



Adenosine A_{2A} Receptor Activation Attenuates Lung Reperfusion Injury Independent of Circulating Leukocytes



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Background

Ischemia Reperfusion (IR) injury occurs following reperfusion of an ischemic organ such as after transplantation.

Pulmonary IR injury:

- Occurs in 15-20% of lung transplants
- Leads to increased ICU, hospital stay, total cost, and mortality
- Independently predicts Bronchiolitis Obliterans and Chronic Rejection²

Contributing Factors to IR Injury:

- Release of reactive oxygen species
- Neutrophil activation and infiltration
- Pulmonary macrophage activation
- Release of cytokines such as TNF- α

Adenosine A_{2A} Receptor:

- Adenosine is produced locally to provide a local anti-inflammatory response to IR injury and inflammation
- Adenosine A_{2A} receptor activation is anti-inflammatory and inhibits neutrophil activation and infiltration.
- It has been shown that A_{2A} receptor agonists reduce IR injury in lungs³, heart⁴, kidney⁵, as well as others, however, most of these studies utilized blood-perfused models, either *in vitro* or *in vivo*.

Hypothesis

Anti-inflammatory actions of A_{2A} receptor agonists have usually been attributed to effects upon circulating neutrophils where activation and sequestration are impaired. We hypothesized that specific activation of A_{2A} receptors (by ATL-303) improves lung function after IR in the absence of circulating leukocytes.



Figure 1

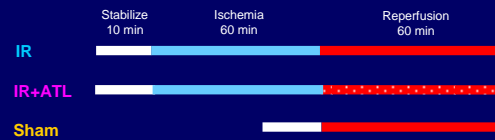
Methods

- Adult, male C57BL6 mice were anesthetized with ketamine & xylazine followed by tracheotomy and intubation, and ventilated with room air (100 br/min).
- Animals were heparinized and exsanguinated.
- We utilized an isolated, buffer-perfused, mouse lung system (Hugo Sachs Elektronik, Germany, Figure 1) as previously described by our laboratory⁶.
- The pulmonary artery was cannulated through the right ventricular outflow tract and the pulmonary circuit was closed by left atrial cannulation.
- Perfusion was at constant flow (2 ml/min) using buffered, isotonic, Krebs Henseleit buffer containing 2% albumin, 0.1% glucose, 0.3% HEPES (335-340 mOsm/kg) at 37°C.
- Lungs were stabilized for 10 min and rendered ischemic by stopping perfusion and ventilating with nitrogen. Reperfusion was initiated with buffer-reperfusion and ventilation with room air.
- Parameters measured include:
 - Pulmonary artery pressure
 - Airway resistance
 - Pulmonary compliance
 - Histology by H&E staining

Study Groups

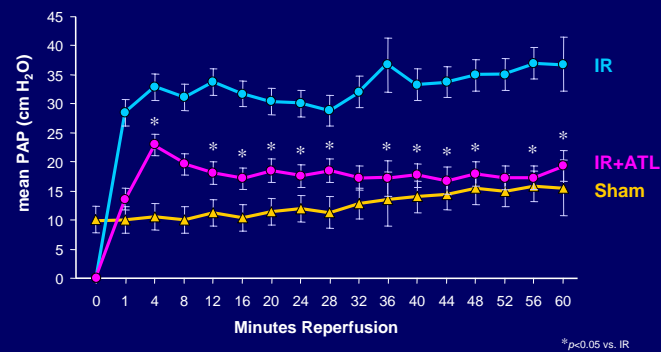
- Sham:** Lungs perfused for 60 min without ischemia (n=10)
- IR:** Lungs ischemic for 60 min followed by reperfusion for 60 min (n=9)
- IR+ATL:** Same as IR except that the reperfusion buffer contained **ATL-303** (100 nM) (n=5).

ATL-303 is a novel, potent and specific adenosine A_{2A} receptor agonist (a gift from Adenosine Therapeutics, LLC, Charlottesville, VA).

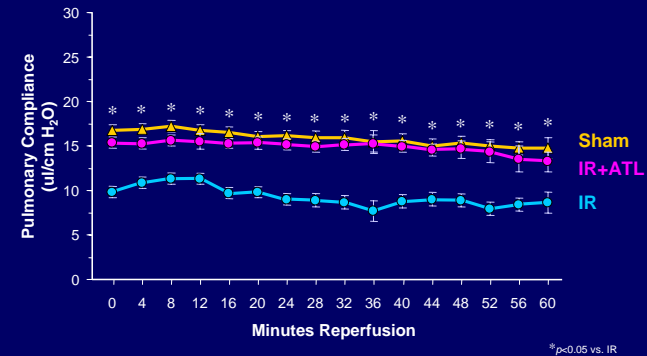


Results

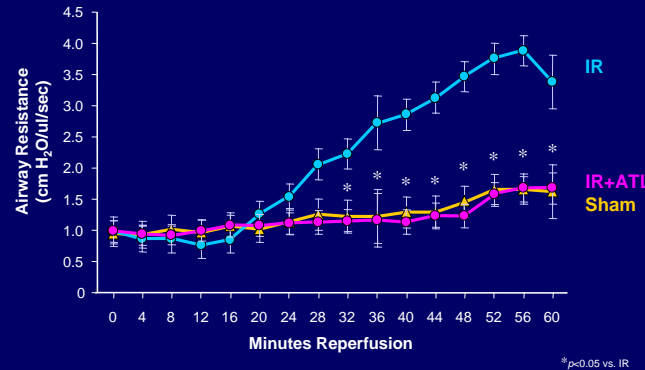
Mean Pulmonary Artery Pressure (mPAP) was elevated throughout reperfusion in the IR group, as expected due to IR injury, and was significantly decreased in the IR+ATL group compared to IR.



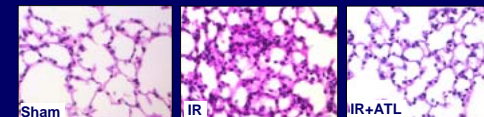
Pulmonary Compliance was severely reduced throughout reperfusion in the IR group and was significantly improved in the IR+ATL group. Pulmonary compliance in IR+ATL was nearly the same as Sham



Airway Resistance (AR) remained similar among all groups during early reperfusion. Significant increases in AR occurred in the IR group, which was completely prevented in the IR+ATL group.



Lung histology (40x magnification, H&E staining) demonstrated fibrinous exudate and thickened septae (edema) in IR lungs and improved histology in IR+ATL lungs.



Summary

- Circulating neutrophils are not required for the initiation of pulmonary IR injury.
- Adenosine A_{2A} receptor activation by ATL-303 improves lung function after IR, even in a non-blood perfused model

Conclusions

These results implicate the important contribution of parenchymal lung cells such as macrophages and endothelial cells, and not just circulating leukocytes such as neutrophils, in A_{2A}-mediated protection from IR injury.

References

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