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ORIGINAL PRE-CLINICAL SCIENCE

Human α_1-antitrypsin improves early post-transplant lung function: Pre-clinical studies in a pig lung transplant model

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BACKGROUND: The translation of novel drugs in lung transplantation is challenged by different physiologic conditions between small animals and humans. Large-animal models provide important pre-clinical evidence and the next step that best informs clinical trials. In the present study, we used a pig lung transplant model to determine whether human α_1-antitrypsin (A1AT), a medication shown to prevent pulmonary ischemia-reperfusion injury in rats, could attenuate reperfusion injury after prolonged hypothermic preservation in a large-animal lung transplant model.

METHODS: Donor lungs were preserved for 24 hours at 4°C, followed by lung transplantation. In a randomized and blinded fashion, intravenous A1AT (240 mg/kg; $n = 5$) or human albumin ($n = 5$) was administered to the recipient before reperfusion. Allograft gas exchange function and lung mechanics were monitored during a 4-hour reperfusion period. Microscopic lung injury, inflammatory response, coagulation activity, and cell death were assessed.

RESULTS: Pulmonary gas exchange was significantly better during the 4-hour reperfusion period in the A1AT group. Treatment with A1AT improved static pulmonary compliance and significantly reduced pulmonary edema and lung permeability. A1AT treatment inhibited inflammatory mediators in the circulation, with reduced activation of nuclear factor-κB and inflammasome, reduced formation of thrombin-antithrombin complex in plasma, and reduced apoptosis in the allografts.

CONCLUSIONS: Administration of human A1AT before reperfusion in recipients improved immediate post-transplant lung function in pigs. A large-animal survival model should be considered to support further advancement toward a clinical trial of A1AT to prevent primary graft dysfunction in lung transplantation.

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In contrast to other solid organ transplants, the number of lung transplants performed continues to rise, a trend that has continued since the first lung transplant operation with successful long-term patient survival.[1] This sustained

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progress is attributable to advances in lung preservation and donor management, use of extended-criteria donor lungs, and the rise of ex vivo lung perfusion strategies.[2]

Despite a significant increase in the number of lung transplants performed and improvements in patient care, the primary causes of death after lung transplantation have remained static during the past decade.[1] In the early post-operative period, primary graft dysfunction (PGD) is the major cause of morbidity and mortality.[3] PGD develops after transplantation in approximately 20% of all lung transplant recipients.[4] Numerous studies have attempted to prevent this dreaded complication; however, to date no preventive or therapeutic options for clinical use are currently available or widely accepted.

The underlying pathogenesis of PGD is multifactorial,[3] with ischemia-reperfusion (IR)– related processes the most common contributing factors for PGD.[5] In solid-organ transplantation, some protection from ischemia is achieved with cold flush preservation. Cellular metabolism is reduced during cold static storage; however, pneumocytes may continue to be injured via oxidative stress, intracellular electrolyte imbalance, and activation of cell death pathways.[6]

Reperfusion aggravates ongoing injury via activation of innate immunity and important plasma cascade systems, such as complement and coagulation.[7] This injury is further provoked during the late phase of reperfusion by the recruitment of recipient neutrophils into the lung tissue, a phenomenon promoted by adhesion molecules. Furthermore, emigrated neutrophils enhance the production of pro-inflammatory cytokines in the allograft together with the externalization of destructive enzymes, including neutrophil elastase.[8] Investigating therapeutic agents that target multiple injurious mechanisms in the pathogenesis of IR-injury related to lung transplantation is therefore desirable and important.

We previously demonstrated beneficial effects of α_1-antitrypsin (A1AT) in reducing IR-induced lung injury in cell culture and in a pulmonary hilar clamping model in rats.[9] In addition to physiologic improvement, treatment with A1AT resulted in the inhibition of inflammatory responses and cell death.[9] A1AT has also been shown to have beneficial effects for IR-induced injury of other organs such as the kidney, heart, and liver.[9] A1AT exerts broad anti-inflammatory properties affecting a wide range of inflammatory cells,[10] most of which are involved in the pathogenesis of PGD.[5]

The A1AT studies performed thus far have largely featured warm ischemic conditions in small animals. Lung transplantation is a major operation with complex requirements involving anesthesia, surgery, and critical care. The physiologic variables (e.g., body size, heart rate, breath rate, hemodynamics, etc.) between small animals and humans are quite different. Thus, the objective of the present study was to test whether pre-treatment of a lung transplant recipient with A1AT improves post-transplant lung function in a large-animal (pig) pre-clinical lung transplant model. The findings, including physiologic, functional, and biologic data, are important steps toward informing the future clinical application of A1AT therapy.

Methods

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996).

Experimental groups

Orthotopic left single-lung transplants were performed in a randomized and blinded fashion. Recipients in the treatment group ($n = 5$) were treated intravenously with 240 mg/kg human A1AT (Zemaira, CSL Behring, King of Prussia, PA) intravenously. Control animals ($n = 5$) received human albumin IV (AlbuRx, CSL Bering) mixed in an equivalent volume to facilitate complete blinding of the investigative team.[11] Infusion duration was 30 minutes and completed 1 hour before reperfusion. This dose was chosen based on a prior pilot dose escalation study in which we tested A1AT at 120 or 240 mg/kg ($n = 2$/dose) derived from current regimens in patients with A1AT deficiency.[12]

We monitored hemodynamic and respiratory function and performed biochemical safety analysis, including hematologic, biochemical, and hemostasis screening tests. No detrimental effects were observed; however, the higher dose (240 mg/kg) was chosen because relatively less microscopic lung injury was found. This dose has also been found to be safe in patients with A1AT deficiency.[13]

Lung transplant procedure

Lung transplants were performed as previously described.[14] Briefly, donor lungs were flushed with 50 ml/kg Perfadex solution (XVIVO Perfusion, Denver, CO), retrieved, and stored at 4°C for 24 hours. The recipient pigs were anesthetized and ventilated with a Servo-i ventilator (Maquet, Solna, Sweden) in pressure-controlled mode with a driving pressure of 15 cm H_2O, positive expiratory-end pressure (PEEP) of 5 cm H_2O, inspiratory-to-expiratory ratio of 1:2, respiratory rate of 15 breaths/min, and fraction of inspired oxygen (F_{IO_2}) of 50%.

After left thoracotomy, the right pulmonary artery was encircled with an umbilical tape to facilitate later clamping, followed by the left pneumonectomy and implantation, with sequential anastomoses of the bronchus, pulmonary artery, and left atrial cuff. The transplanted lung was gently reinflated to a pressure of 25 cm H_2O and then reperfused gradually. During reperfusion, F_{IO_2} was kept at 1 and the driving pressure was kept at 20 cm H_2O.

Plasma, bronchoalveolar wash, blood gas, and allograft tissues sample collection

Blood samples were collected hourly for 4 hours. Plasma was separated, and supernatants were stored at –80°C until analyzed. At the end of 4 hours, a bronchoalveolar wash was performed from the lower lobe using a flexible bronchoscope. Saline (10 ml) was instilled into the endobronchial tree and suctioned back immediately; recovery was approximately 10 ml per animal. The collected wash fluid was centrifuged, and the supernatants were stored at –80°C until analysis.

Gas exchange function was assessed by hourly pulmonary vein blood sampling. After 4 hours of reperfusion, the right pulmonary artery was clamped for 5 minutes to assess oxygenation function of