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Effect of the Inhibition of Phospholipase A_2 in Generation of Free Radicals in Intestinal Ischemia/Reperfusion Induced Acute Lung Injury

Young Man Lee, Yoon-yub Park, Teoan Kim, Hyun G. Cho, Yoon Jeong Lee, and John E. Repine[1]

Department of Physiology, School of Medicine, Catholic University of Taegu-Hyosung, Taegu 705-718, Korea; [1]Webb-Waring Antioxidant Research Institute, Denver, Colorado, USA

The role of phospholipase A_2 (PLA_2) in acute lung leak induced by intestinal ischemia was investigated in association with neutrophilic respiratory burst. To induce lung leak, we generated intestinal ischemia for 60 min prior to the 120 min reperfusion by clamping superior mesenteric artery in Sprague-Dawley rats. Acute lung leak was confirmed by the increased lung leak index and protein content in bronchoalveolar fluid. These changes were inhibited by mepacrine, the non-specific PLA_2 inhibitor. The lung myeloperoxidase (MPO) activity denoting the pulmonary recruitment of neutrophils was increased by intestinal I/R, but decreased by mepacrine. Simultaneously, the number of leukocytes in bronchoalveolar fluid was increased by intestinal ischemia/reperfusion (I/R) and decreased by mepacrine. Gamma glutamyl transferase activity, an index of oxidative stress in the lung, was increased after intestinal I/R but decreased by mepacrine, which implicates that PLA_2 increases oxidative stress caused by intestinal I/R. The PLA_2 activity was increased after intestinal I/R not only in the intestine but also in the lung. These changes were diminished by mepacrine. In the cytochemical electron microscopy to detect hydrogen peroxide, intestinal I/R increased the generation of the hydrogen peroxide in the lung as well as in the intestine. Expression of interleukin-1 (IL-1) in the lung was investigated through RT-PCR. The expression of IL-1 after intestinal I/R was enhanced, and again, the inhibition of PLA_2 suppressed the expression of IL-1 in the lung. Taken together, intestinal I/R seems to induce acute lung leak through the activation of PLA_2, the increase of IL-1 expression associated with increased oxidative stress by neutrophilic respiratory burst.

Key Words: Adult respiratory distress syndrome, Intestinal ischemia-reperfusion, PLA_2, Oxidative stress

INTRODUCTION

Adult respiratory distress syndrome (ARDS) is one of the clinical manifestations of multiple organ failure (MOF) caused by various etiologies, such as trauma, sepsis, burn, multiple fracture, and head injury (Connelly & Repine, 1997). One of the etiologies of MOF, intestinal ischemia/reperfusion (I/R), has been studied along with xanthine oxidase (XO) and the generation of free radicals which causes acute lung injury (Terada et al, 1992). Especially, ARDS has been investigated in relation to free radicals, and XO has been regarded as a prime cause of oxidant generation during ischemia (Hassoun et al, 1998).

Koike et al (1995) demonstrated that the inhibition of PLA_2 had some protective effects on acute lung leak after intestinal I/R, but to date the mechanism has not been elucidated. Some studies (Nagashiro et al, 1997; Turnage et al, 1997) suggested the role of lipid mediators which were responsible for the acute lung injury after intestinal I/R. Among the lipid mediators released by the activation of PLA_2, platelet activating factor (PAF) has been proposed as a highly

Corresponding to: Young Man Lee, Department of Physiology, School of Medicine, Catholic University of Taegu-Hyosung, Taegu 705-718, Korea. (Tel) 053-650-4472, (Fax) 053-621-4106, (E-mail) leeym@cuth.cataegu.ac.kr

probable lipid molecule which causes acute lung leak after intestinal I/R. This is based on the fact that PAF is the byproduct of PLA_2 activation by the hydrolysis of phospholipid. In addition, inflammatory cells, including neutrophils, and endothelial cells are known to produce PAF by activating PLA_2 (Bussolini & Camussi, 1995; Hiroaki et al, 1995). In this regard, PAF has been proposed as a lipid mediator which causes the priming of neutrophils by intestinal I/R.

Although the priming of neutrophils and the infiltration of neutrophils have been identified in the lung, the mechanism of oxidative stress has not been confirmed up to date. Recently, Dana et al (1998) reported a very interesting result regarding the role of PLA_2 and arachidonic acid on the activation of NADPH oxidase in the neutrophils. They demonstrated that arachidonic acid was the principal lipid molecule on neutrophilic respiratory burst by the activation of NADPH oxidase in the phagocytes. Moreover, proinflammatory cytokines, especially interleukin-1 (IL-1), take part in the pathogenesis of ARDS by the generation of free radicals in the lung (Koh et al, 1995). Interestingly, IL-1 recruits neutrophils in the lung and activates PLA_2, which is the rate-limiting enzyme of PAF synthesis (Lee et al, 1997). As IL-1 has been known to cause oxidative stress by activating PLA_2, it is highly probable that lipid mediators, especially PAF might be closely related to the oxidative stress in the lung after intestinal I/R. Because the cytokine network and lipid mediators are thought to be involved in the pathogenesis of ARDS, we investigated the role of PLA_2 in acute lung injury after intestinal I/R in relation to IL-1 and oxidative stress.

METHODS

Sources of reagents

^{125}I-bovine serum albumin and ^{3}H-Dipalmitoylphosphatidylcholine (DPPC) were purchased from NEN, Dupont (Boston, MA). RNA isolation kit was purchased from Gibco-BRL (Gaithersberg, MD). Other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Induction of acute lung injury

Acute lung leak was induced in Sprague-Dawley rats (B.W. 300~350 g) by clamping superior mesenteric artery with a bulldog clamp for 60 min, and reperfusion was permitted for 120 min. For the inhibition of PLA_2, mepacrine (60 mg/kg) was injected intraperitoneally immediately after the clamping of superior mesenteric artery.

Evaluation of lung leak

In separate experiments, rats were anesthetized with ketamine hydrochloride (75 mg/kg) 1.5 hr after reperfusion and then injected with ^{125}I-BSA (1 μCi) intravenously. 30 min later, the trachea cannulated and the rats were ventilated. Immediately thereafter, laparotomy and tracheostomy were performed, and heparin was injected into the right ventricle (200 IU, 0.2 ml). Blood samples were collected (1.0 ml) and the lungs were perfused blood free with PBS before excision. Right lungs and blood samples were counted in the gamma counter. The lung leak index was determined by computing the number per min of ^{125}I in the right lung divided by the number per min of ^{125}I in blood. Left lungs were frozen with liquid nitrogen and stored at -70°C for the analysis of myeloperoxidase (MPO) activities.

Measurement of protein content in bronchoalveolar lavage (BAL) fluid

BAL was carried out to measure phospholipid and protein content. Eight ml of normal saline was infused through the cannula and retrieved three times. Six ml of BAL fluid was obtained from each rat. Protein content was measured by the method of Brown et al (1989) using bicinchoninic acid.

Analysis of lung MPO activity

Lung MPO activity was measured according to the method of Goldblum et al (1985). Briefly, left lungs were thawed and then homogenized in 4.0 ml of potassium phosphate buffer (20 mM, pH 7.4), followed by centrifugation at 30,000 g for 30 min. The pellet was resuspended in 4.0 ml phosphate buffer (50 mM, pH 6.0) with 0.5% hexadecyltrimethyl ammonium bromide. Samples were sonicated for 90 sec on ice, incubated 2 h at 60°C to inactivate tissue myeloperoxidase inhibitors, and then assayed using o-dianisidine as the substrate.

Enumeration of neutrophils in BAL fluid

To count leukocytes in BAL, cytospin was performed at 1,500 rpm for 10 min at 4°C. Supernatant was collected to analyze protein and phospholipid. The cellular pellet was resuspended in 0.5 ml of normal saline and the number of leukocytes was counted with hemocytometer. Thereafter, 100 μl of resuspended solution was cytospin again on slide glass, Wright stained, and differentially counted for the number of neutrophils.

Analysis of pulmonary surfactant

Surfactant was isolated and fractionated according to the method of Putman et al (1995). Briefly, after cellular components were removed, BAL fluid was centrifuged at 40,000 g (Beckman J2M centrifuge) for 15 min at 4°C. Supernatants were collected to analyze light subtype of surfactant, and pellets were resuspended with normal saline to analyze heavy subtype of surfactant. Lipids were isolated by the method of Folch et al (1957) and phospholipid phosphorus was measured in accordance with the method Hess & Derr (1975) used. Phosphorus contents were converted into phospholipid in accordance with the method of Corbet et al (1983) used.

Assay of gamma glutamyl transferase (GGT) in the lung

GGT activity in the lung was measured by employing the method of Meister et al (1981). Briefly, right lungs were thawed, homogenized with 4.0 ml of homogenization buffer (0.15 M KCl, 10 mM Tris-base (pH 7.4), 10 mM EDTA, 50 μg/ml PMSF, 3 μg/ml leupeptin), and sonicated for 90 sec at maximum power. Two substrates of GGT, 0.2 ml of glycylglycine (0.1 M, pH 8.0) and 0.2 ml of L-gamma-glutamyl-p-nitoanilide (5 mM, pH 8.0), were mixed with 0.6 ml of Tris-HCl buffer (0.1 M, pH 8.0) and 0.1 ml of lung homogenate. The mixture was incubated at 37°C for 60 min and the reaction was stopped by adding 0.5 ml of 50 mM serine-borate solution. Light absorbance at 410 nm was proportional to GGT activity. Definition of 1 unit was the production of 1 μmol per min of p-nitroaniline.

Assay of intestinal and pulmonary PLA_2

Intestinal and pulmonary PLA_2 activity was measured by the method of Katsumata et al (1986). Briefly, the blood-free excised lung and small intestine were homogenized with polytron homogenator in 2.0 ml of 20 mM phosphate buffer (pH 7.4), then sonicated for 90 sec. L-α-dipalmitoyl-2 (9,10 (N)^{3}H-palmitoyl) phosphatidylcholine (30 Ci/mmol) was incubated with 100 μl of the intestine and lung homogenate in 880 μl of 100 mM glycine buffer at pH 9.0, containing 10 g/L of BSA, 2.5 mM sodium deoxycholate, 0.1 mM dipalmitoylphosphatidylcholine, 2.0 mM $CaCl_2$ and 1.75 M absolute ethanol containing 200 mM EDTA. The reaction mixture was incubated for 60 min at 37°C. The reaction was stopped by adding 200 μl of 5% Triton X-100. The fatty acids released by hydrolysis were extracted by 5.0 ml of hexane containing 0.1% acetic acid and 2.5 g of Na_2SO_4. After vortexing, the hexane layer was separated and counted in the liquid scintillation counter. Snake venom PLA_2 (*Crotalus adamanteus*) control samples (0.01 U/ml) were assayed with all the other samples to confirm the reproducibility. The one unit was defined as an activity to hydrolyze 1 μmol of substrate per min.

Cytochemical electronmicroscopy of hydrogen peroxide in the lung and intestine

In order to estimate production of hydrogen peroxide in the lung, cytochemical electron microscopy was conducted in a modified version of Hobson et al's method (1991). Briefly, lung samples (1 mm^3) were incubated in cerium chloride media (2.0 mM $CeCl_3$, 10 mM 3-amino-1,2,4-triazol, 0.1 M Tris-maleate buffer, 7% sucrose, 0.0002% Triton X-100) for 60 min. Then, the samples were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer, post fixed, dehydrated, and embedded in Epoxy resin. Counter staining was not performed to increase the contrast between the reaction product and tissue.

RT-PCR

Total RNAs were prepared with an RNA isolation kit (Gibco-BRL, Gaithersberg, MD) in accordance with the manufacturer's instruction, followed by the digestion with RNase-free DNase I.

RT-PCR was performed as described previously

(Doehr et al, 1995). Briefly, for cDNA synthesis, 1 μg of total RNA was heated in a final volume of 10 μl with 2 μg oligo $(dT)_{15}$ primer for 5 min at 60°C, chilled on ice, and reverse transcribed in a final volume of 40 μl containing 1 mM of each dNTP 8 μl 5x M-MLV buffer, 60 units RNase inhibitor, 10 mM DTT, and 400 units of M-MLV reverse transcriptase. Samples were incubated at 37°C for 1 h and subsequently denatured for 10 min at 70°C. PCR primers were synthesized with an Applied Biosystems 391 DNA synthesizer (Weiterstadt, Germany) and purified with NAP-5 columns (Pharmacia, Freiburg, Germany). Primer sequences were from published sources or chosen using a primer selection program (Oligo, National Bioscience, Plymouth, MN) and are given as: beta-actin FP; GTGGGGCGCCCCAGGCACCA, RP; CTCCTTAATGTCACGCACGATTTC, base pair 541 (Albino et al, 1991), IL-beta FP; AAACAGATGAAGGTCTCCTTCCAGG, RP; and TGGAGAACACCACTTGTTCTCCA, base pair 388 (March et al, 1985). PCR reactions were carried out in a final volume of 50 μl containing 2.5 μl RT samples, 5 μl 10x Taq DNA polymerase, and 1 μ Ci[a-^{32}P]dCTP. Amplifications were performed using a DNA thermal cycler (Ericomp Inc., San Diego, CA) for indicated cycles with the following profile: 4 min at 94°C before the first cycle, 1 min for denaturation at 94°C, 1 min for primer annealing, 1 min for primer extension at 72°C and 7 min at 72°C after the last cycle. Linearity of amplification was controlled by three different cycle numbers for one cDNA concentration. PCR products were electrophoresed on 10% (w/v) polyacrylamide gels, and gels were dried and autoradiographed.

RESULTS

Effect of mepacrine on intestinal I/R induced lung leak and BAL protein content

Intestinal I/R increased ($p<0.001$) lung leak index and protein content compared with a sham group. Treatment with mepacrine attenuated lung leak and protein content in BAL fluid ($p<0.01$, $p<0.001$) compared with an intestinal I/R group (Fig. 1, 2).

Effect of mepacrine on intestinal I/R induced increase in lung MPO activity

Intestinal I/R rats had increased ($p<0.001$) lung MPO activity compared with sham rats (Fig. 3). In contrast, rats with intestinal I/R and treatment of mepacrine decreased ($p<0.001$) MPO activity compared with rats with intestinal I/R.

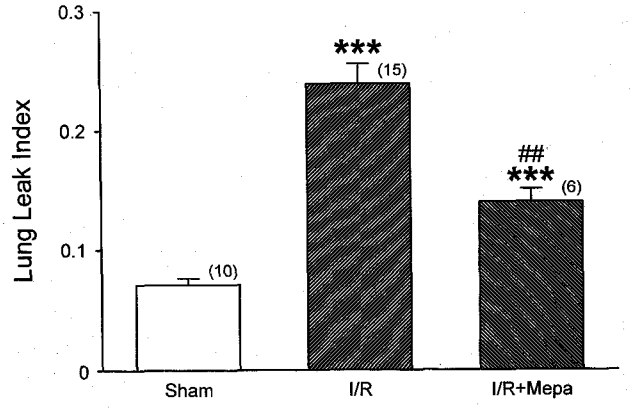

Fig. 1. Lung leak index in gut I/R and gut I/R with mepacrine (Mepa, 60 mg/kg) treated rats. Gut I/R increased lung leak index significantly compared with the lung leak index of gut I/R rats. Each bar represents mean ± SE. The numbers of determinations are shown in the parentheses. ***$p<0.001$, Sham vs. I/R, IR+Mepa, ##$p<0.01$, I/R vs. I/R+Mepa

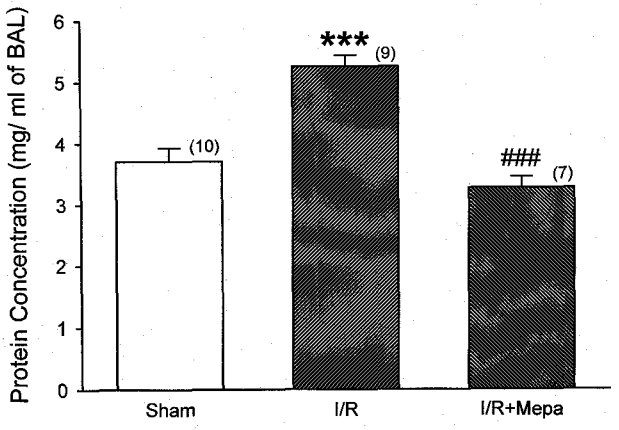

Fig. 2. Effect of gut I/R on the protein contents (mg/ two lungs) in the BAL. Gut I/R increased the protein content in BAL fluid of the lungs of rats. In contrst, treatment of mepacrine significantly decreased the content of protein in the BAL fluid. Each bar represents mean ± SE. The numbers of determinations are shown in the parentheses. ***$p<0.001$. Sham vs. I/R. ###$p<0.001$, I/R vs. I/R+Mepa

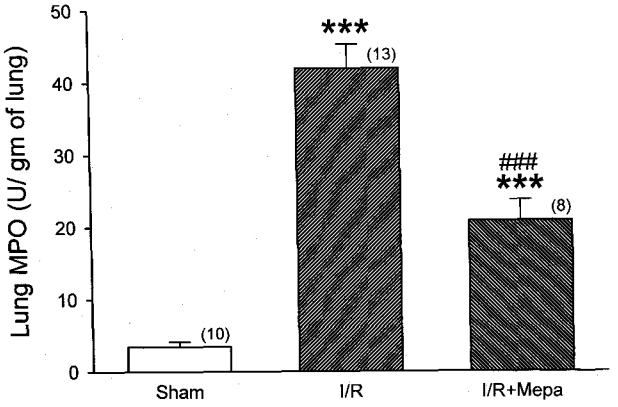

Fig. 3. Effect of mepacrine on gut I/R induced increase in lung MPO activity. Gut I/R increased MPO activity tremendously in the lungs of rats, which signifies the infiltration of neutrophils in the lungs. In contrast, treatment of mepacrine decreased lung MPO activity in gut I/R rats. Each bar represents mean ± SE. The numbers of determinations are shown in the parentheses. ***$p<0.001$, Sham vs. I/R. IR+Mepa. ###$p<0.001$, I/R vs. I/R+Mepa

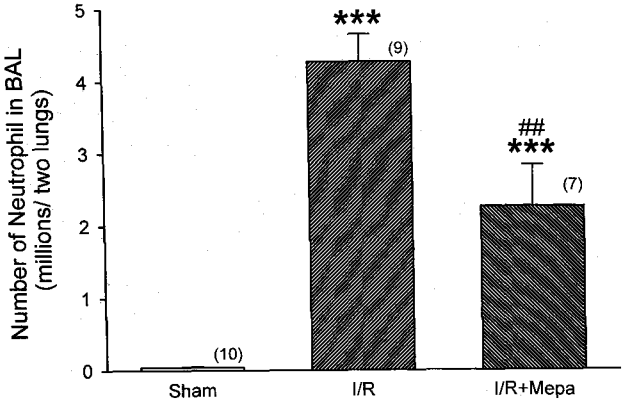

Fig. 4. Numbers of neutrophils in the BAL. Gut I/R increased the numbers of neutrophils in the BAL but after treatment of mepacrine the numbers of neutrophils decreased significantly. Each bar represents mean ± SE. The numbers of determinations are shown in the parentheses. ***$p<0.001$, Sham vs. I/R IR+Mepa, ##$p<0.01$, I/R vs. I/R+Mepa

Effect of mepacrine on the number of neutrophils in BAL fluid

Intestinal I/R rats increased ($p<0.001$) the number of neutrophils in BAL fluid compared with sham rats. Whereas, mepacrine decreased ($p<0.01$) the number of neutrophils in BAL fluid compared with intestinal I/R rats (Fig. 4).

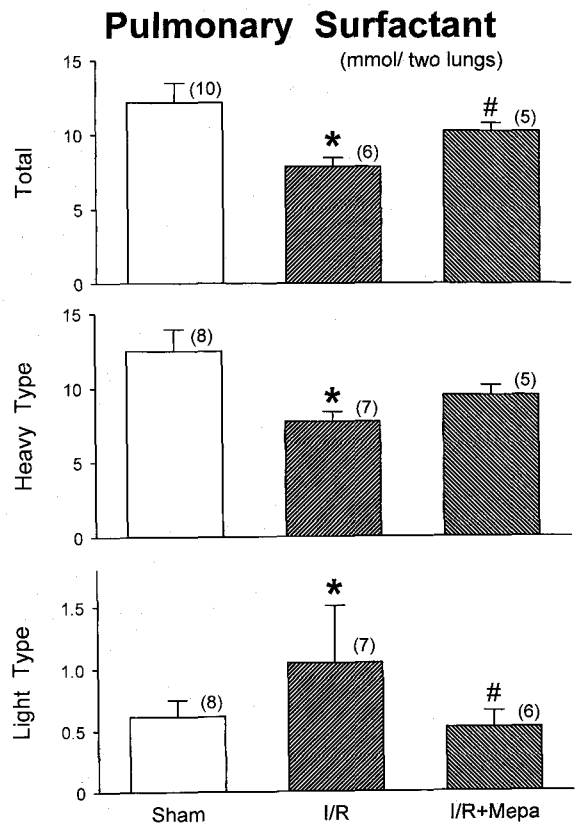

Fig. 5. Effect of mepacrine on the amount of surfactant. The total amount of the surfactant was decreased by gut I/R but it was recovered by treatment of mepacrine up to control. By comparison, the amount of low density surfactant was increased by gut I/R, which denoted the effect of oxidative stress caused by gut I/R in the lung. The amount of heavy density surfactant was decreased by the gut I/R, but it was recovered by treatment of mepacrine up to control level. Each bar represents mean ± SE. The numbers of detrminations are shown in the parentheses. *$p<0.05$, Sham vs. I/R, #$p<0.05$, I/R vs. I/R+Mepa

Effect of mepacrine on the amount of surfactant

Intestinal I/R decreased ($p<0.05$) the amount of total surfactant compared with a sham group, whereas mepacrine recovered ($p<0.05$) the amount of surfactant in intestinal I/R rats. In addition, intestinal I/R decreased ($p<0.05$) the amount of heavy subtype of surfactant, while mepacrine recovered ($p<0.05$) the heavy subtype surfactant up to control level (Fig. 5).

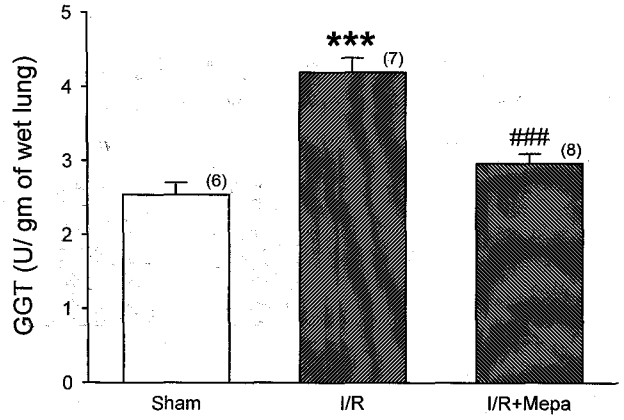

Fig. 6. Changes of GGT activity in the lung after gut I/R and treatment of mepacrine Gut I/R increased GGT activity in the lung compared with control rats, whereas mepacrine decreased GGT activity in gut I/R rats. Each bar represents mean ± SE. The numbers of eterminations are shown in the parentheses. ***$p<0.001$, Sham vs. I/R, ###$p<0.001$, I/R vs. I/R+Mepa

Intestinal I/R increased ($p<0.05$) light subtype of surfactant, while mepacrine treatment after intestinal I/R decreased ($p<0.05$) the amount of light subtype of surfactant to control level.

Effect of mepacrine on the lung GGT activity

Intestinal I/R increased ($p<0.001$) GGT activity in the lung compared with sham rats. In contrast, mepacrine decreased ($p<0.001$) GGT activity in the lung to the control level (Fig. 6).

Effect of mepacrine on intestinal and pulmonary PLA_2 activity

Intestinal I/R increased intestinal and pulmonary PLA_2 activity compared with that of control rats ($p<0.001$). In contrast, mepacrine decreased ($p<0.001$) intestinal and pulmonary PLA_2 activity compared with that of intestinal I/R rats (Fig. 7).

Effect of mepacrine on hydrogen peroxide production in the lung and intestine

After intestinal I/R, large amount of dense deposits of cerrous perhydroxide was found in the microvilli of small intestine compared with those of control rats. And diffuse necrosis of mucosal cells was identified after I/R (Fig. 8, panel a, b). In contrast, mepacrine

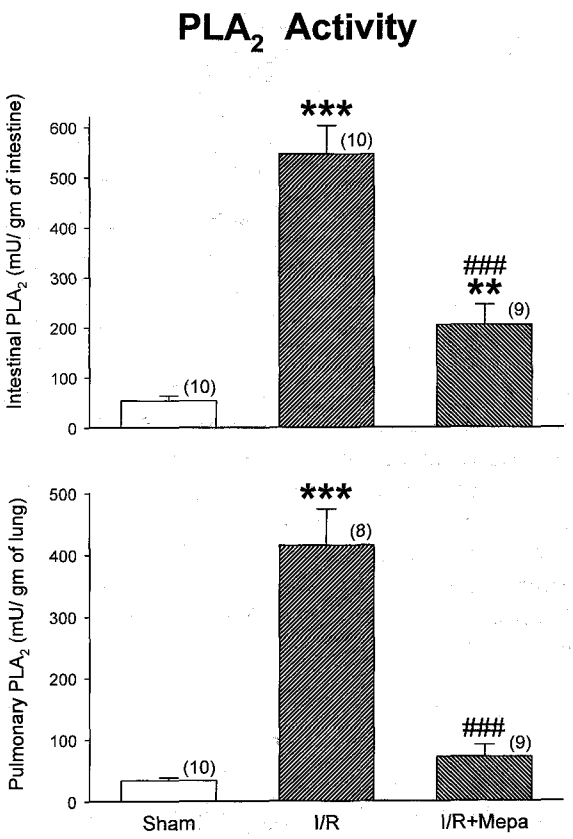

Fig. 7. Effect of mepacrine on the intestinal and pulmonary PLA_2 activity. Gut I/R increased intestinal and pulmonary PLA_2 activity compared with control rats. In contrast, mepacrine decreased intestinal and pulmonary PLA_2 activity compared with gut I/R rats. Each bar represents mean ± SE. The numbers of determinations are shown in the parentheses. ***$p<0.001$, Sham vs. I/R, **$p<0.01$, Sham vs. I/R+Mepa, ###$p<0.001$, I/R vs. I/R+Mepa

decreased the deposits of cerrous perhydroxide and tissue injury (Fig. 8, panel c). In the lung, intestinal I/R increase the deposits of cerrous perhydroxide tremendously compared with those of control rats. In contrast, mepacrine decreased the deposits of cerrous perhydroxide compared with intestinal I/R rats (Fig. 9, panel a, b, c).

Expression of IL-1 mRNA

After intestinal I/R, IL-1 mRNA was increased, while mepacrine decreased the expression of IL-1 effectively after intestinal I/R (Fig. 10).

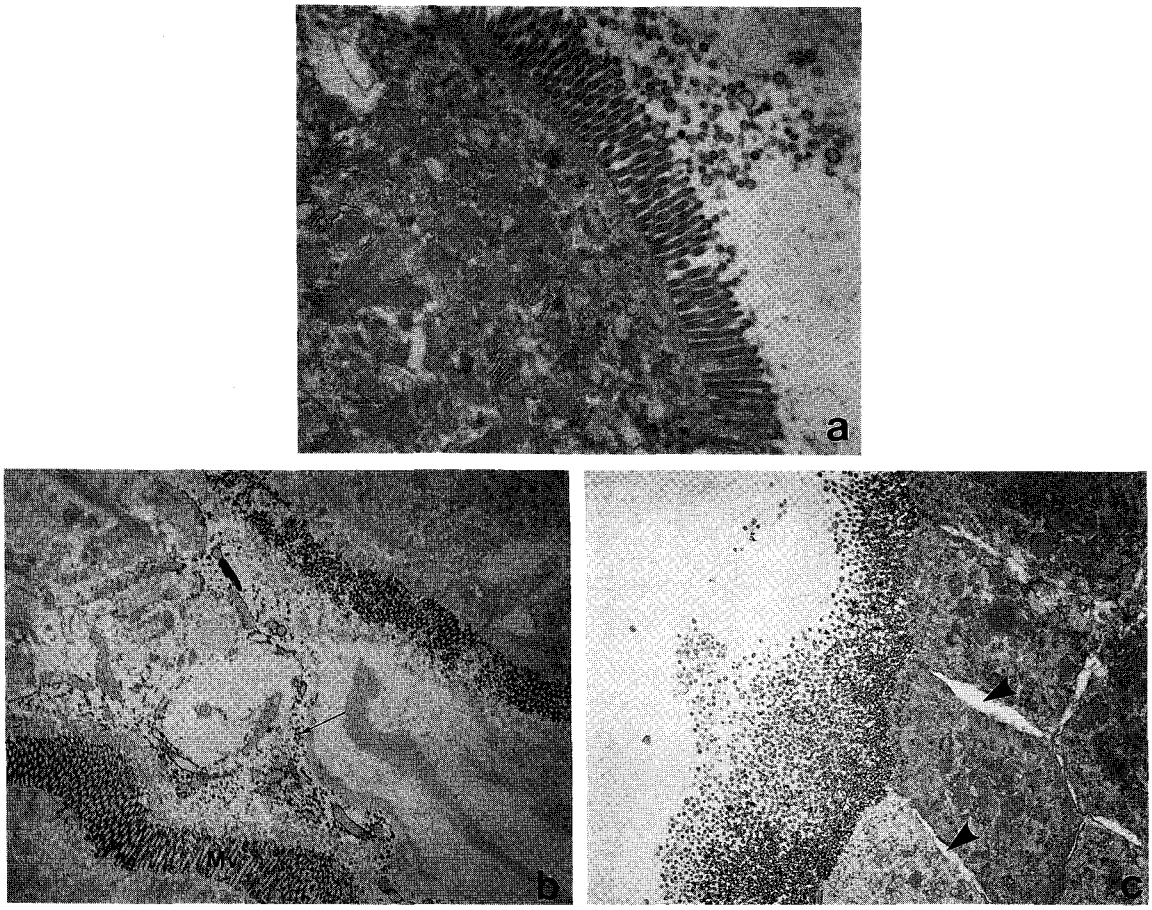

Fig. 8. Effect of mepacrine on the production of hydrogen peroxide in the intestine after gut I/R and mepacrine. Comparing with the deposits of cerrous perhydroxide in the intestinal villi of control rats (panel a, original mag. ×3,000), large amount of cerrous perhydroxide was identified in gut I/R rats (panel b, original mag. ×3,000), even in the lumen of the intestine, the clumping of the cerrous perhydroxide was found (arrow). Mepacrine, however, decreased the deposits of cerrous perhydroxide in the tissue (panel c, original mag. ×3,000). Mepacrine decreased the production of hydrogen peroxide in the intestine effectively and protected submucosal tissues relatively (arrows). Mv: microvilli

DISCUSSION

In an attempt to elucidate the role of PLA_2 in acute inflammatory lung injury induced by intestinal I/R, the inhibition of PLA_2 with mepacrine was done in intestinal I/R induced acute lung injury. Recently Terada et al (1997) argued that XO is the primary cause of acute lung leak after intestinal I/R in rats. Although XO is a well known free radical generator, it is not expounded why the distant organ, the lung, is the target of oxidative stress in case of intestinal ischemia. It has been well confirmed that oxidants are a direct cause of reperfusion injury of the intestine (Deshmukh et al, 1997). Anderson et al (1991) proposed that platelet activating factors and other lipid mediators for the infiltration of neutrophils might be involved in the intestinal injury. In this study, we observed the results of increased lung leak after intestinal I/R, which is consistent with other reports (Xiao et al, 1997; Kim et al, 1998). In addition, the increased infiltration of neutrophils was confirmed by the increased MPO activity and neutrophil count in BAL fluid.

In parallel with these results, GGT activity in the lung was increased by the intestinal I/R. These findings suggested that the acute lung injury induced by intestinal I/R was derived from the neutrophilic oxidative stress. One more interesting finding in our study is that the PLA_2 activity in the intestine and lung was simultaneously increased after intestinal I/R.

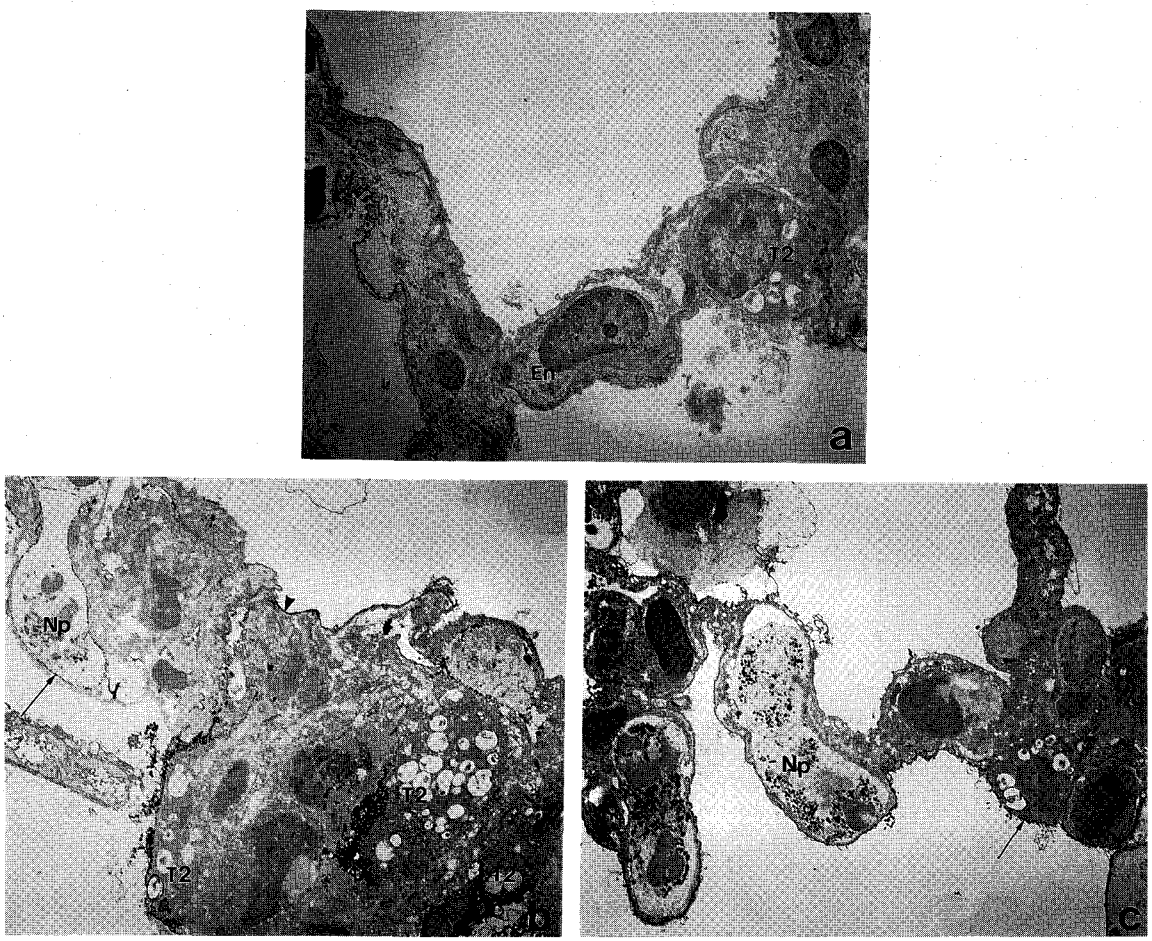

Fig. 9. Effect of the inhibition of the PLA_2 on the generation of hydrogen peroxide in the lung. In control lung, cerrous perhydroxide was not identified (panel a, original mag. ×3,000). whereas after gut I/R dense deposits of cerrous perhydroxide were found in the lung tissue, especially around neutrophils and apical surface of the alveolar type II cells (panel b, original mag. ×3,000). After treatment of mepacrine these changes were diminished although remnants of cerrous perhydroxide was found on the apical surface of the alveolar type II cells (arrows) (panel c, original mag. ×3,000). En: endothelial cell, T2: alveolar type II cell Np: neutrophil

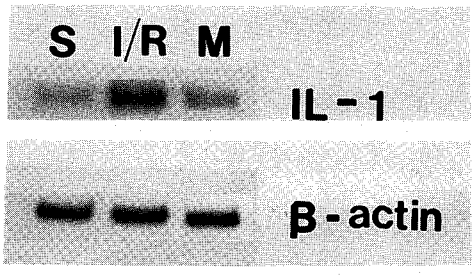

Fig. 10. RT-PCR of interleukin-1 in the lung tissue. Gut I/R increased the expression of the IL-1 in the lung. In contrast, mepacrine decreased the expression of IL-1 in the lungs of rats treated with mepacrine after gut I/R.

According to Koike et al (1995), the increased PLA_2 activity after intestinal I/R was the fundamental trigger of neutrophilic priming in the lung. They demonstrated that mepacrine decreased lung leak after intestinal I/R, and *in vitro*, neutrophilic production of superoxide anion was diminished. In this regard, our data demonstrating the infiltration of neutrophils in the lung and increased GGT activity confirms oxidative stress in the lung after intestinal I/R.

In the lung, extracellular glutathione can be used as antioxidant by the function of GGT. In case of hyperoxic lung injury, GGT activity increased, causing enhanced production of glutathione (Liu et al, 1996). One more confirming fact for the oxidative

stress in the lung is the increased PLA_2 activity in the lung. According to Lee et al (1997) IL-1 increased PLA_2 activity in the lung and released lipid molecules which played as a chemoattractant for neutrophils. Repine (1994) insisted that neutrophils are the principal inflammatory cells to evoke oxidative stress in ARDS. NADPH oxidase in the membrane of neutrophils is the key enzyme in the generation of superoxide anion (Maridonneau-Parini & Tauber, 1986). Some proinflammatory cytokines such as IL-6, TNF, and CINC are synthesized by intestinal ischemia/ reperfusion (Tamion et al, 1997), and these cytokines are involved in the oxidant generation (Jourd'heuil et al, 1997).

In our present study, IL-1 expression was increased by intestinal I/R and this increased expression was inhibited by mepacrine. In association with the role of PLA_2, IL-1 and TNF activate receptors in cell membrane and protein kinase C causing the activation of PLA_2 (Pfeilschifter et al, 1989). Therefore, the increased expression of IL-1 might be related to the activation of PLA_2 in the lung after intestinal I/R.

Increased lipid mediators appear to evoke the activation of the NADPH oxidase (Dana et al, 1994). As it is evident that the activation of PLA_2 increases the production of lipid mediators, especially PAF, the increased level of PAF might trigger the generation of free radicals in the lung. Another possibility is that the increased production of the PAF releases a large amount of oxidants, which activated PLA_2 in the lung. In conjunction with the role of PLA_2 in intestinal I/R, some reports insisted a pivotal role of oxidative stress caused by PLA_2 in intestinal I/R (Nagashiro et al, 1997; Turnage et al, 1997).

In the present study, increased production of hydrogen peroxide was confirmed by the cytochemical electron microscopy. These data indicate the increased production of hydrogen peroxide by the pulmonary PLA_2 activity. This interpretation can be supported by the effect of mepacrine, the non-specific PLA_2 inhibitor, on the production of hydrogen peroxide in the lung and in the intestine. The extent of oxidative stress induced by intestinal I/R comprises the alveolar lumen and endothelial cells. The diffuse infiltration of neutrophils and endothelial necrosis are the hallmark of the acute inflammatory lung injury by the intestinal I/R.

Moreover, the surfactant synthesis and the function of alveolar type II cells were affected in the present study. As a protective system against alveolar flooding, the role of alveolar barrier has been demonstrated (Matthay et al, 1996). For instance, the intact alveolar type II cells and surfactant system keep the alveolar lumen dry by the active transport mechanism of ions and the lowering of surface tension of alveolar surface. These normal functions of alveolar barrier are disrupted by the activation of PLA_2 and oxidants. By the activation of PLA_2, lysophospholipids and PAF injure alveolar barrier through a cytotoxic effect and oxidant generation (Arbibe et al, 1998). In the present study, we found that the composition of surfactant was changed. According to Putman et al (1995), the change in the composition of surfactant is the result of the abnormal metabolism of phospholipid during acute lung injury. Lewis et al (1990) suggested the malfunction of surfactant during ARDS could be derived from the change in the ratio of subtypes of pulmonary surfactant. In this study, the total amount of surfactant was decreased but light subtype was increased, in consistence with Putman et al's report (1995). In contrast, heavy subtype of surfactant was decreased. One more suggestive finding for the disruption of alveolar barrier is the dense deposits of hydrogen peroxide on the apical surface alveolar type II cells. In a cytochemical study, dense deposits of cerrous perhydroxide was found, which implicates the direct effect of oxidants on alveolar type II cells. The peculiar findings of alveolar type II cells in patients with ARDS are the vacant vacuoles in the cytoplasm of type II cells, hypertrophy of lamella bodies, and these are the response of hyperoxic stimulus in the lung (Martensson et al, 1989). Importantly, these pathological findings of ARDS by oxidative stress were diminished by the inhibition of PLA_2 in the present study.

The experimental results presented in our study exhibited a determinant role of PLA_2 as a key enzyme for the induction of neutrophilic respiratory burst in the intestinal I/R-induced acute lung injury. Conclusively, PLA_2 plays a key role in the induction of oxidative stress in the lung, which may be caused through the increased plasma lipid molecules. The increased lipid molecules activate PLA_2 in the lung, which was affected by the oxidative stress produced by NADPH oxidase in the neutrophils.

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