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Precision-cut lung slices for profiling of inhaled compounds

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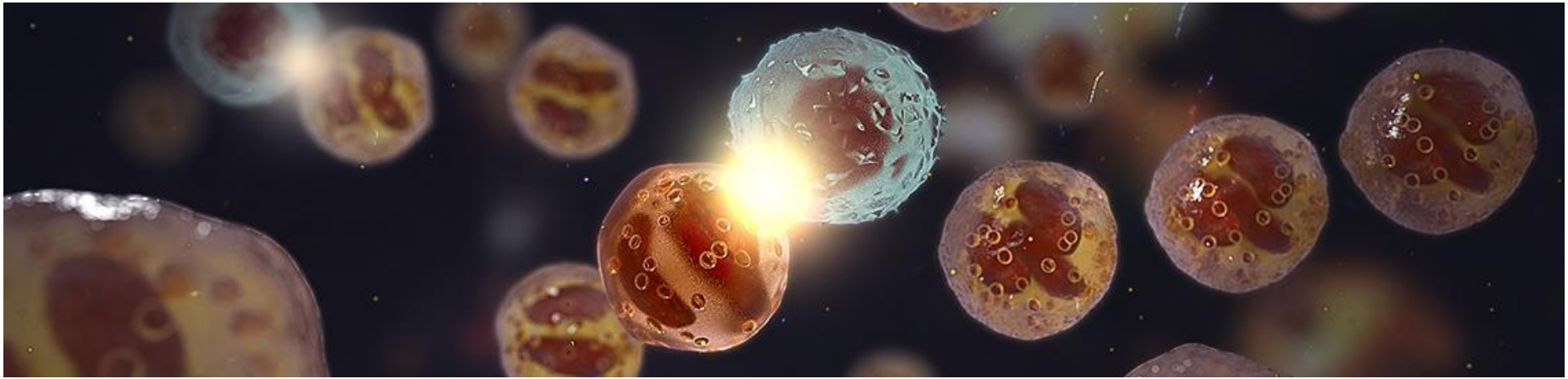
Workshop on Drug Transporters in the Lungs

September 22nd 2016



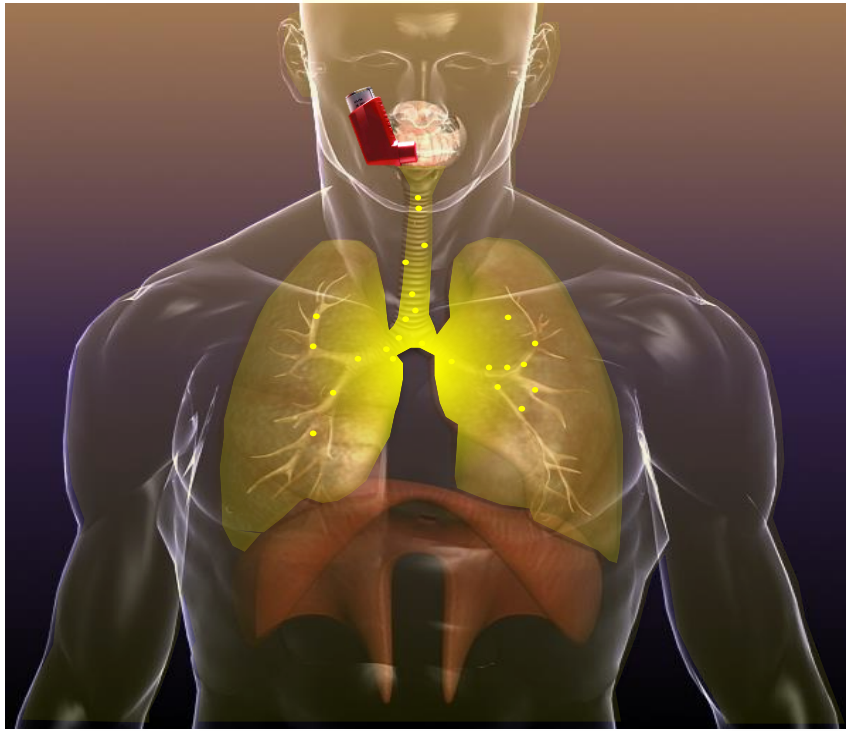
Goals of inhaled drug development

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

Beta$_2$-agonists[1]	Anticholinergics[2]	Corticosteroids[4]
• Hypokalemia • Tachycardia • Tremor	• Dry mouth[2] • Glaucoma[3] • Urinary retention[2]	• Cortisol suppression • Growth suppression • Osteoporosis

1. Lulich KM et al. *Med Toxicol* 1986; 1: 286–99; 2. Scullion JE. *Int J Chron Obstruct Pulmon Dis* 2007; 2: 33–40;
3. Spiriva® Respimat® 2.5 µg, inhalation solution. Summary of Product Characteristics. Available from: https://www.medicines.org.uk/emc/medicine/20134l;
4. Dahl R. *Respir Med* 2006; 100: 1307–17.

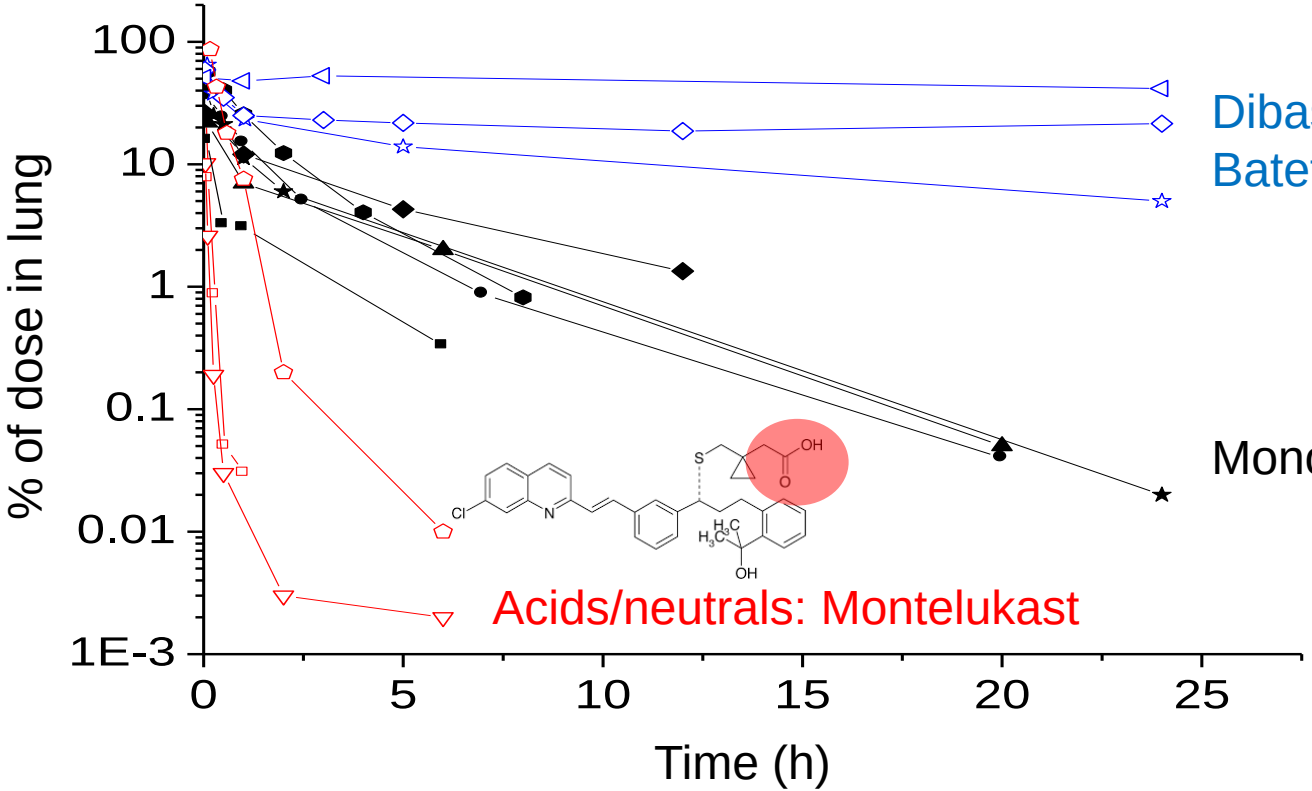
Profiling inhaled drugs in lung slices



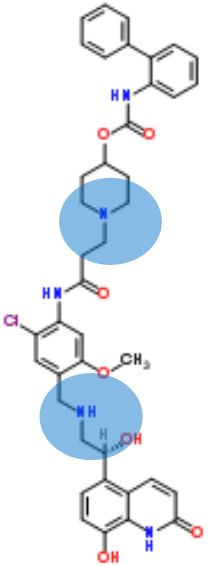
- Drug uptake and binding in lung tissue
- Carrier-mediated cellular uptake
- Prediction of lung retention
- A model for pulmonary metabolism

Lung retention is driven by compound basicity

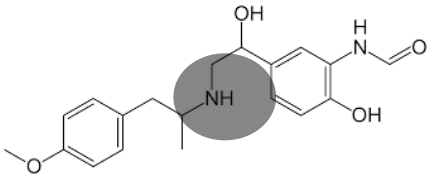
Intratracheal drug administration to rats with terminal lung sampling



Dibases: Batefenterol, etc.

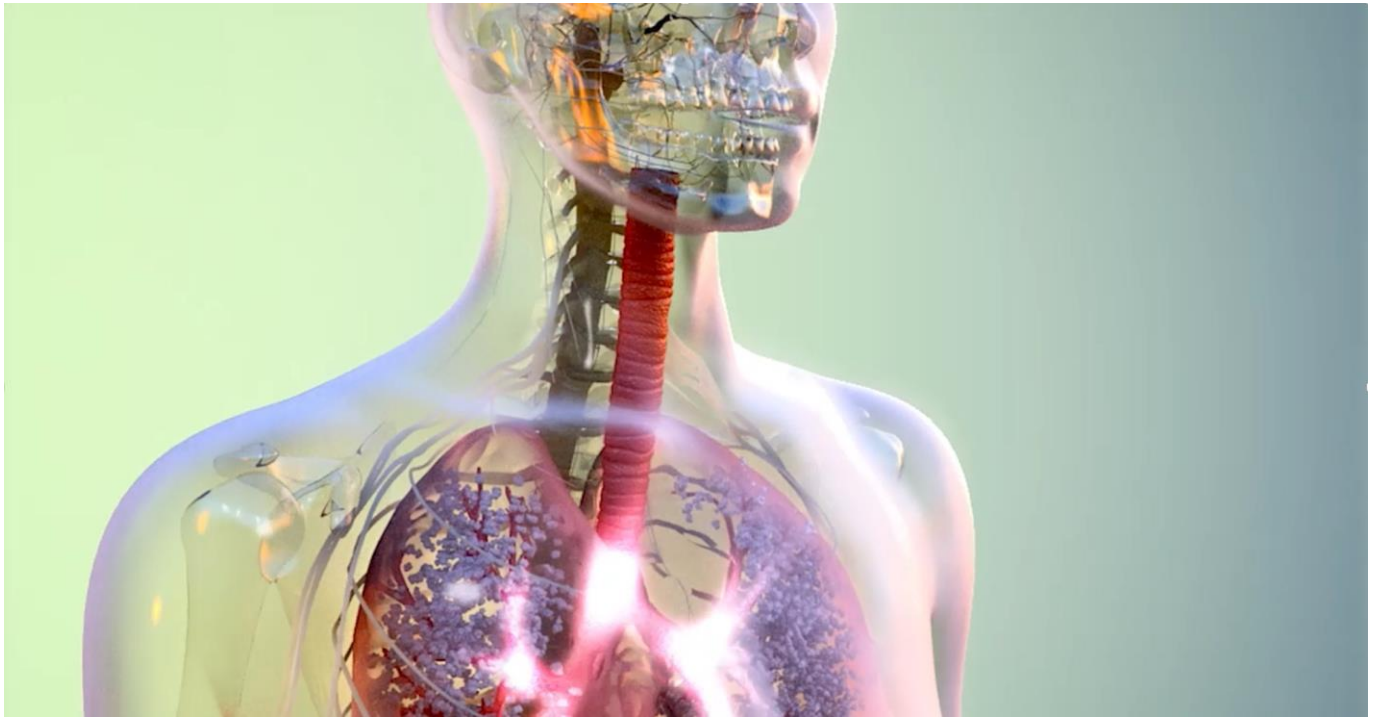


Monobases: Formoterol, etc.

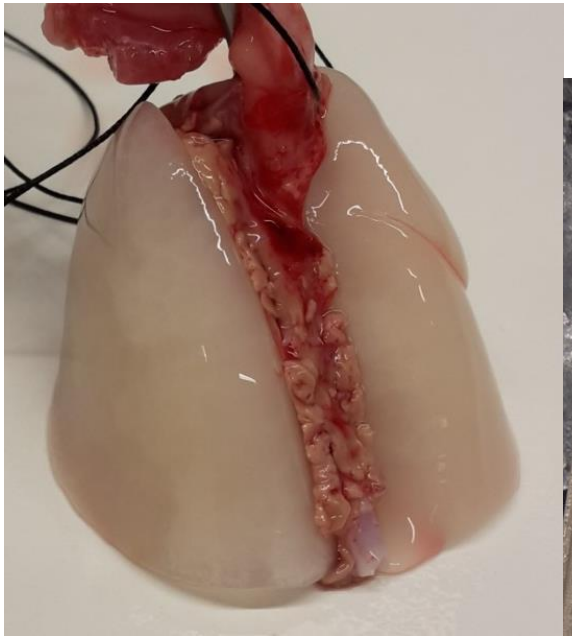


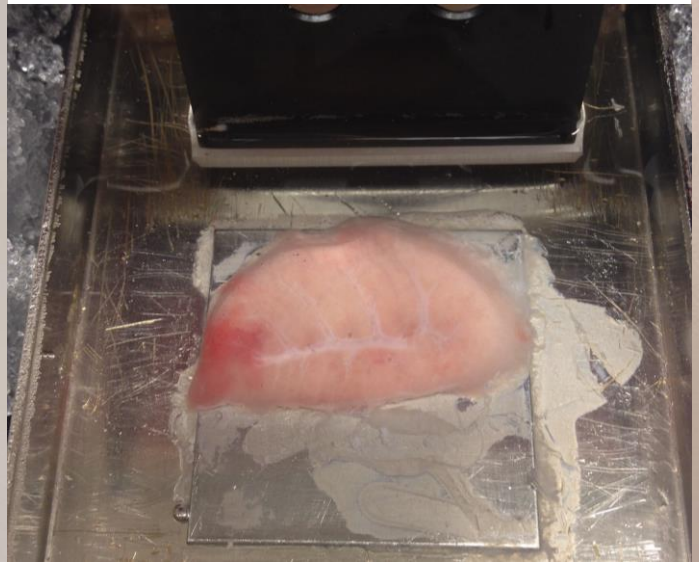
Cooper A et al. *Curr Drug Metab* 2012; 13: 457–73

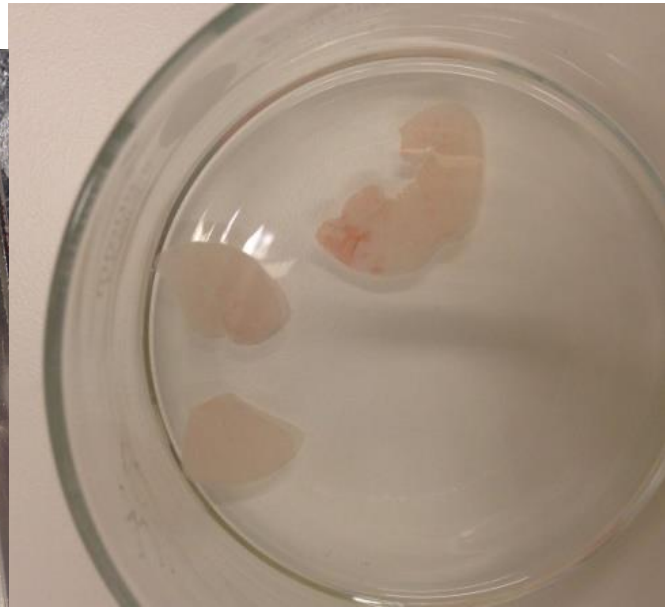
Lung disposition of soluble bronchodilators (salmeterol)



Preparation of slices of agarose inflated rat lung







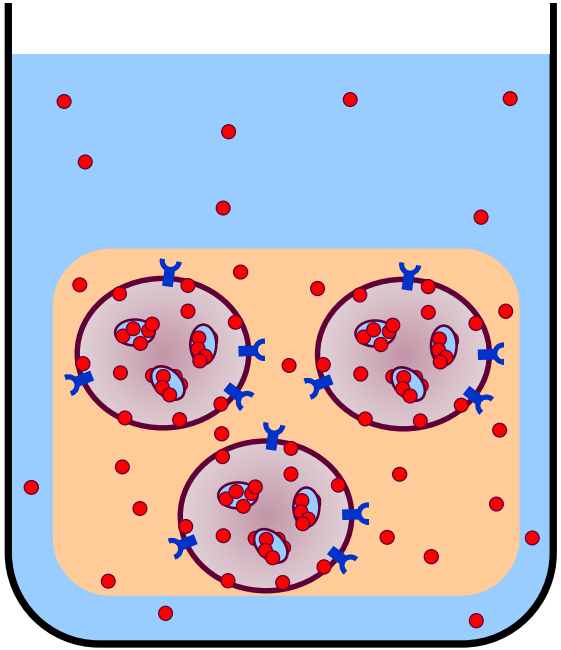
One lung (5 lobes) yields ~40 lung slices with 500 µm thickness

Backstrom E., et al. J Pharm Sci 2016, 105:838-845

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

- At equilibrium of the system C_{buffer}=$C_{u,lung,tissue}$
- $V_{u,lung}$ defined as drug amount tissue (mol/g tissue) (total conc, C_{slice}) divided by the unbound drug concentration in the interstitium ($C_{u,lung,tissue}$)

$$V_{u,lung}(mL/g\ tissue) = \frac{C_{slice} - V_0 * C_{buffer}}{(1 - V_0) * C_{buffer}}$$

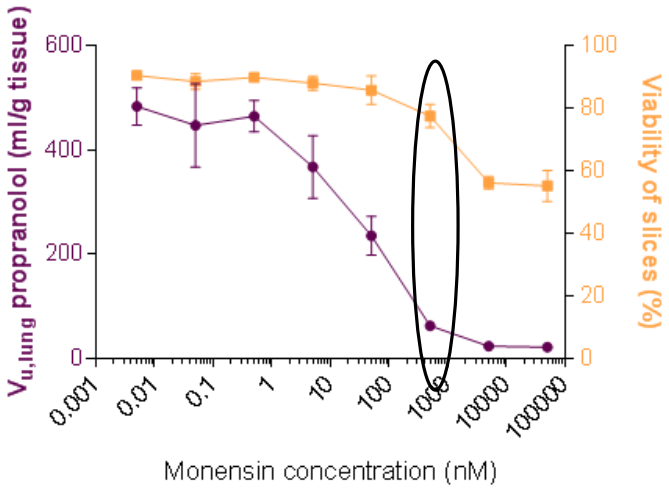


$V_{u,lung}$ quantifies the extent of cellular uptake "tissue binding" at steady-state

Backstrom E., et al. J Pharm Sci 2016, 105:838-845

Lysosomal trapping drives $V_{u,lung}$ for propranolol

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



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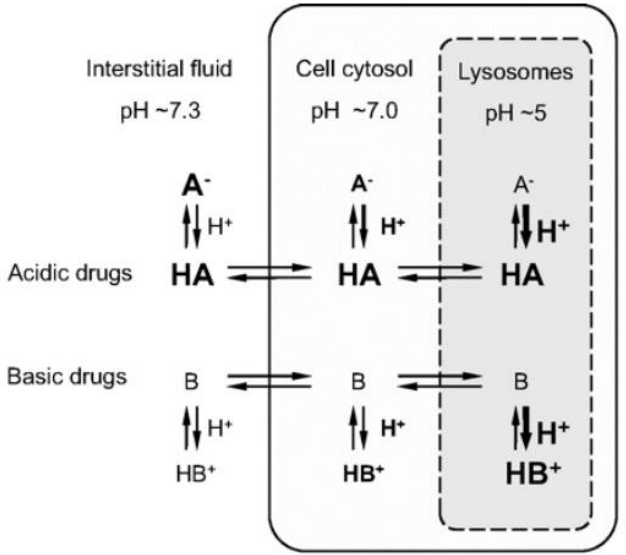


Fridén M., et al. Drug Metab Dispos. 2011 Mar;39(3):353-62.

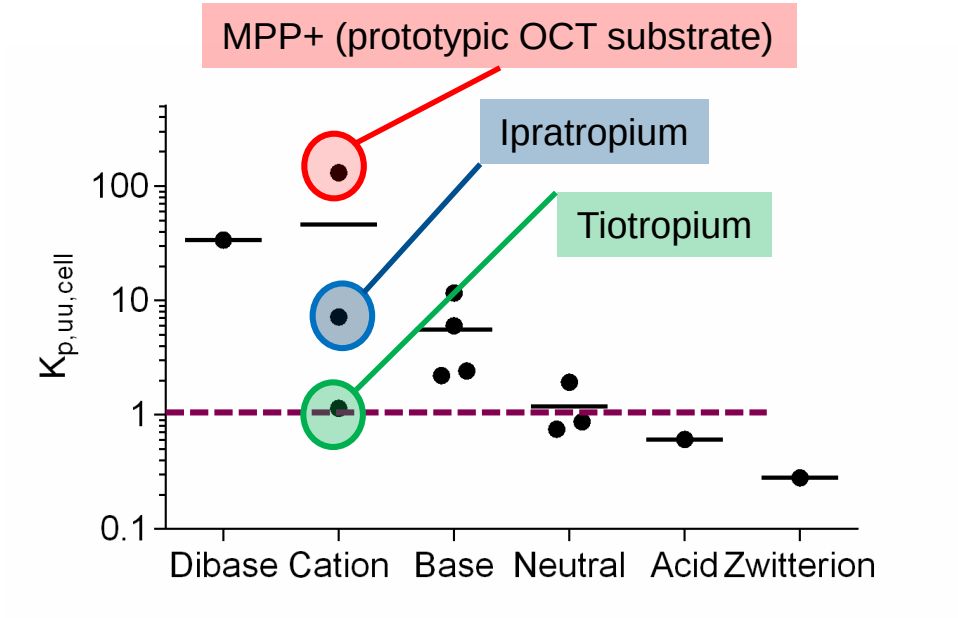
90% of pulmonary propranolol is in the lysosomes

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

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

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

- $K_{p,uu,cell}$ ~ 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

Backstrom E., et al. J Pharm Sci 2016, 105:838-845

$V_{u,lung}$ of inhaled compounds

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

- Long-acting β-agonists average of 1000 mL/g lung tissue
- Short-acting β-agonist 2.2 mL/g lung tissue

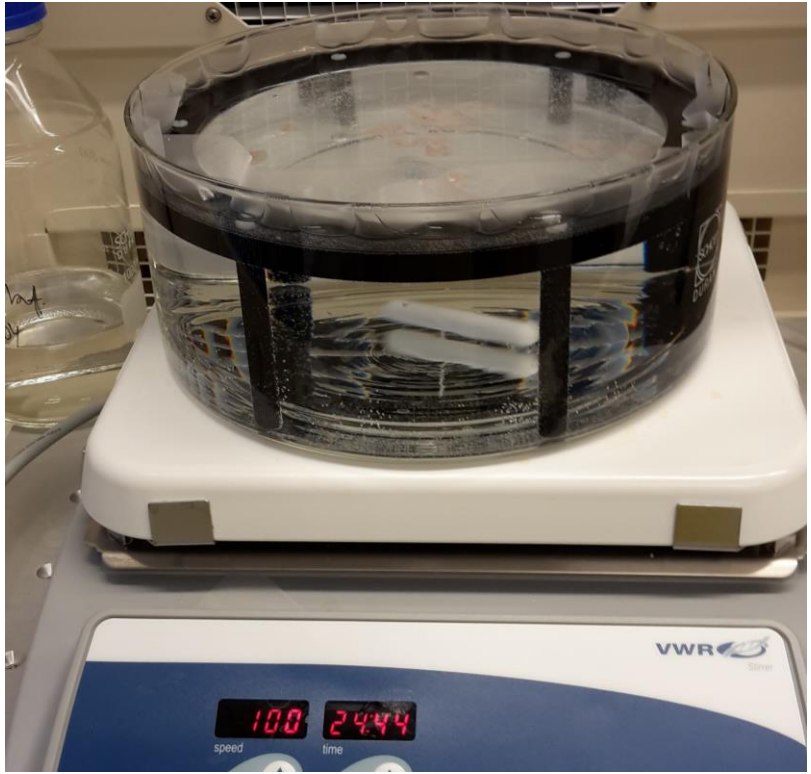
$V_{u,lung}$ appears correlated with bronchodilatory effect duration

Bäckström, E., et al. J Pharm Sci. 2015 Jul 15.

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



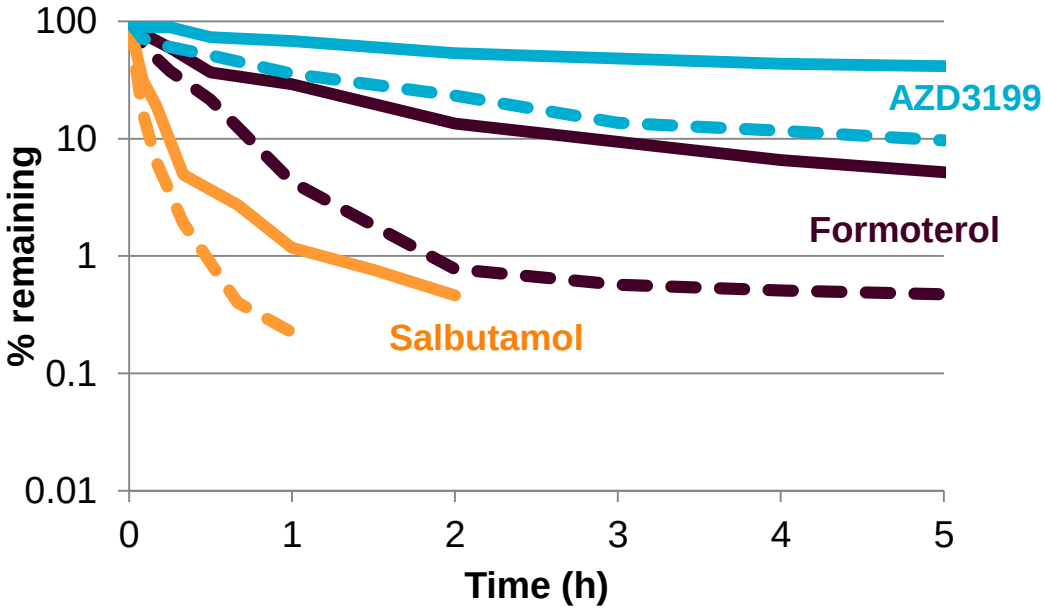
Backstrom E., et al. J Pharm Sci 2016 (manuscript accepted)

Monensin inhibits drug $V_{u,lung}$ and retention in slices

with monensin

without monensin

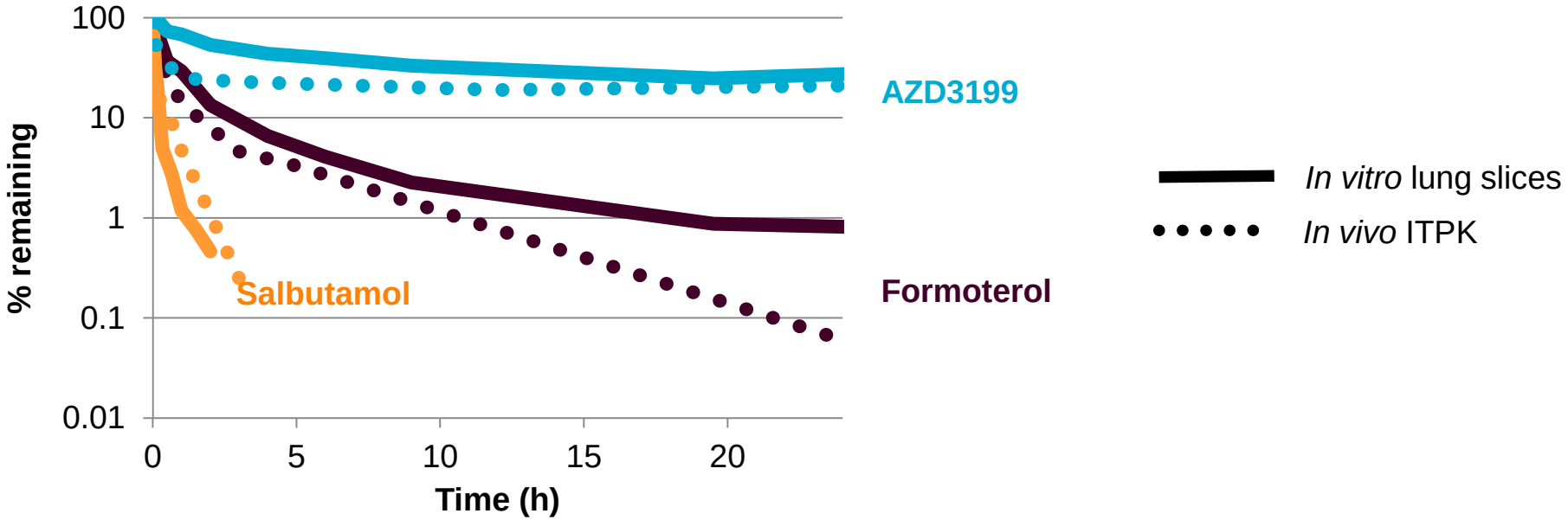
Compound	$V_{u,lung}$ 24 h incubation	$V_{u,lung}$ 5 h incubation	$V_{u,lung}$ 5 h incubation with monensin	% reduction with monensin
AZD3199	3000±43	400±24	110±7.1	73
Formoterol	37±1.3	32±4.6	9.2±1.3	71
Salbutamol	2.2±0.23	3.3±0.51	2.5±1.2	N.S.



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

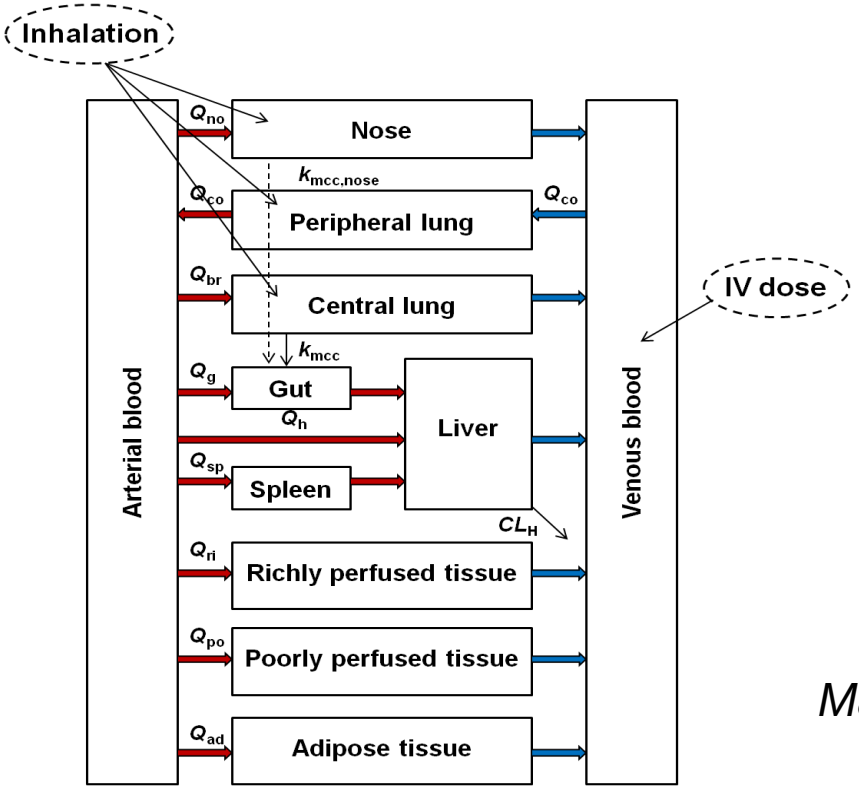


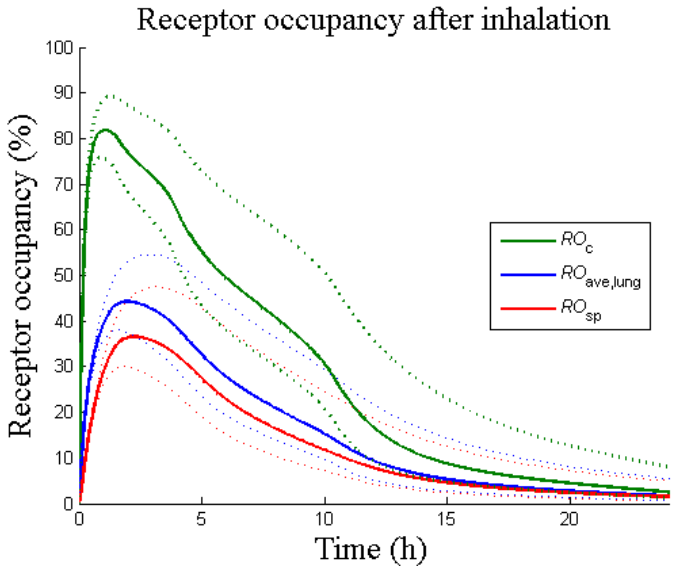
Lung slices represent an integrated experimental system to predict lung retention

Discrepancies between long in vivo t½ of and shorter (initial) t½ 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





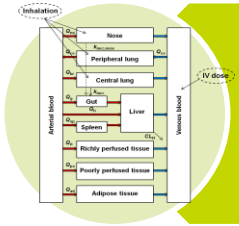
Mathematical modelling provides insight into unobservable compartments of the lung

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

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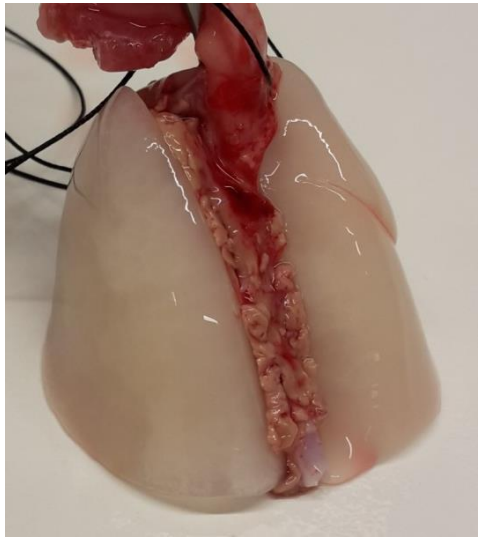
Pär Ewing, Elin Boger, and many others

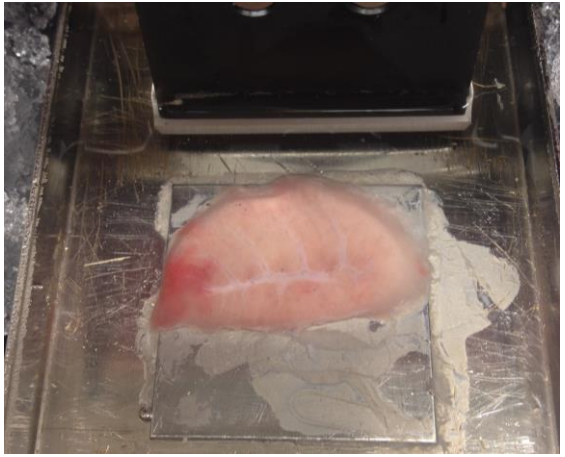
Confidentiality Notice

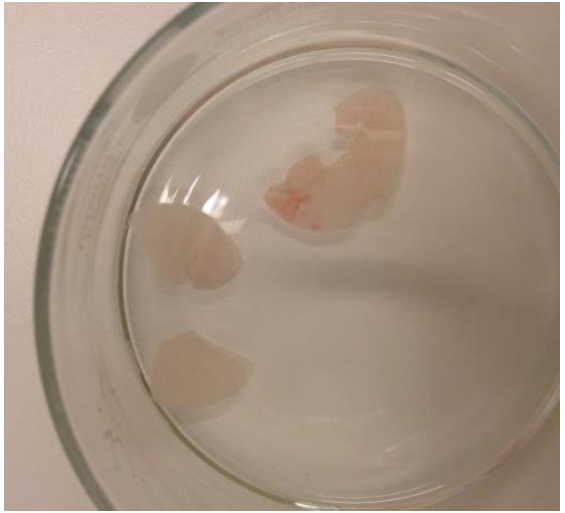
Back-ups

Methodology

Rat lung slices

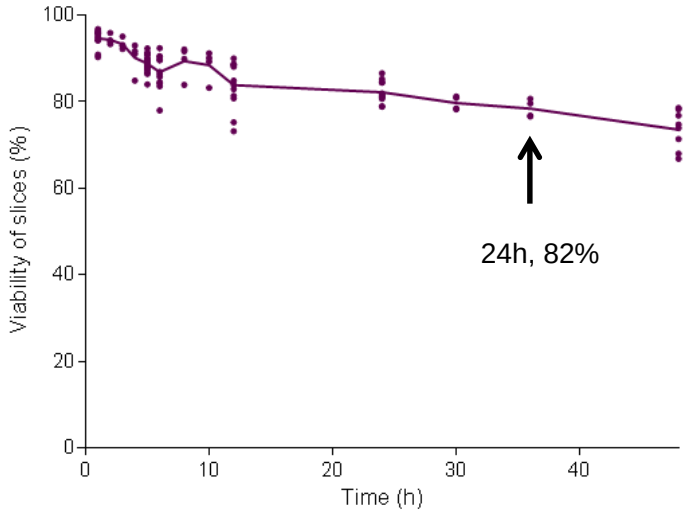




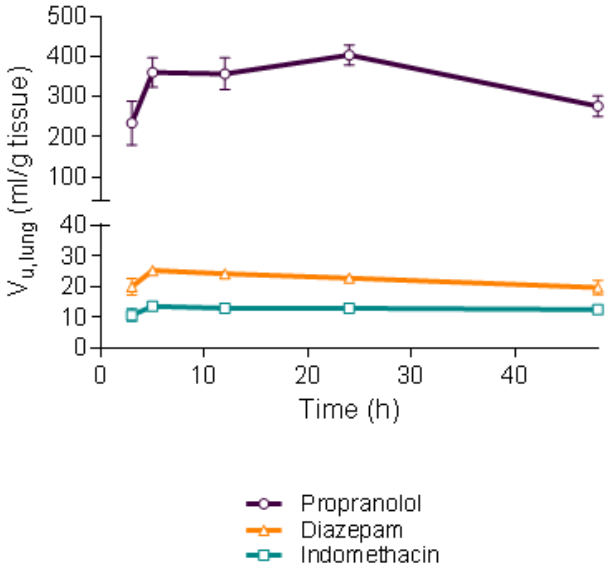


Viability of lung slices and time to equilibrium

• Lactate dehydrogenase (LDH) release to determine the viability of the slices



• Time to equilibrium for different ion classes



Bäckström, E., et al. J Pharm Sci. 2015 Jul 15.