Preventive Effect of Tranilast on Oleic Acid-Induced Lung Injury in Guinea Pigs

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Abstract

Acute respiratory distress syndrome or acute lung injury (ARDS)/(ALI) involve the severe lung injury with pulmonary vascular hyper-permeability and hypoxemia induced by inflammatory reactions. Since ARDS/ALI carries high mortality, the development of new drugs against ARDS/ALI is required. We examined the effect of tranilast, an anti-allergic drug, on vascular hyper-permeability in the lungs and airways, and on hypoxemia, in oleic acid (OA)-induced acute lung injury, an animal model of ARDS/ALI. The increase in pulmonary and airway vascular permeability and the decrease in partial oxygen pressure of arterial blood induced by an intravenous injection of OA were drastically ameliorated by the oral administration of tranilast in a dose-dependent manner. This is the first report to prove that tranilast prevents pulmonary and airway vascular permeability and hypoxemia induced by OA. These results suggest that tranilast may be a candidate drug for the treatment of ARDS/ALI.

Key words tranilast; lung injury; pulmonary vascular hyper-permeability; hypoxemia; oleic acid

Acute respiratory distress syndrome (ARDS) or acute lung injury (ALI) are among the most severe forms of acute lung injuries with severe pulmonary edema and hypoxemia, which are seen in patients with sepsis, severe trauma, fat embolism and so on.1) The mortality of ARDS/ALI is still high (approximately 40%), despite recent advances in intensive care, because there are only a few drugs available for the prevention and treatment of ARDS/ALI.1,2) Therefore, the development of new available drugs against ARDS/ALI is very important.

In ARDS/ALI, non-cardiogenic edema develops as a result of pulmonary vascular hyper-permeability induced by inflammatory reactions. Recent studies have suggested that ARDS/ALI arises from multiple inflammatory mediators, such as cytokines, reactive oxygen species, proteases and eicosanoids, which are released from inflammatory cells (e.g. polymorphonuclear leukocytes (PMN)), possibly through complex mechanisms.3)

Tranilast (N-3,4-dimethoxycinnamoyl-anthranilic acid) has been used clinically to treat allergic diseases, such as bronchial asthma, allergic rhinitis, atopic dermatitis, and hypertrophic scarring and keloid formation. Tranilast seems to have multiple anti-inflammatory effects, through inhibiting the production or releasing cytokines (e.g. tumor necrosis factor-α, interleukin-1β, and -8) or prostanoids (thromboxane A2 and prostaglandin E2) from monocytes4) and superoxide5) from PMNs. Nagai et al.5) reported that tranilast ameliorated the pulmonary vascular hyper-permeability in lipopolysaccharide (LPS)-induced lung injury, one of the animal models of ARDS/ALI. Therefore, these anti-inflammatory effects of tranilast may work against ARDS/ALI caused by LPS. To extend the application of tranilast to the ARDS/ALI caused by factors other than LPS, it is necessary to examine the effects of tranilast on other animal models of acute lung injury.

The injection of oleic acid (OA), an endogenous unsaturated fatty acid, can produce lung injury with pulmonary vascular hyper-permeability and hypoxemia as well as ARDS/ALI. Since pathophysiological changes induced by OA are similar to those in patients with ARDS/ALI, OA-induced lung injury is known as one of the models of these diseases.7) OA-induced lung injury resembles specific forms of ARDS/ALI that follow pancreatitis,8) long bone fractures9) and meconium aspiration,10) all of which are thought to be caused by the toxicity of fatty acids. However, no research has been done to examine the effect of tranilast on lung injury induced by OA. The purpose of this study was to determine whether tranilast ameliorates pulmonary vascular hyper-permeability and hypoxemia induced by OA.

MATERIALS AND METHODS

Animals   Hartley strain guinea pigs (male, 650±85 g) were used. This study was approved by the Animal Care and Use Committee of Kumamoto University, and was performed in accordance with National Institutes of Health guidelines for the care and handling of animals. An operation was performed as described before.11)

Animal Treatment   Animals were divided randomly into the following groups: 1) tranilast (Kissei Pharmaceutical Co., Nagano, Japan), (100, 200 or 400 mg/kg)+OA (ICN Biochemicals Inc., Aurora, OH, U.S.A.) (15 μl/kg)-treated group (n=6), 2) 5% carboxymethyl cellulose (CMC) aqueous solution (2 ml/kg)+OA (15 μl/kg)-treated group (n=6) and, 3) 5% CMC (2 ml/kg)+saline (15 μl/kg)-treated group (n=6).

Determination of Pulmonary and Airway Vascular Permeability   Pulmonary and airway vascular permeability were measured with Evans blue dye as an extravasated serum albumin marker. All animals were anesthetized with pentobarbital sodium (Nembutal®, Dainabott Co., Osaka, Japan)
(25 mg/kg, i.p.), and procaine (Sigma, St. Louis, MO, U.S.A.) was employed for local anesthesia during the operation. A catheter (1.1 mm outer diameter) was inserted into the subclavian vein for the injection of OA and Evans blue (Sigma) (30 mg/kg), which was administered 1 min before the OA injection. Ninety minutes after the OA injection, the chest cavity was opened. Pulmonary intravascular Evans blue was washed out by perfusing saline. This procedure was accomplished by inserting a 13-gauge blunt cannula through the right ventricle into the pulmonary artery, and perfusate outflow came out from the dissected left atrium. The lungs were perfused with 100 ml of saline at a rate of 3 ml/min using a pump (EYELAMicro Tube Pump MP-3, Rikakikai Co., Tokyo, Japan). After the perfusion, the airways and lungs were removed and weighed. The airways were separated into the trachea, main bronchus, and intrapulmonary bronchus. The intrapulmonary bronchus was further cut in two in the middle and divided into the proximal bronchus and the distal bronchus. After measurement of the wet weight, whole right lungs were cut into several sections (about 1 cm thick). Evans blue dye was extracted in 20 ml (for the pieces from the whole right lungs) and 2 ml (for the airways) of 100% formamide solution (Sigma) at 37°C for 18 h, and its concentration was determined by light absorbance at 620 nm with a spectrophotometer (U 3200, Hitachi Ltd., Tokyo, Japan). Interpolation of the data was performed using a standard curve for absorbance from 100 ng/ml to 5 μg/ml. The amounts of Evans blue dye extravasated from the tissues were then expressed as ng/mg of wet weight of tissue.

**Measurement of Partial Oxygen Pressure of Arterial Blood** To examine the effect of tranilast on OA-induced hypoxemia, we measured the partial oxygen pressure of arterial blood (Pao2). Animals were anesthetized, a catheter (1.1 mm outer diameter) was inserted into the subclavian artery for blood sampling, and the other catheter was inserted into the subclavian vein for the injection of OA. Tranilast (200 or 400 mg/kg) or 5% CMC was administered orally 2 h before blood sampling, and the other catheter was inserted into the subclavian vein for the injection of OA. Tranilast (200 or 400 mg/kg) or 5% CMC was administered orally 2 h before the OA injection. Two hundred microliters of arterial blood was collected 5, 10 and 15 min before, and 6, 10, 15, 35, 55, and 75 min after the OA injection, and analyzed with a blood gas analyzer (ABL 300, RADIOMETER Ltd., Copenhagen, Denmark). The mean value of the Pao2 before the OA injection was defined as the value at 0 min.

**Statistical Analysis** Results were expressed as mean±S.E. Multiple comparisons were made to examine the statistical significance of the data. When uniform variance of data was identified by Bartlett’s analysis (p<0.05), one-way analysis of variance (ANOVA) was used to test for statistical differences. When significant differences (p<0.05) were identified, then the data were further analyzed by Dunnett’s multiple range test for significant differences between the tranilast group and the OA control group. The data were analyzed using Student’s unpaired t test to compare the OA control group with the saline control group.

**RESULTS**

**Changes in Pulmonary Vascular Permeability** An injection of OA significantly increased the vascular permeability in the lungs and airways, as indicated by the extravasation of Evans blue given intravenously (Figs. 1, 2). In addition, distinctive patches of intense hemorrhage with Evans blue on the surface of the lungs were observed. Tranilast ameliorated the OA-induced increase in vascular permeability in the lungs and airways. Two hundred and 400 mg/kg tranilast significantly prevented vascular hyperpermeability in the whole right lungs (Fig. 1), and 400 mg/kg tranilast significantly prevented that in the proximal and distal bronchus (Fig. 2).

**Changes in Pao2** As shown in Fig. 3, OA significantly decreased the Pao2. The maximum decrease in Pao2, approximately 45% of the value at 0 min, was observed 6 min after the OA injection. Seventy-five minutes after the OA injection, Pao2 was recovered to approximately 90% of the value at 0 min. Tranilast significantly prevented the decrease in Pao2 induced by OA, in a dose-dependent manner (Fig. 3).

**DISCUSSION**

An intravenous injection of 15 μl/kg OA caused a significant immediate decrease in Pao2 within 10 min after the injection. The decrease in Pao2 was recovered to nearly nor-
suppressed neutrophils, inhibited OA-induced lung injury.\(^\text{16}\) Pulmonary hyperpermeability in OA-induced lung injury is hypoxemia induced by OA injection.\(^\text{17,18}\) Tranilast can inhibit the superoxide production from activated PMNs, while the drug does not have a “scavenging effect”.\(^\text{9}\) Therefore, a probable explanation for the main mechanism of tranilast for the attenuation of the lung injury by OA seems to be the inhibition of superoxide production from inflammatory cells.

In addition, other inflammatory mediators such as proteases,\(^\text{14}\) eicosanoids,\(^\text{7}\) cytokines,\(^\text{15}\) from PMNs and macrophages are likely to play an important part in the development of OA-induced pulmonary and airway vascular hyper-permeability, as well as ARDS/ALI. Tranilast seems to have favorable pharmacological effects against inflammatory reactions, because the drug is shown to inhibit the release and/or production of inflammatory mediators, such as histamine, eicosanoids and cytokines from PMNs\(^\text{19}\) and monocytes.\(^\text{20}\) These effects may be involved in the attenuation of pulmonary vascular hyper-permeability by OA.

Tranilast itself did not have any effects on \(\text{Pao}_2\) and pulmonary permeability (data not shown). In terms of pulmonary vascular permeability, the effect of tranilast seems to have a ceiling effect, because there was not a significant difference between the effect of 200 mg/kg and that of 400 mg/kg. However, since there seems to be a dose dependence on the effect of tranilast on \(\text{Pao}_2\), tranilast may have some mechanism other than the prevention of hyperpermeability, such as the facilitation of HPV or pulmonary lymph flow. Further studies will be needed to prove the mechanisms of the inhibitory effect of tranilast.

Hypoxemia is a critical state which can result in brain damage and/or death, yet it is extremely difficult to improve the hypoxemia in patients with ARDS/ALI. Therefore, if tranilast has preventive effects against hypoxemia in the clinical area, it is intriguing as a candidate against ARDS/ALI. In this study, we proved that tranilast ameliorated the decrease in \(\text{Pao}_2\) induced by OA. In our previous reports, we described that one of the main mechanisms of the decrease in \(\text{Pao}_2\) in our system was an increase in pulmonary vascular permeability based on the effects of tranexamic acid, an inhibitor of plasmin,\(^\text{11}\) and carbazochrome, a drug for the treatment of hemorrhage,\(^\text{19}\) on OA-induced hypoxemia. Therefore, the preventive effect of tranilast on the decrease in \(\text{Pao}_2\) by the OA injection, at least in part, may be through inhibition of the increase in pulmonary vascular permeability.

In our preliminary experiments, 20 and 50 mg/kg of tranilast did not show any effects on OA lung injury. Clinically, tranilast is administered at a dose of around 2 mg/kg. Therefore, the dose used in this experiment (100—400 mg/kg) is much higher compared with the clinical dose. However, “species difference” should be taken into consideration, so we cannot directly extrapolate the doses in animal experiments to clinical doses. In addition, in some animal experiments, 200—400 mg/kg doses were used.\(^\text{5,12}\) So the dose used in our experiment does not seem to be ridiculously high.

In addition, there is an interesting report about the effect of
tranilast on a lung disease. Mori et al.\textsuperscript{20) reported that tranilast suppressed bleomycin-induced fibrosing alveolitis in mice, an animal model of pulmonary fibrosis. In the case of ARDS/ALI, pulmonary fibrosis, which tends to be seen around 1 week after the initiation of ARDS, can lead to a severe reduction of lung compliance, and can be fatal.\textsuperscript{3) It may be an attractive effect of tranilast against ARDS/ALI. Considering these pharmacological properties, tranilast is a noteworthy drug that could be a candidate for the prevention and treatment of ARDS/ALI. We hope that the results of this study will lead to novel therapeutic strategies to prevent or ameliorate ARDS/ALI.

REFERENCES