A homo-dimer of annexin V protects against ischemia reperfusion injury in lung transplantation


Latner Thoracic Surgery Research Laboratories
Division of Thoracic Surgery, Toronto General Hospital
University of Toronto
Disclosure

Diannexin for use in this study was provided by Astellas Pharma Inc.
Primary Graft Dysfunction (PGD) - a significant challenge in lung transplant

Mechanism: ISCHEMIA REPERFUSION INJURY

- 10-20% incidence
- 5x increase in early post-op mortality
- Inferior long term survival
Cell death is an important contributing factor in ischemia reperfusion injury

**Early phase apoptosis**
- Phosphatidylserine (PS) externalization
- Caspase activation

**Late phase apoptosis**
- DNA fragmentation (TUNEL positive)
- Membrane breakdown (PI positive)
Cell death markers (M30 and HMGB-1) in ex vivo lung perfusion perfusate predicts PGD 3

AUC=0.78

AUC=0.86

Hashimoto / Keshavjee J Heart Lung Transpl April 2015.
Higher cell death signals detected during EVLP are associated with worse post transplant survival.

*\( p=0.03 \)

Hashimoto / Keshavjee *J Heart Lung Transpl* April 2015.
Hypothesis

Diannexin can ameliorate ischemia reperfusion induced acute lung injury in lung transplantation by blocking progression of apoptosis.
Impaired Microcirculation

Role of phosphatidylserine (PS) in ischemia reperfusion

Capillary
Blood flow ⇒
Endothelial cells
Anoxia / Apoptosis
Shielding of PS may prevent tissue injury after reperfusion

Diannexin = Di (two) - Annexin V
Syngeneic single lung transplant in rats

Pulmonary flush solution

Donor → 12 h cold ischemic time → Transplant

Recipient

Reperfusion

2 hours
Study groups

- **Diannexin Group** (DN group, n = 10)
- **Control Group** (C group, n = 10)

Pulmonary flush solution

**Donor**
- 12 h cold ischemic time

**Recipient**
- Reperfusion
- 2 hours
Study groups

**Diannexin Group**
(DN group, n = 10)

- Pulmonary flush solution + **Diannexin**
- 12 h cold ischemic time

**Control Group**
(C group, n = 10)

- Reperfusion
- 2 hours

**Recipient**

- I.V. **Diannexin**

- transplant

Diannexin: 1000 μg/kg
Study groups

**Diannexin Group**
(DN group, n = 10)

**Control Group**
(C group, n = 10)

Pulmonary flush solution + *Saline*

Donor → 12 h cold ischemic time → Recipient

Reperfusion

2 hours

*Saline*

I.V.
Diannexin Improved Gas Exchange

Pulmonary vein pO₂  Pulmonary vein pCO₂

<table>
<thead>
<tr>
<th></th>
<th>C group</th>
<th>DN group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

n = 10 each  *p<0.01, **p=.04
Improved peak airway pressure with diannexin treatment

N = 10 each
*p<0.05
Diannexin binding was demonstrated in the lung grafts.

At 2h after reperfusion

Green: Diannexin; Blue: DAPI = nucleus
Reduced alveolar fibrin deposit by diannexin

* $p=0.04$

$n = 10$ each
Reduced cell death with diannexin treatment

- **TUNEL staining**: p=0.15
  - n =10 each

- **Plasma M30 (caspase cleaved cytokeratin 18)**: *p=0.01
  - n =10 each

- **PARP cleavage in the graft**: *p<0.01
  - n =6 each
Lower pro-inflammatory cytokine expression in the diannexin treated group

**mRNA**

- **IL-6**
  - C group: 0.05
  - DN group: 0.02
  - *p=0.01*

- **MIP-2**
  - C group: 1.0
  - DN group: 0.5
  - *p=0.03*

**Protein**

- **IL-6**
  - C group: 0.3
  - DN group: 0.2
  - *p<0.01*

- **MIP-2**
  - C group: 0.2
  - DN group: 0.15
  - *p=0.84*

**n =10 each**
Conclusion

• Apoptotic cell death is an important component of ischemia reperfusion injury

• Shielding of exposed PS with diannexin:
  o Decreased apoptotic cell death
  o Improved lung injury
  o Reduced the secondary inflammatory cytokine response
  o Significantly improved post transplant lung function

• A promising potential therapy to be applied prior to or at the time of implantation to prevent and treat primary graft dysfunction
Acknowledgements

- Supervisor: Dr. S Keshavjee
- Co-PI: Dr. M Liu, Dr. M Cypel

- Latner Thoracic Surgery Research Laboratories
- Keio University School of Medicine
- Ishidsu Shun Memorial Scholarship
- Mitsukoshi Health and Welfare Foundation
- Obradovich Family Thoracic Surgery Research Scholarship
The Toronto Lung Transplant Program