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ORIGINAL PRE-CLINICAL SCIENCE

Human recombinant apyrase therapy protects against canine pulmonary ischemia-reperfusion injury

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BACKGROUND: There is accumulating evidence that extracellular adenosine triphosphate (eATP) promotes many of the underlying mechanisms that exacerbate acute lung injury. However, much of these data are from inbred rodent models, indicating the need for further investigation in higher vertebrates to better establish clinical relevance. To this end we evaluated a human recombinant apyrase therapy in a canine warm pulmonary ischemia-reperfusion injury (IRI) model and measured eATP levels in human lung recipients with or without primary lung graft dysfunction (PGD).

METHODS: Warm ischemia was induced for 90 minutes in the left lung of 14 mongrel dogs. Seven minutes after reperfusion, the apyrase APT102 (1 mg/kg, $n = 7$) or saline vehicle ($n = 7$) was injected into the pulmonary artery. Arterial blood gases were obtained every 30 minutes up to 180 minutes after reperfusion. Bronchioalveolar lavage fluid (BALF) was analyzed for eATP concentration, cellularity, and inflammatory mediator accumulation. Thirty bilateral human lung transplant recipients were graded for immediate early PGD and assessed for BALF eATP levels.

RESULTS: APT102-treated dogs had progressively better lung function and less pulmonary edema during the 3-hour reperfusion period compared with vehicle-treated controls. Protection from IRI was observed, with lower BALF eATP levels, fewer airway leukocytes, and blunted inflammatory mediator expression. Human lung recipients with moderate to severe PGD had significantly higher eATP levels compared with recipients without this injury.

CONCLUSIONS: Extracellular ATP accumulates in acutely injured canine and human lungs. Strategies that target eATP reduction may help protect lung recipients from IRI.

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Ischemia-reperfusion injury (IRI) is the major driver of primary lung graft dysfunction, a form of acute lung injury that limits short-term and long-term survival of lung transplant recipients.[1–4] Pathologic features of lung IRI are

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most prominent in the alveoli, where neutrophil congestion, coagulopathy, and hemorrhaging, lead to the hypoxemia and impaired fluid clearance characteristic of patients with primary graft dysfunction (PGD).[5]

Although the underlying mechanisms of IRI have not been completely elucidated, it is well established that the accumulation of danger-associated molecular patterns (DAMPs) can induce the inflammatory mediator production and leukocyte extravasation associated with acute lung injury.[3,6,7] In an attempt to find DAMPs that play a key role in exacerbating lung transplant–mediated IRI, we recently reported that there is a significant rise of the DAMP extracellular adenosine triphosphate (eATP) in the bronchioalveolar lavage fluid (BALF) from rat lung isografts injured by prolonged cold preservation.[8]

In healthy tissues, eATP is normally maintained in very low concentrations by ectonucleotidases such as triphosphate diphosphohydrolase 1 (CD39), alkaline phosphatases, and 5'-ectonucleotidase (CD73). In particular, CD39 and CD73 work in tandem to hydrolyze ATP and adenosine diphosphate to adenosine monophosphate and ultimately generate adenosine, a purine that enforces tissue homeostasis by negatively regulating immune cell activation.[9,10] However, ATP is released in high amounts in injured tissues, which seemingly overwhelms the pyrophosphatase activity of the endogenous ectonucleotidase network. This in turn leads to eATP engagement of P2 family purigenic receptor family members that promote inflammatory mediator expression.[11] For example, eATP stimulation of the $P2X_7$ receptor triggers Nod-like receptor, pyrin domain containing 3 (NLRP3) inflammasome activation leading to interleukin (IL)-1β post-translational modification and release.[12–14]

Data from experimental models of acute lung injury have been important to decipher the relationship between ATP release and pulmonary function. In mouse airways, eATP levels were elevated in models of acute respiratory distress syndrome and ventilation-induced airway injury.[15,16] Our group has also demonstrated that a modified soluble form of the human ecto-ATP diphosphohydrolase CD39L3 (APT102) is effective at depleting eATP and preventing lung transplant–mediated IRI in rats.[8] In higher vertebrates, however, whether eATP accumulates in lungs damaged by IRI or whether it exacerbates pulmonary dysfunction remains unclear. In this study, we measured BALF eATP levels in a model of canine pulmonary IRI and in human lung recipients with varying degrees of PGD severity. Our results show that BALF eATP levels are elevated in dog and human lungs exposed to IRI. Furthermore, prevention of eATP accumulation with APT102 administration protects dogs from lung IRI.

Methods

The Washington University School of Medicine Animal Studies Committee approved all surgical and experimental procedures in this study. 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 National Academy of Sciences and published by the National Institutes of Health.

Animals and reagents

The experiments used 14 mongrel dogs (Oak Hill Genetics, Ewing, IL), weighing 22 to 30 kg. APT Therapeutics (St. Louis, MO) supplied APT102, a recombinant soluble form of the modified human ecto-ATP diphosphohydrolase CD39L3.

Operative procedure and APT102 treatment

For surgery, dogs were sedated with propofol (5–7 mg/kg), intubated, and mechanically ventilated at a tidal volume average of 20 ml/kg and a rate of 12 breaths/min to maintain a physiologic partial pressure of carbon dioxide (P_{CO_2}). A positive end-expiratory pressure of 5.0 cm H_2O, and a fraction of inspired oxygen (F_{IO_2}) of 1.0 was maintained. Anesthesia during surgery was administered with inhalation of between 1% and 3% isoflurane and intravenous hydromorphone hydrochloride (7–15 μg/kg/h).

The left carotid artery was dissected and catheterized for continuous pressure monitoring and arterial blood gas sampling. The right jugular vein was dissected and catheterized for continuous monitoring of right heart pressure and serum sampling. Periodic palpebral reflex and spontaneous movements were observed to monitor the depth of anesthesia. A left thoracotomy was performed. The chest incision was made between the fourth and fifth intercostal space for access to the left main bronchus, pulmonary veins, and artery. Hilar stripping of the left lung was performed, along with complete transection of the nerves, bronchial arteries, and lymphatics to prevent possible collateral circulation.

Stripping of the right pulmonary artery was performed, and warm ischemia was induced for 90 minutes by clamping the left pulmonary artery (LPA) and veins and by excluding the left lung from ventilation by insertion of a bronchial blocker (Phycon, Hamberg Germany). After the LPA was unclamped and the bronchial blocker was removed, the right pulmonary artery was clamped for 15 minutes to normalize blood flow into the left lung due to transient pulmonary resistance after ischemia.

After 7 minutes of reperfusion to the left lung, APT102 (1 mg/kg; $n = 7$) or saline ($n = 7$), along with 250 mg methylprednisolone (Solu-Medrol; Pfizer, New York, NY) was administered into the LPA. For single-lung ventilation, peak airway pressures were initiated at an average of 20 cm H_2O for no longer than 20 minutes at the start of single-lung ventilation and then kept near 10 cm H_2O for the duration of the procedure. Minute ventilation adjustments were also made to maintain CO_2 levels at 35 mm Hg. The average tidal volume ultimately used for the study was 15 ± 5 ml/kg.

Sodium bicarbonate was administered as needed at 30 minutes after reperfusion to counter hyperkalemia. Sodium heparin was also administered to keep the activated clotting time above 350 seconds. Left BALF and biopsy specimens were obtained at 90 and 180 minutes after reperfusion. At 180 minutes after reperfusion, saturated KCl was administered for euthanization while under deep anesthesia.

Measurement of blood gas

Arterial blood samples were taken for blood gas analysis before ischemia and every 30 minutes after reperfusion. The arterial partial pressure of oxygen (P_{aO_2}) was determined with a blood-gas analyzer (Stat Profile, pHOxPlusC; Nova Biomedical Corp, Waltham MA).

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