animal models [16] and clinical lung transplantation [17]. Similar results have been reported with PAF acetylhydrolase (PAF-AH) [18].

3-Methyl-1-phenyl-2-pyrazoline-5-one (MCI-186, edaravone), a free radical scavenger, has been recently approved in Japan for the treatment of acute ischemic stroke [19] and has also exhibited protective effects in acute myocardial infarction [20]. Animal models have reported that edaravone attenuates IR; however, its effect on IR lung injury is still not characterized.

The present study was designed to test the following hypothesis in an isolated rat lung model: edaravone ameliorates IR-induced lung injury by suppressing ROS formation and inhibiting activation of PLA₂ and its downstream cascade, which otherwise leads to edema formation and leukocyte extravasation, partially mediated through stimulation of the PAF-R.

2. Material and methods

2.1. Experimental protocol

Animals received humane care in compliance with the ‘Guide for the Care and Use of Laboratory Animals (National Institute of Health Publication, 1985)’. The Institutional Committee for Experimental Research of our University approved this experiment.

Isolated lungs were assigned to five groups (n = 7, each): in the sham and vehicle group, lungs were immediately washed after thoracotomy or perfused for 2 h without an ischemic period, respectively. In two ischemic groups, 10 mg/kg of MCI-186 from Mitsubishi Pharma Co., Osaka, Japan (edaravone group) or saline (control group) was i.v. infused via the external jugular vein 20 min before lung harvest. Lungs were then flushed with low-potassium dextran (LPD) solution, stored for 18 h, and reperfused for 2 h. The utilized dose and timing of the administration of edaravone were applied on the basis of preliminary experiments.

2.2. Lung harvest, preservation, and reperfusion

The procedure has been reported previously [21]. Briefly, male Sprague–Dawley (SD) rats (350–450 g) from Sankyo Labo Service, Tokyo, Japan were anesthetized (50 mg/kg of ketamine hydrochloride i.p.) and mechanically ventilated through a tracheostomy with a FiO₂ 0.95, a TV of 3 mL, a PEEP of 8 cmH₂O and ventilated as above. The rate of perfusion was gradually increased to 8 mL/min in the first 15 min then kept constant throughout the experiment. Drained blood from the lungs was deoxygenated to adjust the PO₂ to 40–50 mmHg using a rat model membrane oxygenator (Senko Ika, Tokyo, Japan) ventilated with 95% N₂ + 5% CO₂. At the end of the perfusion period, lungs were washed with saline solution.

2.3. Monitoring

During reperfusion, blood gas tension was determined at 15, 30, 60, 90, and 120 min (STAT PROFILE pHox, Nova Biomedical, Waltham, MA, USA). Mean pulmonary artery pressure (mPAP) and peak airway pressure (pAwP) were recorded continually.

2.4. Lung wet-to-dry ratio

The right apical and accessory lobes were excised, weighed together, and desiccated at 70 °C for 1 week. The weight immediately after reperfusion and the stable dry-weight after 1 week were used to calculate the lung wet-to-dry weight ratio. The remaining lobes (middle and inferior) were snap frozen in liquid nitrogen and stored at −80 °C.

2.5. Bronchoalveolar lavage (BAL)

Left side lungs were injected four times with 2.5 mL of 0.01 M phosphate buffer (pH 7.4), with three sets of instillations and withdrawals each. At least 90% of the total injected volume was recovered. BAL fluid (BALF) was centrifuged at 1000 rpm for 10 min and the protein concentration was measured by the bicinchonic acid (BCA) method.

2.6. Malondialdehyde (MDA)

To assess lipid peroxidation, the thiobarbituric acid test (TBA) was used for measuring levels of tissue MDA. Lungs were homogenized in 1.15% KCl and 0.2 mL of 8.1% sodium dodecylsulfate (SDS), 1.5 mL of 20% acetate acid, 1.5 mL of TBA solution, and 0.7 mL of distilled water were added to the sample. The mixture was kept in a boiling water bath for 1 h, cooled in a tap-water system for 10 min, then 1 mL of distilled water and 5 mL of N-butanol/pyridine were added. The mixture was centrifuged at 3000 rpm for 15 min, and the absorbance read. Results are expressed as nanogram per gram of tissue.

2.7. Myeloperoxidase (MPO) activity

To assess neutrophil infiltration, lung tissue (50 mg) was homogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB) in 50 mM phosphate buffer (pH 6.0) at a ratio of 1:10 (w/v) for 20 s, and centrifuged at 17,000 rpm for 20 min. The pellet was resuspended in HTAB, freeze—thawed (20 min at −80 °C), homogenized for 60 s, sonicated three times for 30 s, and centrifuged at 17,000 rpm for 20 min. The test
sample was mixed with 100 mM phosphate buffer (pH 6.0) containing 1 mM of o-dianisidine dihydrochloride and 0.005% of H₂O₂, and absorbance read at 450 nm.

2.8. Histological evaluation

Additional experiments were performed (n = 3, per group). The right lungs were fixed with 10% formalin and left lungs were embedded with Tissue-Tek® compound (Sakura Finetechnical Co., Tokyo, Japan) and stored at −80 °C. Formalin-fixed tissues were sliced into 4-μm thick sections and processed for hematoxylin—eosin stain (H&E).

2.9. Phospholipase A₂ study

2.9.1. Lung cPLA₂ and sPLA₂ activity

The assay was performed using a commercially available PLA₂ assay kit (765021, Cayman, Ann Arbor, MI, USA). The lungs (50 mg) were homogenized according to the manufacturer and supernatants were further centrifuged at 14,000 × g for 40 min using a cellulose membrane filter with a cut-off of 30 kDa (Microcon YM-30, Millipore, MA, USA) to separate sPLA₂ forms. To avoid any measurement of iPLA₂ in the sample, the specific inhibitor bromoenol lactone was used. Results are expressed as nanomoles per milligram per milliliter.

2.9.2. Immunoblotting for phosphorylated cPLA₂

Lung homogenates (50 μg) were electrophoresed on 6% SDS-PAGE, transferred onto PVDF membranes, and blocked for 1 h with 5% skin milk. The primary antibody (polyclonal anti-phospho-cPLA₂, Cell Signaling Tech., MA, USA, at 1:200 or monoclonal anti-phosphorylated cPLA₂, Santa Cruz at 1:500) were incubated at RT for (rabbit polyclonal anti-phospho-cPLA₂, Cell Signaling or anti-phosphorylated cPLA₂, 2831, Cell Signaling Tech., MA, USA, at 1:200 or monoclonal anti-phospho-cPLA₂, Cell Signaling Tech., MA, USA, at 1:30,000) was incubated overnight and the secondary antibody (anti-rabbit or anti-mouse IgG HRP from Protein Block Serum-Free (X0909, DakoCytomation, CA, USA) for 5 min. Blots were recorded in LAS-1000 camera (Fujifilm, Tokyo, Japan). HeLa whole cell lysates (D1604, Santa Cruz, CA, USA) served as positive control.

2.9.3. Immunofluorescence for phosphorylated cPLA₂ and PAF receptor (PAF-R)

Frozen sections (4 μm) fixed with 1% paraformaldehyde were blocked with Protein Block Serum-Free (X0909, DakoCytomation, CA, USA) for 5 min. Primary antibodies (rabbit polyclonal anti-phospho-cPLA₂, Cell Signaling or anti-PAF-R, sc-20732, Santa Cruz at 1:30) were incubated at RT for 2 h and anti-rabbit IgG FITC-conjugated at 1:500 for 1 h. Rabbit serum without first antibody was used as negative control. Mounted slices were visualized within 2 h on a Zeiss Axiophot microscope.

2.9.4. Leukotriene B₄ (LTB₄) measurement

LTB₄ level in BAL fluid was determined by enzyme immunoassay (EIA) kit (RNP 223, Amersham). The detection limit of the EIA assay for LTB₄ was 6 pg/mL.

2.9.5. Platelet activator factor acetylhydrolase (PAF-AH) activity

Lung PAF-AH activity was measured using a commercially available kit (760901, Cayman) according to the provider. Results are expressed as nanogram per milliliter per minute.

2.10. Pharmacological intervention

To assess if IR-induced expression of PAF-R mediated lung injury, we chose to study the effect of a PAF-R antagonist (ABT-491). In an additional group of rats (n = 7), 1 mg/kg of ABT-491 from Sigma (ABT-491 group) was i.v. administered 20 min before lung harvest. Lungs were preserved and reperfused as outlined. The lung function, wet-to-dry ratio, proteins in BALF, and MPO activity were determined as above.

2.11. Data analysis

Data are expressed as mean ± SEM. Lung functional data (PO₂, pAwP, and PAP) were analyzed by repeated measures of ANOVA and remaining data by one-way ANOVA. If ANOVA showed an overall difference, post hoc comparisons were performed with the Turkey test. P < 0.05 was considered statistically significant.

3. Results

3.1. Lung functional parameters

Throughout reperfusion, the PO₂ level in the edaravone group remained similar to the vehicle group, whereas the control group showed the poorest values compared to the other groups (p < 0.001, respectively) (Fig. 1). Additionally, the control group exhibited the highest pAwP level compared to the other groups (p < 0.05, respectively). Although the mPAP at the end of the reperfusion was not different among the groups, the edaravone group displayed a faster hemodynamic recovery compared to control group (Table 1).

3.2. Lipid peroxidation, edema formation, and leukocyte extravasation

After IR, the control group showed a significantly increased MDA level (a lipid peroxidation marker, Fig. 2),
wet-to-dry weight ratio (an index of pulmonary edema), total protein in BALF (an index of microvascular permeability), and MPO activity compared to the other groups. Although edaravone treatment significantly decreased all these parameters compared to control group, protein leakage, and pulmonary edema were increased compared to the sham group ($p < 0.05$, Table 2).

Histological examination was consistent with these results. In control lungs, IR induced increased alveolar thickening and marked neutrophil infiltration. In contrast, the alveolar architecture was well preserved and histological changes were minimal in edaravone-treated lungs, resembling the vehicle or sham lungs (Fig. 3).

### 3.3. Study of phospholipase A$_2$

IR induced an increased cPLA$_2$ and sPLA$_2$ activity and LTB$_4$ formation in the control compared to the edaravone group ($p < 0.05$). Of note, PAF-AH activity was increased in the edaravone group compared to the control group ($p < 0.01$); however, no significant difference between the sham and control group was observed (Table 3). Consistently, control lungs showed an increased expression of phosphorylated cPLA$_2$, the active form of the enzyme, mainly via alveolar macrophages and PAF-R mainly in alveolar cells compared to sham or vehicle groups (Fig. 4), whereas edaravone treatment significantly attenuated the activation of cPLA$_2$ ($p < 0.001$) and PAF-R expression (Fig. 5).

### 3.4. Pharmacological intervention (Fig. 5)

The physiological and biochemical results are summarized in Fig. 1 and Tables 1 and 2. As shown, pharmacological inhibition of the PAF-R by ABT-491 significantly improved oxygenation capacity and reduced neutrophil infiltration, protein leakage, and edema formation resembling edaravone action.

#### Table 1

Mean pulmonary artery pressure and peak airway pressure during reperfusion

<table>
<thead>
<tr>
<th>Time-course of reperfusion (min)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>Vehicle 7.3 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>7.3 ± 0.2</td>
<td>7.5 ± 0.2</td>
<td>7.7 ± 0.2</td>
</tr>
<tr>
<td>Control 11.3 ± 0.3*</td>
<td>10.2 ± 0.6*</td>
<td>9.2 ± 0.4</td>
<td>8.8 ± 0.3</td>
<td>9.0 ± 0.3*</td>
<td></td>
</tr>
<tr>
<td>Edaravone 9.5 ± 0.4*</td>
<td>8.8 ± 0.4</td>
<td>8.0 ± 0.4</td>
<td>8.0 ± 0.4</td>
<td>7.8 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>ABT-491 10.3 ± 0.4*</td>
<td>9.2 ± 0.5*</td>
<td>8.3 ± 0.5</td>
<td>8.3 ± 0.5</td>
<td>8.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Peak airway pressure (cmH$_2$O)</td>
<td>Vehicle 7.6 ± 0.3</td>
<td>7.9 ± 0.3</td>
<td>8.1 ± 0.3</td>
<td>8.7 ± 0.3</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>Control 10.7 ± 0.6***</td>
<td>11.1 ± 0.7***</td>
<td>11.9 ± 0.6***</td>
<td>12.1 ± 0.7***</td>
<td>12.9 ± 0.6***</td>
<td></td>
</tr>
<tr>
<td>Edaravone 8.9 ± 0.1</td>
<td>8.9 ± 0.1</td>
<td>9.6 ± 0.2</td>
<td>10.4 ± 0.3</td>
<td>10.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>ABT-491 8.5 ± 0.2</td>
<td>8.5 ± 0.2</td>
<td>9.3 ± 0.2</td>
<td>9.8 ± 0.1</td>
<td>10.5 ± 0.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± SEM ($n = 7$, per group). *$p < 0.05$ versus vehicle. **$p < 0.001$ versus vehicle. ***$p < 0.05$ versus edaravone and ABT-491.

#### Table 2

Edema formation and neutrophil extravasation after ischemia–reperfusion

<table>
<thead>
<tr>
<th>Wet-to-dry ratio</th>
<th>Proteins in BALF (mg/mL)</th>
<th>MPO activity (OD/(mg min))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 6.64 ± 0.33*</td>
<td>0.2 ± 0.03*</td>
<td>0.44 ± 0.06*</td>
</tr>
<tr>
<td>Vehicle 6.91 ± 0.22*</td>
<td>0.2 ± 0.03*</td>
<td>0.57 ± 0.05*</td>
</tr>
<tr>
<td>Control 10.70 ± 0.30***</td>
<td>1.0 ± 0.07</td>
<td>1.21 ± 0.15</td>
</tr>
<tr>
<td>Edaravone 8.15 ± 0.46***</td>
<td>0.4 ± 0.03***</td>
<td>0.64 ± 0.10***</td>
</tr>
<tr>
<td>ABT-491 7.47 ± 0.08**</td>
<td>0.2 ± 0.02*</td>
<td>0.72 ± 0.07**</td>
</tr>
</tbody>
</table>

Values are mean ± SEM ($n = 7$, per group). BALF, bronchoalveolar lavage fluid; MPO, myeloperoxidase. *$p < 0.05$ versus control. **$p < 0.05$ versus sham. ***$p < 0.05$ versus ABT-491.
4. Discussion

We have investigated the effect of edaravone on preventing the lung injury resulting from cold storage followed by warm reperfusion in an isolated rat model. The results demonstrate that administration of edaravone before lung harvest suppresses IR lung injury and preserves better functional capacity. These results validate the protective effect of edaravone against lung IR injury for which no clinical agent are currently available.

After IR, the data demonstrate an impaired gas exchange and increased lung compliance in control lungs, together with an increased lipid peroxidation. These findings were significantly attenuated by edaravone treatment in this isolate model. For the mechanism of action, the better preservation of the alveolar-capillary membrane prevented the drop in PO₂ in the edaravone group. Thus, these results confirm the reported effect of edaravone as a potent antioxidant agent [22] and its protective effect on IR injury [19,20].

Several strategies have evolved that attempt to improve lung transplantation outcome. Thus, LPD solution was specifically developed for lung preservation [1,2]. It is reported that LPD solution decreases oxidative damage, pulmonary vasoconstrictors, and thrombus formation, allowing a relatively safe extension of the ischemic time in animal models [1,2]. In our study, conversely to the reported literature, the mPAP at the end of the experimental period remained similar among the groups. Thus, the hemodynamic improvement observed in the control group could be due at least in part to the action of the LPD solution, which likely has partially preserved the endothelial integrity after IR. Nevertheless, our data showed an extended protection with edaravone, as reflected by a better gasses exchange, faster hemodynamic recovery, and decreased edema formation. Supporting these findings, Kozower et al. [23] reported an attenuated IR lung injury when endothelium-targeted catalase is administered before lung ischemia.

A critical step in the amplification of the initial IR injury is the interaction between neutrophils and endothelium [2,6].

Table 3
Effects of edaravone on phospholipase A₂ cascade (PLA₂) after IR

<table>
<thead>
<tr>
<th></th>
<th>cPLA₂ activity (nmol/(mg min))</th>
<th>sPLA₂ activity (nmol/(mg min))</th>
<th>LTB₄ in BALF (pg/mL)</th>
<th>PAF-AH activity (ng/(mL min))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.87 ± 0.14 †</td>
<td>0.17 ± 0.02 ‡</td>
<td>5.53 ± 1.27 †</td>
<td>44.0 ± 0.63 ††‡‡‡</td>
</tr>
<tr>
<td>Vehicle</td>
<td>0.94 ± 0.13 †</td>
<td>0.18 ± 0.03 ‡</td>
<td>5.96 ± 1.06 †</td>
<td>53.6 ± 2.42 †</td>
</tr>
<tr>
<td>Control</td>
<td>1.68 ± 0.23 ‡</td>
<td>0.35 ± 0.03 ‡</td>
<td>29.76 ± 3.33 ‡</td>
<td>47.2 ± 1.77 ‡‡‡</td>
</tr>
<tr>
<td>Edaravone</td>
<td>1.06 ± 0.06 ‡</td>
<td>0.23 ± 0.02 ‡</td>
<td>15.04 ± 2.54 †</td>
<td>57.6 ± 1.29 †</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 7, each group). cPLA₂ and sPLA₂, cytosolic and soluble PLA₂; LTB₄, leukotriene B₄; PAF-AH, platelet-activating factor acetylhydrolase.

† p < 0.05 versus control.
‡ p < 0.01 versus edaravone.
‡‡ p < 0.05 versus vehicle.
We have also observed an increased neutrophil infiltration in control lungs, together with an increased expression of PAF-R and LTB4 level. Additionally, treatment with ABT-491 decreased neutrophil infiltration. Neutrophil infiltration in the lung was confirmed by MPO assay and histology. The present results suggest that a major mediator of neutrophil infiltration is a PLA2 product, most probably PAF, which acts synergically with products of the arachidonic acid pathway, such as LTB4. It is known that PAF is displayed on the surface of stimulated endothelial cells acts as a juxtacrine signal for the activation and adhesion of neutrophils [14]. Furthermore, recent evidence has shown LTB4 to be an important mediator of neutrophil-mediated lung injury [24].

PLA2 is a key enzyme that plays an important role in modulating the host inflammatory response in lung injury [10]. We have demonstrated that edaravone reduces cPLA2 and sPLA2 activity and leukotriene formation after IR. Since edaravone has been thus far classified exclusively as a free
recently Nakos et al. [12] reported an increased expression of pulmonary endothelial cells [7,8]. In support of our findings, arachidonate mobilization in human lung epithelial or endothelial cells was determined. In vitro, ROS increases the cPLA2 activation and edaravone decreases the activation of PLA2 remains to be determined signals. However, the exact mechanism by which ROS plays a role and contribute to the physiological alteration of the PAF-R. Furthermore, it is also likely that ROS might amplify lung injury through inactivation of PAF-AH, a key regulator of PAF metabolism. Supporting our findings, Ambrosio et al. [25] reported that ROS can rapidly and irreversibly inactivate plasmatic PAF-AH. In addition, Kim et al. [18] reported that administration of PAF-AH in the preservation solution and before reperfusion attenuates IR injury.

In the present study, IR-induced increases in pAwP, proteins in BALF, leukocyte infiltration and LTB4 were significantly attenuated but not eliminated by the edaravone treatment. These observations indicate that factors other than ROS may play a role and contribute to the physiological alteration of the PAF-R. Furthermore, we do acknowledge certain limitations and weaknesses in the model and hence this study. The model is an ex vivo model with the inherent limitations of not being a true system. Nevertheless, the baseline conditions established by the sham group allowed an evaluation of the effect of the isolated system on lung injury. Furthermore, before clinical application of edaravone based on the findings reported here, the results should be re-studied an in vivo lung transplantation model. We take the current results to suggest that such further study is much needed.

In summary, this study shows in an ex vivo model that edaravone attenuates IR-induced lung injury and preserves lung function by decreasing ROS formation and PLA2 activation. Pulmonary edema formation and leukocyte extravasation appear to be partially mediated through PAF receptor activation.

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References


Fig. 5. Effect of edaravone on IR-induced cPLA2 activation. (A) Representative immunoblot from lung homogenates using a specific anti-phospho-cPLA2 antibody. Phosphorylated cPLA2 was detected as described in Section 2. Alpha-tubulin was used to determine loaded protein and HeLa cell lysate as positive control. (B) Lung phosphorylated cPLA2 content as determined by densitometric analysis of blotted membranes (n = 6, each group). Results are expressed as mean ± SEM. *p < 0.001 versus other groups, respectively.


