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Rho-kinase (ROCK-1 and ROCK-2) upregulation in oleic acid-induced lung injury and its restoration by Y-27632

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Abstract

The possible contribution of Rho/Rho-kinase signalling in oleic acid (100 mg kg^{-1}, i.v., for 4 h)-induced lung injury was investigated in rats. Furthermore, the possible protective effect of the administration of a Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632, 0.5–5 mg kg^{-1}, i.v., 15 min before the administration of oleic acid), was also examined. Western blot analysis as well as histopathological examination revealed that Rho-kinase (ROCK-1 and ROCK-2) was upregulated in lungs obtained from oleic acid-administrated rats. In addition, the markers of oxidative and nitrosative stress, i.e., malondialdehyde, myeloperoxidase, 3-nitro-L-tyrosine and nitrite/nitrate, in serum and lung tissue were also increased in the injury group. Treatment of rats with 5 mg kg^{-1} Y-27632 reversed the oleic acid-induced lung damage, which was demonstrated by histopathological assessment and confirmed in Western blot experiments: ROCK-blots were more intense in the oleic acid group than in control and Y-27632 treatment reversed ROCK upregulation. In addition, malondialdehyde, myeloperoxidase, 3-nitro-L-tyrosine and nitrite/nitrate were also normalized after the administration of Y-27632 (0.5 mg kg^{-1} and 5 mg kg^{-1}). These findings suggest that ROCK-1 and ROCK-2 are involved in oleic acid-induced lung damage in rats, and that inhibition of this enzyme by Y-27632 may have a protective effect against such damage. Consequently, Rho kinase inhibitors may be potential therapeutic agents in the treatment of acute respiratory distress syndrome (ARDS).

Keywords: Lung injury; Nitric oxide; Oleic acid; Rho-kinase; Y-27632

1. Introduction

Oleic acid-induced lung injury is a good experimental model (Dickey et al., 1981) of acute respiratory distress syndrome (ARDS), which is an important cause of morbidity and mortality (Demling, 1995). It is characterized by diffuse interstitial and alveolar oedema with focal haemorrhage and vascular congestion, and by interstitial and alveolar infiltration of leukocytes (Beilman, 1995). Different kinds of stimuli can increase alveolocapillary permeability and this results in oedema, atelectasis and hypoxaemia, a condition known as acute lung injury (Davidson et al., 2000). Endothelial cells form a major part of the capillary permeability barrier and changes in the cells are associated with increased capillary permeability. Microfilaments and cytoskeletal actin are the major structures involved in maintaining endothelial cell shape (Golbidi et al., 2003). Therefore, endothelial cells may produce constriction, analogous to smooth muscle cell contraction, with the help of these elements. In inflammatory reactions, gaps between endothelial cells could open up and this may lead to extravasation of fluid and macromolecules. Airway epithe-

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lial cells can also contribute to inflammation by releasing inflammatory mediators such as interleukin-8, and this event is governed by protein kinase C, phosphatidic acid and Rho signalling (Cummings et al., 2002). Oleic acid, an 18-carbon lipid, has been reported to inhibit endothelium-dependent vasodilatation (Egan et al., 1999) and to stimulate smooth muscle proliferation (Lu et al., 1996). Therefore, this fatty acid may have an important role in the pathogenesis of endothelial dysfunction (Park et al., 2003).

In addition to the Ca^{2+}-dependent mechanisms of endothelial cell contraction, which is taken place by the mediation of myosin light chain kinase (MLCK), there may be another pathway which is not dependent on intracellular free calcium concentration, i.e., Ca^{2+} sensitization (van Nieuw Amerongen et al., 2000). In this phenomenon, the phosphorylation of myosin light chain (MLC) and the force of contraction are not dependent on the intracellular concentration of Ca^{2+} (Somlyo and Somlyo, 1994). Rho is a small GTPase which involved in the cytoskelatal responses to extracellular signals, such as various agonists including thrombin and lysophosphatidic acid (van Nieuw Amerongen et al., 2000; Ridley and Hall, 1992). Rho and its downstream effectors have been implicated in various cellular functions, such as regulation of vascular and non-vascular smooth muscle tone (Uehata et al., 1997; Büyükafşar and Levent, 2003; Büyükafşar et al., 2003), cell motility (Saurin et al., 2002; Takaishi et al., 1994), cytokinesis (Kishi et al., 1993) and non-muscle cell contraction (Essler et al., 1998; van Nieuw Amerongen et al., 1998).

Although several mechanisms, such as increased permeability, polymorphonuclear leukocyte recruitment and inflammation, have been implicated in the pathogenesis of oleic acid-induced lung injury, its detailed cellular mechanisms still remain to be elucidated. Therefore, in the present study, we investigated the possible involvement of Rho kinase in an experimental lung injury model. In addition, we tested the possible protective effect of the Rho-kinase inhibitor, (+)-(*R*)-*trans*-4-(1-aminoethyl)-*N*-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate, Y-27632, on oleic acid-induced lung injury. We evaluated malondialdehyde, myeloperoxidase, 3-nitro-L-tyrosine and nitrite/nitrate as injury markers. Furthermore, we also examined tissue specimens histopathologically. Finally, we measured the expression of ROCK (both isoforms, i.e., ROCK-1 and ROCK-2) in the sham (saline-treated), oleic acid and Y-27632 groups by Western blot analysis.

2. Materials and methods

2.1. Experimental protocol

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of Mersin University Centre for Experimental Medicine. Both sexes of Wistar rats weighing 150–200 g were randomly separated into the following six groups.

Group 1 (n=6): saline (0.3 ml 200 g^{-1}) was injected serving as control.
Group 2 (n=6): oleic acid was administered by intravenous injection at the dose of 100 mg kg^{-1}.
Group 3 (n=5): oleic acid (100 mg kg^{-1}, i.v.) plus Y-27632 (5 mg kg^{-1}, i.v.) was administered.
Group 4 (n=6): oleic acid (100 mg kg^{-1}, i.v.) plus Y-27632 (0.5 mg kg^{-1}, i.v.) was administered.
Group 5 (n=4): Y-27632 (5 mg kg^{-1}, i.v.) was administered.
Group 6 (n=5): Y-27632 (0.5 mg kg^{-1}, i.v.) was administered.

All drugs and saline were injected into the tail vein via a 24-G polyethylene catheter under light anaesthesia with ketamine. Acute lung injury was induced by intravenous administration of 100 mg kg^{-1} of oleic acid (*cis*-9-octadecanoic acid). Oleic acid was initially diluted in ethanol and saline was added to a final concentration of 25 mg ml^{-1} of oleic acid. This was infused intravenously at a constant flow over 5 min.

2.2. Collecting blood and tissue samples

Four hours after the administration of the drugs, the rats were anaesthetized with a high dose of ketamine (80 mg kg^{-1}, i.m.), the thorax was opened and blood samples were taken by cardiac puncture for malondialdehyde, myeloperoxidase, 3-nitro-L-tyrosine and nitrite/nitrate analysis. Thereafter, the both lungs were harvested. Some pieces of lungs were preserved in formaldehyde solution (10%) for histopathologic evaluation, and other pieces were used for biochemical examination and Western blotting.

2.3. Measurement of myeloperoxidase

Myeloperoxidase activity was measured by detecting leukosequestration. Tissue (300 mg) was homogenized in 0.02 M ethylenediamine tetra acetic acid (EDTA, pH=4.7) in a Teflon Potter homogenizer. Homogenates were centrifuged at 20,000×g for 15 min at +4 °C. Thereafter, the pellet was re-homogenized in 1.5 ml 0.5% hexadecyltirimethylammonium bromide (HETAAB) prepared in 0.05 M K_3PO_4 (pH=6) buffer and then it was re-centrifuged at 20,000×g for 15 min at +4 °C. The determination of sera and supernatant tissue myeloperoxidase activity depends on the fact that myeloperoxidase reduces *o*-dianozidine, and hence reduced *o*-dianozidine was measured at 410 nm with a spectrophotometer.

2.4. Measurement of malondialdehyde

The tissues were homogenized in 0.15 mM KCl for malondialdehyde determination. After the homogenate was centrifuged at 3000×*g*, the malondialdehyde levels in supernatant and sera, as an index of lipid peroxidation, were determined using thiobarbituric acid. The principle of the method depends on the measurement of the pink colour produced by interaction of barbituric acid, which is formed as a result of lipid peroxidation. The coloured reaction product, 1,1,3,3-tetraetoxypropane, was used as the primary standard.

2.5. Measurement of nitrite and nitrate

The levels of nitrite and nitrate were determined by using a photometric endpoint determination. Nitrate is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of the enzyme, nitrate reductase. The nitrite formed reacts with sulphanilamide and *N*-(1-naphthyl)-ethylenediamine dihydrochloride to give a red-violet diazo dye. The diazo dye is measured on the basis of its absorbance in the visible range at 550 nm.

2.6. Determination of 3-nitro-L-tyrosine

The tissues were homogenized in ice-cold phosphate-buffered saline (pH=7.4). Equivalent amounts of each sample were hydrolyzed in 6 N HCl at 100 °C for 18–24 h, then the samples were analyzed on a HP 1049 high-performance liquid chromatography (HPLC) apparatus. The analytical column was a 5-μm pore size Spherisorb ODS-2 C_{18} reverse-phase column (4.6–250 nm; Alltech, Deerfield, IL, USA). The guard column was a C_{18} cartridge (Alltech). The mobile phase was 50 mmol sodium acetate/50 mmol citrate/8% methanol, pH=3.1. HPLC analysis was performed under isocratic conditions at a flow rate of 1 ml min^{-1} and an ultraviolet (UV) detector was set at 274 nm. 3-Nitro tyrosine peaks were determined according to their retention times and the peaks were confirmed by spiking samples with added exogenous 3-nitro tyrosine (10 μmol), which was reduced to aminotyrosine.

2.7. Histopathological examination

The specimens were fixed in 10% formalin for 24 h, and standard dehydration and paraffin-wax embedding procedures were used. Haematoxylin–eosin-stained slides were prepared by using standard methods. Light microscopic analyses of lung specimens were done by blinded observation to evaluate pulmonary architecture, tissue oedema formation and infiltration of the inflammatory cells. The results were classified into four grades, where grade 1 represented normal histopathology, grade 2 indicated minor neutrophil leukocyte infiltration, grade 3 represented moderate neutrophil leukocyte infiltration, perivascular oedema formation and partial destruction of pulmonary architecture, and grade 4 represented dense neutrophil leukocyte infiltration, abscess formation and complete destruction of the pulmonary architecture.

2.8. Western blotting for ROCK-1 and ROCK-2

Lungs were immediately removed and homogenized with lysis buffer (composition in mM: Tris–HCl (pH=7.4) 50 mM, NaCl 400 mM, EGTA 2 mM, EDTA 1 mM, dithiothreitol 1 mM, phenylmethylsulfonyl fluoride 10 μM, leupeptin 10 μg ml^{-1}, pepstatin 1 μg ml^{-1}, benzamidine 1 mM). The homogenate was centrifuged at 3000×*g* for 10 min at 4 °C and the supernatant was removed. It was then used for protein analysis (with Lowry method) and Western blotting. Thereafter, the homogenate was loaded in wells, electrophoresed on 8% polyacrylamide-sodium dodecyl sulphate (SDS) gels and then transferred to a nitrocellulose membrane. The membrane was blocked with the blocking agent of ECL advance chemiluminescense kit in Tris-buffered solution (TBS) containing 0.05% Tween-20 (TBS-T). It was then probed with a primary antibody raised against ROCK-1 (ROKβ) and ROCK-2 (ROKα, Polyclonal IgG, Santa Cruz Biotechnology, CA, USA) at 1:200 dilution, followed by horseradish peroxidase-conjugated secondary antibody (donkey antigoat, 1:1000, Santa Cruz Biotechnology, CA, USA). The blots were then detected with the ECL Advance Kit (Amersham Biosciences, Freiburg, Germany).

2.9. Drugs and chemicals

3-Nitro tyrosine and oleic acid were obtained from Sigma Chemical (St. Louis, MO, USA). Y-27632 was purchased from Tocris Cookson (Bristol, UK). Primary and secondary antibodies were obtained from Santa Cruz Biotechnology (CA, USA) and ECL from Amersham Bioscience (Freiburg, Germany). All organic solvents were HPLC grade. Y-27632 was dissolved in saline. Oleic acid was initially dissolved in ethanol and then diluted with saline in a saline/ethanol ratio of 9:1. This was applied by slow intravenous bolus injection into the tail vein.

2.10. Statistical analysis

Biochemical data are expressed as means±standard errors of the mean for *n* observations. Histopathological scores are expressed as medians±quartiles. For comparison, analysis of variance (ANOVA) followed by Bonferroni post hoc test or Kruskal–Wallis and Dunn tests were used. A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Effects of oleic acid administration on lung histopathology, serum or tissue nitrite/nitrate, 3-nitro tyrosine, myeloperoxidase and malondialdehyde levels

Histopathological examination revealed that pulmonary histology was normal (grade 1) in the sham group (control) (Fig. 1). However, oleic acid induced pronounced acute lung damage (Fig. 1). The lung tissue was much darker red in the oleic acid group than in the other groups. Furthermore, an increase in congestion, neutrophil infiltration and even derangement of pulmonary architecture were observed under light microscopy (grade 3). Oleic acid treatment also increased serum and tissue nitrite/nitrate, 3-nitro tyrosine, myeloperoxidase and malondialdehyde levels (Figs. 2–4). Western blot analysis indicated that oleic acid administration significantly upregulated the expression of Rho-kinase (ROCK-1 and ROCK-2, Fig. 5).

3.2. Preventive effect of Y-27632 on oleic acid-induced lung injury, and restoration of oxidative and nitrosative stress markers after the administration of Y-27632

Administration of Y-27632 (0.5 mg kg^{-1}) did not cause a significant improvement in lung histology (data not shown). However, a higher dose of Y-27632 (5 mg kg^{-1}) prevented the lung injury induced by oleic acid although there was some focal alveolar oedema (Fig. 1). The colour of the lung in the treatment group was pink, similar to that in the sham group. A box plot of histopathological scores is shown as an inset in Fig. 1. Pretreatment of the rats in the oleic acid group with Y-27632 (5 mg kg^{-1}) normalized serum nitrite/nitrate, myeloperoxidase and malondialdehyde as well as tissue 3-nitro tyrosine, myeloperoxidase and malondialdehyde levels (Figs. 2–4). Western blot analysis also confirmed the restorative effect of Y-27632 (Fig. 5). The Rho-kinase inhibitor alone had no effect on the pulmonary histopathology at either dose (data not shown), and neither did

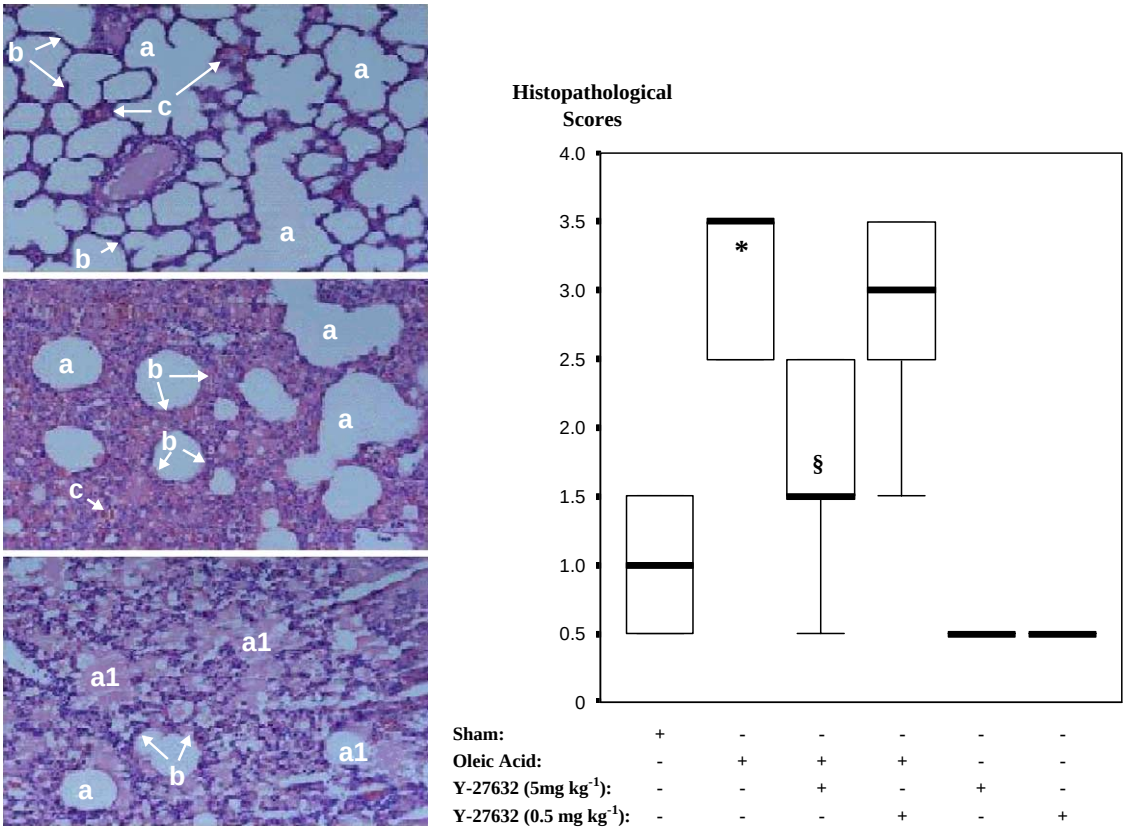

Fig. 1. Histopathology of lung tissues. Normal pulmonary histology was observed in the sham group (upper panel, grade 1). ×200. Oleic acid group (100 mg kg^{-1}, i.v., for 4 h) revealed extensive oedema and haemorrhage and a large build-up of neutrophils (central panel, grades 3–4). ×200. In the treatment group (Y-27632, 5 mg kg^{-1}), there were attenuated histopathological features including alveolar oedema, but relatively little evidence of haemorrhage and greatly reduced alveolar and interstitial neutrophil content (lower panel, grade 2). ×200. a: alveolar spaces, b: alveolar walls, c: capillary vessels, a1: an alveolus filled with proteinous oedema fluid. Y-27632 (0.5 mg kg^{-1}) did not improve effect on the pulmonary histopathology (data not shown). In the inset are the histopathological scores, which are expressed as medians±quartiles of four to six observations. For comparison, Kruskal–Wallis and Dunn tests were used. *P<0.01, different from the control group; $^{§}P$<0.01, different from oleic acid group.

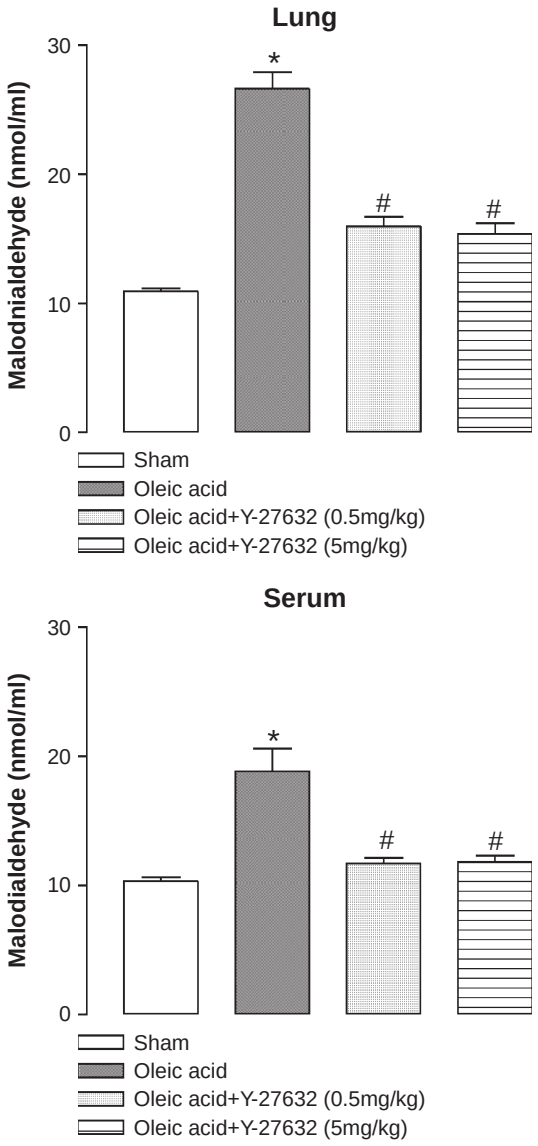

Fig. 2. Tissue (upper) and serum (lower) malondialdehyde levels in the sham, oleic acid and treatment groups. Oleic acid administration increased malondialdehyde levels and Y-27632 prevented this increase. Data represent means±S.E.M. of four to six observations. For comparison, analysis of variance (ANOVA) followed by Bonferroni post hoc test was used. *P<0.001, different from sham group; $^{\#}P$<0.001, different from oleic acid group.

it significantly change serum and tissue oxidative and nitrosative stress markers (data not included).

4. Discussion

The major finding of the present study is the fact that oleic acid, which is used for inducing acute lung injury in experimental animal models increased the expression of Rho-kinase (ROCK-1 and ROCK-2) protein, which could possibly mediate its deleterious effect on the lung. In addition, pretreatment of rats with Y-27632 (5 mg kg^{-1}), a selective Rho-kinase inhibitor, prevented all pathological signs such as increased nitric oxide metabolites, 3-nitro tyrosine, myeloperoxidase and malondialdehyde levels both in serum and in tissue. It also prevented histopathological changes in the lung. Furthermore, Y-27632 inhibited the upregulated expression of ROCK, an enzyme one of the most important pathways of Ca^{2+} sensitization in smooth muscle and non-muscle cells (Fukata et al., 2001).

Oleic acid can inhibit endothelium-dependent vasodilatation (Egan et al., 1999) and also facilitate smooth muscle proliferation (Lu et al., 1996). Therefore, it is possible that this fatty acid can induce endothelial dysfunction (Park et al., 2003). Furthermore, a potent

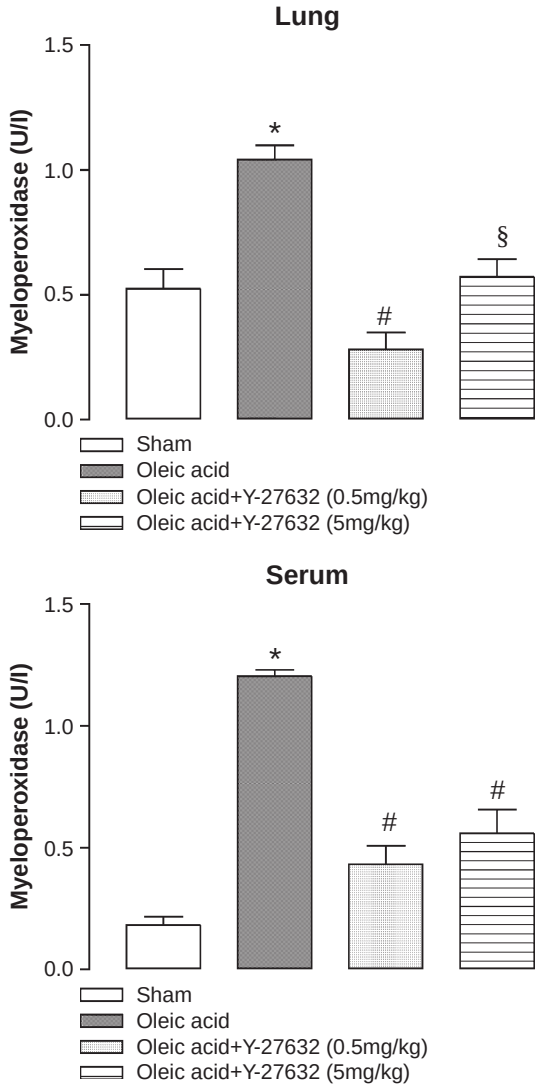

Fig. 3. Tissue (upper) and serum (lower) myeloperoxidase levels in the sham, oleic acid and treatment groups. Oleic acid administration increased myeloperoxidase levels and Y-27632 prevented this increase. Data represent means±S.E.M. of four to six observations. For comparison, analysis of variance (ANOVA) followed by Bonferroni post hoc test was used. *P<0.001, different from sham group; $^{\#}P$<0.001, $^{\S}P$<0.01, different from oleic acid group.

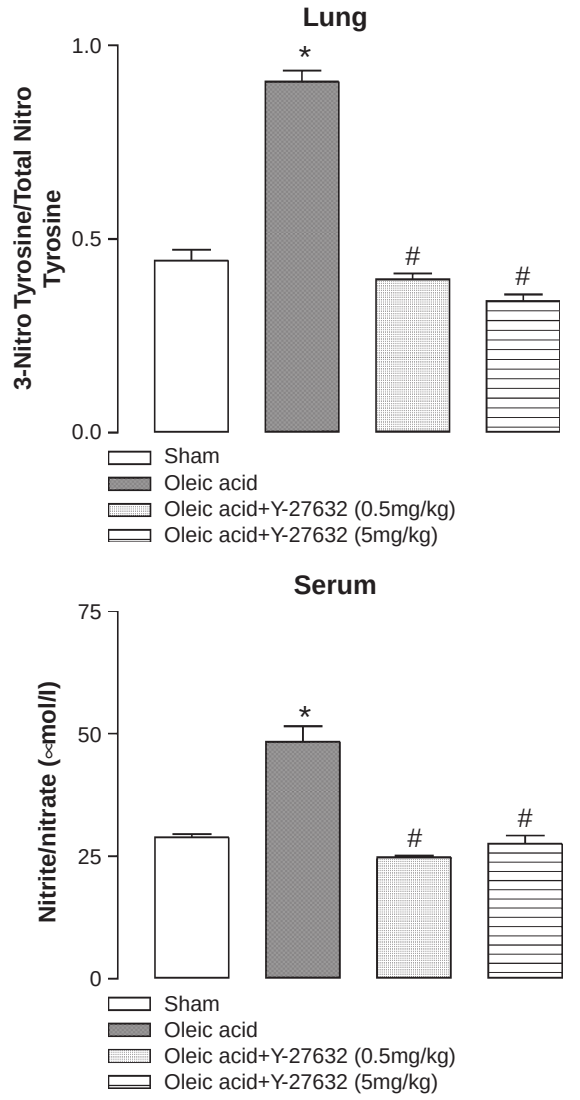

Fig. 4. Tissue level of 3-nitro tyrosine (upper panel) and serum level of nitrite/nitrate (lower panel) in the sham, oleic acid and treatment groups. Oleic acid administration increased 3-nitro tyrosine and nitrite/nitrate levels and Y-27632 prevented these increases. Data represent mean±S.E.M. of four to six observations. For comparison, analysis of variance (ANOVA) followed by Bonferroni post hoc test was used. *P<0.001, different from sham group; $^{\#}P$<0.001, different from oleic acid group.

vasoconstrictor agent and an enhancer of endothelial permeability, thromboxane A_2 is also involved in oleic acid-induced lung injury (Ishitsuka et al., 2004). Oleic acid-induced lung injury mimics the acute inflammation of the ARDS (Dickey et al., 1981). Therefore, this experimental model is useful in order to assess therapeutic approaches to the treatment of ARDS. It also helps us explore possible pathophysiological mechanisms of acute lung injury. The compound induces a massive and rapid influx of polymorphonuclear leukocytes within 1 h, with a peak inflammatory response at 4 h (Hussain et al., 1998). Therefore, we used 4 h of oleic acid challenge.

In the present study, the malondialdehyde level increased after oleic acid administration, indicating that leukocyte recruitment and oxidative stress were induced. It has been demonstrated that superoxides are released from polymorphonuclear leukocytes after application of oleic acid (Moriuchi et al., 1998). This fatty acid is supposed to act by binding to the endothelium, triggering the lipid peroxidation chain reaction in the membrane (Davidson et al., 2000). Oxidant injury may also cause changes in endothelial cell morphology, i.e. changes in endothelial cell shape are associated with increased capillary permeability. This may be evoked by the activation of Rho/Rho-kinase signalling (Breslin and Yuan, 2004). Furthermore, the endothelial activation and barrier dysfunction elicited by cytokines are implicated in the pathogenesis of the pulmonary oedema associated with acute lung injury syndromes (Petrache et al., 2003). Therefore, inhibition of ROCK by Y-27632 could inhibit the increased endothelial permeability. In this study, ROCK activation may have triggered oleic acid-induced lung injury. Once the pathological state is initiated, ROCK expression might increase, because several cellular events which are responsible for oleic acid-induced lung injury involve ROCK activation, i.e., neutrophil movement, endothelial permeability increase, etc. This may explain why Y-27632 inhibits ROCK-1 and ROCK-2 expression although it is known to be a ROCK enzyme inhibitor.

It has been shown that under inflammatory conditions there is an abundant release of NO, which is derived from inducible and Ca^{2+}-independent nitric oxide synthase (iNOS). Accordingly, in the present study, the production of NO metabolites increased in the serum of oleic-administered rats. Interestingly, it has been suggested that endogenous NO may also affect the Ca^{2+} sensitization phenomenon, which is mainly governed by Rho/Rho-kinase signalling by upregulating RhoA activation (Sauzeau et al., 2000; Etter et al., 2001). The expression of Rho-kinase, the downstream target of RhoA, was found increased in this study. This may reflect cross-talk between Rho-signalling and NO/L-arginine pathways. It has been reported that Rho-GTPase signalling can modulate the inducible form of nitric oxide in airway epithelial cells (Kraynack et al., 2002). Apart from superoxide anion, NO is also another noxious stimulus for lung inflammation (Zeidler et al., 2004). In the present study, both nitrosative and oxidative stress were induced because serum and tissue levels of nitric oxide metabolites, myeloperoxidase, 3-nitro tyrosine and malondialdehyde were increased. The interaction of excess NO with superoxide leads to the generation of a highly toxic species, peroxynitrite, which inhibits cellular respiration (Moncada, 2000), exacerbating the condition. The marker of peroxynitrite formation, 3-nitro tyrosine, also increased in the lung tissue and serum obtained from oleic acid-treated groups.

MLC phosphorylation plays a crucial role in smooth muscle contraction (Somlyo and Somlyo, 1994) and in the actin–myosin interaction in stress fibres and in contraction of non-muscle cells, such as endothelial and airway

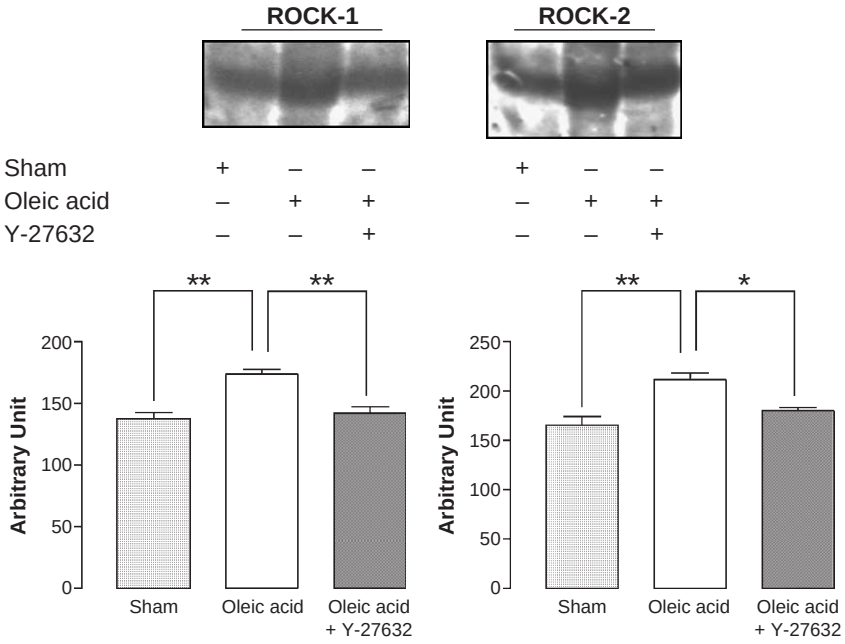

Fig. 5. Expression levels of Rho-kinase (ROCK-1 and ROCK-2) in the lung injury. The lungs obtained from saline, oleic acid and oleic acid plus Y-27632 (5 mg kg^{-1}, i.v.)-treated rats were homogenized. The lung homogenates were then submitted to SDS-PAGE with 8% polyacrylamide and transferred to a nitrocellulose membrane. The membrane was blocked with the blocking agent of ECL Advance Chemiluminescence Kit for 1 h. It was then probed with a primary antibody raised against ROCK-1 and ROCK-2 polyclonal Immunoglobin G (Santa Cruz Biotechnology, USA) at 1:200 dilution, followed by horseradish peroxidase-conjugated secondary antibody (donkey antigoat, 1:1000). The blots were then detected with an Advance Chemiluminescence Detection Kit (Amersham Biosciences, Germany). Relative density of the protein bands was evaluated with a computer program (Scion image, USA). Data represent means±S.E.M. of four observations. For comparison, analysis of variance (ANOVA) followed by Bonferroni post hoc test was used. *$P<0.05$, **$P<0.01$.

epithelial cells (Wysolmerski and Lagunoff, 1991; Moore et al., 2003). Moreover, it has been suggested that in addition to protein kinase C and phosphatidic acid, Rho is also an important signalling component in the inflammation of human bronchial epithelial cells (Cummings et al., 2002). This strengthens our main finding that Rho-kinase could be involved in oleic acid-induced lung injury. However, oleic acid has been demonstrated to activate protein kinase C via the formation of diacylglycerol in endothelial (Park et al., 2003) and smooth muscle cells (Yu et al., 2001). Apart from Rho kinase, protein kinase C is also another Ca^{2+}-sensitizing protein through the activation of CPI-17 protein, which phosphorylates and inhibits myosin phosphatase, counteracting smooth muscle contraction (Fukata et al., 2001).

We do not know how oleic acid causes the activation of Rho-kinase or whether it activates the enzyme through a receptor-dependent or independent mechanism. Further studies are needed to answer these questions. It has been reported that oleic acid causes the release of endothelin-1 upon activation of nuclear factor-kB (Park et al., 2003). Endothelin-1-induced post-receptor events involve at least the activation of Rho/Rho-kinase signalling (Sakurada et al., 2001). Like oleic acid, another lipid-like compound, lisophosphatidic acid, is considered a classical activator of RhoA protein (Fleming et al., 1996). Consequently, oleic acid may activate Rho proteins and subsequently its downstream target, Rho-kinase. However, we did not measure the activation of this enzyme. As for upregulation of ROCK by oleic acid challenge, the endothelial cell activation and barrier dysfunction implicated in the pathogenesis of pulmonary oedema in the acute lung injury induced by oleic acid might be associated with an increase in ROCK expression, because ROCK is an important protein for endothelial cell constriction, which leads to oedema formation and other inflammatory events (Breslin and Yuan, 2004).

In conclusion, this is the first report indicating the involvement of Rho/Rho-kinase signalling in oleic acid-induced lung injury in rats. Furthermore, treatment of rats with a Rho-kinase inhibitor, Y-27632, prevented the lung injury. This may provide a new therapeutic approach to the treatment of acute respiratory distress syndrome.

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