“What is New in Lung Ischemia-Reperfusion”

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Ischemia-Reperfusion Injury (Primary Graft Dysfunction)

IR injury = acute graft dysfunction in absence of rejection, technical, or infx.

Occurs in 20-30% of transplant recipients

Results in increased hospital stay, cost, & mortality

Leads to increased risk of Bronchiolitis Obliterans

### Primary Graft Dysfunction Grading

<table>
<thead>
<tr>
<th>GRADE</th>
<th>$\text{PO}_2/\text{FiO}_2$</th>
<th>X-ray</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$&gt;300$</td>
<td>NO Diffuse Infiltrates</td>
</tr>
<tr>
<td>1</td>
<td>$&gt;300$</td>
<td>Diffuse Infiltrates</td>
</tr>
<tr>
<td>2</td>
<td>$200 &lt; \text{P/F} &lt; 300$</td>
<td>Diffuse Infiltrates</td>
</tr>
<tr>
<td>3</td>
<td>$&lt;200$</td>
<td>Diffuse Infiltrates</td>
</tr>
</tbody>
</table>
Primary Graft Dysfunction and Bronchiolitis Obliterans Syndrome

Daud et al. AJRCCM. 175 (5): 507-13, 2007
Lung IR Injury - Pathophysiology

- Restoration of blood flow---> oxidative stress
- Endothelial cell dysfunction
- Synthesis of adhesion molecules
- Leukocyte activation
- INFLAMMATION
- Biphasic response:
  - Primary phase - macrophage activation, NKT cell activation
  - Secondary phase - neutrophil activation & infiltration
The "Big" picture
(Currently)

Prevention
Controlled reperfusion
WBC filters
Inhaled NO
Early ECMO
Lung Preservation

- LPD (Perfadex)
- Retrograde Flush
- Avoid Hyperinflation
Controlled Reperfusion

- Lower FiO2 at time of reperfusion to 40%
- Incrementally increase flow over 10-15 minutes to newly implanted lung
Treatment of IRI

• Diuresis & ventilatory support
  - ARDS/NETT ventilation, optimized positive end-expiratory pressure (PEEP)

• Inhaled nitric oxide
  - Decreases pulmonary artery pressure and improves PaO2 / FiO2 ratio

• Inhaled prostacyclin

• Sedation/Paralysis

• ECMO

• Most cases will resolve over 24-48 hours

Shargall and colleagues. J Heart Lung Transplant 2005; 24: 1489-1500
Treatment for IRI

- Complement Inhibition

- Pulmonary Surfactant

- Platelet-activating Factor Antagonist
ECMO

• ECMO
  - Severe cases

• Early institution may lead to diminished mortality
  - Severe Grade 3 PGD
  - Early (< 24 hours) compared to late (>24 hours) institution: 1-year survival 67% versus 0% respectively

• Long-term survival of patients with PGD requiring ECMO was inferior to that of patients who did not require ECMO

Mode of ECMO

• Venovenous
  - IJ-femoral vein

• Venoarterial
  - Extrathoracic (femoral vein and artery)
  - Intrathoracic (right atrium and aorta)

Venovenous ECMO improved results
(Hartwig and colleagues. Ann Thorac Surg 2005; 80; 1872-80.)
# Improved Mortality with ECMO

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Early Era (n=136)</th>
<th>Current Era (n=151)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 day mortality</td>
<td>11.8%</td>
<td>3.9%</td>
<td>0.02</td>
</tr>
<tr>
<td>Incidence of RI</td>
<td>25%</td>
<td>22.6%</td>
<td>0.73</td>
</tr>
<tr>
<td>Mortality with RI</td>
<td>38.2%</td>
<td>11.4%</td>
<td>0.02</td>
</tr>
<tr>
<td>Mortality of RI treated with ECMO</td>
<td>80%</td>
<td>25%</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean duration of ECMO</td>
<td>89 ± 29.8 hrs</td>
<td>30.6 ± 8 hrs</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Ailawadi and colleagues JTCVS 2009 137(3) 688-94.
The Potential “Big” picture

**Prevention**
- EVLP

**Treatment**
- Anti-oxidants
- NF-κB blockage
- Macrophage depletion
- NKT cell depletion
- Anti-TNF-α agents
- WBC filters
- Anti-IL-17
- A$_{2A}$AR agonists
- A$_{2B}$AR antagonists
TCV Lab at UVA
Adenosine

- Adenosine is a protective agent with anti-inflammatory effects
- Mediates its effects through four adenosine receptors (AR):
  - $A_1$AR
  - $A_2A$AR
  - $A_2B$AR
  - $A_3$AR
$A_{2A}AR$

- Anti-inflammatory
- Predominantly expressed on inflammatory cells (neutrophils, macrophages, monocytes, T cells)
- Inhibits neutrophil activation and infiltration
- Agonists reduce IR injury in various organs
Lung IR injury and $A_2A$AR activation: 3 animal models
\( A_{2A} AR \) - Mouse Lung IR

- Isolated, buffer-perfused mouse lungs
- 1 hr ischemia → 1 hr reperfusion +/- ATL313

Ashish Sharma et al.
*J Thorac Cardiovasc Surg* 139(2)
474-82, 2010.
A$_{2A}$AR – Rabbit Lung IR

- Isolated blood-perfused rabbit lungs
- ATL313 administration:
  - Pretreatment (1hr prior to ischemia, 100nM)
  - During reperfusion (100nM)
  - Pretreatment + Reperfusion

A$_{2A}$AR - Pig

- Pig left lung transplant model
- Most clinically relevant model
- 6 hrs cold ischemia $\rightarrow$ 4 hrs reperfusion +/- ATL-146e

Summary

$A_{2A}AR$ activation:

- Improves lung function
- Reduces lung injury & cytokine expression

Treatment with $A_{2A}AR$ agonist could prevent acute graft dysfunction in lung transplant patients
Pro and anti-inflammatory

- Macrophages/dendritic cells
  - Increases IL-10 and decreases IL-12 (anti-inflammatory)
- Type II alveolar epithelial cells
  - Increases IL-19 → increases monocytes TNF-α (pro-inflammatory)
- Lung fibroblasts
  - Increases IL-6 (induces differentiation to myofibroblasts; profibrotic) (IL-6 with TGF-β drives Th17 cells; increases immune response)
- Mast cells
  - Increases IL-4 and IL-8 (pro-inflammatory)
- Bronchial smooth muscle cells
  - Increases IL-6 and MCP-1 (pro-inflammatory) (IL-6 with TGF-β drives Th17 cells; increases immune response)
### Pulmonary Artery Pressure (cm H₂O)

<table>
<thead>
<tr>
<th>Group</th>
<th>Value (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Sham</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>WT IR</td>
<td>12 ± 1.2</td>
</tr>
<tr>
<td>KO IR</td>
<td>5 ± 0.2</td>
</tr>
</tbody>
</table>

*Significant difference compared to WT Sham (p < 0.05)

### Pulmonary Compliance (µl/cm H₂O)

<table>
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<th>Group</th>
<th>Value (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Sham</td>
<td>5 ± 0.5</td>
</tr>
<tr>
<td>WT IR</td>
<td>8 ± 1.2</td>
</tr>
<tr>
<td>KO IR</td>
<td>7 ± 0.3</td>
</tr>
</tbody>
</table>

*Significant difference compared to WT Sham (p < 0.05)

### Airway Resistance (cm H₂O/µl/sec)

<table>
<thead>
<tr>
<th>Group</th>
<th>Value (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT Sham</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>WT IR</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>KO IR</td>
<td>1.5 ± 0.1</td>
</tr>
</tbody>
</table>

*Significant difference compared to WT Sham (p < 0.05)
A2BAR KO Mice Bone Marrow into WT
No decrease in IRI

WT Mice Bone Marrow into A2BAR KO
Decrease in IRI

NOT the BM derived cells that are important
Summary

$A_{2B}AR$ antagonism:

- Improves lung function
- Reduces lung injury & cytokine expression

Treatment with $A_{2B}AR$ antagonist could prevent acute graft dysfunction in lung transplant patients
The Potential “Big” picture

**Prevention**

- EVLP

**Treatment**

- Anti-oxidants
- NF-κB blockage
- Macrophage depletion
- NKT cell depletion
- Anti-TNF-α agents
- WBC filters
- Anti-IL-17
- $A_{2A}$AR agonists
- $A_{2B}$AR antagonists

**ISCHEMIA**

**REPERFUSION**

- Oxidative Stress
- CD4+ T cell
- Epithelial cell
- Macrophage
- Neutrophil activation
- Lung Injury
- IL-17
- TNF-α
- KC (IL-8)
Ex Vivo Lung Perfusion (EVLP)

- Isolated circuit at normothermia
- Method to assess the quality of donor lungs and potentially repair the lungs.
  - Short term (1-2 hour) = Assessment
  - Long term (up to 12 hours) = Maintenance (preservation and treatment)
- Use of *ex-vivo* IL-10 gene therapy
  - Human lungs unsuitable
  - Intra-airway delivery of adenoviral vector encoding human interleukin-10
    - Significant improvement in function
    - Favorable shift from pro-inflammatory to anti-inflammatory cytokine expression
    - Recovery of alveolar-blood barrier integrity.

Methods

• **Prime:**
  - Steen Solution® (2 Lit)
  - Cefazolin (500 mg)
  - Heparin (10,000 IU)
  - Methylprednisolone (500 mg)

• **Control group (n=3)**
  - 14 hrs cold ischemia
  - EVLP with Steen Solution® only
  - Perfusion for 5 hours

• **Drug group (n=3)**
  - 14 hrs cold ischemia
  - EVLP with Steen Solution® and Adenosine A\textsubscript{2A} receptor agonist (CGS21680)
  - Drug provided (10µM) during the perfusion time, at 0 and 3 hr

Solution Used: Steen Solution® (Vitrolife, Sweden)

- Human serum albumin
- Dextran 40
- Glucose
- Sodium bicarbonate
- Calcium chloride
- Magnesium chloride
- Sodium chloride
- Potassium chloride
- Sodium dihydrogen phosphate
**Oxygenation Index**

- EVLP
- EVLP + A2AR agonist

* p-value: 0.04

**Wet to Dry weight ratio**

- EVLP (n=3)
- EVLP + A2AR Agonist (n=3)

* p-value: 0.02

**Mean airway pressure**

- EVLP (n=3)
- EVLP + A2AR Agonist (n=3)

* p-value: 0.03
Conclusions

• Ischemia-reperfusion injury remains a substantial problem in lung transplantation

• Current methods may help prevent and treat lung ischemia-reperfusion
  - WBC filters
  - Controlled reperfusion
  - Early use of inhaled NO
  - Early ECMO

• Novel preventions and treatments are being actively explored
  - Adenosine compounds approaching clinical trial implementation
  - *Ex-vivo* lung perfusion