FR901451, A NOVEL INHIBITOR OF HUMAN LEUKOCYTE ELASTASE FROM *Flexibacter* sp.

II. PHARMACOLOGICAL EFFECT OF FR901451

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Intratracheal (i.t.) or intravenous (i.v.) administration of FR901451, a potent inhibitor of human leukocyte elastase (HLE) prevented HLE-induced lung hemorrhage in hamsters with ED_{50} values of 10.5 μg/site and 8.1 mg/kg, respectively. α1-Antitrypsin (α1-AT) also showed inhibitory effect in this model. However, the ED_{50} value by i.t. injection of FR901451was 20-fold lower than that of α1-AT. Moreover, FR901451 i.t. significantly modulated porcine pancreas elastase (PPE)-induced changes of the respiratory mechanics in hamsters. The ED_{50} values were 529 μg/site and 244 μg/site, which were expressed by static lung compliance (Cst) and vital capacity (VC) of the lungs, respectively. These results suggest that FR901451 could be clinically useful agent for the treatment of the destructive lung disease such as pulmonary emphysema.

FR901451, a product of *Flexibacter* sp. No.758 is a potent and competitive inhibitor of leukocyte elastase *in vitro*[1]. In order to investigate the *in vivo* effects, FR901451 was evaluated on animal models of elastase-induced lung injury. In addition, α1-AT, an endogenous inhibitor of leukocyte elastase was also evaluated in these models for comparison.

Materials

Human sputum elastase (HSE) and porcine pancreas elastase (PPE) were purchased from Elastin Products, Pacific, MO, USA. HSE was used as HLE without further purification[2]. Human α1-AT was purchased from Sigma Chemicals, St Louis, MO, USA. Archronium Chloride (Dialferin) was purchased from Japan Roche, Tokyo, Japan. Male golden Syrian hamsters, weighing approximately 120 g, were obtained from Japan SLC, Shizuoka, Japan. All parts used to construct a constant volume, whole-body plethysmograph were purchased from Nihon Kohden, Tokyo, Japan.

Methods

The lung hemorrhage was induced by i.t. injection of HLE (50 μg/site) by a minor modified method reported previously[3]. Briefly, hamsters were anesthetized by intraperitoneal injection of 40 mg/kg of pentobarbital. Saline or HLE was instilled i.t. *via* a small incision in the ventral neck region by using a syringe. The incisions were closed with surgical quick set adhesive. FR901451 or α1-AT in saline was administered i.t. at 5 minutes and i.v. at 3 minutes before HLE injection. Three hours after HLE injection, the animals were sacrificed by CO_2 asphyxiation. The trachea was exposed, and a 16 gauge needle inserted and held in place using surgical suture. The lungs were then lavaged with 2.5 ml saline in a syringe by gently expanding the lungs and then withdrawing the saline , yielding a final volume of approximately 1.5 ml bronchoalveolar lavage (BAL) fluid from each animal. 250 μl of the BAL was centrifuged at 3,000 rpm for 10 minutes. The supernatant was removed by aspiration, and 2 ml of distilled water was added to cause cell disruption, and centrifuged at 1,000 rpm for 5 minutes. Then, the supernatant was measured

spectrophotometrically at 541 nm with a spectrophotometer, and hemoglobin contents were expressed as OD at 541 nm.

The lung mechanics were measured by the system according to the methods of Koo *et al.*[4] with a minor modification. Briefly, hamsters were anesthetized intraperitoneally with pentobarbital. PPE (100 μg/site, i.t.) in 0.2 ml of saline was instilled through the oral cavity. FR901451 in 0.2 ml saline was administered i.t. at 5 minutes before PPE. Three weeks after PPE instillation, the hamsters were anesthetized with pentobarbital and respiratory mechanics of the animals were studied using a whole body, constant-volume, variable-pressure plethysmograph. A water-filled esophageal catheter was used to estimate pleural pressure. Quasi-static deflation pressure-volume (P-V) curve were obtained under paralysis by Dialferin. The inflation of the lungs to a transpulmonary pressure (PL) of 30 cmH_2O permitted slow deflation to a PL of 0 cmH_2O and gently aspilated to a PL of -20 cmH_2O. Quasi-static lung compliance (Cst) was defined as the slope of the steep portion of the deflation P-V curve in the mid-volume range. Vital capacity (VC) was defined as the difference in lung volume between total lung capacity (volume at PL = 25 cmH_2O) and residual volume (volume at PL = -20 cmH_2O).

Statics

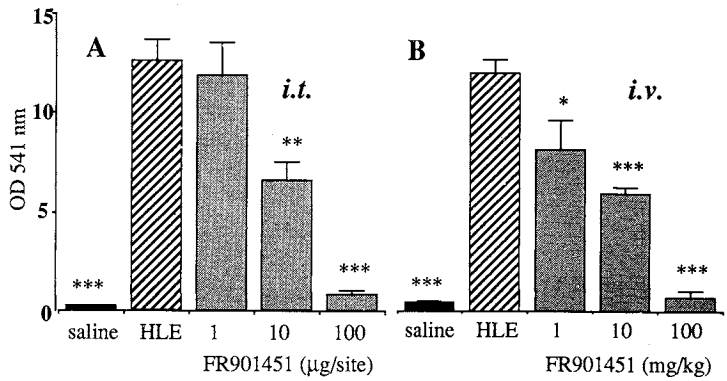
Data were analyzed by Student's t-test. P values were considered significant if $p<0.05$. ED_{50} values were calculated by Probit method.

Results

Effects of FR901451 and α1-AT on HLE-induced Hemorrhage

HLE at a dose of 50 μg/site i.t. induced a marked lung hemorrhage in hamsters at 3 hours after HLE. FR901451 i.t. at doses of 10 and 100 μg/site with 5 minutes prior to HLE injection significantly prevented the lung hemorrhage. The ED_{50} value was 10.5 μg/site (Fig. 1A). When FR901451 was administerd i.v. into the animals with 3 minutes prior to HLE injection, the compound significantly inhibited the lung hemorrhage with an ED_{50} value of 8.1 mg/kg (Fig. 1B). Human α1-AT (i.t.) also showed significant inhibitory effect in this model, however, the ED_{50} value (228 μg/site) was 20-fold higher than that of FR901451 (Fig. 2A). α1-AT i.v. did not show any inhibitory effect on the lung hemorrhage at dose of up to 100 mg/kg (Fig. 2B).

Fig. 1. Effect of FR901451 on HLE-induced lung hemorrhage in hamsters.



Fifty μg of HLE was instilled i.t. to anesthetized hamsters. Drug or saline was administered i.t. at 5 minutes and i.v. at 3 minutes before HLE injection. Three hours after HLE injection, the animals were sacrificed and BAL fluid were harvested, and the hemoglobin contents were measured. Results are expressed as the absorbance (mean ± SEM, OD at 541 nm) from n = 6 hamsters. Aristerisks indicate a significant difference from the vehicle/HLE level: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Fig. 2. Effect of α1-AT on HLE-induced lung hemorrhage in hamsters.

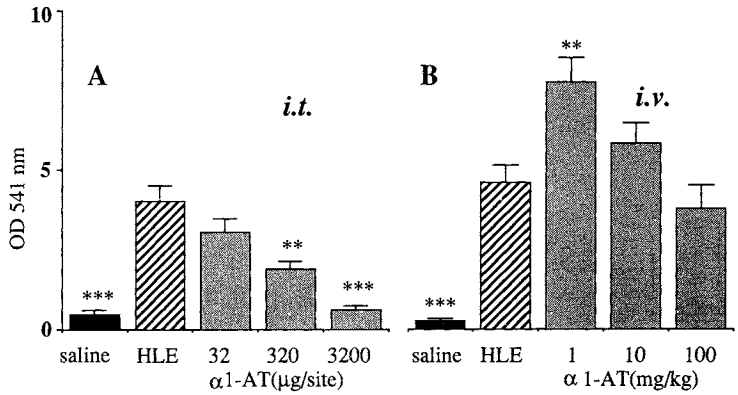
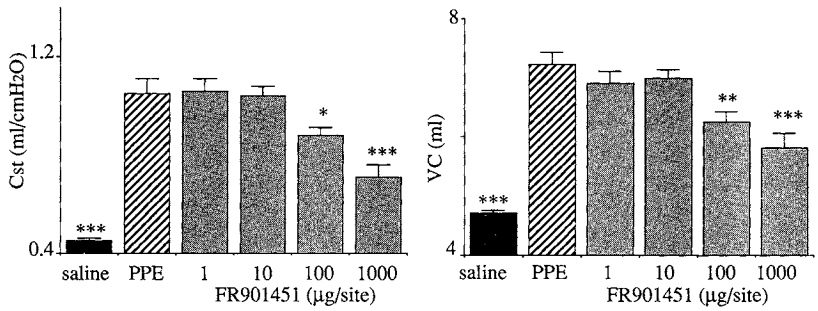
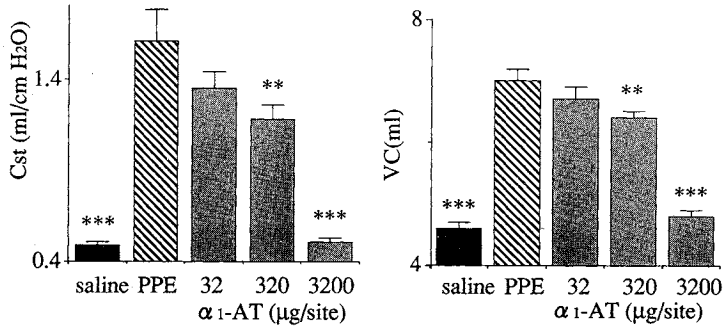


Fig. 3. Effect of FR901451 on elastase-induced emphysema.



A hundred μg of PPE was instilled i.t. to anesthetized hamsters. Three weeks after PPE injection, the respiratory mechanics of the hamsters were measured and Cst (ml/cmH_2O) and VC (ml) values were calculated. Results are expressed as the mean $\pm$ SEM from n = 8 hamsters. Aristerisks indicate a significant difference from the vehicle/PPE level: * $P<0.05$, ** $P<0.01$ and *** $P<0.001$.

Fig. 4. Effect of α1-AT on elastase-induced emphysema.



Effects of FR901451 and α1-AT on PPE-induced Emphysema

Empysematous changes of lung in hamster was induced by an i.t. injection of PPE. PPE (100 μg/site, i.t.) to anesthetized hamsters resulted in marked changes of respiratory mechanics at three weeks after PPE injection. The lung of PPE treated hamsters inflated largely and deflated steeply compared with saline treated hamsters' lung. These changes were expressed as increases in Cst and VC. FR901451 i.t. at 100

and 1,000 μg/site with 5 minutes before PPE significantly protected against PPE-induced emphysematous changes in both Cst and VC. The ED_{50} value which was expressed by Cst was 529 μg/site (Fig. 3). Likewise, α1-AT i.t. at doses of 320 and 3,200 μg/site also protected against emphysema in both Cst and VC (Fig. 4).

Discussion

In our previous report, we showed that FR901451 is a potent inhibitor for HLE and PPE *in vitro*[1]. To assess the *in vivo* effect of FR901451, the compound was evaluated in animal models of HLE-induced acute lung hemorrhage and PPE-induced pulmonary empysema. In hamsters, FR901451 i.t. prevented lung hemorrhage induced by i.t. instillation of HLE at very low doses, and the ED_{50} value was much lower than that of α1-AT. FR901451 i.v. was also active (ED_{50}; 8.1 mg/kg) in this model. However , α1-AT was not active at doses of up to 100 mg/kg.

Intratracheal instillation of elastase to animal also causes damage to the elastic fibers of the alveolar walls manifesting as airspace enlargement and increased static compliance. As these changes are similar to human emphysema, and this animal model has been commonly used as experimental pulmonary emphysema to investigate the pathogenesis of emphysema and to evaluate the effect of elastase inhibitors. The PPE-induecd emphysematous changes of respiratory mechanics was monitored by a simple constant volume, whole-body plethysmograph. In this animal model for emphysema, FR901451 i.t. significantly modulated the physiological changes of lung mechanics assessed by Cst and VC. α1-AT (i.t.) also showed inhibitory effect in this model. Although the ED_{50} value of both compounds on Cst change were similar, the value of α1-AT (924 μg/site) on VC change was higher than that of FR901451 (244 μg/site).

Extracellular HLE released from the leukocytes is normally inhibited by endogenous inhibitors such as α1-AT, so, its physiological action is restricted. Recently, it has been postulated that pulmonary emphysema occurs as a result of a local elastase-antielastase imbalance by oxidative inactivation or genetic deficiency of α1-AT[5~7]. α1-AT has been available in treating for patients with pulmonary emphysema due to α1-AT deficiency since 1988[8]. Potent and low molecular weight inhibitors of elastase such as FR901451 may have a number of potential advantages in the treatment of pulmonary emphysema over α1-AT replacement therapy. Moreover, FR901451 prevented several tissue damages induced by exogenously administered elastases, suggesting that FR901451 could be clinically useful agent in treating the destruvtive process in which leukocyte elastases are involved.

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