Pharmacologic Modulation of Alveolar Liquid Clearance in Transplanted Lungs by Phentolamine and FK506

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Background. **The lung's capacity to clear alveolar fluid can determine the severity of the edema seen after transplantation. We recently observed that alveolar liquid clearance was decreased in transplanted lungs. This study evaluates the ability of phentolamine and FK506 to modulate the severity of lung injury and the decline in alveolar liquid clearance after transplantation.**

Methods. **A canine orthotopic single-lung transplantation model was used. The lungs were preserved with a low-potassium-dextran solution (50 mL/kg) and transplanted after 3 hours of cold ischemia. The experimental protocol included a control group, a phentolamine group, in which donor lungs were infused with phentolamine (2 mg/kg), and a FK506 group, in which the animals received FK506 (25 mg/kg per hour) intravenously during reperfusion. After 4 hours of reperfusion, alveolar liquid clearance, wet-to-dry ratios, lung epithelial Na^+ channel expression, and the response to β-adrenergic stimulation were measured.**

Results. **The increase in wet-to-dry ratios of transplanted lungs was less pronounced in the phentolamine and FK506 groups. The FK506 treatment led to improvement of alveolar liquid clearance. Neither phentolamine nor FK506 influenced lung epithelial Na^+ channel expression in transplanted lungs or preserved alveolar cell ability to respond to β-adrenergic stimulation.**

Conclusions. **Phentolamine or FK506 treatment during reperfusion improves alveolar liquid clearance and decreases the severity of lung injury.**

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Primary graft failure after lung transplantation manifests itself by noncardiogenic pulmonary edema. This phenomenon represents a significant cause of morbidity and mortality [1, 2]. Clinical observations suggest that dysfunction of sodium (Na^+) transport and alveolar liquid clearance might contribute to the development of pulmonary edema in the posttransplant period [3]. Recently, we reported that alveolar liquid clearance was significantly inhibited in a canine model of lung transplantation [4]. Alveolar liquid clearance is mediated by unidirectional Na^+ transport from the apical to the basolateral side of the alveolar epithelium, which creates an osmotic gradient that leads to pulmonary edema resolution [5]. Sodium enters the cells by the epithelial sodium channel (ENaC) located at the apical surface and is extruded by sodium-potassium-adenoside triphosphatase (Na^+-K^+-ATPase) at the basolateral surface [5]. Modulation of Na^+ transport and lung liquid clearance across the epithelium can be achieved by modulating the activity or the expression of ENaC or Na^+-K^+-ATPase [5–7].

Although the dysfunction in alveolar liquid clearance depends on the severity of the injury [8], the mechanism leading to this dysfunction is not completely understood. Studies using other lung injury models have shown that oxidative stress might be involved [9]. It has also been shown that the α-adrenergic blocker phentolamine can modulate the oxidative stress and alveolar liquid clearance in hemorrhagic shock–induced lung injury [10]. It has been also reported that FK506, an immunosuppressant utilized in solid organ transplantation, can decrease the severity of lung injury [11–13]. Based on these observations, we decided to test the hypothesis that alveolar liquid clearance and the severity of injury of transplanted lungs can be modulated by phentolamine or FK506.

Material and Methods

Experimental Protocols and Surgical Procedures

Orthotopic left lung transplantations were performed in 34 dogs (18 to 26 kg). These experiments were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health) as applied by the Animal Care Committee of our research center.

Three groups of animals were studied. In the control group (n = 6), the donor lungs were flushed with a low-potassium–dextran solution, 50 mL/kg (Perfadex; Vitrolife, Englewood, CO), but no pharmacologic treatment was given to the animals.

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Table 1. Physiologic Variables

			After Surgery			
	Before Surgery	Immediately After Surgery	60 Minutes	120 Minutes	180 Minutes	240 Minutes
pH						
Control	7.42 ± 0.04	7.36 ± 0.04	7.37 ± 0.03	7.38 ± 0.03	7.41 ± 0.03	7.38 ± 0.04
Phentolamine	7.48 ± 0.03	7.46 ± 0.05	7.45 ± 0.02	7.45 ± 0.03	7.43 ± 0.03	7.43 ± 0.02
FK506	7.49 ± 0.04	7.40 ± 0.02	7.41 ± 0.03	7.39 ± 0.03	7.39 ± 0.03	7.39 ± 0.03
Control	119 ± 9	111 ± 4	117 ± 3	105 ± 6	111 ± 3	109 ± 3
Heart rate, beats/min						
Phentolamine	127 ± 10	104 ± 7	98 ± 6[a]	98 ± 6	93 ± 6[a]	96 ± 6
FK506	121 ± 5	108 ± 6	106 ± 5	112 ± 2	104 ± 6	101 ± 6
Control	80 ± 6	73 ± 3	70 ± 3	76 ± 4	82 ± 3	80 ± 5
MAP, mm Hg						
Phentolamine	74 ± 8	76 ± 5	68 ± 3	65 ± 3[a]	67 ± 3[a]	69 ± 3[a]
FK506	76 ± 6	67 ± 4	69 ± 3	70 ± 4	70 ± 5[a]	66 ± 4[a]
Control	7 ± 1	9 ± 2	9 ± 1	10 ± 1	9 ± 1	9 ± 1
MPAP, mm Hg						
Phentolamine	7 ± 2	7 ± 2	7 ± 2	7 ± 2	8 ± 1	7 ± 1
FK506	6 ± 1	8 ± 2	8 ± 2	8 ± 2	7 ± 1	5 ± 1[a]

[a] $p < 0.05$ versus control.

MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure.

In the phentolamine group (n = 6), phentolamine (Paladin Labs, Montreal, Canada), 2 mg/kg, was infused over a 2-minute period into the lung vasculature of the donor animal through a pulmonary artery catheter before harvesting of the lungs. This phentolamine dose was used to inhibit increased vascular permeability of the lungs [14] and was greater than that administered to improve graft function after renal transplantation [15].

In the FK506 group (n = 5), FK506 (Tacrolimus; Fujisawa Canada, Markham, Canada) was infused intravenously (25 $mg \cdot kg^{-1} \cdot h^{-1}$), starting 30 minutes before reperfusion in the recipient animals and running until the end of the 4-hour reperfusion period. This FK506 dose was shown to inhibit increased vascular permeability and decrease the severity of injury in an endotoxin lung injury model [11].

Detailed surgical procedures for both harvesting donor lungs and transplantation have been described elsewhere [4]. After flushing, the donor lungs were inflated with 100% oxygen, excised, and placed in crushed ice. The recipient animals were placed under general anesthesia for 30 minutes before the left lung transplantation. After anastomoses of the left mainstem bronchus, pulmonary artery, and left atrium, the lungs were reperfused, and ventilation was reestablished. Total ischemic time was 192 ± 4 minutes. Mean arterial pressure, mean pulmonary arterial pressure, heart rate, and arterial blood gas were recorded during anesthesia and monitored for 4 hours after transplantation.

Gravimetric Measurements and Pulmonary Myeloperoxidase Activity

To determine the severity of lung injury in our model, we measured the wet-to dry ratio of the native and transplanted lungs as well as the neutrophil sequestration as measured by the myeloperoxidase (MPO) activity assay [4]. After 4 hours of reperfusion, the transplanted left lung and native right lung were divided into three lobes on each side. The middle lobes were frozen in liquid nitrogen, and stored

Table 2. Wet-to-Dry Ratio

	Control (C)	Phentolamine (P)	FK506 (F)	
Native right lungs	4.4 ± 0.3	4.3 ± 0.4	4.0 ± 0.3	C versus P: NS C versus F: NS P versus F: NS
Transplanted left lungs	7.2 ± 0.6	5.9 ± 0.8	5.3 ± 0.7	C versus P: $p < 0.01$ C versus F: $p < 0.001$ P versus F: NS

Right versus left: $p < 0.001$, $p < 0.001$, $p < 0.01$, respectively.

NS = not significant.