Selective adrenergic receptor modulators and acute lung injury

Jean-Francois Pittet, M.D.
Department of Anesthesiology
University of Alabama at Birmingham, USA
- I have no competing interest to declare.
- This work has been funded by the National Institute of Health.
Vectorial ion transport across the alveolar epithelium
β-adrenergic agonists-stimulated ion transport across the alveolar epithelium
Inhibition of alveolar epithelial ion and fluid transport in association with higher mortality in patients with acute lung injury

Ware LB, Am J Respir Crit Care Med 2001, 163:1376
Rationale

- Intravenous or airspace β-adrenergic agonists stimulate alveolar epithelial fluid transport in multiple studies including normal animal and ex-vivo human lungs via a cAMP-dependent mechanism.

- Phase 2 clinical trial (BALTI study) showed that sustained treatment with intravenous beta-agonists reduces extravascular lung water in patients with acute lung injury.
  
  **Perkins GD, Am J Respir Crit Care Med 2006, 173:281**

- Phase 3 clinical trials: ALTA NIH/ARDS Network (β-agonist I.T. to ALI/ARDS patients) and BALTI-2 studies were negative.
  
  **Matthay MA, Am J Respir Crit Care Med 2011 184:561**  
  **Smith G, Lancet 2012 379:229**

- Additionally, BALTI Prevent study (β2-agonist I.T. in patients undergoing esophagectomy to prevent ARDS) was negative.
  
  **Perkins GD et al, Am J Respir Crit Care Med 2014 189:674**
Hypothesis

Inflammatory mediators released during the initial phase of the inflammatory response may inhibit the β-adrenergic agonist-stimulated alveolar epithelial fluid transport in patients with acute lung injury
IL-8, a mediator and marker of mortality in ARDS patients

- Predominant neutrophil chemokine in distal airspace of patient with ARDS. Marker of mortality.
  
  *Amat A, Crit Care Med 2000, 28:57*

- Critical mediator of ARDS (acid aspiration or smoke inhalation, and hemorrhagic shock).
  
  *Laffon M, Am J Respir Crit Care Med 1999, 160:1443  

- Marker of mortality in patients with ARDS.
  
  *Parsons PE, Crit Care Med 2005; 33:1*
Relationship between pulmonary edema fluid levels of IL-8 and alveolar fluid clearance in patients with acute lung injury

A

B

Pulmonary Edema Fluid IL-8 (pg/ml)

Alveolar Fluid Clearance (%/h)

Alveolar Fluid Clearance (%/h)

IL-8 <4000 pg/ml

IL-8 >4000 pg/ml
CINC-1 decreases $\beta_2$AR agonist-stimulated alveolar epithelial fluid transport in an in vivo model of hemorrhagic shock in rat.
**CINC-1 decreases β_2AR agonist-stimulated CFTR-specific Cl\(^-\) transport across primary rat ATII cells**

**A**

![Graph showing short-circuit current over time](image)

**B**

![Bar graph showing epinephrine-dependent Cl\(^-\) transport](image)

**C**

![Bar graph showing epinephrine-dependent Cl\(^-\) transport](image)

**D**

![Bar graph showing terbutaline-dependent Cl\(^-\) transport](image)

---

<table>
<thead>
<tr>
<th>CINC-1 (ng/ml)</th>
<th>0</th>
<th>1</th>
<th>3</th>
<th>10</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>CINC-1 Ab</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CINC-1 Ab</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

---

<table>
<thead>
<tr>
<th>CINC-1 (10ng/ml)</th>
<th>-</th>
<th>0.5h</th>
<th>0.5h</th>
<th>6h</th>
<th>6h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont IgG</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>CINC-1 Ab</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
CINC-1 decreases β₂AR agonist-stimulated CFTR-specific Cl⁻ transport across primary human ATII cell monolayers

E.

F.

[Graph showing the decrease in epinephrine-dependent Cl⁻ transport with IL-8 treatment over time and concentration]
CINC-1 abolishes $\beta_2$AR agonist-induced whole cell currents in rat ATII cells

A

B

Epinephrine (200µM)  -  -  -  +  +  +
CFTR (inh)-172  -  +  -  -  +  -
CINC-1 (10ng/ml)  -  -  +  -  -  +
Exposure to CINC-1 inhibits the β₂AR-dependent activation of the cAMP/PKA pathway in primary rat ATII cell monolayers

A

B

C

Exposure to CINC-1 inhibits the β₂AR-dependent activation of the cAMP/PKA pathway in primary rat ATII cell monolayers
Hypothesis
Short exposure (30min) to CINC-1 decreases $\beta_2$AR agonist-stimulated Cl- transport across primary rat ATII cell monolayers via a PI3K-dependent mechanism.
Inhibition of PI3 kinase restores $\beta_2$AR agonist-stimulated alveolar fluid in a rat model of hemorrhagic shock

![Graph showing epinephrine-dependent AFC (% of epinephrine-treated control) for different conditions.](image)
Exposure to CINC-1 decreases $\beta_2$AR agonist-stimulated Cl- transport across primary rat ATII cell monolayers via a GRK2-dependent mechanism.

A

<table>
<thead>
<tr>
<th>Condition</th>
<th>GRK2 (membrane)</th>
<th>Densitometry Units (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CINC-1 (10ng/ml)</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Cont IgG (3µg/ml)</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>CINC-1 Ab (3µg/ml)</td>
<td>-</td>
<td>300</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>Condition</th>
<th>Densitometry Units (% of Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CINC-1 (10ng/ml)</td>
<td>0</td>
</tr>
<tr>
<td>p-$\beta_2$AR (Ser355)</td>
<td>400</td>
</tr>
</tbody>
</table>

* $p<0.05$ compared to control
Short exposure (30 min) to CINC-1 decreases β₂AR agonist-stimulated Cl⁻ transport across primary rat ATII cell monolayers via a GRK2-dependent mechanism.

**Figure C**

Epinephrine-dependent Cl⁻ transport (%)

- CINC-1 (10ng/ml)
- GRK2inh (75µm)

**Figure D**

Epinephrine-dependent Cl⁻ transport (%)

- CINC-1 (10ng/ml)
- NP-PKCζ (2.5µg/ml)
- P-PKCζ (2.5µg/ml)

**Figure E**

125I-ICYP specific binding (fmol/mg)

- Control
- CINC-1 (10ng/ml, 30')
Prolonged exposure (6h) to CINC-1 inhibits the \( \beta_2 \)AR-dependent activation of the PKA and CFTR promoter activity, gene expression, and function, via a downregulation of the \( \beta_2 \)-adrenergic receptor at the cell membrane.
CINC-1-induced heterologous internalization of the $\beta_2$AR in L2 cells is PI3-kinase-dependent.
IL-8/CINC1 inhibits β-adrenergic agonist-stimulated ion transport across the alveolar epithelium via a heterologous desensitization and downregulation of the β2-adrenergic receptors.

Our data indicate that inhibition of inflammatory mediators, such as IL-8, or their signaling pathway combined with LOW-DOSE β2-adrenergic agonists may stimulate net alveolar fluid clearance in patients with ARDS.
Ongoing work

IL-8 and TGF-β have a synergistic inhibitory effect on the cAMP-stimulated ion transport across the alveolar epithelium via the activation of two different PI3K isoforms.
ACKNOWLEDGMENTS

Investigators:
- Jeremie Roux, PhD, UCSF
- Michel Carles, MD, PhD, Nice
- Carmel M. McNicholas, PhD, UAB
- Brant Wagener MD, PhD, UAB

Collaborators:
- Kevan Shokat, PhD, UCSF
- Michael Matthay, MD, UCSF
- Jae-Woo Lee, MD, UCSF
- Sadis Matalon, PhD, UAB