Chronic pulmonary artery occlusion increases alveolar fluid clearance in rats

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Objective: We had observed that pulmonary artery ligation for 14 days did not induce lung infiltration in a patient who had undergone a lobectomy for lung cancer. Our hypothesis was that long-term pulmonary artery ligation decreased lung water volume and/or increased alveolar fluid clearance. We determined the mechanism responsible for lung water balance in rats with chronic pulmonary artery occlusion for 14 days.

Methods: Sprague–Dawley rats ($n = 45$) were used. Through a left thoracotomy, the left pulmonary artery was ligated for 14 days. Then, we measured lung water volume, alveolar fluid clearance, the effects of β -adrenergic agonist and antagonist, mRNA expression, and protein expression in the lungs.

Results: Chronic left pulmonary artery occlusion increased both lung water volume and alveolar fluid clearance in the left lungs, but not in the right lungs with pulmonary perfusion. Neither a β -agonist nor a β -antagonist changed the increase in alveolar fluid clearance. Real-time polymerase chain reaction revealed an increase in α_1 -Na,K-ATPase mRNA and a decrease of β_2 -adrenoreceptor mRNA, but no change in β_1 -Na,K-ATPase mRNA and α -, β -, γ -epithelial sodium channel mRNA, in the left lung without pulmonary perfusion. Western blot analysis revealed an increase in α_1 -Na,K-ATPase subunit, but no change in β_1 -Na,K-ATPase subunit.

Conclusion: Chronic pulmonary artery occlusion increases alveolar fluid clearance via α_1 -Na,K-ATPase overexpression in rats.

Surgery is the most common form of treatment for non–small cell lung cancer. However, there are some complications during surgery. While silicotic hilar lymph nodes (11R inferior) were dissected from the pulmonary artery, we had observed an unexpected hemorrhage from a distal pulmonary artery that was thereafter ligated at the base of the branches to stop the hemorrhage. Fortunately, no abnormal infiltration such as pulmonary edema was found in the lung lobe with pulmonary artery ligation by a chest x-ray film and a computed tomographic scan 14 days after the operation. However, questions remained regarding lung water balance, especially whether lung water volume decreased or whether alveolar fluid clearance increased.

The amount of alveolar fluid volume is determined by the balance between formation and clearance of alveolar fluid.¹ If the formation exceeds the clearance of

Abbreviations and Acronyms

ATPase	= adenosine triphosphatase
cDNA	= complementary deoxyribonucleic acid
DNA	= deoxyribonucleic acid
ENaC	= epithelial sodium channel
GAPDH	= glyceraldehyde-3-phosphate dehydrogenase
Na,K-ATPase	= sodium-potassium-adenotriphosphatase
PCR	= polymerase chain reaction
RNA	= ribonucleic acid

alveolar fluid, as in cases of hydrostatic pulmonary edema and acute lung injury, the amount of alveolar edema fluid increases and oxygen exchange deteriorates across the alveolar epithelial barrier.² The mechanisms responsible for alveolar fluid clearance have been studied in the past 2 decades.³ Alveolar epithelial type I and II cells drive Na⁺ from the alveolar spaces through the apical epithelial sodium channel (ENaC) and basolateral sodium-potassium-adenotriphosphatase (Na,K-ATPase).⁴ Ion gradients generated with transported ions drive fluid out of the alveolar spaces.¹ Inasmuch as stimulation of β -adrenergic receptors augmented the rate of alveolar fluid clearance and accelerated the resolution of pulmonary edema,^{5,6} some β_2 -adrenergic agonists have been considered to be an effective medicine for patients with pulmonary edema.⁷

The impairment of pulmonary blood flow sometimes occurs in patients with pulmonary edema and acute respiratory distress syndrome; therefore, the effect of pulmonary perfusion on alveolar fluid clearance has been studied in *in vivo* and *ex vivo* models. Acute pulmonary ischemia did not affect alveolar fluid clearance in sheep.^{8,9} Although the rate of alveolar fluid clearance is impaired in the isolated lungs than as *in vivo* lungs,¹⁰ alveolar fluid clearance continued in the isolated lungs without any pulmonary perfusion in several species including human lungs.^{6,10,11} Interestingly, β -adrenergic agonists increase alveolar fluid clearance in these *ex vivo* and *in vivo* lungs.¹⁰ However, the duration of pulmonary ischemia was no longer than 8 hours in those studies. Chronic pulmonary artery occlusion occurs in several circumstances in clinical practice. For example, pulmonary artery obstruction can occur as a result of anastomotic stenosis, kinking, or extrinsic compression in a recipient of lung transplantation. Branches of the pulmonary artery are often obstructed by the invasion of lung cancer. Postoperative pulmonary thromboembolism obstructs the stem and branches of pulmonary arteries. It is uncertain whether chronic pulmonary artery occlusion affects the rate of alveolar fluid clearance.

The first objective in the current study was to determine whether chronic pulmonary artery occlusion affected lung

water volume and alveolar fluid clearance. Inasmuch as alveolar fluid clearance increased in the rat lungs with chronic pulmonary artery occlusion, the second objective was to determine the mechanism responsible for the increase in alveolar fluid clearance. The third objective was to determine whether a β_2 -adrenergic agonist increased alveolar fluid clearance in the rat lungs with chronic pulmonary artery occlusion.

Materials and Methods

Propranolol (a nonselective β -adrenergic antagonist) and terbutaline (a β_2 -adrenergic agonist) were obtained from Sigma Chemical Company (St Louis, Mo). RNA probes (α_1 -, β_1 -Na,K-ATPase, α -, β -, γ -ENaC, β_2 -adrenoreceptor) and antibodies (α_1 -, β_1 -Na,K-ATPase) were obtained from Applied Biosystems (Foster, Calif).

General Protocol

This study was approved by the Committee on Animal Experiments at Kanazawa Medical University. Specific pathogen-free male Sprague-Dawley rats (250–300 g, Japan SLC Inc, Hamamatsu, Japan) received humane care in compliance with guidelines from the University Committee on Animal Resources. Rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg). The rats were orally intubated with Surflo Teflon IV catheters (18G; Terumo, Tokyo, Japan), placed in the right lateral decubitus position, and ventilated with an animal respirator (Harvard Apparatus, Dover, Mass) with 100% oxygen at a peak airway pressure of 7 cm H₂O combined with a positive end-expiratory pressure of 2 cm H₂O. The tidal volume and respiratory rate were 2.0 mL and 70 cycles/min, respectively. Body temperature was maintained at 37°C $\pm$ 1°C. Through the fourth intercostal space, a left thoracotomy was performed. The apex of the left lung was pressed with a cotton pad gently toward the diaphragm, and the left pulmonary artery was separated from the left main bronchus gently and then ligated with a 3–0 silk suture. The lung was inflated and the thoracotomy incision was closed.

After the operation, the rats were awakened from the anesthesia and allowed free access to rat chow and water *ad libitum* for 14 days. After left pulmonary artery occlusion for 14 days, alveolar fluid clearance was measured in the isolated rat lungs.^{12–14} In brief, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (50 mg/kg) and exsanguinated via the abdominal aorta. Blood samples were obtained for the measurement of plasma catecholamine levels. The trachea, bilateral lungs, and heart were excised en bloc through a median sternotomy. Isotonic saline solution (37°C) containing 5% bovine albumin was instilled separately into the left lung (6 mL/kg) and right lung (8 mL/kg). Because the right lung is larger than the left lung, a larger volume of albumin solution was instilled into the right lungs. Neither instilled volume (ranging from 6–8 mL/kg) nor oxygen concentration used to inflate lungs had an effect on alveolar fluid clearance.^{11–14} The lungs were placed in a humid incubator at 37°C and inflated with 100% oxygen at an airway pressure of 7 cm H₂O over 1 hour. Alveolar fluid was aspirated from the right lung and then from the left lung separately 1 hour after instillation. Protein concentrations in the instilled and aspirated alveolar fluid samples

were measured with the pyrogallol red protein dye-binding method.¹³

Specific Protocol

Group 1. The effect of left pulmonary artery occlusion on lung water volume. Lung water volume was estimated by the ratio of lung water to dry lung weight. The lung water/dry lung weight ratio was measured in rats with left pulmonary artery occlusion for 14 days ($n = 6$). As a control, the lung water/dry lung weight ratio was measured in rats that underwent left thoracotomy without left pulmonary artery occlusion 14 days before the measurement ($n = 4$).

Group 2. The effect of left pulmonary artery occlusion on alveolar fluid clearance. Alveolar fluid clearance was measured in rats with left pulmonary artery occlusion for 14 days ($n = 7$). As a sham control, alveolar fluid clearance was measured in rats that underwent left thoracotomy without left pulmonary artery occlusion 14 days before the measurement ($n = 4$).

Group 3. The effects of propranolol and terbutaline on alveolar fluid clearance in rats with left pulmonary artery occlusion. Inasmuch as alveolar fluid clearance increased in the left lungs in rats with pulmonary artery occlusion, we determined whether endogenous catecholamine played a role in the increase in alveolar fluid clearance. To inhibit the effect of endogenous catecholamine, we instilled an isotonic 5% albumin solution containing propranolol (10^{-4} mol/L) separately into the right and left lungs from rats that underwent left pulmonary artery occlusion for 14 days ($n = 4$). Because propranolol did not inhibit the increase in alveolar fluid clearance in rats with pulmonary artery occlusion for 14 days, we determined whether chronic pulmonary artery occlusion preserved the effect of terbutaline, a β_2 -adrenergic agonist, on alveolar fluid clearance. An isotonic 5% albumin solution containing terbutaline (10^{-6} mol/L) was instilled separately into the right and left lungs from rats that underwent left pulmonary artery occlusion for 14 days ($n = 5$). As a control, an isotonic 5% albumin solution was instilled into the individual lungs from rats that underwent sham left thoracotomy without left pulmonary artery occlusion 14 days before the measurement ($n = 4$).

Group 4. Real-time polymerase chain reaction and Western blot analysis. Because chronic pulmonary artery occlusion increased alveolar fluid clearance, we determined the mechanisms responsible for the increase in alveolar fluid clearance. First, we measured the expression levels of ENaC mRNA, Na,K-ATPase mRNA, and β_2 -adrenoreceptor mRNA by real-time polymerase chain reaction (RT-PCR) (5 rats with left pulmonary artery occlusion, 4 sham rats without pulmonary artery occlusion). Second, since the expression of α_1 -Na,K-ATPase mRNA increased, we measured the Na,K-ATPase protein levels by Western blot analysis (2 rats with pulmonary artery occlusion, 2 sham rats without pulmonary artery occlusion, and 2 control rats without thoracotomy).

Measurements

Lung water volume. Left and right lungs were excised separately and weighed immediately for the measurement of wet lung weight. Thereafter, the lungs were dried in an oven (65°C) for 4 days for the measurement of dry lung weight. The lung water (LW)/dry lung (DL) weight ratio was calculated as follows¹³:

$$\text{LW/DL} = (\text{Wet lung weight} - \text{Dry lung weight}) / \text{Dry lung weight} \quad (1)$$

Alveolar fluid clearance. Alveolar fluid clearance was estimated by the progressive increase of the albumin concentration in the alveolar spaces.^{6,10,11} Alveolar fluid clearance (AFC) was calculated as follows:

$$\text{AFC} = ([V_i - V_f]/V_i) \times 100 \quad (2)$$

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

$$V_f = (V_i \times P_i)/P_f \quad (3)$$

where P represents the concentration of albumin in the instilled solution (i) and in the final alveolar fluid (f).

Plasma catecholamine. Catecholamine (epinephrine, norepinephrine, and dopamine) levels in plasma were measured as reported in prior studies.¹²

Real-time quantitative PCR. After left pulmonary artery occlusion for 14 days, rats were exsanguinated under anesthesia with pentobarbital sodium. The distal lung tissue samples were freshly frozen in liquid nitrogen and stored at -80°C . Total RNA was extracted from the lung tissue with RNA isolative reagent (Isogen; Wako, Osaka, Japan) according to the manufacturer's manual. cDNA was synthesized from 5 μg of total RNA in the DNA engine (PTC-200; MJ Research, Watertown, Mass). Then 3.5 μL cDNA was performed with a 1-step RT-PCR reagent (TaqMan; Applied Biosystems) in a final volume of 20 μL containing 1 μL TaqMan probes, diethylpyrocarbonate water 5.5 μL , and TaqMan universal PCR master mixture 10 μL , at 50°C 2 minutes, 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute, totally 40 cycles in sequence detection system (ABI PRISM 7700; Applied Biosystems). Oligonucleotide primers and TaqMan probes of α -, β -, γ -ENaC, α_1 -, β_1 -Na,K-ATPase, β_2 -adrenoreceptor, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes were purchased from Assays-On Demand Gene Expression Products (Applied Biosystems). We picked the sample with lowest Ct value* in all of samples as the standard sample. The standard curve was made of the degressive concentration of standard samples at 5-fold. Gene expression levels, quantified by the standard curve method according to the manufacturer's instructions and standardized with the expression levels of GAPDH gene, were used to analyze the relative amount of target mRNA expressions.

Western blot analysis. Proteins were extracted from the lung tissues with a lysis buffer containing 50 mmol/L Tris-HCl (pH 7.6), 10% glycerol, 5 mmol/L magnesium acetate, 0.2 mmol/L ethylenediamine tetraacetic acid, 1 mmol/L phenylmethylsulfonyl fluoride, and 1% sodium dodecylsulfate. Protein extract was quantified by the Bradford method (Bio-Rad, Hercules, Calif) and 20 μg of the extracted protein was applied to electrophoresis with a 15% polyacrylamide gel and then transferred to a nitrocellulose membrane (AtoH, Tokyo, Japan). After blocking with 5% nonfat milk, 0.05% Tween-20 in Tris-buffered saline, the nitrocellulose membrane was reacted with anti- α_1 - and β_1 -Na,K-ATPase monoclonal antibodies (1:2000) overnight at 4°C . Blots were washed

*The number of cycles at which fluorescence goes over the cutoff value.

and incubated with peroxidase-labeled rabbit anti-mouse antibodies (1:2000) for 1 hour at room temperature. After incubation, blots were washed 3 times with blocking solution with 0.05% Tween-20 in Tris-buffered saline. The membranes were incubated with chemiluminescence luminol reagent (Supersignal; Pierce, Rockford, Ill) and immunoreactive bands were visualized and photographed digitally by ATTO Light-Capture (AE-6971; ATTO Corporation, Tokyo, Japan).

Morphologic examination. The lung were fixed by injection of 10% formalin solution through the trachea at a pressure of 20 cm H₂O and immersed in 10% formalin solution. A 3-mm thick section was obtained from the center of the each piece of both lungs. These sections were embedded in paraffin and then sectioned serially at 5 μ m and stained with hematoxylin and eosin. All sections were coded randomly. Microscopic fields using magnifications of $\times 100$ and $\times 200$ were examined in each section.

Statistics

Data are summarized as the mean and standard error (mean $\pm$ SE). The data were analyzed by a 1-way analysis of variance with the Student–Newman–Keuls post hoc test (GraphPad Prism 4; GraphPad Software Inc, San Diego, Calif).

Results

Left Pulmonary Artery Occlusion Increased Lung Water Volume and Alveolar Fluid Clearance

The lung water/dry lung weight ratio was 10% greater in the left lungs with left pulmonary artery occlusion (4.32 ± 0.10 g/g; $P < .05$) than in the right lungs in the same rats (4.00 ± 0.03 g/g) and in left lungs in sham rats without pulmonary artery occlusion (3.90 ± 0.03 g/g) (Figure 1, A). In sham rats, there was no difference between the lung water/dry lung weight ratios in the left lungs and in the right lungs. Alveolar fluid clearance significantly increased in the left lungs in rats with left pulmonary artery occlusion ($13.6\% \pm 1.0\%$; $P < .05$) than in the right lungs in the same rats ($9.6\% \pm 0.5\%$) and in the left lungs in sham rats without pulmonary artery occlusion ($8.6\% \pm 0.5\%$) (Figure 1, B). In sham rats, there was no difference between alveolar fluid clearance in the left lungs and that in the right lungs. RT-PCR revealed that the expression level of α_1 -Na,K-ATPase mRNA (Figure 1, C), but not β_1 -Na,K-ATPase mRNA (Figure 1, D), increased in the left lungs in rats with pulmonary arterial occlusion. Thoracotomies in sham rats did not change the expression levels of α_1 - and β_1 -Na,K-ATPase mRNA. Western blotting revealed that the α_1 -Na,K-ATPase subunit protein level increased in the left lungs in rats with pulmonary artery occlusion (Figure 1, E), whereas a statistical analysis was impossible because of small number of samples. However, the β_1 -Na,K-ATPase subunit protein level did not change in the left lungs in rats with pulmonary artery occlusion. Neither a thoracotomy nor

Figure 1. The effect of pulmonary artery occlusion on lung water volume (A), alveolar fluid clearance (B), on Na,K-ATPase mRNA (C and D), and protein levels (E). Left pulmonary artery occlusion increased both the lung water/dry lung weight ratio (A) and alveolar fluid clearance (percent of instilled volume over 1 hour, B) in the left lungs, but not in the right lungs, in rats with left pulmonary artery occlusion. RT-PCR demonstrated that left pulmonary artery occlusion increased the α_1 -Na,K-ATPase mRNA levels (C), but not β_1 -Na,K-ATPase mRNA levels (D), in the left lungs in rats with left pulmonary artery occlusion. The protein levels of α_1 -Na,K-ATPase, but not β_1 -Na,K-ATPase, increased in the left lung in rats with pulmonary artery occlusion (occlusion, $n = 2$) compared with the left lungs in sham control (control, $n = 2$) (E). * $P < .05$ vs sham control and right lungs in rats with pulmonary artery occlusion. † $P < .01$ vs values of Na,K-ATPase mRNA over GAPDH mRNA in the right lungs in rats with left pulmonary artery occlusion and in sham rats without pulmonary artery occlusion. Means $\pm$ standard errors. GAPDH, Glyceraldehyde-3-phosphate dehydrogenase.

pulmonary artery occlusion changed the expression levels of α -, β -, and γ -ENaC mRNA.

The Effects of Propranolol and Terbutaline on Alveolar Fluid Clearance in Rats With Left Pulmonary Artery Occlusion

Propranolol (10^{-4} mol/L) did not change alveolar fluid clearance in bilateral lungs in rats with left pulmonary artery occlusion (Figure 2, A). Terbutaline (10^{-6} mol/L) increased alveolar fluid clearance in the right lungs in rats with pulmonary artery occlusion. However, terbutaline did not increase alveolar fluid clearance in the left lungs in rats with left pulmonary artery occlusion. Plasma catecholamine levels 14 days after surgery were not different from the normal values.¹³ The expression levels of a β_2 -adrenergic receptor mRNA decreased in the left lung in rats with pulmonary artery occlusion (Figure 2, B).

Figure 2. The effects of terbutaline and propranolol on alveolar fluid clearance in rats with left pulmonary artery occlusion (A). Terbutaline (10^{-6} mol/L) increased alveolar fluid clearance in the right lungs, but not in the left lungs, in rats with left pulmonary artery occlusion. Propranolol (10^{-4} mol/L) had no effect on alveolar fluid clearance in both lungs in rats with left pulmonary artery occlusion. * $P < .05$ vs the rates of alveolar fluid clearance in the right lungs in rats with pulmonary artery occlusion. **None,** No treatment of propranolol or terbutaline. The effect of pulmonary artery occlusion on β_2 -adrenoceptor mRNA level (B). RT-PCR demonstrated that the level of β_2 -adrenoceptor mRNA over GAPDH mRNA decreased in the left lungs in rats with left pulmonary artery occlusion. * $P < .05$ vs the mRNA levels in the right lungs in rats with left pulmonary artery occlusion and in bilateral lungs in control rats. Means $\pm$ standard errors. **GAPDH,** Glyceraldehyde-3-phosphate dehydrogenase; **RT-PCR,** real-time polymerase chain reaction; **mRNA,** messenger ribonucleic acid.

Morphologic Examination

The formation of edema fluid was absent in the right lungs in rats with left pulmonary artery occlusion and in the bilateral lungs in sham rats (Figure 3, A). Although the formation of edema fluid was absent in the alveolar spaces, perivascular cuffing was present in the left lungs in rats with pulmonary artery occlusion for 14 days (Figure 3, B and C). Pulmonary infarction and thrombus formation were absent in the lungs with pulmonary artery occlusion.

Discussion

In this study, we found that pulmonary artery occlusion for 14 days increased lung water volume by 10% and also alveolar fluid clearance by 40%. Although α -, β -, γ -ENaC mRNA and β_1 -Na,K-ATPase mRNA levels did not increase in the lungs in rats with pulmonary artery occlusion, mRNA and protein levels of α_1 -Na,K-ATPase increased. We also found that pulmonary artery occlusion for 14 days abolished the effect of a β_2 -adrenergic agonist on alveolar fluid clearance.

Inasmuch as pulmonary artery occlusion decreases the hydrostatic force in the Starling equation,¹ we hypothesized that lung water volume would decrease in the rat lungs

without pulmonary perfusion. However, lung water volume measured by a gravimetric method increased. There may be two explanations for the increase in lung water volume because perivascular cuffing was present around the ligated pulmonary artery. First, because lung interstitial fluid drains primarily through the pathway via pulmonary circulation,⁸ the decrease in pulmonary blood flow impaired the clearance capacity of interstitial fluid and resulted in the increase in lung water volume. Second, whereas lung lymph flow also plays a role in the drainage of interstitial lung fluid and the rate of lung lymph flow is impaired in lungs without pulmonary perfusion,^{9,15} it is also likely that decreased lung lymph flow impaired the drainage capacity and resulted in the increase in lung water volume. Because there was no fluid accumulation around bronchi, it is unlikely that bronchial circulation played an important role in the increase in lung water volume.

An amiloride-sensitive sodium channel and basolateral Na,K-ATPase play a primary role in alveolar fluid clearance.¹⁶ We determined whether pulmonary arterial occlusion changed the expression of the sodium channel and Na,K-ATPase. First, since the sodium channel consists of three homologous subunits: α -, β -, and γ -ENaC,¹⁷ we measured the mRNA levels of three subunits and found that RT-PCR did not reveal a significant change in the mRNA levels. Second, although the overexpression of β_1 -Na,K-ATPase increased alveolar fluid clearance,¹⁸ we found that pulmonary arterial occlusion did not change the expression of β_1 -Na,K-ATPase. In this study, because pulmonary artery occlusion increased both mRNA and protein expressions of α_1 -Na,K-ATPase, it is likely that α_1 -Na,K-ATPase played a role in the increase in alveolar fluid clearance. Although we did not measure the activity of Na,K-ATPase, our data are supported by a report that the overexpression of α_1 -Na,K-ATPase by catecholamine correlated with the increase in the of Na,K-ATPase activity.¹⁹

Endogenous catecholamines,^{20,21} exogenous β_2 -adrenergic agonists,^{5,6} and the overexpression of β_2 -adrenoceptors²² increased alveolar fluid clearance. Therefore, we determined whether the mechanism mediated by a β -adrenoceptor played a role in the increase in alveolar fluid clearance in the lungs with pulmonary artery occlusion. First, because the endogenous catecholamine levels in plasma did not increase in rats with pulmonary artery occlusion, it is unlikely that the increase in plasma catecholamine was responsible for the increase in alveolar fluid clearance. Second, although it was reported that propranolol inhibited the effect of endogenous catecholamine on alveolar fluid clearance in rats with shock,²⁰ propranolol did not inhibit the increase in alveolar fluid clearance in this study. Third, although there was a possibility that terbutaline, a potent β_2 -adrenergic agonist, did not stimulate alveolar fluid clearance because of a plateau of alveolar fluid clearance, it is unlikely because the increased alveolar fluid clearance in this

Figure 3. Morphologic examination of the lungs in rats with pulmonary artery occlusion for 14 days. Periarterial cuffing was absent in the right lungs (A) but present in the left lungs (B, $\times 100$, C, $\times 200$) in rats without pulmonary perfusion. Arrows, Perivascular cuffing.

study was smaller than the plateau of alveolar fluid clearance.¹⁴ Finally, because the expression of β_2 -adrenoceptor mRNA decreased, the decreased expression may be associated with the impaired effect of terbutaline. The impaired function of β -adrenoceptors has been indicated in hemorrhagic shock,²¹ hyperoxic lung injury,²³ and ventilator-induced lung injury.²⁴ However, it is uncertain whether the impaired function of β -adrenoceptors is due to downregulation of β -adrenoceptor, the defect of receptor signaling, or some combination of impaired alveolar epithelial function.²⁵

There are several limitations in this study. First, the role of bronchial circulation and lymph flow that may play an important role in lung fluid balance was not determined. Second, the activity of Na,K-ATPase was not determined and the number of lungs used for protein analysis is small. Third, the protein level, trafficking, and signaling of β -adrenoceptors were not determined.

In clinical relevance in thoracic surgery, the results of this study indicate that a treatment increasing alveolar fluid clearance should be considered to eliminate alveolar edema fluid in the lung with pulmonary artery occlusion. In addition, it is unlikely that a β_2 -adrenergic agonist is a useful agent for resolution of pulmonary edema in the lung without pulmonary blood flow.

In conclusion, chronic pulmonary artery occlusion increases alveolar fluid clearance via the overexpression of α_1 -Na,K-ATPase in rats.

References

- Matthay MA, Folkesson HG, Clerici C. Lung epithelial fluid transport and the resolution of pulmonary edema. *Physiol Rev.* 2002;82:569-600.
- Frank JA, Wang Y, Osorio O, Matthay MA. Beta-adrenergic agonist therapy accelerates the resolution of hydrostatic pulmonary edema in sheep and rats. *J Appl Physiol.* 2000;89:1255-65.
- Matthay MA, Landolt CC, Staub NC. Differential liquid and protein clearance from the alveoli of anesthetized sheep. *J Appl Physiol.* 1982;53:96-104.
- Borok Z, Liebler JM, Lubman RL, Foster MJ, Zhou B, Li X, et al. Na transport proteins are expressed by rat alveolar epithelial type I cells. *Am J Physiol Lung Cell Mol Physiol.* 2002;282:L599-608.
- Berthiaume Y, Staub NC, Matthay MA. Beta-adrenergic agonists increase lung liquid clearance in anesthetized sheep. *J Clin Invest.* 1987;79:335-43.
- Sakuma T, Okaniwa G, Nakada T, Nishimura T, Fujimura S, Matthay MA. Alveolar fluid clearance in the resected human lung. *Am J Respir Crit Care Med.* 1994;150:305-10.
- Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med.* 2001;163:1376-83.
- Jayr C, Matthay MA. Alveolar and lung liquid clearance in the absence of pulmonary blood flow in sheep. *J Appl Physiol.* 1991;71:1679-87.
- Sakuma T, Pittet JF, Jayr C, Matthay MA. Alveolar liquid and protein clearance in the absence of blood flow or ventilation in sheep. *J Appl Physiol.* 1993;74:176-85.
- Sakuma T, Folkesson HG, Suzuki S, Okaniwa G, Fujimura S, Matthay MA. Beta-adrenergic agonist stimulated alveolar fluid clearance in ex vivo human and rat lungs. *Am J Respir Crit Care Med.* 1997;155:506-12.
- Sakuma T, Suzuki S, Usuda K, Handa M, Okaniwa G, Nakada T, et al. Preservation of alveolar epithelial fluid transport mechanisms in re-warmed human lung after severe hypothermia. *J Appl Physiol.* 1996;80:1681-6.
- Sakuma T, Hida M, Nambu Y, Osanai K, Toga H, Takahashi K, et al. Effects of hypoxia on alveolar fluid transport capacity in rat lungs. *J Appl Physiol.* 2001;91:1766-74.
- Sakuma T, Sagawa M, Hida M, Nambu Y, Osanai K, Toga H, et al. Time-dependent effect of pneumonectomy on alveolar epithelial fluid clearance in rat lungs. *J Thorac Cardiovasc Surg.* 2002;124:668-74.
- Sakuma T, Tuchihaara C, Ishigaki M, Osanai K, Nambu Y, Toga H, et al. Denopamine, a β_1 -adrenergic agonist, increases alveolar fluid clearance in ex vivo rat and guinea pig lungs. *J Appl Physiol.* 2001;90:10-6.
- Fukuda N, Folkesson HG, Matthay MA. Relationship of interstitial fluid volume to alveolar fluid clearance in mice: ventilated vs. in situ studies. *J Appl Physiol.* 2000;89:672-9.
- Matalon S, O'Brodovich H. Sodium channels in alveolar epithelial cells: molecular characterization, biophysical properties, and physiological significance. *Annu Rev Physiol.* 1999;61:627-61.

17. Eaton DC, Chen J, Ramosevac S, Matalon S, Jain L. Regulation of Na⁺ channels in lung alveolar type II epithelial cells. *Proc Am Thorac Soc*. 2004;1:10-6.
18. Azzam ZS, Dumasius V, Saldias FJ, Adir Y, Sznajder JI, Factor P. Na,K-ATPase overexpression improves alveolar fluid clearance in a rat model of elevated left atrial pressure. *Circulation*. 2002;105:497-501.
19. Azzam ZS, Adir Y, Crespo A, Comellas A, Lecuona E, Dada LA, et al. Norepinephrine increases alveolar fluid reabsorption and Na,K-ATPase activity. *Am J Respir Crit Care Med*. 2004;170:730-6.
20. Pittet JF, Wiener-Kronish JP, McElroy MC, Folkesson HG, Matthay MA. Stimulation of lung epithelial liquid clearance by endogenous release of catecholamines in septic shock in anesthetized rats. *J Clin Invest*. 1994;94:663-71.
21. Modelska K, Matthay MA, Brown LA, Deutch E, Lu LN, Pittet JF. Inhibition of beta-adrenergic-dependent alveolar epithelial clearance by oxidant mechanisms after hemorrhagic shock. *Am J Physiol*. 1999;276:L844-57.
22. Mutlu GM, Adir Y, Jameel M, Akhmedov AT, Welch L, Dumasius V, et al. Interdependency of beta-adrenergic receptors and CFTR in regulation of alveolar active Na⁺ transport. *Circ Res*. 2005;96:999-1005.
23. Saldias FJ, Comellas A, Ridge KM, Lecuona E, Sznajder JI. Isoproterenol improves ability of lung to clear edema in rats exposed to hyperoxia. *J Appl Physiol*. 1999;87:30-5.
24. Frank JA, Pittet JF, Lee H, Godzich M, Matthay MA. High tidal volume ventilation induces NOS2 and impairs cAMP-dependent air space fluid clearance. *Am J Physiol Lung Cell Mol Physiol*. 2003;284:L791-8.
25. Mutlu GM, Koch WJ, Factor P. Alveolar epithelial beta 2-adrenergic receptors: their role in regulation of alveolar active sodium transport. *Am J Respir Crit Care Med*. 2004;170:1270-5.