SCHOOL OF PHARMACY
UNIVERSITY of WASHINGTON

Pursuing the holy grail of predicting and verifying tissue drug concentrations: A proteomics and PET imaging approach

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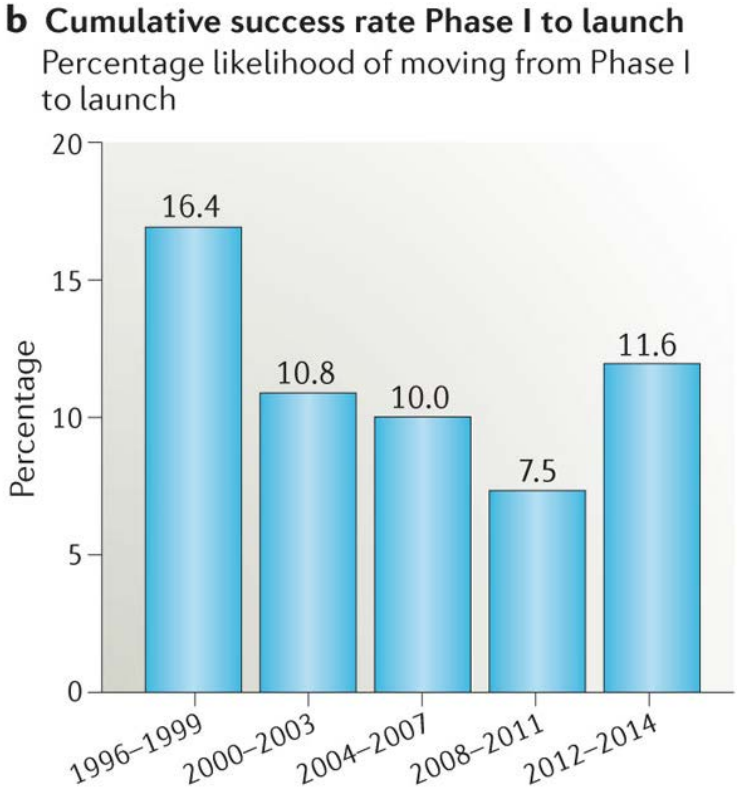
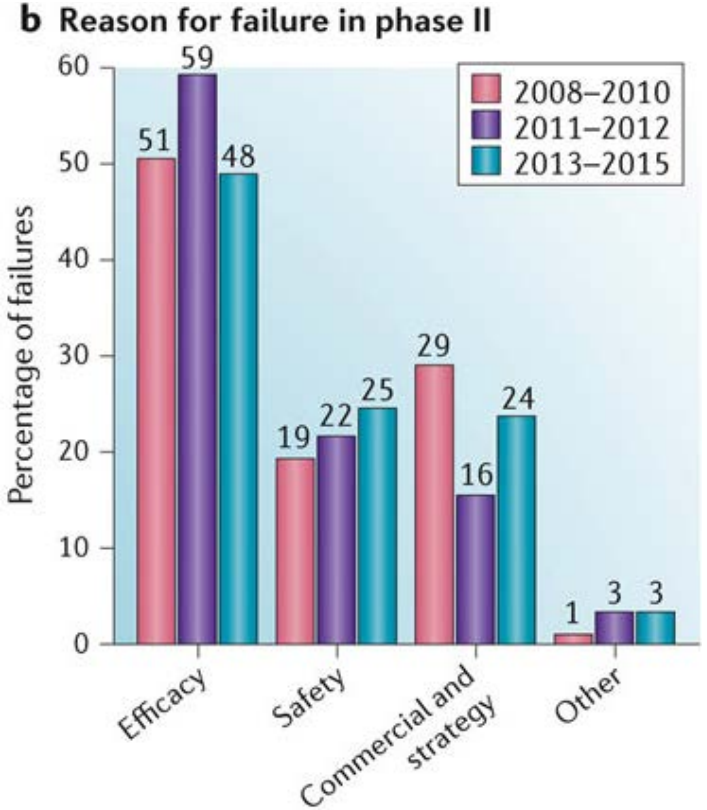
Failure Rate and Reasons for Failure in Drug Development





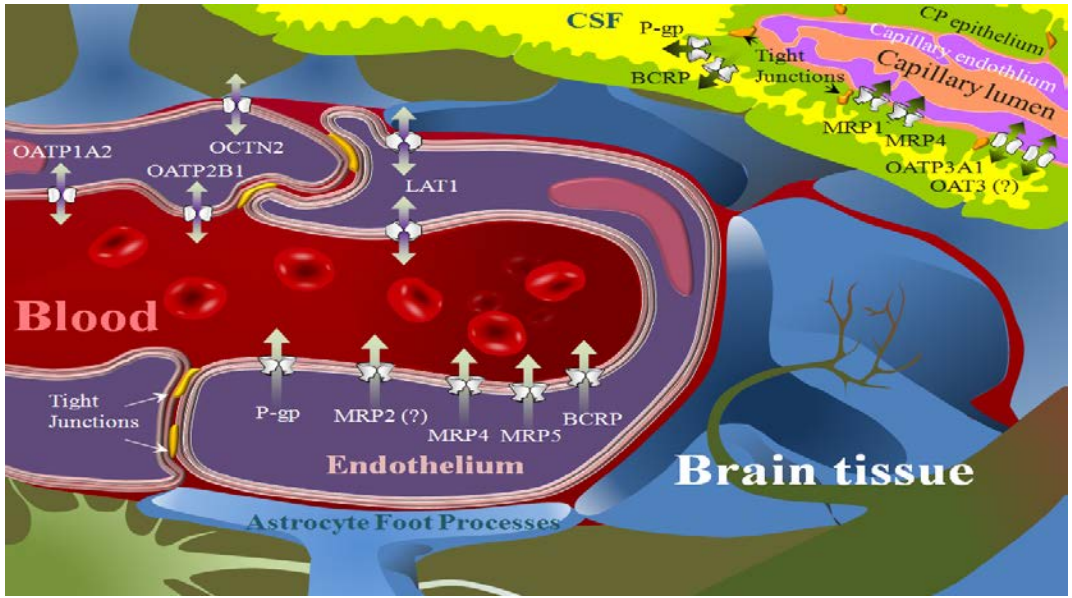
Smietana et al., Nature Reviews Drug Discovery 15, 379–380 (2016)

Harrison, Nature Reviews Drug Discovery 15, 817–818 (2016)

One Possible Reason for Lack of Drug Efficacy & Safety

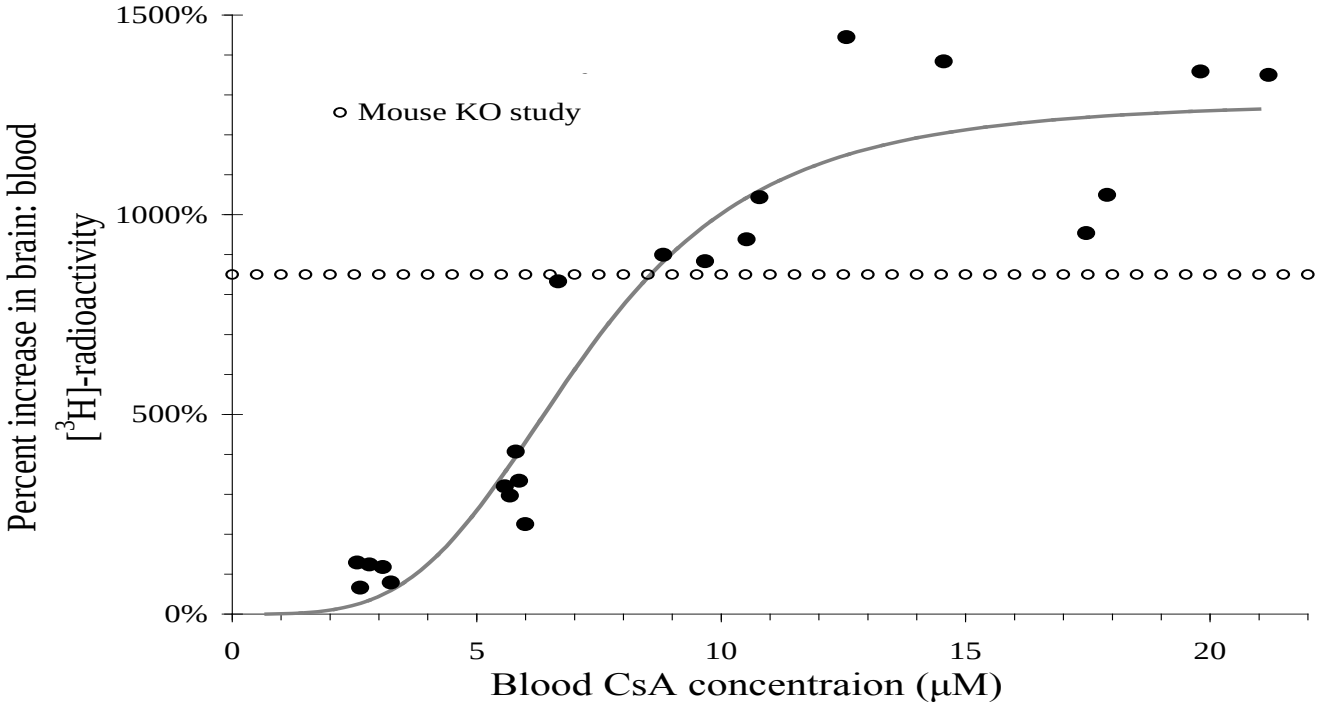
- Unable to measure or predict tissue conc. of drugs
- Unbound plasma conc. ≠ unbound tissue conc. if transporters are involved, i.e. asymmetry between blood and tissue drug conc. (e.g. liver:blood due to OATPs)

Asymmetry In Brain:Blood Drug Conc. Due to Blood-Brain Barrier Efflux Transporters



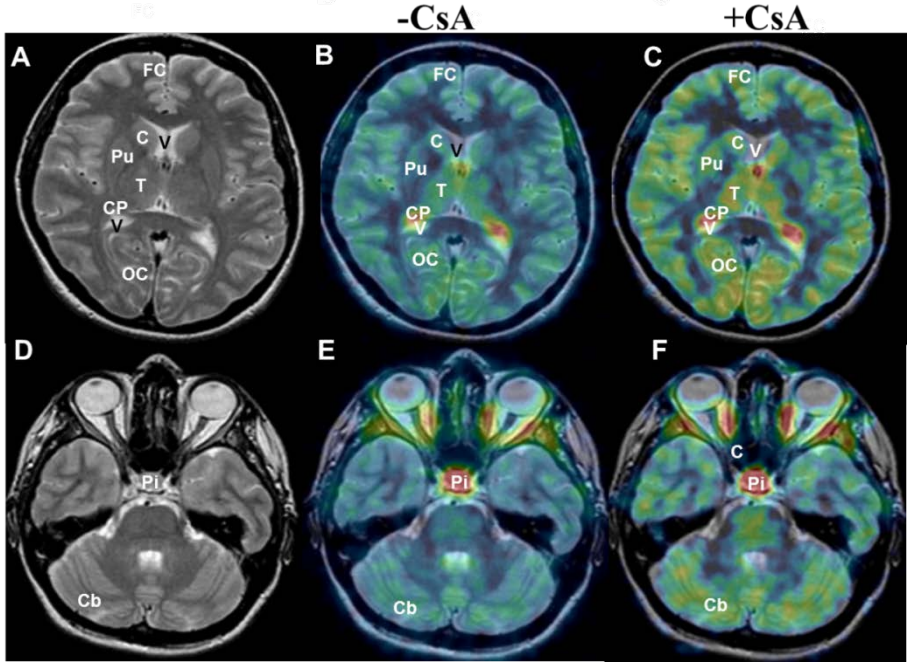
Eyal, Hsiao & Unadkat Pharmacol. Ther., 2009

P-gp at the Rat BBB Results in Asymmetry in Brain:Blood Conc. of Verapamil (P-gp substrate)

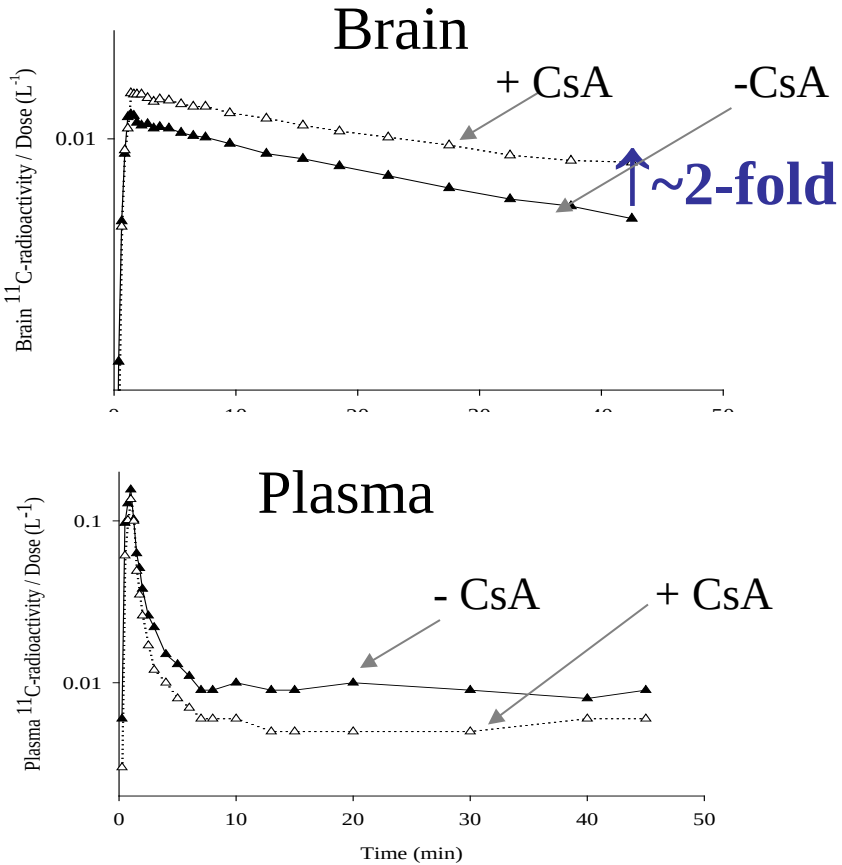


Hsiao et al J Pharmacol Exp Ther. 2006

Asymmetry in Drug Conc. at the Human Brain:Blood Barrier: P-gp Efflux of ^{11}C-Verapamil



^{11}C-verapamil AUC_{brain}:AUC_{blood} (20 min) - 0.42±0.04



Eyal et al., *Clin Pharmacol Ther.* 2010

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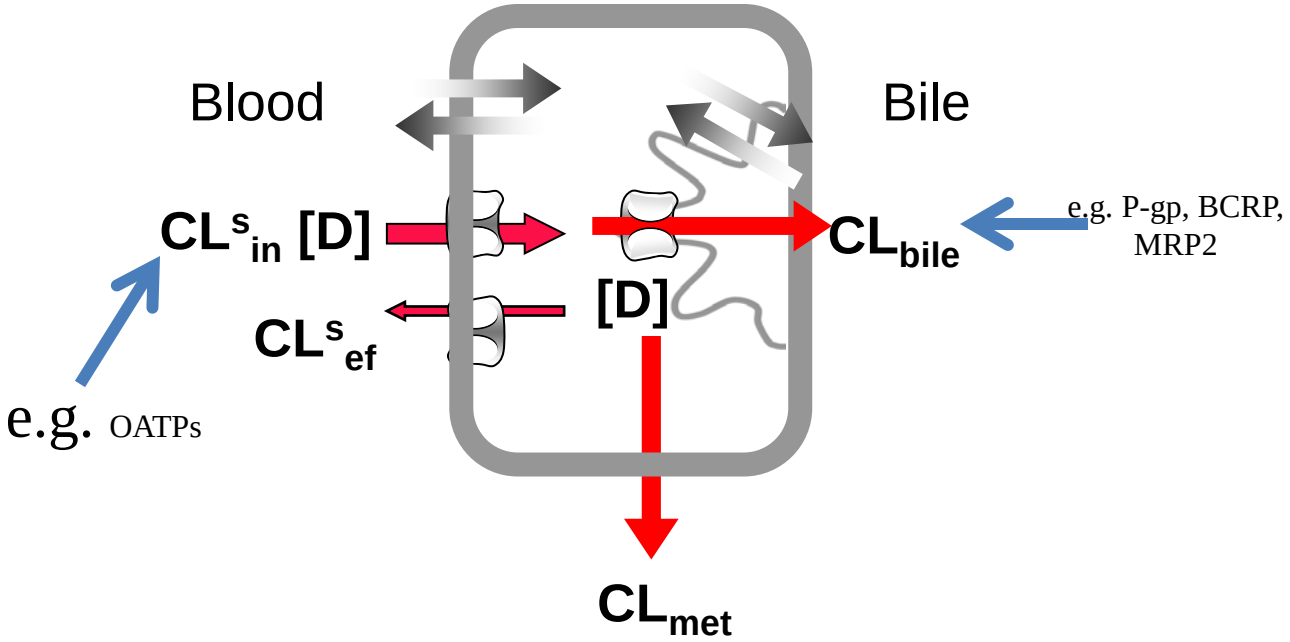
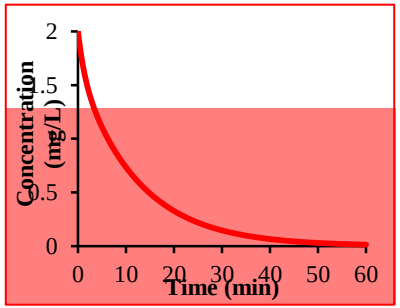
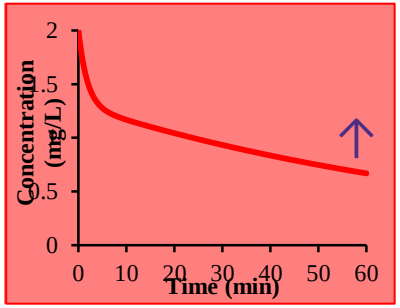
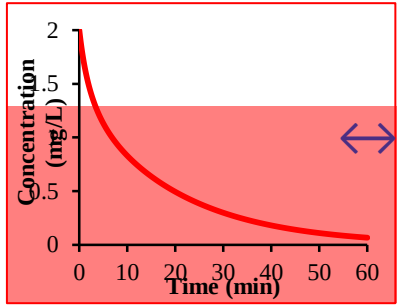
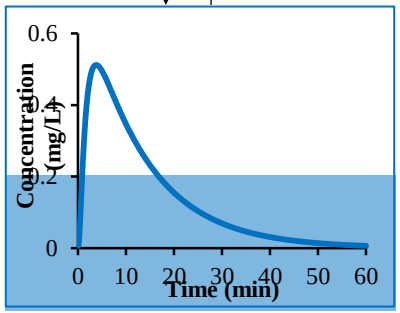
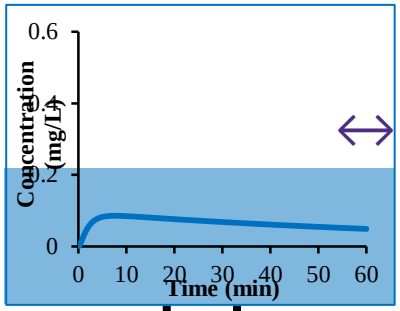
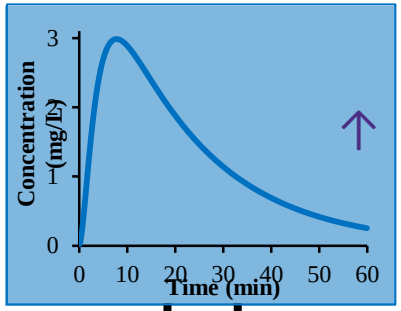
Asymmetry in Tissue:Blood Drug Conc. - Liver



Asymmetry in Liver Tissue:Plasma Conc. when Cl^{s}_{in} is the Rate-Determining Step

i.e. $CL_{met+bile} >> CL^{s}_{ef}$

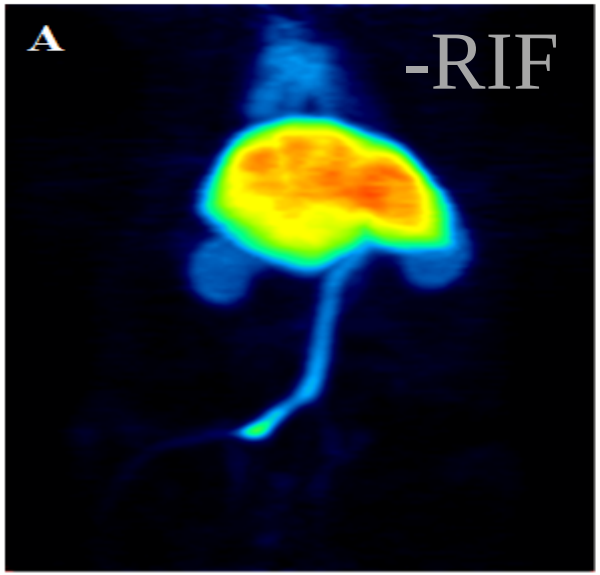
	No inhibition	Inhibition of sinusoidal uptake CL	Inhibition of metabolism and biliary efflux CL
Blood			
	CL^{s}_{in} ↓ ↑ CL^{s}_{ef}	CL^{s}_{in} ✕ ↑ CL^{s}_{ef}	CL^{s}_{in} ↓ ↑ CL^{s}_{ef}
Liver			
	CL_{bile} ↓ CL_{met} ↓	CL_{bile} ↓ CL_{met} ↓	CL_{bile} ✕ ✕ CL_{met}

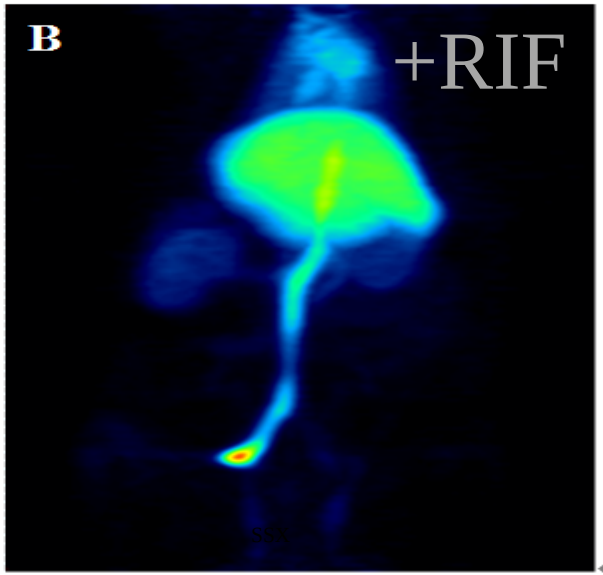
Patilea-Vrana & Unadkat. CPT. 2016

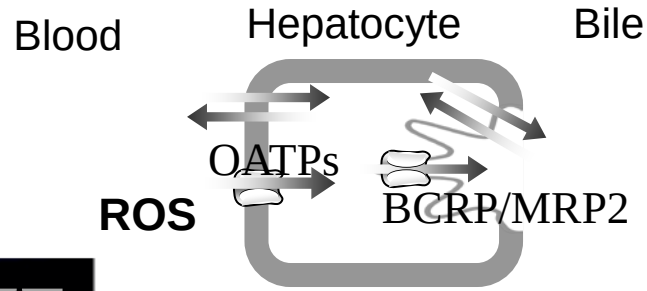
Assumptions:
> Liver is the only eliminating organ
> 90% inhibition

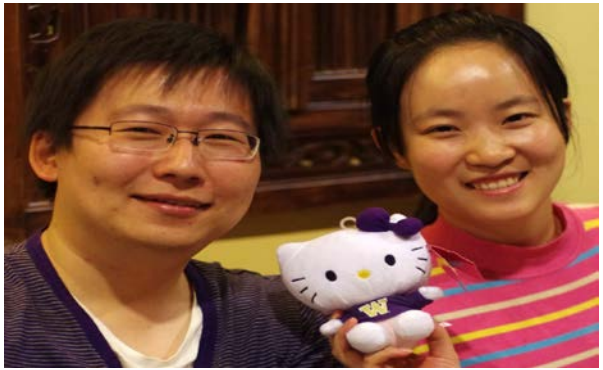
Asymmetry in Hepatic:Blood Conc. of ^{11}C-Rosuvastatin in the Rat

Coronal 2 min SUV images of ^{11}C-Rosuvastatin

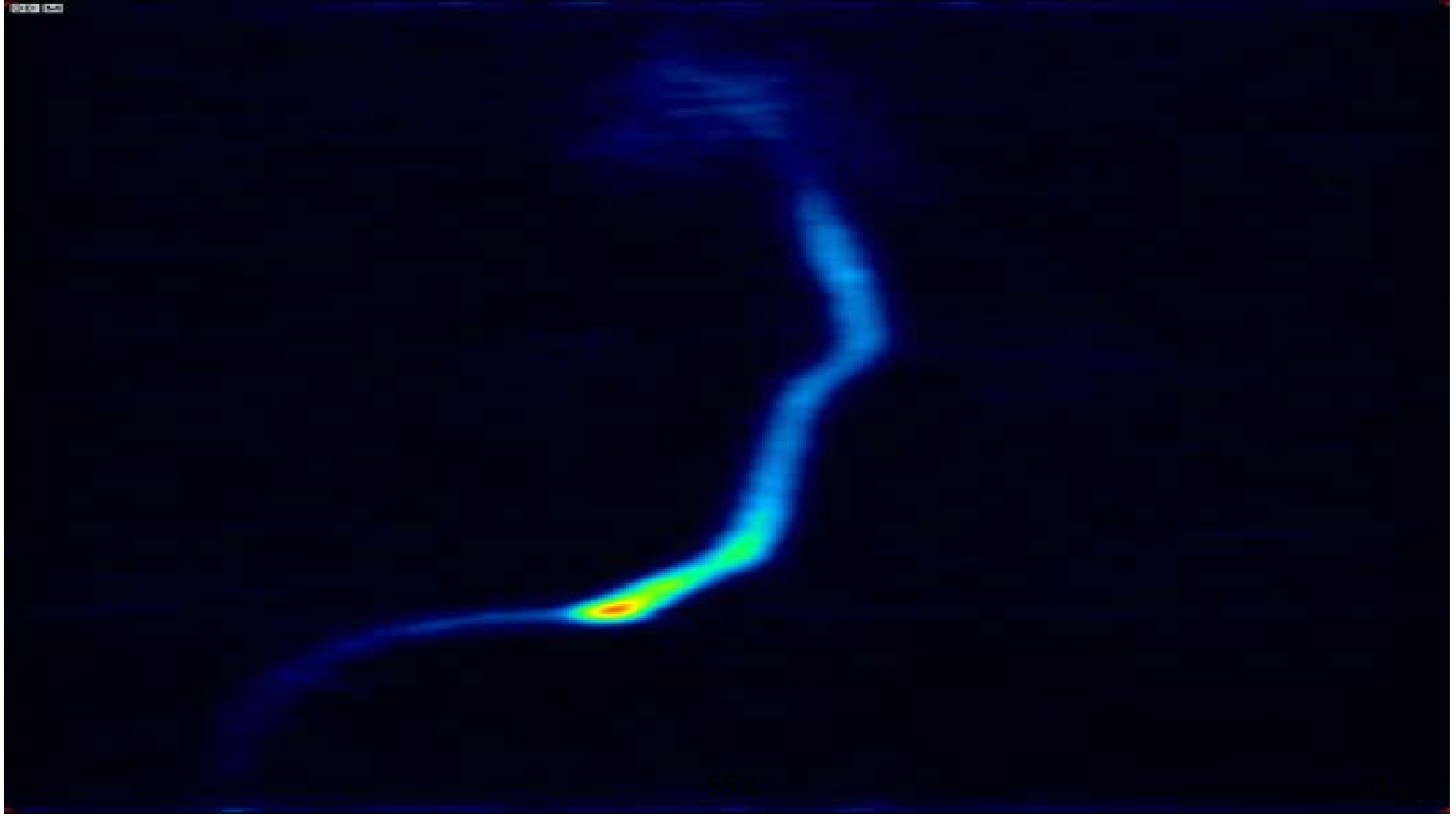








He et al., Mol Pharm., ‘14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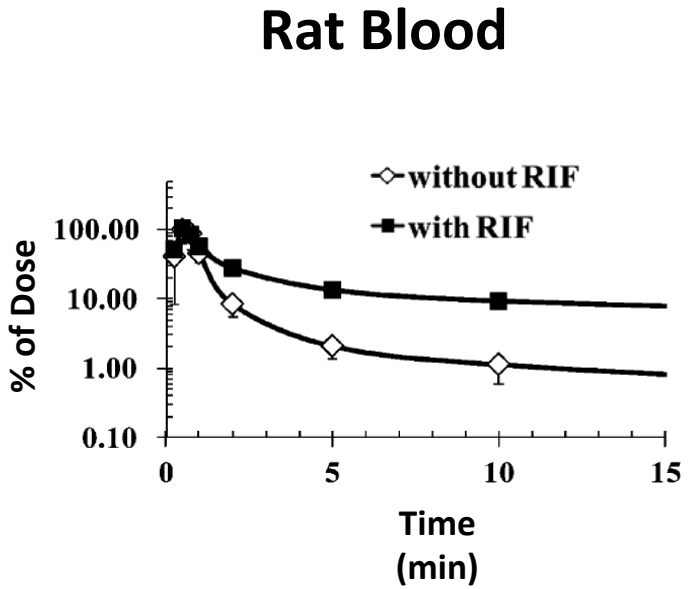
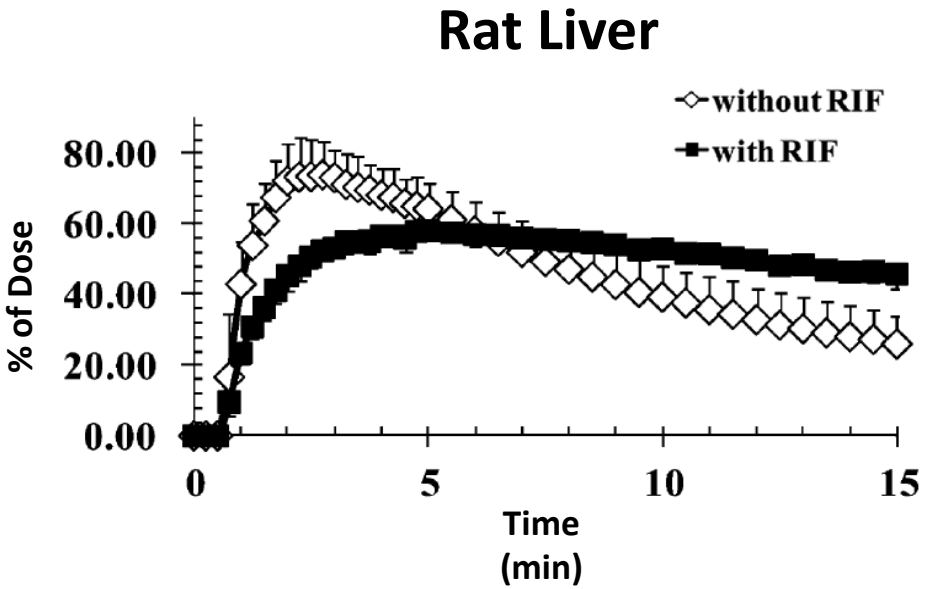
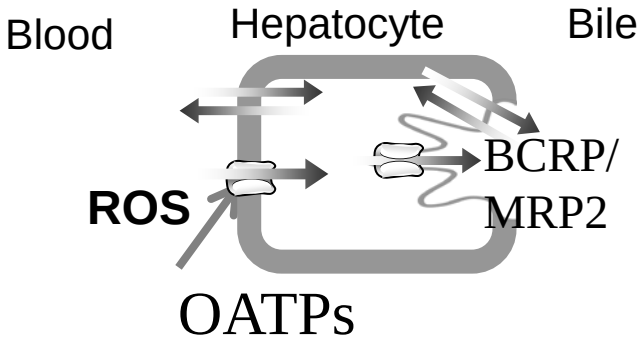
Changes in Rat Liver Exposure to Rosuvastatin ± Rifampin





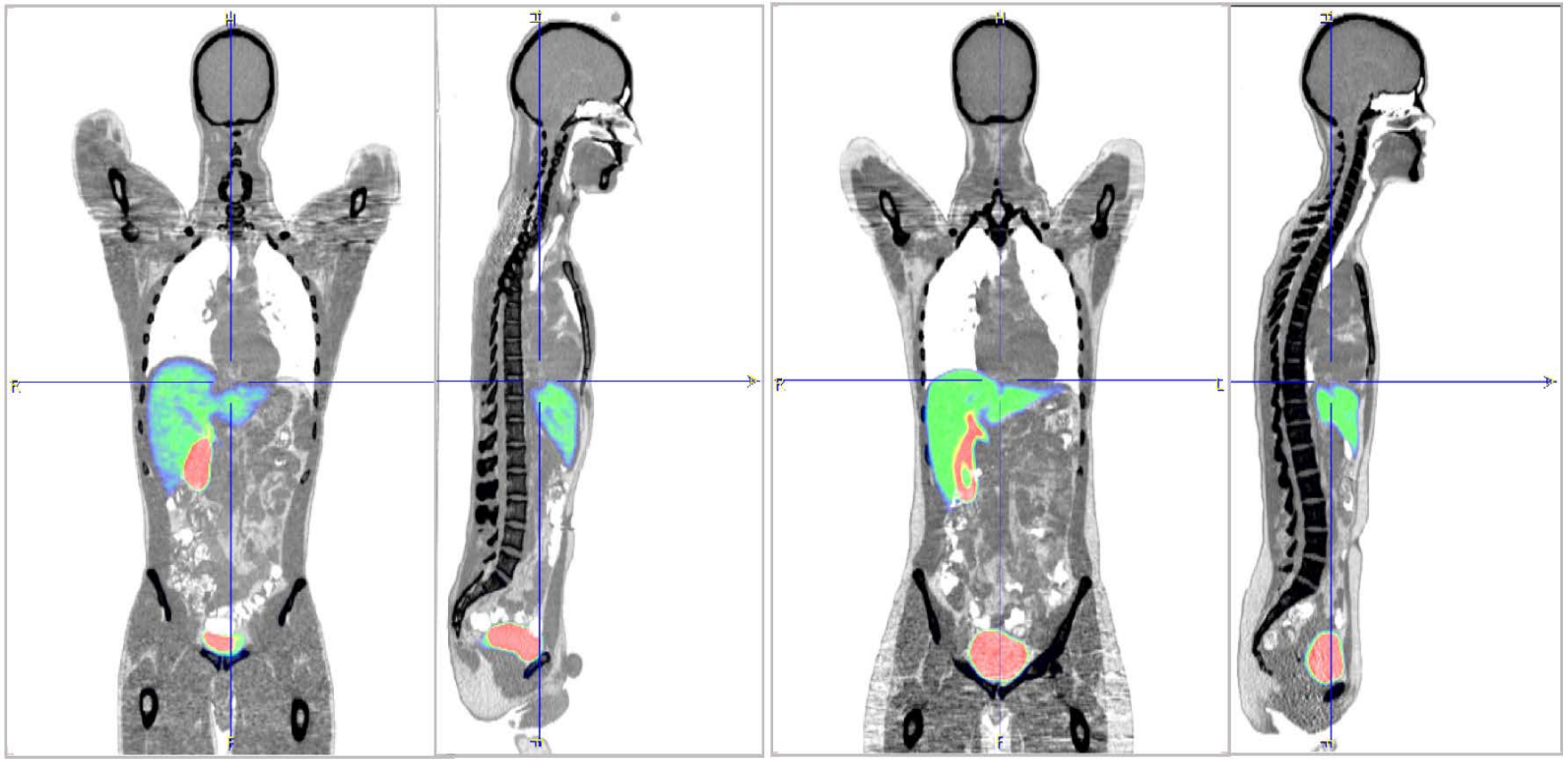


2.3 fold increase in RSV plasma AUC but
NO significant increase in RSV liver AUC

SSX

He et al., Mol Pharm. 2014

Biodistribution of ^{11}C-rosuvastatin in humans



Billington et al., In progress

How Can we Predict Tissue Drug Conc. in Humans?

- PET imaging (MRI and other imaging modalities do not have the required sensitivity):
  - Requires sophisticated equipment and radiochemistry
  - Costly (about $20-40K/experiment/subject)
- Therefore we need alternative methods that will allow us to predict tissue conc. of drugs in humans

APPLICATION OF PROTEOMICS DATA TO PREDICT PK AND TISSUE CONC. OF DRUGS

Hypothesis: Predict transporter-mediated in-vivo CL and tissue concentration of drugs in humans from in-vitro studies

Transporter-expressing cells

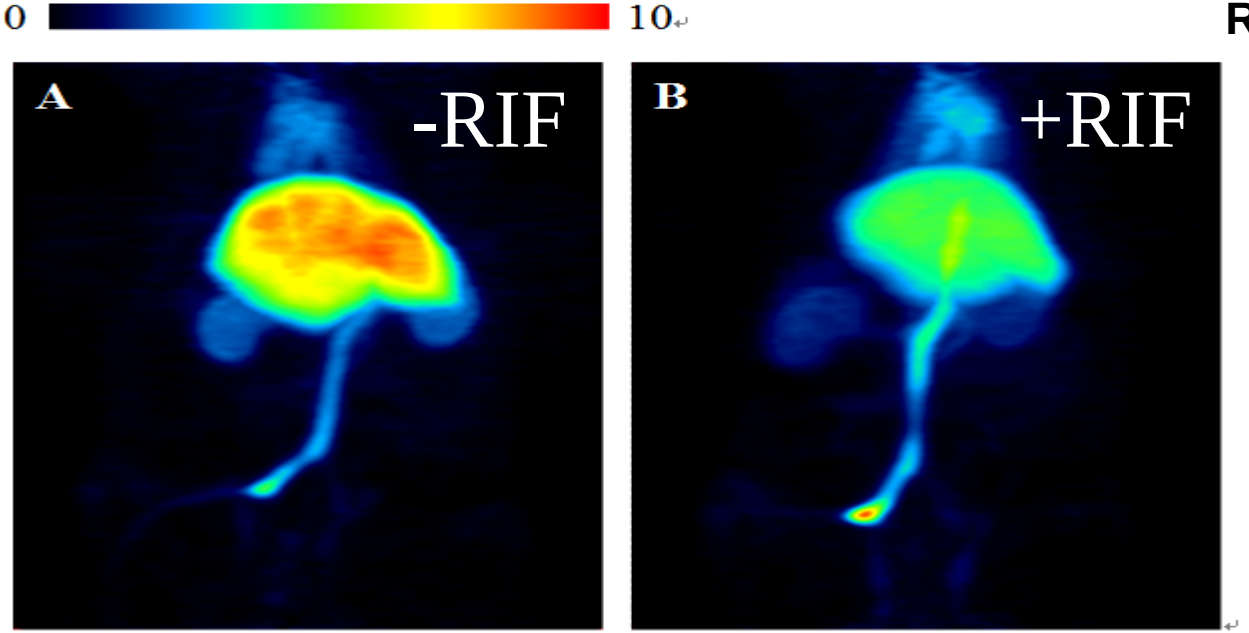
1. transporter-mediated drug CL
2. transporter abundance using quantitative proteomics
3. Obtain transporter scaling factor

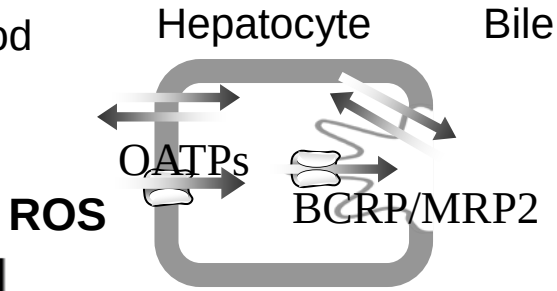
Predict *in-vivo* CL and tissue conc. using transporter scaling factor

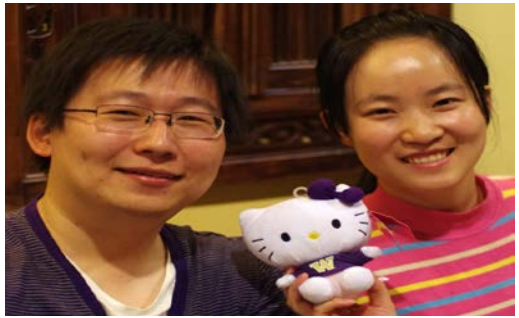
Verify predictions using PET imaging

Hepatic Uptake and Biliary Excretion of ^{11}C-Rosuvastatin in the Rat

Coronal 2 min SUV images of ^{11}C-Rosuvastatin

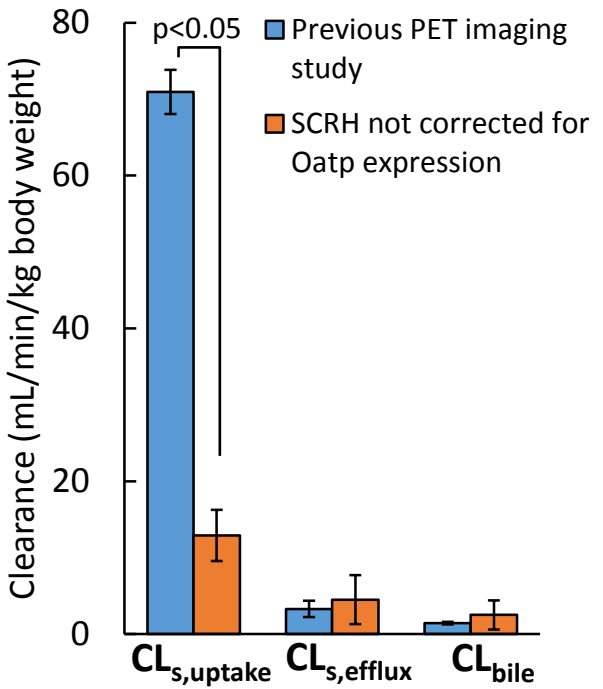




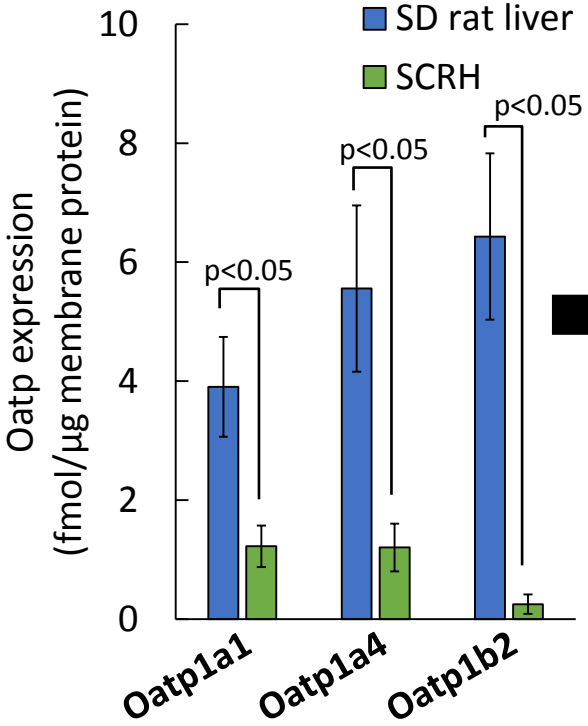


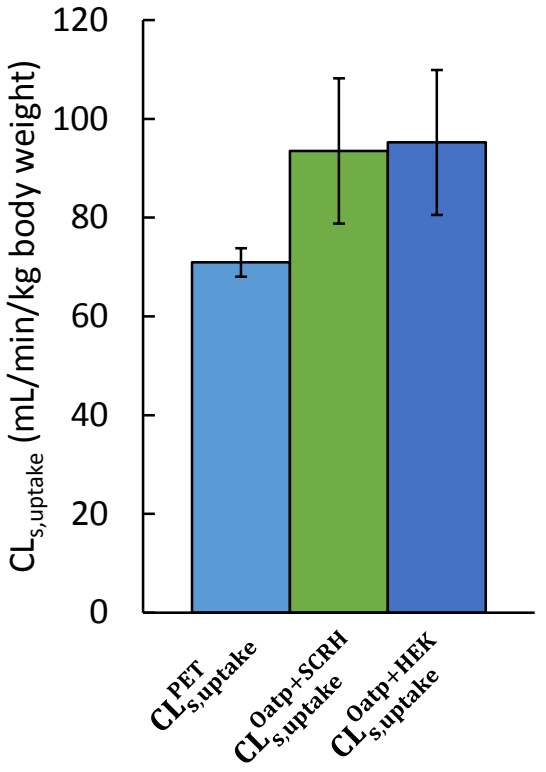
He et al., Mol Pharm., ‘14

Successful prediction of the hepatobiliary clearance of rosuvastatin using cell lines, sandwich-cultured rat hepatocytes and quantitative proteomics



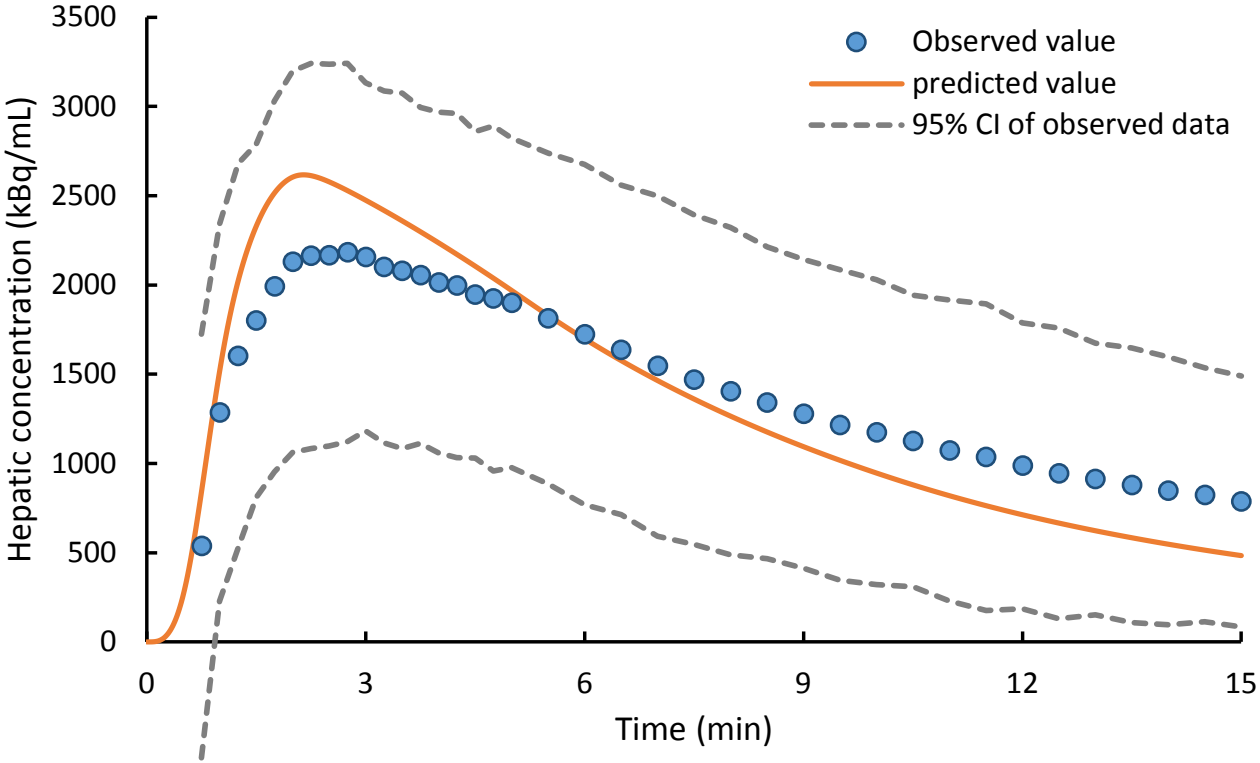
$CL_{s,uptake}$; sinusoidal uptake
$CL_{s,efflux}$; sinusoidal efflux
CL_{bile}; canalicular efflux





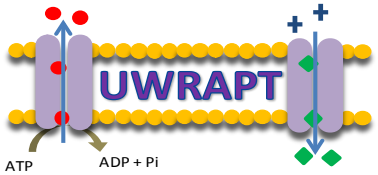
Ishida et al., DMD, 2018

Rat Hepatic Rosuvastatin Conc. well Predicted



Ishida et al., DMD 2018

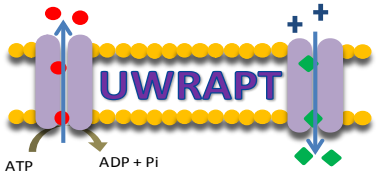
Can Rosuvastatin Hepatobiliary CL and Hepatic Conc. be Predicted in Humans?



Total transporter abundance in suspended (SH), plated (PH), sandwich-cultured (SCH) hepatocytes and liver tissue

Data removed as not published

Kumar et al.,
Unpublished data



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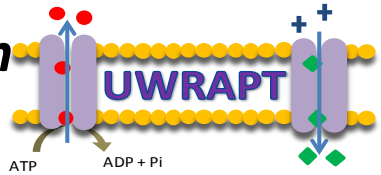
Plasma membrane transporter abundance in suspended (SH), plated (PH), sandwich-cultured (SCH) hepatocytes cf liver tissue

Kumar et al.,
Unpublished data

Transporter-expressing cells better predict in-vivo (IV) human rosuvastatin hepatic uptake clearance than hepatocytes



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$$CL_h = \frac{Q_h \cdot f_u \cdot CL_{s,uptake}}{Q_h + f_u \cdot CL_{s,uptake}}$$

*Assuming sinusoidal uptake is the rate determining step in RSV plasma CL

Patilea-Vrana G and Unadkat JD, *Clin Pharmacol Ther*, 2016

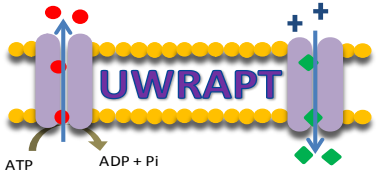
RSV uptake CL scaled on the basis of hepatocellularity *(Classical method)

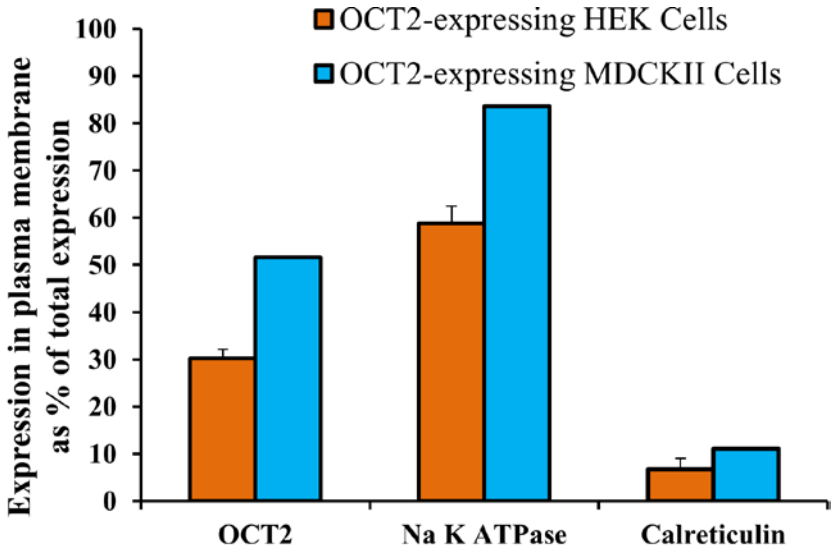
*88 mg protein/g human liver

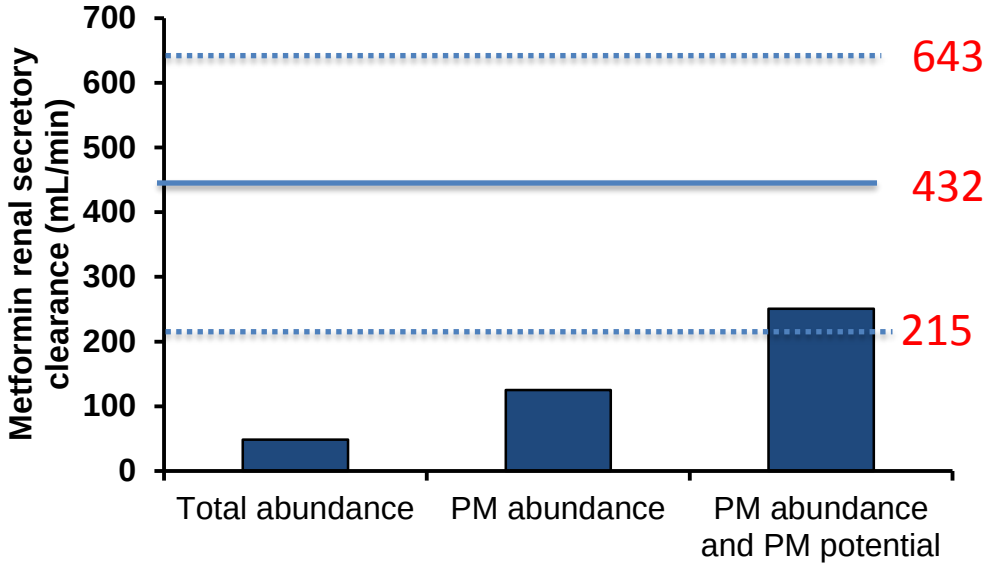
Martin et al., Clin Ther. 2003 Oct;25(10):2553-63.

Karlgren et al., J Med Chem. 2012 May 24;55(10):4740-63.

Metformin renal clearance is reasonably well-predicted using OCT2 expressing cells







Observed metformin renal secretory clearance in humans: 432 (range 215-643) mL/min

Kumar et al., DMD 2018

Summary

- Predicting tissue concentration and therefore efficacy and toxicity of a drug is the next frontier in ADME research
- The hepatic ECL model clarifies when transporters will or will not affect the systemic and tissue PK of a drug
- Tissue conc. measurement is possible using PET. However, this method cannot be routinely applied
- IVIVE using transfected cells and quantitative transporter proteomics is a promising technique to predict tissue drug conc.
- These predictions should be validated using PET imaging probes that interrogate multiple drug transporters

Major Contributors

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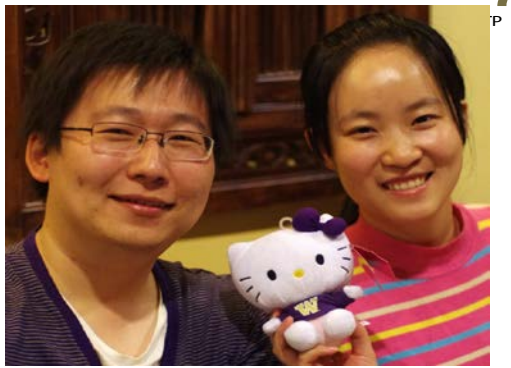
Sarah Billington



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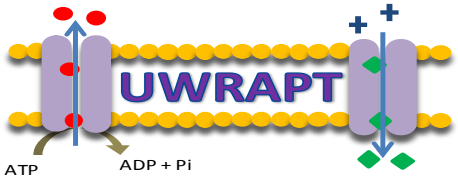

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Dept. of Radiology: Jeanne Link, David Mankoff, Todd Richards, Janet Eary, Satoshi Minoshima, Ken Maravilla, Mark Muzi, Steve Shoner, David Lewis, Jean Lee and the PET suite team

Dept. of Medicine: Ann Collier and her team; Scott Lee and his team

Dept. of Anesthesiology: Karen Domino, Matthew Pennington

Dept. of Pharmaceutics: Bhagwat Prasad, Edward Kelly, Carol Collins, Joanne Wang

Kidney Research Institute: Jonathan Himmelfarb

Univ. of Greifswald: Stefan Oswald and team

Children's Mercy Hospitals: Steven Leeder and team

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UWRAPT
ATP
ADP + Pi