Adenosine A₃ Receptor Activation Attenuates Lung Ischemia-Reperfusion Injury via a Neutrophil-Dependent Mechanism


Department of Surgery
University of Virginia Health System
Charlottesville, VA
Presenter Disclosure

ASHISH K. SHARMA

The following relationships exist related to this presentation:

No Relationships to Disclose
Lung Ischemia-Reperfusion (IR) Injury: The clinical problem after transplantation

- Primary graft dysfunction
- 20-30% of recipients
- Increased risk of Bronchiolitis Obliterans

Left lung transplant
IR Injury - Pathophysiology

- Restoration of blood flow:
  - Oxidative stress
  - Proinflammatory cytokines
  - Inflammation

- Innate immune response:
  - Macrophage
  - T cell
  - Neutrophils
Role of Adenosine in Inflammation

- Adenosine
  - Increased secretion in inflammation
  - Cytoprotective effects
  - Four receptors: $A_1$, $A_{2A}$, $A_{2B}$ and $A_3$

- Adenosine $A_3$ Receptor ($A_3$R)
  - $G_i$ protein-coupled receptor
  - ↓ cAMP
  - Expressed in:
    - Cells (PMNs, T cells, epithelial cells etc.)
    - Tissue (Lung, Liver, Kidney etc.)

- $A_3$R plays a pivotal role in cardiac, skeletal muscle, intestinal and kidney ischemia-reperfusion injury
Hypothesis

- $A_3R$ activation attenuates lung IR injury
  
  Effects occur in part via a neutrophil-dependent mechanism
In vivo mouse left lung hilar clamp model:

- 1 hr ischemia followed by 2 hrs reperfusion (IR)
- WT and A3R+/- mice:
  - Sham
  - IR
  - IR+Cl-IB-MECA (A3R agonist; 100 µg/kg i.v.)

Pulmonary function

- Airway Resistance
- Pulmonary Compliance
- Pulmonary Artery Pressure

Cytokine expression – BAL fluid

Myeloperoxidase (MPO) – BAL fluid

Edema – Lung wet/dry weight

Neutrophil infiltration – Immunohistochemistry
A$_3$R agonist improves lung function after IR injury

- Pulmonary Compliance (µL/cm H$_2$O)
- Pulmonary Artery Pressure (cm H$_2$O)
- Airway Resistance (cm H$_2$O/µL/sec)

* p<0.05 vs. Sham
# p<0.05 vs. IR
A3R agonist attenuates lung inflammation after IR

* p<0.05 vs. Sham
# p<0.05 vs. IR
A$_3$R agonist decreases lung edema after IR

* p<0.05 vs. Sham
# p<0.05 vs. IR
A₃R agonist decreases neutrophil infiltration \textit{in vivo}

- **MPO (ng/mL)**
  - **WT**
  - **A₃R⁻⁻**

- **Neutrophils/HPF**
  - **WT-Sham**
  - **WT-IR**
  - **WT-IR+MECA**

* * p<0.05 vs. Sham
# # p<0.05 vs. IR
A₃R agonist decreases neutrophil activation *in vitro*

**In vitro model:** Hypoxia/Reoxygenation (3/1 hr)

![Graph showing MPO (ng/mL) across different conditions: Normoxia, HR, HR+MECA, HR, HR+MECA.](image)

- * p<0.05 vs. Normoxia
- # p<0.05 vs. HR
A<sub>3</sub>R agonist decreases neutrophil chemotaxis *in vitro*

![Graph showing the effect of A<sub>3</sub>R agonist on neutrophil chemotaxis](chart.png)

- Media + Vehicle
- 10% FBS + Vehicle
- 10% FBS + MECA

* p<0.05 vs. Media + Vehicle
# p<0.05 vs. 10% FBS + Vehicle
Conclusions

- $A_3R$ activation attenuates lung dysfunction and inflammation after lung IR.

- The protective effects of $A_3R$ activation are due, at least in part, to attenuation of neutrophil activation and chemotaxis.

- The use of $A_3R$ agonists may be a novel therapeutic strategy to prevent lung IR injury after transplantation.
Acknowledgments

Kron / Laubach Lab Members

Vanessa Hajzus
Tony Herring
Sheila Hammond
Cynthia Dodson

Funding:

- NIH R01: HL092953
- NIH T32: HL007849-I0 11A