

Full Paper

Uridine 5'-Triphosphate Stimulates Alveolar Fluid Clearance in the Isolated Rat Lungs

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Abstract. Uridine 5'-triphosphate (UTP) increases chloride secretion followed by fluid movement into the proximal airspaces. However, little is known about whether UTP affects fluid movement in the distal airspaces. We studied the effect of UTP on basal and stimulated alveolar fluid clearance in the isolated rat lungs. Isosmotic 5% albumin solution was instilled into the alveolar spaces of isolated rat lungs, which were then inflated with 100% oxygen at an airway pressure of 7 cmH₂O. Alveolar fluid clearance was measured by the progressive increase in albumin concentrations over 1 h. Although UTP (10⁻⁹–10⁻⁶ M) did not increase alveolar fluid clearance, UTP (10⁻⁵–10⁻³ M) and isoproterenol (10⁻⁵ M), a β -adrenergic agonist, increased alveolar fluid clearance by 40% and 120% of the basal values, respectively. A combined treatment of UTP (10⁻⁴ M, 10⁻³ M) and isoproterenol increased alveolar fluid clearance by 280% of the basal value. The effects of UTP in the presence and absence of isoproterenol were abolished by blockers of a P2 purinoceptor and chloride channels. These results indicate that UTP stimulates alveolar fluid clearance in the distal airspaces of rat lungs.

Keywords: β -adrenergic agonist, purinoceptor, chloride channel, alveolar epithelium

Introduction

The mechanisms responsible for alveolar fluid clearance have been studied over the past two decades (1). The initial step in alveolar fluid clearance is to move alveolar sodium ions through apical sodium channels and basolateral Na⁺-K⁺ ATPase on the alveolar epithelia (2, 3). Osmotic gradients created by these transported ions drive alveolar fluid from the alveolar spaces (4) and may result in the resolution of alveolar edema (5).

β -Adrenergic agonists stimulate alveolar fluid clearance in the normal and pathological lungs (1). The prevailing idea was that activation of adrenergic-receptors increases the open probability of sodium channels, leading to an increase in apical membrane sodium permeability and an increase in sodium and fluid uptake from the alveolar space (6). Recent studies indicated that the effect of β -adrenergic agonists was primarily mediated by chloride ion transport (7). A series of complementary approaches using wild-type

and cystic fibrosis Δ F508 mice, as well as in the isolated human lungs, defined the role of Cl⁻ transport in fluid clearance in the distal airspaces of intact mouse and human lungs (8). However, little is known about the identity and role of chloride channels in alveolar fluid clearance (9).

Extracellular uridine 5'-triphosphate (UTP) is an agonist of the P2Y₂ receptor that is found in type II alveolar epithelial cells (10–12). UTP has several effects on the proximal airway epithelia. For example, UTP was effective in vivo chloride secretagogues in the nasal epithelia of patients with cystic fibrosis (CF) (13). UTP inhibited sodium transport in non-CF and CF airways in human upper airway epithelial cells (14). Luminal UTP stimulates Cl⁻ secretion by a Ca²⁺-independent mechanism and inhibits Na⁺ absorption by a Ca²⁺-dependent mechanism in intact distal bronchi isolated from porcine lungs (15). Therefore it was expected that UTP might increase chloride secretion in combination with the decrease in sodium absorption and then resulted in the accumulation of fluid in the airways (13). If UTP has these effects in the distal

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airspaces, UTP would inhibit alveolar fluid clearance and deteriorate the resolution of alveolar edema.

Therefore, our first objective was to determine if UTP has effect on basal alveolar fluid clearance in the isolated rat lungs. Our second objective was to determine if UTP has effect on isoproterenol, a β -adrenergic agonist, to stimulate alveolar fluid clearance in the isolated rat lungs. Our third objective was to determine the mechanisms responsible for the effects of UTP on basal alveolar fluid clearance and isoproterenol to stimulate alveolar fluid clearance.

Materials and Methods

Materials

UTP was obtained from Kirin Co., Ltd. (Tokyo). Glibenclamide (a blocker of cystic fibrosis transmembrane conductance regulator CFTR), isoproterenol (a β -adrenergic agonist), 5-nitro-2-(3-phenylpropyl-amino) benzoate (NPPB, a non-selective chloride channel antagonist), propranolol (a β -adrenergic antagonist), and suramin (a non-selective P2 purinergic antagonist) were obtained from Sigma (St. Louis, MO, USA).

Experimental protocol

All rats received humane care and this study was approved by the Committee for Animal Experiments at Kanazawa Medical University. Alveolar fluid clearance was measured in the isolated rat lungs in the absence of pulmonary perfusion or ventilation (16, 17). Briefly, male Sprague-Dawley rats (200–250 g; Japan SLC, Inc., Hamamatsu) were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg). An endotracheal tube was inserted through a tracheostomy. The rats were exsanguinated via the abdominal aorta and the trachea, bilateral lungs, and heart were excised en bloc through a median sternotomy. Warmed isotonic saline solution (7 ml/kg, 37°C) containing 5% bovine albumin was instilled into both lungs, followed by 4 ml oxygen to deliver all the instilled fluid into the alveolar spaces. The lungs were placed in a humid incubator at 37°C and inflated with 100% oxygen at an airway pressure of 7 cmH₂O. Alveolar fluid was aspirated 1 h after instillation.

Effect of UTP on basal alveolar fluid clearance: To determine if UTP changed basal alveolar fluid clearance, albumin solution containing UTP (10^{-9} M, n = 4; 10^{-8} M, n = 4; 10^{-7} M, n = 4; 10^{-6} M, n = 4; 10^{-5} M, n = 6; 10^{-4} M, n = 6; 10^{-3} M, n = 6) was instilled into the alveolar spaces of isolated rat lungs. Since UTP increased basal alveolar fluid clearance, we examined if the effect of UTP on basal alveolar fluid clearance was mediated via a P2 purinoceptor or chloride channels. Suramin

(10^{-3} M, n = 4), NPPB (10^{-4} M, n = 4), or glibenclamide (10^{-3} M, n = 4) was added to albumin solution in the presence of UTP (10^{-4} M) and instilled into the distal airspaces of rat lungs. As a control, albumin solution was instilled into the alveolar spaces of isolated rat lungs (n = 16). In addition, we examined if basal alveolar fluid clearance was mediated via a P2Y₂ purinoceptor or chloride channels. Suramin (10^{-3} M, n = 4), NPPB (10^{-4} M, n = 4), or glibenclamide (10^{-3} M, n = 4) was added to albumin solution and instilled into the alveolar spaces of rat lungs. Samples of alveolar fluid were collected 1 h after instillation.

Effect of UTP on alveolar fluid clearance in the presence of isoproterenol: We previously reported that 10^{-5} isoproterenol increased alveolar fluid clearance in the isolated rat lungs and ICI-118,551, a selective β_2 -adrenergic antagonist, inhibited the effect of isoproterenol to stimulate alveolar fluid clearance (18). Therefore, we determined if UTP changed the effect of isoproterenol to stimulate alveolar fluid clearance. Albumin solution containing isoproterenol (10^{-5} M) and UTP (10^{-7} M, n = 4; 10^{-6} M, n = 4; 10^{-5} M, n = 6; 10^{-4} M, n = 6; 10^{-3} M, n = 12) was instilled into the alveolar spaces of isolated rat lungs. As a control, albumin solution containing isoproterenol (10^{-5} M, n = 12) was instilled into the alveolar spaces of isolated rat lungs. Samples of alveolar fluid were collected 1 h after instillation.

Effect of suramin, NPPB, glibenclamide, and propranolol on alveolar fluid clearance in the presence of isoproterenol: We examined if the effect of isoproterenol on alveolar fluid clearance was mediated through a P2Y₂ purinoceptor and chloride channels. Suramin (10^{-3} M, n = 4), NPPB (10^{-4} M, n = 4), or glibenclamide (10^{-3} M, n = 4) was added to albumin solution in the presence of isoproterenol (10^{-5} M) and instilled into the distal airspaces of rat lungs. In addition, to examine if the effect of isoproterenol was mediated through β -adrenoceptors, propranolol (10^{-4} M) was added to albumin solution in the presence of isoproterenol (10^{-5} M) and instilled into the distal airspaces of rat lungs (n = 4). Samples of alveolar fluid were collected 1 h after instillation.

Mechanisms responsible for the effect of UTP to enhance alveolar fluid clearance in the presence of isoproterenol: Since UTP enhanced the effect of UTP to stimulate alveolar fluid clearance, we examined if the effect of UTP on isoproterenol to stimulate alveolar fluid clearance was mediated via a P2Y₂ purinoceptor, a non-selective chloride channel, or CFTR. Suramin (10^{-3} M, n = 4), NPPB (10^{-4} M, n = 4), or glibenclamide (10^{-3} M, n = 4) was added to albumin solution in the presence of UTP (10^{-4} M) and isoproterenol (10^{-5} M),

and instilled into the distal airspaces of rat lungs. Samples of alveolar fluid were collected 1 h after instillation.

Measurements

Alveolar fluid clearance: The protein concentrations in the instilled and aspirated solutions were measured by the pyrogallol red protein dye-binding method (SRL, Inc., Tokyo). Alveolar fluid clearance was estimated by the progressive increase in the concentration of albumin (16, 17). Alveolar fluid clearance (AFC) was calculated as follows:

$$\text{AFC} = [(V_i - V_f) / V_i] \times 100$$

where V is the volume of the instilled albumin solution (i) and the final alveolar fluid (f).

$$V_f = (V_i \times P_i) / P_f$$

where P is the concentration of protein in the instilled albumin solution (i) and the final alveolar fluid (f). The term alveolar does not imply that all reabsorption occurs across the alveolar epithelial cells because the distal bronchial epithelia can also transport sodium.

Osmolality: Osmolality in albumin solution was measured by a freezing point depression method using an osmometer (Fiske One-Ten Osmometer; Fiske Associates, Norwood, MA, USA).

Statistics

Data are summarized as the mean and standard deviation. The data were analyzed by a one-way analysis of variance (ANOVA) with the Student-Newman-Keuls post hoc test when multiple comparisons were needed. Differences with a *P* value of <0.05 were regarded as significant.

Results

An addition of 10^{-3} M UTP to albumin solution did not change osmolality levels (296 ± 4 mOsm/kgH₂O in the solution containing 10^{-3} M UTP and 295 ± 2 mOsm/kgH₂O in the control solution).

Basal alveolar fluid clearance was $6.9 \pm 2.2\%$ of the instilled volume in the isolated rat lungs. UTP (10^{-5} – 10^{-3} M) increased alveolar fluid clearance to approximately 1.4-fold the basal value, although UTP (10^{-9} – 10^{-6} M) did not (Fig. 1). Suramin, NPPB, and glibenclamide did not change basal alveolar fluid clearance (Fig. 2). However, these agents abolished the effect of UTP to stimulate basal alveolar fluid clearance.

Isoproterenol (10^{-5} M) increased alveolar fluid clearance to 2.2-fold the basal value (Fig. 3). An additional treatment of UTP ranging from 10^{-5} to 10^{-3} M increased alveolar fluid clearance in a dose-dependent fashion. A combined treatment of UTP (10^{-4} M, 10^{-3} M) and

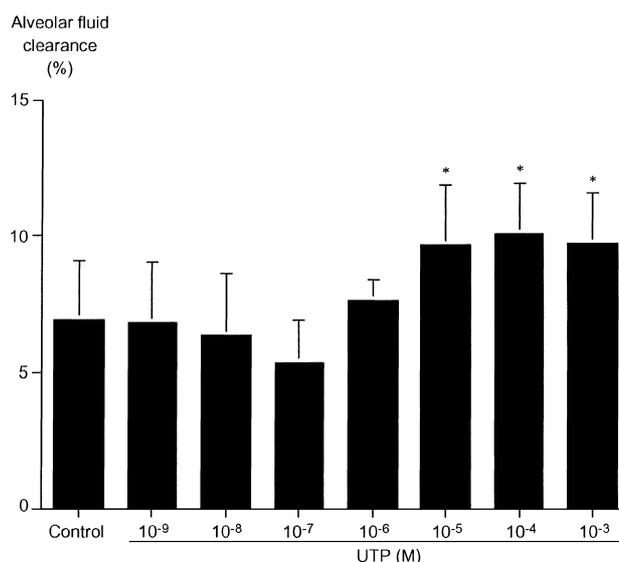


Fig. 1. Dose-dependent effect of UTP on basal alveolar fluid clearance. **P*<0.05 vs control value.

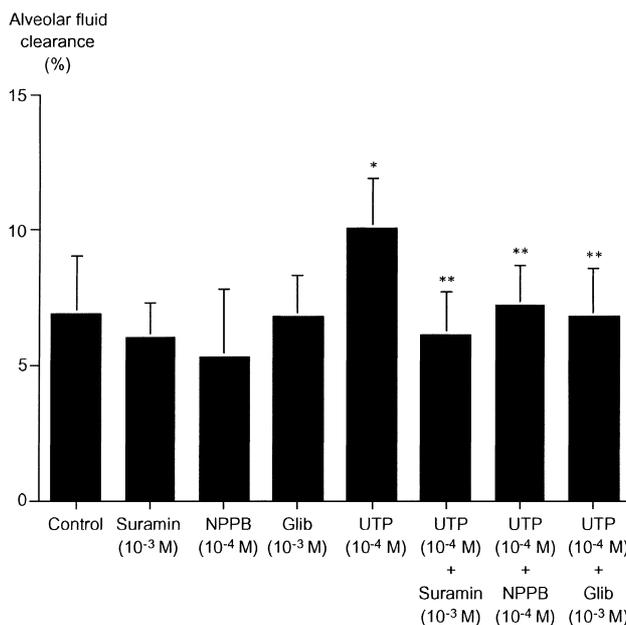


Fig. 2. Mechanisms responsible for the effect of UTP to stimulate basal alveolar fluid clearance. **P*<0.05 vs control value. ***P*<0.05 vs value in the presence of UTP. Glib: glibenclamide.

isoproterenol increased alveolar fluid clearance to 3.8-fold basal value.

Suramin, NPPB, and glibenclamide had no effect on isoproterenol to stimulate alveolar fluid clearance (Fig. 4). Propranolol abolished the effect of isoproterenol to stimulate alveolar fluid clearance.

Suramin, NPPB, and glibenclamide inhibited the

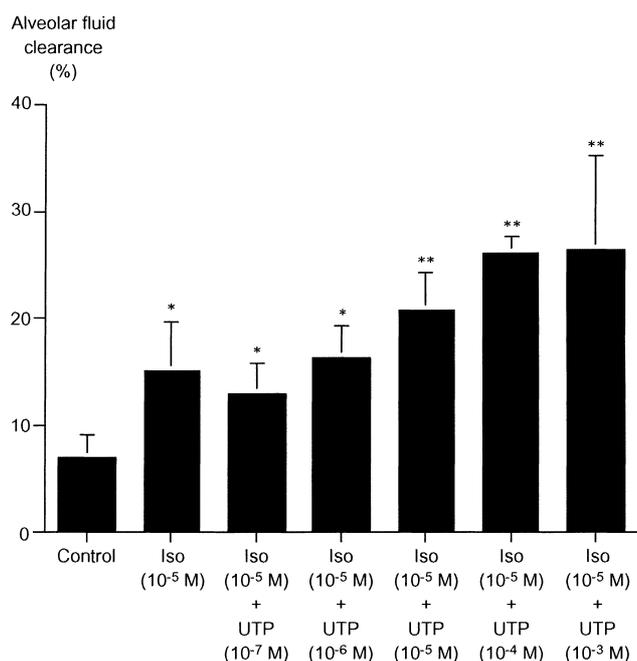


Fig. 3. Dose-dependent effect of UTP on isoproterenol to stimulate alveolar fluid clearance. * $P < 0.05$ vs control value. ** $P < 0.05$ vs value in the presence of isoproterenol. Iso: isoproterenol.

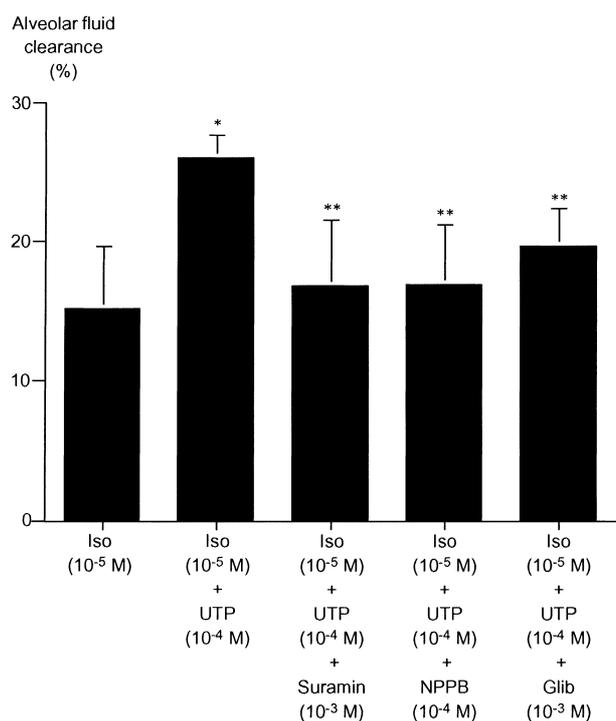


Fig. 5. Mechanisms responsible for the effect of UTP to stimulate alveolar fluid clearance. * $P < 0.05$ vs value in the presence of isoproterenol. ** $P < 0.05$ vs value in the presence of isoproterenol and UTP. Iso: isoproterenol, Glib: glibenclamide.

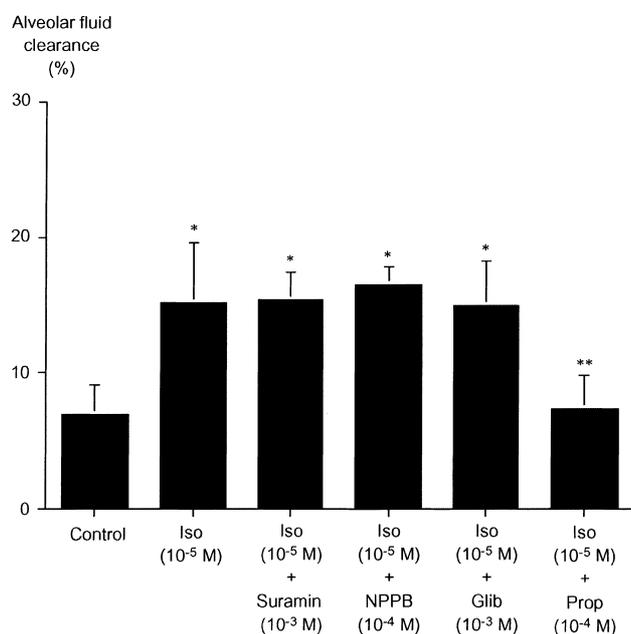


Fig. 4. Effect of suramin, NPPB, glibenclamide, and propranolol on isoproterenol to stimulate alveolar fluid clearance. * $P < 0.05$ vs control value. ** $P < 0.05$ vs value in the presence of isoproterenol. Iso: isoproterenol, Glib: glibenclamide, Prop: propranolol.

effect of UTP to stimulate alveolar fluid clearance in the presence of isoproterenol (Fig. 5).

Discussion

UTP increased basal alveolar fluid clearance and also enhanced the effect of isoproterenol to stimulate alveolar fluid clearance in rat lungs. To determine the mechanisms responsible for these findings, we tested three hypotheses. First, we tested whether the effect of UTP was mediated via a P2 purinoceptor. Suramin, a non-selective P2 purinoceptor antagonist, was administered in combination with UTP and/or isoproterenol. Although suramin had no effect on isoproterenol to stimulate alveolar fluid clearance, suramin abolished the effect of UTP in the presence and absence of isoproterenol. Therefore, the effect of UTP was mediated via a P2 purinoceptor. Second, we tested if the effect of UTP to stimulate alveolar fluid clearance was mediated via chloride channels. The results revealed that NPPB did not inhibit either basal alveolar fluid clearance or the effect of isoproterenol on alveolar fluid clearance, but inhibited the effect of UTP to stimulate basal alveolar fluid clearance. In addition, NPPB abolished the effect of UTP on isoproterenol to stimulate alveolar fluid clearance. NPPB is a potent inhibitor of chloride channels, but a non-selective inhibitor (7). Therefore, we tested the effect of glibenclamide that is a more

specific inhibitor of chloride ion transport via CFTR (8). Glibenclamide had the similar effects as NPPB. Therefore, it is likely that the effect of UTP was mediated via a glibenclamide-sensitive chloride channel.

Previously it was reported that there was a cumulative effect of keratinocyte growth factor and β -adrenergic agonist on alveolar fluid clearance (19). In contrast, when alveolar fluid clearance was increased by catecholamine-independent mechanisms, an additive effect with β -adrenergic therapy was not achieved (20). In the present study, there was a cumulative effect of UTP and isoproterenol in alveolar fluid clearance. NPPB abolished the effect of UTP on isoproterenol to stimulate alveolar fluid clearance. In addition, glibenclamide, a selective blocker of CFTR abolished the effect of UTP on isoproterenol to stimulate alveolar fluid clearance. Therefore, it is likely that chloride channels play a role in the cumulative effect of UTP in the presence of isoproterenol.

The effect of UTP on alveolar epithelial cells may be different from that on bronchial epithelial cells. In bronchial airways, the increase of intracellular Ca^{2+} stimulate chloride secretion into the luminal side in the bronchus (21). Epinephrine, a β -adrenergic agonist, increased bronchial cell membrane permeability to chloride and probably stimulated a specific chloride pump (22). β -Agonists stimulate chloride ion secretion in canine airway epithelial cells, suggesting that β -agonists increase water secretion into the airways (23). In contrast, in distal airways, chloride movement via CFTR plays an important role in isoproterenol- and terbutaline-stimulated alveolar fluid clearance (8). An adrenergic stimulation of transepithelial sodium absorption across the alveolar epithelium occurs indirectly by activation of apical chloride channels, resulting in hyperpolarization and an increased driving force for sodium uptake through amiloride-sensitive sodium channels (6). These reports support our results.

The results in this study are inconsistent with the previous findings from other laboratories. First, glibenclamide did not inhibit the effect of isoproterenol in this study. However, glibenclamide inhibited the effect of isoproterenol and terbutaline in mice and human lungs, respectively (8). Since the rate of alveolar fluid clearance in rat was lower than that in mouse, it is possible that the low rate of alveolar fluid clearance has contributed to the absence of the effect of glibenclamide on alveolar fluid clearance in the presence of isoproterenol. In the human lung study, the lungs were exposed to severe hypothermia and thereafter rewarmed before the measurements (8). Therefore, the difference in experimental preparation might have induced the discrepancy between the effects of glibenclamide in the

rat and human lungs. Second, Davis et al. reported that UTP decreased alveolar fluid clearance in mice (24). Although it is unclear how the difference was induced, their results seem to be consistent with the effect of UTP on the proximal airways. Further studies are needed to clarify whether there is a species difference in the effect of UTP on alveolar fluid clearance and in the effect of glibenclamide on alveolar fluid clearance in the presence of β -adrenergic agonist. Especially, the effect of UTP on alveolar fluid clearance should be examined in the human lungs.

Was the effect of UTP taking place in the proximal airways? First, the instilled albumin solution was delivered into the distal airways by the following injection of 4 ml of oxygen. Second, Evans blue dye bound with albumin revealed that the instilled solution was delivered into the distal airspaces in the rat lungs (16). In addition, protein concentration in liquid aspirated with a catheter wedged from the distal air spaces is a good reflection of alveolar fluid protein concentration (25). Third, if the instilled solution remained in the proximal airways, UTP should decrease alveolar fluid clearance, because luminal UTP stimulated Cl^- secretion and inhibits Na^+ absorption from porcine lungs (15). However, the results were the opposite. Therefore, it is unlikely that the effect of UTP took place in the proximal airways.

In summary, UTP increased basic alveolar fluid clearance and enhanced the effect of isoproterenol to stimulate alveolar fluid clearance in isolated rat lungs. The effect of UTP was inhibited with a blocker of P2 purinoceptor and by blockers of chloride channels. These results indicate that UTP increases net alveolar fluid clearance in the distal air spaces.

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