How can human models of ALI inform clinical trials

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How can human models of ALI inform clinical trials

• Limitations of animal models
• Human models of ALI
  – Ex-vivo lung perfusion
  – LPS inhalation
  – Surgical models of ALI
• Identifying novel therapies using human models
Stepwise approach

- Phase 3 clinical trial
- Phase 2 clinical trial
- Proof of concept clinical trial
- Human models of ALI
- Observational data
- Animal data
- In vitro data
Cytokine Storm in a Phase 1 Trial of the Anti-CD28 Monoclonal Antibody TGN1412

The simple truths

• Human cells are not
  – Animal cells
  – Cancer or virally transformed cell lines

• Humans are not
  – Animals
Monocyte depletion reduces pulmonary inflammation in a murine ALI model

Dhaliwal et al. AJRCCM (in press)
Monocyte depletion does NOT reduce pulmonary inflammation in a human ALI model.

- MD arm n=15
- Sham arm n=14

**Graph 1:**
- % neutrophils in BALF
- MD arm: 40-60%
- Sham arm: 42-60%
- p=0.69

**Graph 2:**
- BAL protein (g/L)
- MD arm: 0.25-0.35 g/L
- Sham arm: 0.25-0.40 g/L
- p=0.30
New models of ALI

Phase 3 clinical trial
Phase 2 clinical trial
Proof of concept clinical trial
Human models of ALI
Observational data
Animal data
In vitro data

Surgical models
LPS model
Human Ex-Vivo Lung Perfusion
Human ex-vivo lung perfusion
Human ex-vivo lung perfusion

Peristaltic pump

Perfusate Reservoir

37°C C water bath

Lung

CPAP 10 cm H₂O
95% O₂, 5% CO₂

Pulmonary Artery
Pressure = 10 - 12 mm Hg

Left Atrial
Pressure = 0 mm Hg

Lee JW et al. PNAS 2009 106:16357-62
Human ex-vivo lung perfusion

Surgical preparation of lung
Begin perfusion without blood

Intervention

- AFC and BAL in RUL or LUL (Control)
- 1 hr
- Lung temp 36°C
- Apply CPAP

AFC and BAL in RML or LLL (Endotoxin)
- 4 hrs
- Add 100 ml fresh whole blood to perfusate
- Instill 0.1 mg/kg of endotoxin into the airspaces of the RML or LLL
Histological evidence of ALI

Absolute Neutrophil Counts

Control Lung Lobe: $9 \pm 6 \times 10^6$ cells
LPS Lung Lobe: $25 \pm 25 \times 10^6$ cells
Impaired alveolar fluid clearance

Alveolar fluid clearance (%/h)

- Control
- No Blood
- Blood

+ Endotoxin

* Indicates significant difference
Increased pulmonary oedema is associated with mortality in ALI

Craig et al. CCM 2011
Cytokine response in ex-vivo lung consistent with ALI
Mesenchymal stem cells (MSC) improves pulmonary inflammation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Absolute Neutrophil Counts</th>
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<tbody>
<tr>
<td>Control Lung Lobe</td>
<td>9 ± 6 x 10^6 cells</td>
</tr>
<tr>
<td>LPS Lung Lobe</td>
<td>25 ± 25 x 10^6 cells</td>
</tr>
<tr>
<td>LPS + MSC conditioned media Lung Lobe</td>
<td>13 ± 11 x 10^6 cells</td>
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<tr>
<td>LPS + MSC Lung Lobe</td>
<td>6 ± 5 x 10^6 cells</td>
</tr>
</tbody>
</table>

* P = 0.1017

P = 0.0171
Surgical preparation of lung
Perfusion

- Lung temp 36°C
- Apply CPAP

1 hr

Baseline AFC
(Control)

4 hrs

AFC
(Treatment)

Intervention

Human ex-vivo lung perfusion
In vivo lung injury
MSCs improve alveolar fluid clearance and is mediated by KGF

* P significant by ANOVA vs. Control
√ P significant by ANOVA vs. MSC IV
Inhaled LPS as an *in vivo* model to study pulmonary inflammation in healthy subjects

- FEV1
- BAL
- FEV1
- Plasma

50 µg LPS inhalation
E. Coli serotype O26:B6 (Sigma)
Breath activated dosimeter
Inhaled LPS induces inflammatory cytokines in pulmonary compartment

- TNF (pg/ml) $p = 0.0006$, unpaired t test
- IL-1β (pg/ml) $p = 0.0056$, unpaired t test
- IL-8 (pg/ml) $p < 0.0001$, unpaired t test
Inhaled LPS induces pulmonary epithelial activation/injury
Increased BAL protein as a measure of alveolar barrier function
Simvastatin in the inhaled LPS model of ALI

Treatment with a clinically relevant dose of simvastatin will reduce pulmonary inflammation induced by LPS inhalation in humans.

Day 1-3
Simvastatin (n=20) or placebo (n=10)

Day 4
Simvastatin or placebo under supervision

1 hr 6 hr 18 hr
Plasma FEV1 LPS inhalation FEV1 BAL FEV1 Plasma

Shyamsundar et al. AJRCCM 2009 179:1107-1114
<table>
<thead>
<tr>
<th>TIME</th>
<th>Baseline</th>
<th>Day 4</th>
<th>Day 4 Following LPS inhalation</th>
<th>Day 4 Prior to bronchoscopy</th>
<th>Day 5 24 hrs after LPS inhalational</th>
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<tbody>
<tr>
<td>Symptom assessment</td>
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<td>Vital signs and pulse oximetry</td>
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<td>Routine haematology and renal function</td>
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<td>Creatinine kinase (CK)</td>
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<td>Liver function tests</td>
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<tr>
<td>Lung function (FEV1 and FVC)</td>
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<tr>
<td>Adverse event assessment</td>
<td>*</td>
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</tbody>
</table>
Simvastatin decreases pulmonary TNF following LPS inhalation

* $p < 0.05$ vs placebo
Lovastatin decreases pulmonary inflammation measured by FDG PET following LPS instillation

Chen et al. AJRCCM 2009 180:533-539
Elective surgery as a cause of acute lung injury

- ALI/ARDS associated with:
  - Oesophagectomy 15-40%
  - Lung resection 1-7%
  - Cardiopulmonary bypass (CPB) 1.3%
  - Elective AAA repair
One lung ventilation as a model of direct pulmonary injury

- Overdistension
- Hyperperfusion
- Hyperoxia

- Ischaemia-reperfusion
- Atelectotrauma
- Surgical trauma (contusion & lymphatic disruption)

Local and systemic release of inflammatory mediators
Pulmonary inflammation associated with one lung ventilation

Cooling tube and insulating jacket
Connection to patient via endo-tracheal tube

Expiratory flow to ventilator
RTube, Respiratory Research Inc
One-way valve

Inspiratory flow from ventilator

Moloney et al. 2004 AJRCCM 169:64
Simvastatin decreases alveolar epithelial and systemic endothelial injury during one lung ventilation.

Two way ANOVA, $p = 0.02$

Mann Whitney U, $p = 0.01$
Cardiopulmonary bypass as a systemic model causing pulmonary inflammation

Moloney et al. 2004 AJRCCM 169:64

![Graph showing CRP and BAL neutrophils](image)

- CRP mg/L
  - Pre operative
  - Post operative day 0
  - Post operative day 1
  - Post operative day 2
  - Post operative day 3

- BAL neutrophils (%)
  - Pre CABG
  - Post CABG

P < 0.0001
n = 20

***
AAA repair induces systemic and pulmonary inflammation.

**IL-8 (ng/ml)**

- **Pre-op**
- **Post-op**

* $p < 0.001$

**EBC pH**

- **Pre Clamp**
- **Post Clamp**

$ p = 0.008$
AAA repair associated with impaired oxygenation

![Box plot comparing PF Ratio before and after clamp](image)

- Pre-Clamp
- Post-Clamp

$p=0.008$
Limitations of models

• Model specific mechanisms
  – LPS induced inflammation
  – Ischaemia reperfusion
• Short-term
• Need to use to test treatment as well as pre-treatment interventions
Conclusions

- Variety of human models characterised by pulmonary and systemic injury reflecting ALI
- Useful in testing novel therapeutic agents to identify ineffective therapy
- Improve design of subsequent clinical trials of pharmacological agents