Precision-cut lung slices for profiling of inhaled compounds

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Workshop on Drug Transporters in the Lungs

September 22\textsuperscript{nd} 2016
Goals of inhaled drug development

Maximising patient benefit and safety: Targeted delivery by clever design of drug molecule and formulation

- Beta$_2$-agonists$^1$
  - Hypokalemia
  - Tachycardia
  - Tremor

- Anticholinergics$^2$
  - Dry mouth$^2$
  - Glaucoma$^3$
  - Urinary retention$^2$

- Corticosteroids$^4$
  - Cortisol suppression
  - Growth suppression
  - Osteoporosis

Profiling inhaled drugs in lung slices

- Drug uptake and binding in lung tissue
- Carrier-mediated cellular uptake
- Prediction of lung retention
- A model for pulmonary metabolism
Lung retention is driven by compound basicity

*Intratracheal* drug administration to rats with terminal lung sampling

Cooper A et al. *Curr Drug Metab* 2012; 13: 457–73
Lung disposition of soluble bronchodilators (salmeterol)
Preparation of slices of agarose inflated rat lung

One lung (5 lobes) yields ~40 lung slices with 500 µm thickness

Unbound drug volume of distribution in the lung \((V_{u,\text{lung}})\)

- At equilibrium of the system \(C_{\text{buffer}} = C_{u,\text{lung,tissue}}\)

- \(V_{u,\text{lung}}\) defined as drug amount tissue (mol/g tissue) (total conc, \(C_{\text{slice}}\)) divided by the unbound drug concentration in the interstitium \((C_{u,\text{lung,tissue}})\)

\[
V_{u,\text{lung}}(\text{mL/g tissue}) = \frac{C_{\text{slice}} - V_0 \ast C_{\text{buffer}}}{(1 - V_0) \ast C_{\text{buffer}}}
\]

\(V_{u,\text{lung}}\) quantifies the extent of cellular uptake “tissue binding” at steady-state
Lysosomal trapping drives $V_{u,\text{lung}}$ for propranolol

- Monensin transports ions across the cell membrane, reducing the pH-gradient

90% of pulmonary propranolol is in the lysosomes


Estimation of intracellular free drug accumulation: $K_{p,uu,cell}$

$$K_{p,uu,cell} = \frac{C_{u,cell}}{C_{u,lung ISF}} = f_{u,lung} \times V_{u,lung}$$

The unbound drug partition coefficient of the cell ($K_{p,uu,cell}$):

- $K_{p,uu,cell} \sim 1$ suggest that tissue binding to tissue is the main distribution mechanism
- $K_{p,uu,cell}$ values $>1$ indicate carrier-mediated influx or lysosomal trapping
- $K_{p,uu,cell}$ values $<1$ indicates cellular efflux

High $K_{p,uu,cell}$ for MPP+ and ipratropium suggests (OCT(N)) mediated uptake
\( V_{u,\text{lung}} \) of inhaled compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ion class</th>
<th>( pK_a )</th>
<th>( V_{u,\text{lung}} ) (mL/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD3199</td>
<td>Dibase</td>
<td>6.9, 8.1</td>
<td>2970 (43)</td>
</tr>
<tr>
<td>Formoterol</td>
<td>Base</td>
<td>7.7</td>
<td>36.9 (1.3)</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>Base</td>
<td>8.3</td>
<td>109 (12)</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>Cation</td>
<td>12.9</td>
<td>12.9 (0.7)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>Base</td>
<td>9.5</td>
<td>500 (15)</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>Base</td>
<td>9.2</td>
<td>2.21 (0.23)</td>
</tr>
<tr>
<td>Salmeterol</td>
<td>Base</td>
<td>9.1</td>
<td>864 (46)</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>Cation</td>
<td>3.87</td>
<td>3.87 (0.20)</td>
</tr>
</tbody>
</table>

- Long-acting \( \beta \)-agonists average of 1000 mL/g lung tissue
- Short-acting \( \beta \)-agonist 2.2 mL/g lung tissue

\( V_{u,\text{lung}} \) appears correlated with bronchodilatory effect duration
Prediction of lung retention using rat lung slices

Modified experimental setup

• Pre-load slices with compound
• Transfer to large vessel to study rate of drug release
• Monensin can be included to inhibit lysosomal trapping
• Allows comparison with IT PK data of % of dose remaining in lung

Backstrom E., et al. J Pharm Sci 2016 (manuscript accepted)
Monensin inhibits drug $V_{u,lung}$ and retention in slices

Lysosomal trapping is a plausible mechanism for lung retention and prolonged effect duration of beta-agonist bronchodilators

Backstrom E., et al. J Pharm Sci 2016 (manuscript accepted)
In vitro lung retention can be predicted by lung slices

Lung slices represent an integrated experimental system to predict lung retention

Discrepancies between long in vivo $t\frac{1}{2}$ of and shorter (initial) $t\frac{1}{2}$ may point towards vectorial transport across epithelial that is not captured in slices.

Backstrom E., et al. J Pharm Sci 2016 (manuscript accepted)
Physiologically-based pharmacokinetic modelling helps the design of well tolerated and effective medicines.

Mathematical modelling provides insight into unobservable compartments of the lung.

Boger E. et al. CPT pharmacometrics and systems pharmacology 2016 (In press)
Conclusions on utility of lung slices

Determine extent and mechanisms of inhaled drug tissue distribution and metabolism

Determine the dynamics of drug release as an integrated experiments to predict lung retention

PBPK modelling should be used to contextualize and to explore the role of measured properties
Acknowledgments

Erica Bäckström, Post-Doc at Uppsala University, (currently AstraZeneca Gothenburg)

Margareta Hammarlund-Udenaes, Uppsala University

Anders Lundquist, Bioanalyst at AstraZeneca Gothenburg

Per Bäckman, inhalation biopharmaceutics at AstraZeneca Gothenburg (currently Mylan)

Pär Ewing, Elin Boger, and many others
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Back-ups
Methodology

Rat lung slices
Viability of lung slices and time to equilibrium

- Lactate dehydrogenase (LDH) release to determine the viability of the slices
- Time to equilibrium for different ion classes

24h, 82%