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JTE-607, a cytokine release blocker, attenuates acid aspiration-induced lung injury in rats

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Abstract

The present study was designed to clarify the effects of (-)-ethyl *N*-{3,5-dichloro-2-hydroxy-4-[2-(4-methyl-piperazin-1-yl)ethoxy]benzoyl}-L-phenylalaninate dihydrochloride (JTE-607), a novel multiple cytokine inhibitor, on hydrochloric acid (HCl) aspiration lung injury in rats. HCl (0.1 N, 2 ml kg^{-1}) was instilled into male Sprague–Dawley rats that were pretreated with or without JTE-607 (30 or 75 mg kg^{-1} h^{-1}). As a control, normal saline (2 ml kg^{-1}) was instilled in rats. All the animals were anesthetized with intraperitoneally injected pentobarbital sodium (40 mg kg^{-1}). Bronchoalveolar lavage was performed 5 h (h) after HCl or normal saline instillation. In bronchoalveolar lavage fluid, the increases in total nuclear cell counts, neutrophil counts, optical density at 412 nm as an indication of pulmonary hemorrhage, concentrations of albumin, tumor necrosis factor (TNF)-α, interleukin (IL)-6 and cytokine-induced neutrophil chemoattractant induced by HCl instillation were significantly reduced by JTE-607 pretreatment. The level of expression of tumor necrosis factor-α and interleukin-6 mRNA in lung tissue was analyzed. The mean expression level of tumor necrosis factor-α and interleukin-6 mRNA in the JTE-607 group was lower than that in the HCl and NS groups. The wet-to-dry weight ratio was also determined, and JTE-607 at the dose of 75 mg kg^{-1} h^{-1} significantly attenuated the increased wet-to-dry weight ratio induced by HCl. These results suggest that JTE-607 can inhibit the production of inflammatory cytokines such as tumor necrosis factor-α, interleukin-6 and cytokine-induced neutrophil chemoattractant and attenuate acid-induced lung injury in rats. This agent might be therapeutically useful for lung injury.

Keywords: Inflammatory cytokine; Neutrophil; Pulmonary edema; Bronchoalveolar lavage fluid

1. Introduction

Acid aspiration lung injury is an acute inflammatory process that causes extensive alterations in lung structure and function. It is characterized by increased permeability-induced pulmonary edema and infiltration of neutrophils into air spaces. Although the precise mechanism of lung injury is not fully understood, several cytokines have been implicated in the development of lung injury. In human studies, high levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and interleukin-8 have been found in plasma and bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome (Chollet-Martin et al., 1993, 1996; Milar et al., 1989; Miller et al., 1992). In animal studies, the concentrations of interleukin-6 and cytokine-induced neutrophil chemoattractant in bronchoalveolar lavage fluid increased and the expression of RNA for these cytokines was up-regulated in the tissue of lung as damaged by acid (Davidson et al., 1999; Nishina et al., 1998). Anti-interleukin-8 antibody prevented acid-induced abnormalities in oxygenation, extravascular lung water formation and lung endothelial permeability in rabbits (Folkesson et al., 1995). Anti-tumor necrosis factor-α treatment reduced protein permeability and improved blood oxygenation in acid-induced lung injury (Davidson et al., 1999). These findings support an important role of pro-inflammatory cytokines in acid-induced lung injury. So for, however, there are no agents to prevent cytokine production without affecting other mediators in the inflammatory process. In addition, there is a possibility that anti-inflammatory agents have adverse effects by inhibiting host immunity and increasing the risk of secondary infection. JTE-607 is a multiple cytokine inhibitor which can suppress the production of several inflammatory cytokines without causing immunosuppression (Kakutani et al., 1999). Iwamura et al. (2002)

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reported that JTE-607 has an inhibitory effect on lipopolysaccharide-induced lung inflammation by reducing cytokine-induced neutrophil chemoattractant production. It has been shown that tumor necrosis factor-α and macrophage inflammatory protein-2, another member of the cytokine-induced neutrophil chemoattractant family and a murine homologue of human interleukin-8, are decreased by JTE-607 in lung and in plasma in burn-induced acute lung injury of endotoxemic mice (Sasaki et al., 2003). JTE-607 may be a useful anti-inflammatory agent for the treatment of cytokine-mediated disease. The present study was designed to evaluate the effect of JTE-607 on acid aspiration-induced lung injury in rats.

2. Materials and methods

This study was carried out in accordance with the European Community Guideline for the use of Experimental Animals and was approved by the ethics committee of the Shinshu University School of Medicine.

2.1. Animals and drugs

Six-week-old male Sprague–Dawley rats weighing 250–280 g were purchased from Japan SLC (Hamamatsu, Japan). JTE-607, a novel inflammatory cytokine synthesis inhibitor, (-)-ethyl *N*-{3,5-dichloro-2-hydroxy-4-[2-(4-methyl-piperazin-1-yl)ethoxy]benzoyl}-L-phenylalaninate dihydrochloride, was provided by Japan Tobacco (Tokyo, Japan). Five percent mannitol was used to dissolve the agent.

2.2. Experiments and protocols

The present study was performed as the following two experiments.

2.2.1. Experiment 1

Bronchoalveolar lavage was performed to collect bronchoalveolar lavage fluid 5 h after hydrochloric acid (HCl) or normal saline instillation. The lungs of each animal were lavaged with 5 ml of normal saline four times ($n = 8$ in each group).

2.2.2. Experiment 2

Lung tissue samples were taken to evaluate histopathologic changes, to assess pulmonary edema and to analyze the quantitative mRNA level of tumor necrosis factor-α and interleukin-6, using real-time reverse transcription polymerase chain reaction (RT-PCR) ($n = 8$ in each group).

In each experiment, animals were anesthetized with intraperitoneally injected pentobarbital sodium (40 mg kg^{-1}). The right jugular vein was established for administration of drugs and fluid maintenance. A tracheostomy was performed. Normal saline or JTE-607 was infused continuously over 5.5 h at a rate of 1 ml h^{-1} via the right jugular vein, using an infusion pump (TERUMO TE-312, Tokyo, Japan). At 30 min after the start of the infusion, normal saline (2 ml kg^{-1}) or HCl (0.1 N 2 ml kg^{-1}) was instilled into the trachea via the tracheostomy. Animals were then mechanically ventilated (SHINANO Respirator Model SN-480-7, Tokyo, Japan) at a tidal volume (V_t) of 10 ml kg^{-1}, a breathing frequency of 50 breaths min^{-1}, a fraction of inspired O_2 (FiO_2) of 1.0.

In each experiment, animals were allotted to five groups: (1) JTE-607 alone group: 75 mg kg^{-1} h^{-1} JTE-607 treatment and normal saline instilled into the trachea. (2) NS group: normal saline infusion and normal saline instilled into the trachea. (3) HCl group: normal saline infusion and HCl instilled into the trachea. (4) Low-dose JTE treatment group: 30 mg kg^{-1} h^{-1} JTE-607 treatment and HCl instillation. (5) High-dose JTE treatment group: 75 mg kg^{-1} h^{-1} JTE-607 treatment and HCl instillation.

2.3. Measurements

2.3.1. Bronchoalveolar lavage fluid

Total nuclear cell counts were measured using a hemocytometer (Sysmex F-520). Cell monolayers were prepared by cytocentrifugation for neutrophil counts. Differential counts were performed on 200 cells from smears stained with May-Giemsa. The absorbance of bronchoalveolar lavage fluid was measured to determine the extent of pulmonary hemorrhage. It was analyzed by the following method: 0.75 ml distilled water was added to 0.75 ml bronchoalveolar lavage fluid in a test tube. This test tube was gently inverted and incubated at room temperature for 30 min, and centrifuged at $3000 \times g$ for 10 min. The optical density of the supernatant was read at 412 nm, using a Double Beam Spectrophotometer (UVIDEC-610A, Japan Spectroscopic, Tokyo, Japan). The albumin concentration in bronchoalveolar lavage fluid was determined by nephelometric immunoassay. Tumor necrosis factor-α and interleukin-6 concentrations in bronchoalveolar lavage fluid were determined by Enzyme Linked-Immuno-Sorbent Assay (ELISA, Immunoassay Kit by TFB, Tokyo, Japan). First, 50 μl of standard diluent buffer was added to onto eight-well plates. The samples, including standards, controls and unknowns, were pipetted into appropriate wells. Then, 50 μl of biotinylated antibody specific for rat tumor necrosis factor-α or rat interleukin-6 was added and incubated for 1.5 h at room temperature. The wells were washed four times with wash buffer. Then, 100 μl of streptavidin-peroxidase (enzyme) was added and incubated for 45 min at room temperature. The wells were washed four times and 100 μl of stabilized chromogen (substrate solution) was added to each well and incubated for 30 min at room temperature in the dark. One hundred microliters of stop solution was added and the color of the solution in the wells changed from blue to yellow. The optical density of the colored product at 450 nm was

directly proportional to the concentration of cytokines present in the specimen.

Cytokine-induced neutrophil chemoattractant was measured by enzyme immunoassay, using a rat GRO/CINC-1 measurement kit IBL (Immuno-Biological Laboratories, Gunma, Japan). Briefly, 100 μl standards, controls and samples were added to wells, respectively. After 1 h at 37 °C, the wells were washed. One hundred microliters of biotinylated antibody specific for rat GRO/CINC-1 was added and incubated. After a washing step, tetramethyl benzidine (TMB) was added. The samples were incubated in the dark for 30 min, 100 μl of stop solution was added, and then the optical density at 450 nm was read.

2.3.2. Histopathology findings and assessment of pulmonary edema

Eight rats in each group were used to evaluate the wet-to-dry weight ratio of the lung and the histopathologic findings. After the animals were killed, the lungs were removed immediately. The right lung was weighed and then heated at 80 °C to a constant weight in a convection oven (Programmable incubator IC-300P, Iuchi Osaka, Japan) for 72 h and the residue was weighed. The left lung was fixed in 10% formalin solution for 10 h. The tissue blocks obtained from midsagittal slices of the lungs were embedded in paraffin. The samples were stained with hematoxylin and eosin for microscopic examination.

2.3.3. Real-time quantitative RT-PCR

In the NS, HCl and JTE-607 (75 mg kg^{-1} h^{-1}) groups, 10 mg lung tissue from each rat was homogenized. Total RNA of this sample was isolated. After the density of RNA was measured, 100 ng total RNA from each sample was reverse transcribed. These samples were kept at −80 °C until the real-time PCR analysis. In the present study, tumor necrosis factor-α and interleukin-6 mRNA were quantitatively analyzed. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the expression level of tumor necrosis factor-α and interleukin-6 mRNA. Eight 10-fold dilutions of cDNA were amplified to obtain standard curves and to determine the amplification efficiency of GAPDH, tumor necrosis factor-α and interleukin-6 cDNAs. Each PCR reaction contained primer, probe, commercial PCR Master mix (TaqMan Universal PCR Master mix, Applied Biosystems, Foster City, CA) and 10 μl of the diluted cDNA sample. Samples of GAPDH, tumor necrosis factor-α and interleukin-6 from each group were placed in a 96-well plate and amplified in an automated fluorometer (ABI Prism 7700 Sequence Detection System, PE Applied Biosystems) (Shimizu et al., 2003). All the samples were preheated at 95 °C for 10 min, amplified for 50 cycles of 95 °C for 15 s, followed by heating at 60 °C for 1 min. The relative level of expression of tumor necrosis factor-α and interleukin-6 was defined by the tumor necrosis factor-α/GAPDH and interleukin-6/GAPDH mRNA ratio. Primers and probes were designed using the Primer Express Software (Applied Biosystems). The sequences of the primers and probes are listed in Table 1.

2.4. Statistical analysis

All data are expressed as means ± S.E. The difference between the means of two groups was evaluated with analysis of variance (ANOVA) followed by Bonferroni's test. P values less than 0.05 were considered significant. STATVIEW 5.0 software (Abacus Concepts, Berkeley, CA) was used for all statistical tests.

3. Results

There were no significant differences in measurable parameters between the JTE-607 alone group and the NS group.

3.1. Bronchoalveolar lavage fluid

Recovery rates of bronchoalveolar lavage fluid were greater than 90% in all groups. There were no significant differences in the recovery rates among the groups.

3.1.1. Nuclear cell counts and neutrophils

The nuclear cell counts (Fig. 1A), neutrophil differential ratio (Fig. 1B) and neutrophil counts (Fig. 1C) in bronchoalveolar lavage fluid are shown in Fig. 1. The nuclear cell counts in the HCl group were significantly higher than that of the NS group. The increased cells in the HCl group consisted mainly of neutrophils. JTE-607 treatment in both high- and low-dose groups significantly decreased the increased nuclear cell counts, neutrophil, differential ratio and neutrophil counts by HCl.

Table 1
Sequence of PCR primers and TaqMan probes specific bovine GAPDH, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α)

Cytokine	Primer	Primer sequence (5′–3′)	Probe	Probe sequence (5′–3′)
GAPDH	GAPDH f	GAAGGTGAAGGTCG GAGT	GAPDH	CAAGCTTCCCGTTCTC AGCC
	GAPDH r	AAGATGGTGATGGG ATTTC		
IL-6	IL-6. 586f	AATGGAGAAGTTAG AGTCACAGAAGGA	IL-6.614t	TGGCTAAGGACCAAGA CCATCCAACTCA
	IL-6.674r	TGACCTTTAGAAAT TCTTCAAGTGCTT		
TNF-α	TNF. 435f	ACAAGGCTGCCCC GACTAT	TNF.467t	ACACCGTCAGCCGATT TGCCACTT
	TNF.521r	GCGGAGAGGAGGC TGACTT		

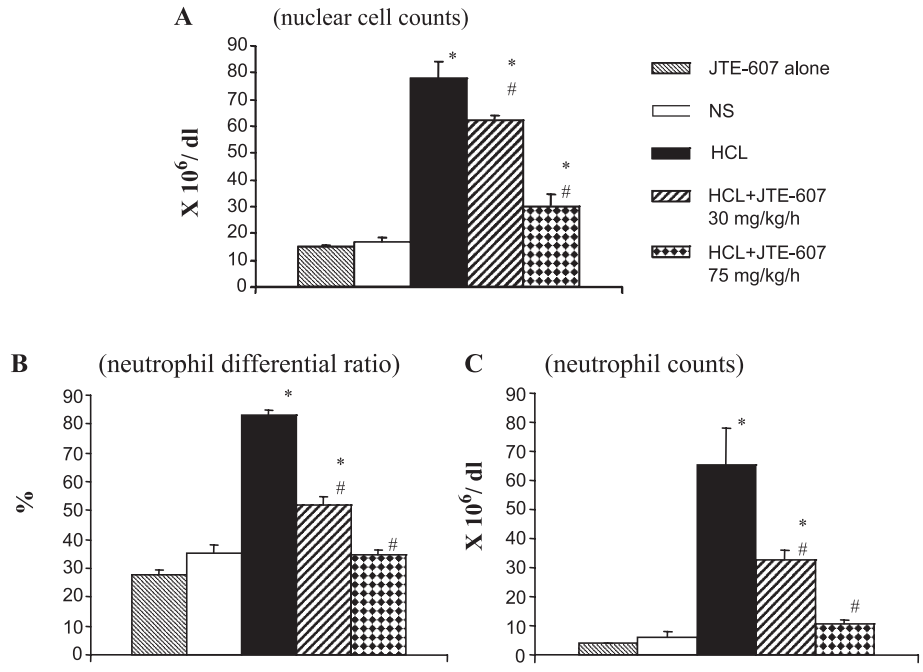

Fig. 1. Nuclear cell counts (A), neutrophil differential ratio (B) and neutrophil counts (C) in bronchoalveolar lavage fluid of all groups. JTE-607 alone group, rats were instilled with normal saline (2 ml kg^{-1}) and treated with 75 mg kg^{-1} h^{-1} of JTE-607 ($n=8$); NS group, rats were instilled with normal saline and treated with normal saline treatment ($n=8$); HCl group, rats were instilled with hydrochloric acid (HCl, 2 ml kg^{-1}) and treated with normal saline ($n=8$); HCl + JTE-607 30 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 30 mg kg^{-1} h^{-1} ($n=8$); HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 75 mg kg^{-1} h^{-1} ($n=8$). Values are expressed as means ± S.E. *$P<0.05$ versus both JTE-607 alone and NS group; #$P<0.05$ versus HCl group.

3.1.2. Absorbance

The optical density at 412 nm of the HCl group was significantly higher than that of the other groups, suggesting marked hemorrhage in the lung tissue. JTE-607 in both high- and low-dose treatment groups significantly attenuated this change, suggesting that JTE-607 diminished the hemorrhage in lung tissue induced by HCl instillation (Fig. 2).

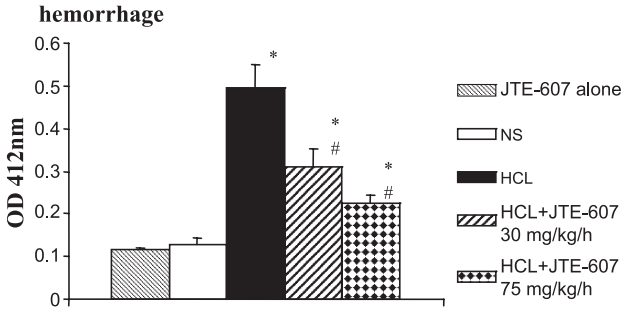

Fig. 2. Optical density of bronchoalveolar lavage fluid at 412 nm, representing the amount of hemoglobin in BALF. JTE-607 alone group, rats were instilled with normal saline (2 ml kg^{-1}) and treated with 75 mg kg^{-1} h^{-1} of JTE-607 ($n=8$); NS group, rats were instilled with normal saline and treated with normal saline treatment ($n=8$); HCl group, rats were instilled with hydrochloric acid (HCl, 2 ml kg^{-1}) and treated with normal saline ($n=8$); HCl + JTE-607 30 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 30 mg kg^{-1} h^{-1} ($n=8$); HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 75 mg kg^{-1} h^{-1} ($n=8$). Values are expressed as means ± S.E. *$P<0.05$ versus both JTE-607 alone and NS group; #$P<0.05$ versus HCl group.

3.1.3. Albumin

Albumin concentrations in bronchoalveolar lavage fluid were significantly higher in the HCl group than in the NS group, suggesting an increase in lung permeability due to HCl instillation. JTE-607 treatment in both high- and low-concentration groups significantly attenuated the increase in permeability (Fig. 3).

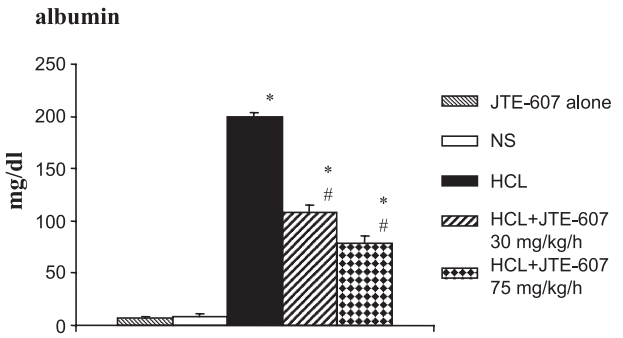

Fig. 3. Albumin concentrations in bronchoalveolar lavage fluid of all groups. JTE-607 alone group, rats were instilled with normal saline (2 ml kg^{-1}) and treated with 75 mg kg^{-1} h^{-1} of JTE-607 ($n=8$); NS group, rats were instilled with normal saline and treated with normal saline treatment ($n=8$); HCl group, rats were instilled with hydrochloric acid (HCl, 2 ml kg^{-1}) and treated with normal saline ($n=8$); HCl + JTE-607 30 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 30 mg kg^{-1} h^{-1} ($n=8$); HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 75 mg kg^{-1} h^{-1} ($n=8$). Values are expressed as means ± S.E. *$P<0.05$ versus both JTE-607 alone and NS group; #$P<0.05$ versus HCl group.

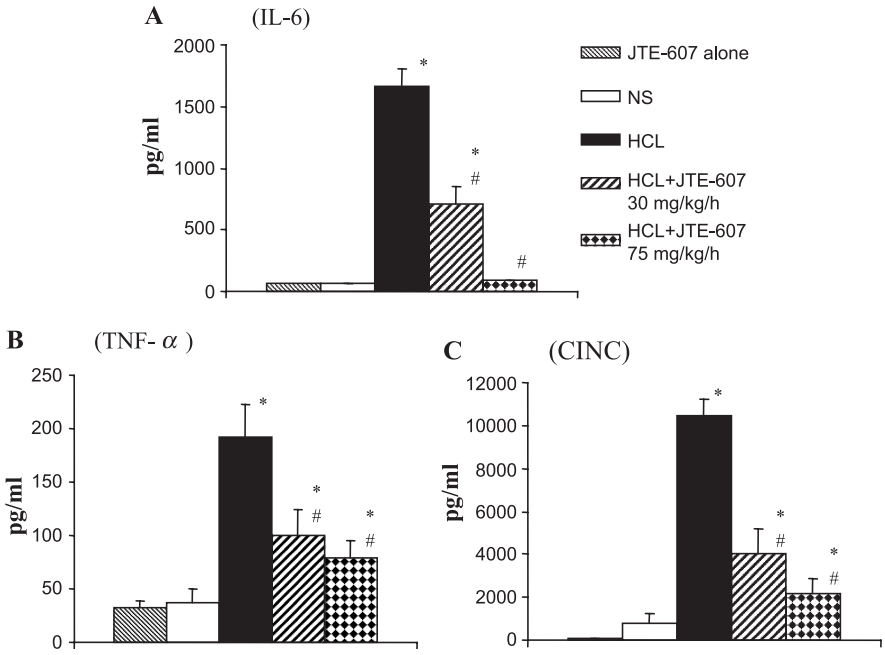

Fig. 4. Cytokine levels in bronchoalveolar lavage fluid of all groups. JTE-607 alone group, rats were instilled with normal saline (2 ml kg^{-1}) and treated with 75 mg kg^{-1} h^{-1} of JTE-607 ($n=8$); NS group, rats were instilled with normal saline and treated with normal saline treatment ($n=8$); HCl group, rats were instilled with hydrochloric acid (HCl, 2 ml kg^{-1}) and treated with normal saline ($n=8$); HCl + JTE-607 30 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 30 mg kg^{-1} h^{-1} ($n=8$); HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 75 mg kg^{-1} h^{-1} ($n=8$). (CINC): Cytokine-induced neutrophil chemoattractant. Values are expressed as means ± S.E. *$P<0.05$ versus both JTE-607 alone and NS group; #$P<0.05$ versus HCl group.

3.1.4. Cytokines

Tumor necrosis factor-α, interleukin-6 and cytokine-induced neutrophil chemoattractant levels in bronchoalveolar lavage fluid significantly increased in rats receiving HCl compared with those in normal saline rats. JTE-607 pretreatment in high- and low-doses was effective in decreasing the production of these pro-inflammatory cytokines (Fig. 4).

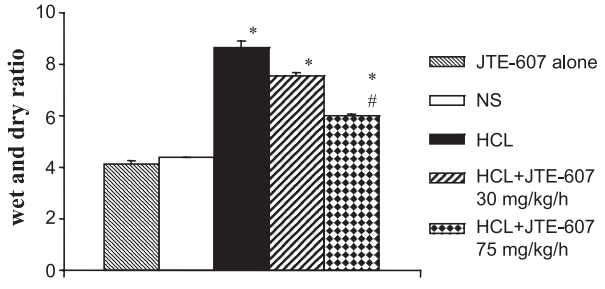

Fig. 5. Wet-to-dry weight ratio of lung of all groups. JTE-607 alone group, rats were instilled with normal saline (2 ml kg^{-1}) and treated with 75 mg kg^{-1} h^{-1} of JTE-607 ($n=8$); NS group, rats were instilled with normal saline and treated with normal saline treatment ($n=8$); HCl group, rats were instilled with hydrochloric acid (HCl, 2 ml kg^{-1}) and treated with normal saline ($n=8$); HCl + JTE-607 30 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 30 mg kg^{-1} h^{-1} ($n=8$); HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 75 mg kg^{-1} h^{-1} ($n=8$). Values are expressed as means ± S.E. *$P<0.05$ versus both JTE-607 alone and NS group; #$P<0.05$ versus HCl group.

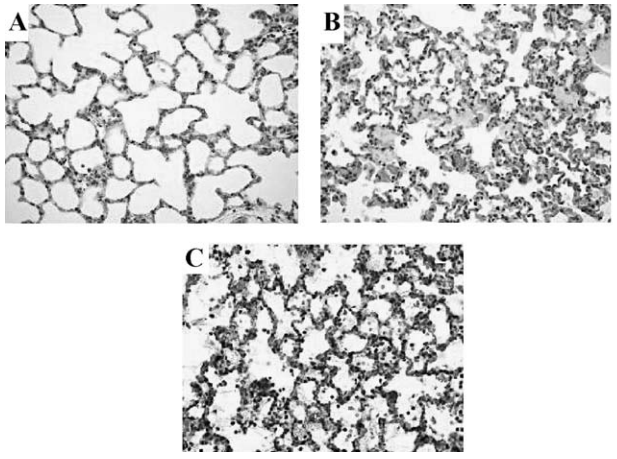

Fig. 6. Photomicrographs of the light microscopic appearance of lung tissue in the groups. (A) NS group. Rats were instilled with normal saline and treated with normal saline. (B) Hydrochloric acid (HCl) group. Rats were instilled with HCl and treated with normal saline. (C) HCl + JTE-607 75 mg kg^{-1} h^{-1} group. Rats were instilled with HCl and treated with JTE-607 at the dose of 75 mg kg^{-1} h^{-1}. Sections were stained with hematoxylin and eosin. The magnification was × 200. In the HCl group, congested alveolar walls, alveolar edema and hyaline membranes with alveolar wall disruption were observed. Numerous neutrophils and red blood cells were present in the alveolar spaces. In JTE-607 at the dose of 75 mg kg^{-1} h^{-1} groups, few neutrophils and red blood cells were present in air spaces. Alveolar edema and hyaline membranes were not observed and the integrity of alveolar walls was preserved.

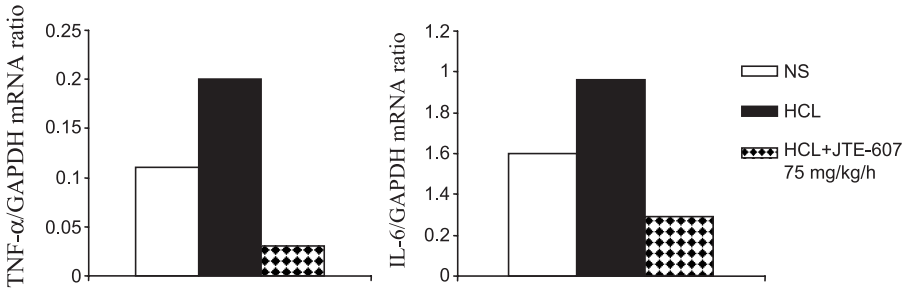

Fig. 7. Tumor necrosis factor-α/GAPDH and interleukin-6/GAPDH mRNA ratio in NS, HCl and HCl + JTE-607 75 mg kg^{-1} h^{-1} group. NS group, rats were instilled with normal saline and treated with normal saline; HCl group, rats were instilled with HCl and treated with normal saline; HCl + JTE-607 75 mg kg^{-1} h^{-1} group, rats were instilled with HCl and treated with JTE-607 at the dose of 75 mg kg^{-1} h^{-1}. Samples are the mix of each rat lung in the same group. Values are expressed as a ratio.

3.2. Wet-to-dry weight ratio

The wet-to-dry weight ratio in the lungs was calculated to assess pulmonary edema. The ratio significantly increased in rats receiving HCl. In the high-dose JTE-607 treatment group, the wet-to-dry weight ratio was significantly reduced (Fig. 5).

3.3. Histology

Microscopic examination of the rat lungs treated with HCl showed that the alveolar wall was thickened and damaged. There were erythrocytes and infiltration of neutrophils in the alveolar spaces; hyaline membrane formation was also found in HCl group. All these changes were attenuated by high-doses of JTE-607(Fig. 6).

3.4. mRNA of TNF-α and IL-6 quantification

The expression of tumor necrosis factor-α and interleukin-6 mRNA in the rat lung of each group as a ratio to that of GAPDH (tumor necrosis factor-α/GAPDH and interleukin-6/GAPDH mRNA ratio) is shown in Fig. 7. The tumor necrosis factor-α/GAPDH mRNA ratios in the JTE group was lower than those in the HCl group and the NS group. The interleukin-6/GAPDH mRNA ratio in the JTE group was lower than those in the HCl group and the NS group.

4. Discussion

The present study was designed to clarify the effects of JTE-607, a novel *N*-benzoyl-L-phenylalanine derivative compound, on acid-induced lung injury. Results of this study demonstrated that JTE-607 not only reduced the concentration of tumor necrosis factor-α, interleukin-6 and cytokine-induced neutrophil chemoattractant in bronchoalveolar lavage fluid but also decreased the expression of tumor necrosis factor-α and interleukin-6 mRNA in lung tissue increased by acid aspiration in rats. In addition, the agent significantly reduced the accumulation of neutrophils in lung tissue and pulmonary vascular permeability. These actions of JTE-607 resulted in the attenuation of lung injury induced by acid aspiration in rats. Our study is the first to report that JTE-607 attenuates acid-induced lung injury in an animal model.

This study showed that acid-aspiration induced a high concentration of tumor necrosis factor-α, interleukin-6 and cytokine-induced neutrophil chemoattractant in bronchoalveolar lavage fluid, as found in other studies (Davidson et al., 1999; Folkesson et al., 1995; Modelska et al., 1999). JTE-607 reduced the levels of these inflammatory cytokines increased by acid-aspiration in the present study. The attenuation of inflammatory cytokines by this agent was observed in other studies. In in vitro studies, JTE-607 could prevent the increase in the mRNA levels of inflammatory cytokines produced by lipopolysaccharide-stimulated human peripheral blood mononuclear cells (Kakutani et al., 1999). These authors also demonstrated that JTE-607 could reduce the release of these inflammatory cytokines in response to lipopolysaccharide from peripheral blood mononuclear cells in humans and other species. The increased levels of tumor necrosis factor-α, interleukin-6, interleukin-8, interleukin-1β and interleukin-10 induced by lipopolysaccharide were significantly suppressed by JTE-607 treatment. Subsequently, Iwamura et al. (2002) reported that JTE-607 suppressed cytokine-induced neutrophil chemoattractant synthesis by lipopolysaccharide-stimulated rat alveolar macrophages. Sasaki et al. (2003) reported in mice that macrophage inflammatory protein-2 levels and gene expression in pulmonary macrophages after lipopolysaccharide administration were attenuated by JTE-607. Similarly, in the present study we found that this agent inhibited lung expression of mRNA for tumor necrosis factor-α and interleukin-6 induced by HCl. Thus, it is likely that JTE-607 suppresses the mRNA levels of these cytokines in inflammatory cells in response to acid instillation.

Numerous studies have demonstrated that interactions between neutrophils and cytokines play a major role in the development of lung injury. Cytokine-induced neutrophil chemoattractant mediates neutrophil chemotaxis and activation in vitro and causes neutrophil influx into lungs in rat models of pulmonary inflammation (Baggiolini et al., 1994; Miller and Krangel, 1992). Tumor necrosis factor-α also

stimulates neutrophils to release toxic substances such as reactive oxygen species, which contribute to the development of lung injury (Berkow et al., 1987). Neutralization of interleukin-8 (Folkesson et al., 1995) or tumor necrosis factor-α (Davidson et al., 1999; Driscoll et al., 1997; Goldman et al., 1990) significantly decreases the magnitude of acid aspiration lung injury by inhibiting neutrophil accumulation in the lung. Furthermore, interleukin-6 participates in the recruitment of neutrophils into tissue sites by induction of interleukin-8 (Hierholzer et al., 1998; Romano et al., 1997). Thus, pro-inflammatory cytokines activate or promote neutrophil recruitment and the interaction leads to an inappropriate release of cytotoxic substances and to the subsequent release of inflammatory cytokines, resulting in tissue damage (Fan and Edgington, 1993; Cassatella, 1995; Chollet-Martin et al., 1996). In this study of acid lung injury, the interaction between neutrophils and cytokines remains unknown. JTE-607 reduced the number of neutrophils in bronchoalveolar lavage fluid induced by acid aspiration. To date, there are no reports about a direct interaction between JTE-607 and neutrophils. We speculate that JTE-607 prevents acid-induced lung injury primarily by inhibition of pro-inflammatory cytokines and secondarily by reduction of neutrophil accumulation. However, further studies are needed to clarify the role of JTE-607 on neutrophil behavior in the development of lung injury.

The IC_{50} value of JTE-607 for inhibiting cytokines from rat peripheral blood mononuclear cells is the highest among humans, monkeys, rabbits, and mice (Kakutani et al., 1999). In other words, the inhibitory potency of JTE-607 on cytokine synthesis in rats is lower than that in humans and other species. In the present study, both low and high doses of JTE-607 significantly reduced the levels of pro-inflammatory cytokines. In addition, the high-dose of JTE-607 (75 mg kg^{-1} h^{-1}) could attenuate the permeability edema induced by acid aspiration. This finding suggests that this dose is enough to prevent acid-induced lung injury in rats. Since the IC_{50} value of JTE-607 in human peripheral blood mononuclear cells is lower than in other species, this agent is a relatively selective cytokine synthesis inhibitor for humans. Further studies are necessary to find the optimal dose of this agent in various experimental models of acute inflammatory reactions.

Although JTE-607 could attenuate the production of inflammatory cytokines in bronchoalveolar lavage fluid induced by acid aspiration, the anti-inflammatory activity against interleukin-6 and cytokine-induced neutrophil chemoattractant appeared to be more potent than that against tumor necrosis factor-α. Kakutani et al. (1999) demonstrated that inhibition of the response of interleukin-6 and interleukin-8 to lipopolysaccharide in human peripheral blood mononuclear cells was observed with a minimum dose of JTE-607. The inhibitory activity for interleukin-6 was 1.5-fold higher than that for tumor necrosis factor-α. These findings, including our present result, suggested that JTE-607 acted as a cytokine inhibitor predominantly for interleukin-6 and interleukin-8. This agent might be more effective against interleukin-6 or interleukin-8 mediated inflammatory disease.

Inhibiting the bioactivity of one pro-inflammatory cytokine may lead to overexpression of another cytokine and cause adverse effects during inflammatory disease (Echtenacher et al., 1996). The inhibitory activity of JTE-607 on type 1 T helper cytokines (interleukin-2 and interferon-γ) is markedly less than that on tumor necrosis factor-α and interleukin-8 from lipopolysaccharide-stimulated peripheral blood mononuclear cells (Kakutani et al., 1999). These type 1 T helper cytokines are also important in the host defense (Huang et al., 1993; Klein et al., 1997). JTE-607 inhibited the pro-inflammatory cytokines without any influence on host immunity (Kakutani et al., 1999). This is different from other anti-inflammatory agents (Modelska et al., 1999; Van Wauwe et al., 1995). Thus, JTE-607 may be a specific anti-inflammatory drug for the treatment of inflammatory cytokine-mediated disease without inducing secondary infection.

In summary, we have shown that inflammatory cytokines participate in the pathogenesis of acid-induced acute lung injury in rats. The prevention of inflammatory cytokine secretion by JTE-607 could attenuate acid aspiration lung injury. Although JTE-607 did not completely prevent acid-induced lung injury, the present findings suggest that this agent as a useful inhibitor of cytokines in acid aspiration induced lung injury. Thus, JTE-607 is a novel and useful cytokine inhibitory agent to investigate cytokine-related inflammation. Further investigations using this agent are warranted to clarify the pathophysiology of inflammation related cytokines.

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