



Attenuation of ischemia-reperfusion injury by adenosine A_{2A} receptor activation on resident lung cells

Ashish K. Sharma¹, Zequan Yang¹, Joel Linden², Irving L. Kron¹ and Victor E. Laubach¹
Departments of ¹Surgery and ²Medicine, University of Virginia Health System, Charlottesville, VA



Abstract

Ischemia-reperfusion (I/R) injury after lung transplantation leads to significant morbidity and mortality, which remains a major obstacle in clinical lung transplantation. In the present study, we investigated the effects of adenosine A_{2A} receptor (A_{2A}AR) activation on pulmonary I/R injury using an isolated, buffer-perfused murine lung model. To assess the protective effects of A_{2A}AR activation (via a specific A_{2A}AR agonist, ATL313), three groups of C57BL/6 mice were studied: a sham group (perfused for 120 min), an I/R group (60 min ischemia + 60 min reperfusion) and I/R + ATL313 group (identical to I/R group except that ATL313 was included in the reperfusion buffer). After I/R, lungs displayed significant dysfunction (increased airway resistance, pulmonary artery pressure and decreased pulmonary compliance) and significant injury (increased vascular permeability and edema). Lung I/R injury and dysfunction were significantly ameliorated by ATL313 treatment. Significant induction of pro-inflammatory cytokines/chemokines TNF- α , KC (CXCL1), MIP-2 (CXCL2) and RANTES (CCL5) occurred in lungs after I/R, which was ameliorated by ATL313 treatment. Lungs from A_{2A}AR KO mice undergoing I/R also displayed significant dysfunction, injury and cytokine/chemokine production, but ATL313 had no effect in these mice. These results suggest that specific activation of A_{2A}AR significantly protects against pulmonary I/R injury via amelioration of the pro-inflammatory signaling cascade. This protection occurs in the absence of circulating blood thereby indicating a prominent role of A_{2A}AR on resident lung leukocytes in acute I/R injury. Specific A_{2A}AR activation may be a promising therapeutic target for the prevention or treatment of pulmonary graft dysfunction in transplant patients.

Materials & Methods

Isolated, buffer-perfused mouse lung I/R model: An isolated, buffer-perfused mouse lung I/R model was used for this study which has been previously described by our group (Zhao *et al.*, *AJP Lung Cell Mol Physiol*, 291: 1018-26). Isolated lungs were subjected to ischemia (95% nitrogen and 5% carbon dioxide) for 60 min followed by 60 min of reperfusion with Krebs-Henseleit buffer. Hemodynamic and pulmonary function parameters were recorded throughout the reperfusion period by the PULMODYN data acquisition system (Hugo Sachs Elektronik).

Bronchoalveolar (BAL) fluid collection: After perfusion, lungs were lavaged with 0.5 ml saline for three times, and the BAL fluid was collected and centrifuged (1500 g for 15 min at 4°C).

Cytokine and chemokine protein analysis: A mouse-specific multiplex cytokine panel assay (Bio-Rad Laboratories) was used to quantify the cytokine and chemokine protein content in BAL fluid.

Lung wet-to-dry ratio: Lung wet-to-dry ratio was used as an indicator of pulmonary edema. The lower lobe of the right lung was harvested, weighed and placed in a vacuum oven (at 58°C) until a stable, dry weight was achieved. The ratio of lung wet weight to dry weight was then calculated.

Vascular permeability assay: At the completion of reperfusion, the perfusion buffer (Krebs Henseleit buffer) was replaced with 30 mg/ml BSA solution, and the lungs were perfused for an additional 5 min at the same flow rate as during reperfusion. As an indicator of lung injury, lung vascular permeability was assessed by measuring the BSA concentration in BAL fluid.

Myeloperoxidase (MPO) measurement: MPO was measured in BAL fluid as an indicator of neutrophil infiltration into alveolar spaces. The quantitative measurement of MPO was calculated by the mean absorbance values of each sample at 450 nm, and the final concentrations are expressed as ng/ml.

Results

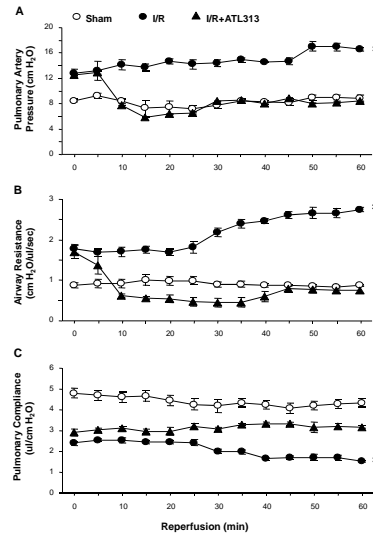


Figure 1. Temporal changes in pulmonary function during reperfusion. Pulmonary artery pressure (A), airway resistance (B) and pulmonary compliance (C) were measured throughout 60 min reperfusion in WT sham and I/R lungs. Lung function is significantly impaired after I/R compared to sham, and ATL313 (30 nM) significantly attenuated lung dysfunction. * p<0.01 I/R vs all.

Figure 3. Lung I/R injury is attenuated by A_{2A}AR activation. (A) Lung vascular permeability was assessed by measuring BSA concentration in BAL fluid. (B) Lung edema was measured by wet-to-dry weight ratio. Significant lung injury (increased vascular permeability and edema) occurred after I/R in WT mice which was attenuated by ATL313 treatment. Significant lung injury also occurred in A_{2A}AR KO mice after I/R, but ATL313 had no effect on A_{2A}AR KO lungs. *p<0.001 I/R vs sham; #p<0.01 WT I/R+ATL313 vs WT I/R.

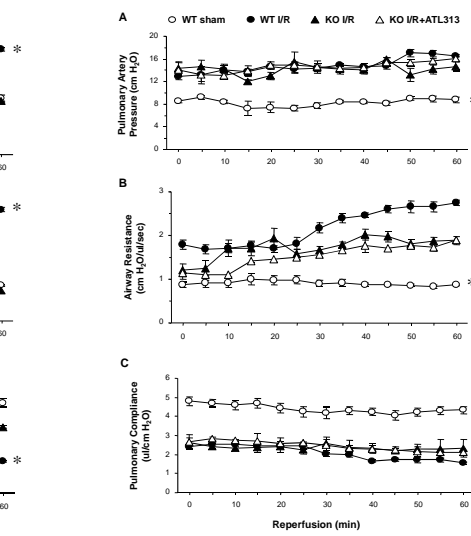
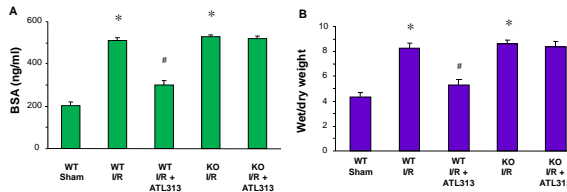


Figure 2. Pulmonary function during reperfusion in A_{2A}AR KO mice. Pulmonary artery pressure (A), airway resistance (B) and pulmonary compliance (C) were measured throughout 60 min reperfusion in A_{2A}AR KO lungs after I/R with and without the administration of ATL313. Lung function was significantly impaired in A_{2A}AR KO mice and ATL313 treatment offered no protection. * p<0.01 WT sham vs all.



Results

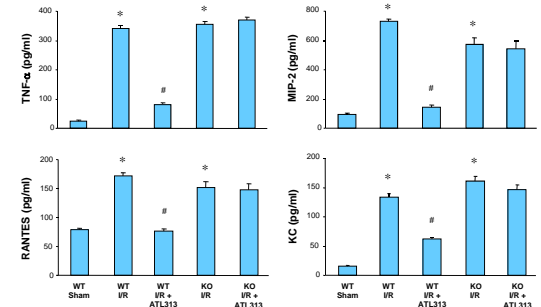


Figure 4. Cytokine/chemokine expression after lung I/R. The expression of TNF- α , MIP-2 (CXCL2), RANTES (CCL5), and KC (CXCL1) in BAL fluid were significantly induced after I/R in WT and A_{2A}AR KO mice (*p<0.01 vs WT sham). Cytokine/chemokine induction was significantly impaired by ATL313 treatment in WT mice but not in A_{2A}AR KO mice (#p<0.001, WT I/R+ATL313 vs WT I/R).

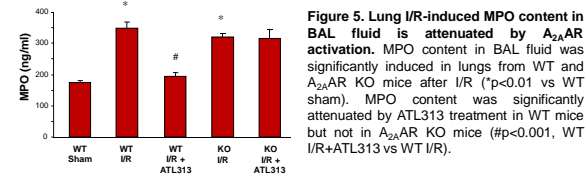


Figure 5. Lung I/R-induced MPO content in BAL fluid is attenuated by A_{2A}AR activation. MPO content in BAL fluid was significantly induced in lungs from WT and A_{2A}AR KO mice after I/R (*p<0.01 vs WT sham). MPO content was significantly attenuated by ATL313 treatment in WT mice but not in A_{2A}AR KO mice (#p<0.001, WT I/R+ATL313 vs WT I/R).

Summary

- Lung I/R injury is attenuated by ATL313, a specific adenosine A_{2A} receptor agonist.
- Adenosine A_{2A} receptor activation on resident lung cells (likely leukocytes or epithelial cells) play an important role in the amelioration of lung I/R injury.
- Adenosine A_{2A} receptor-mediated protection occurs via the attenuation of pro-inflammatory cytokines/chemokines and prevention of subsequent neutrophil activation.