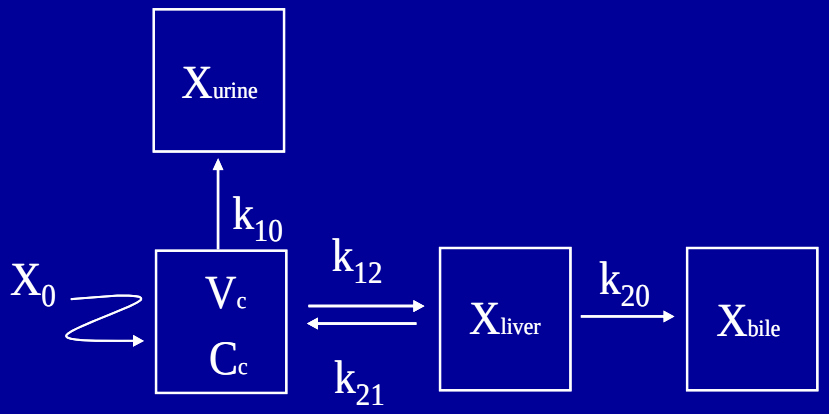
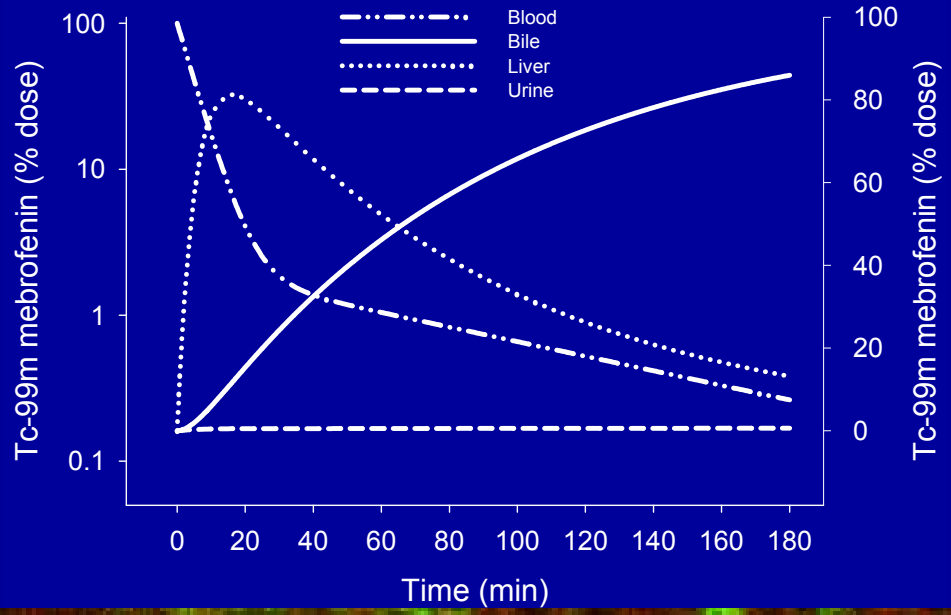
Imaging Techniques and Pharmacokinetic Modeling/Simulation:

Useful Quantitative Tools for the Drug Transport Scientist





Kim L. R. Brouwer

Eshelman School of Pharmacy,
University of North Carolina at Chapel Hill

Imaging Techniques and Pharmacokinetic Modeling/Simulation

- Available Tools
  - Utility
  - Requirements
  - Advantages
  - Limitations
- When should these tools be used? What questions can be addressed?
- How can in vivo, in situ and in vitro drug transport data, coupled with modeling and simulation, be used to predict the impact of altered transport on drug disposition?

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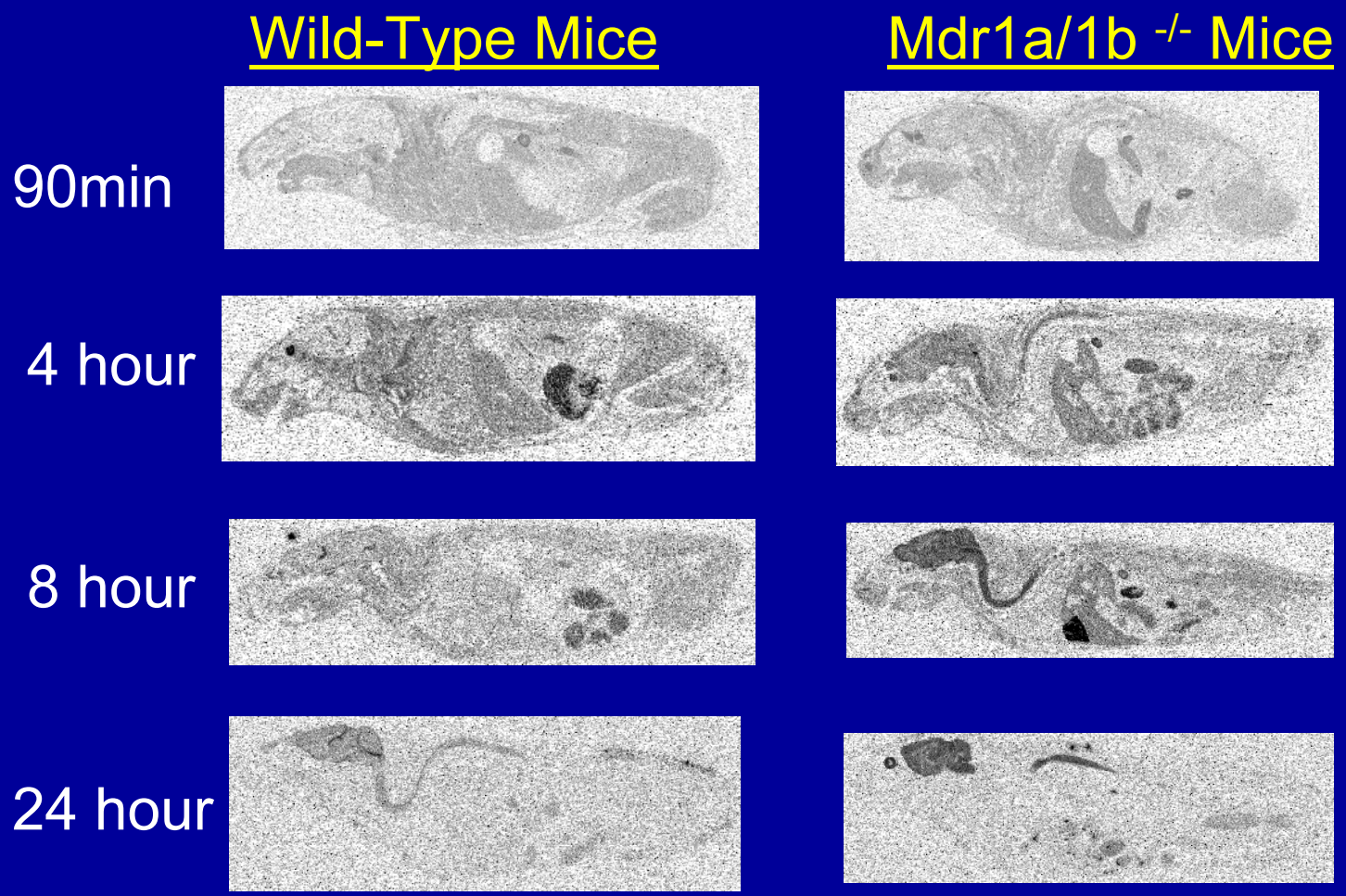
Whole Body Autoradiography (WBA)

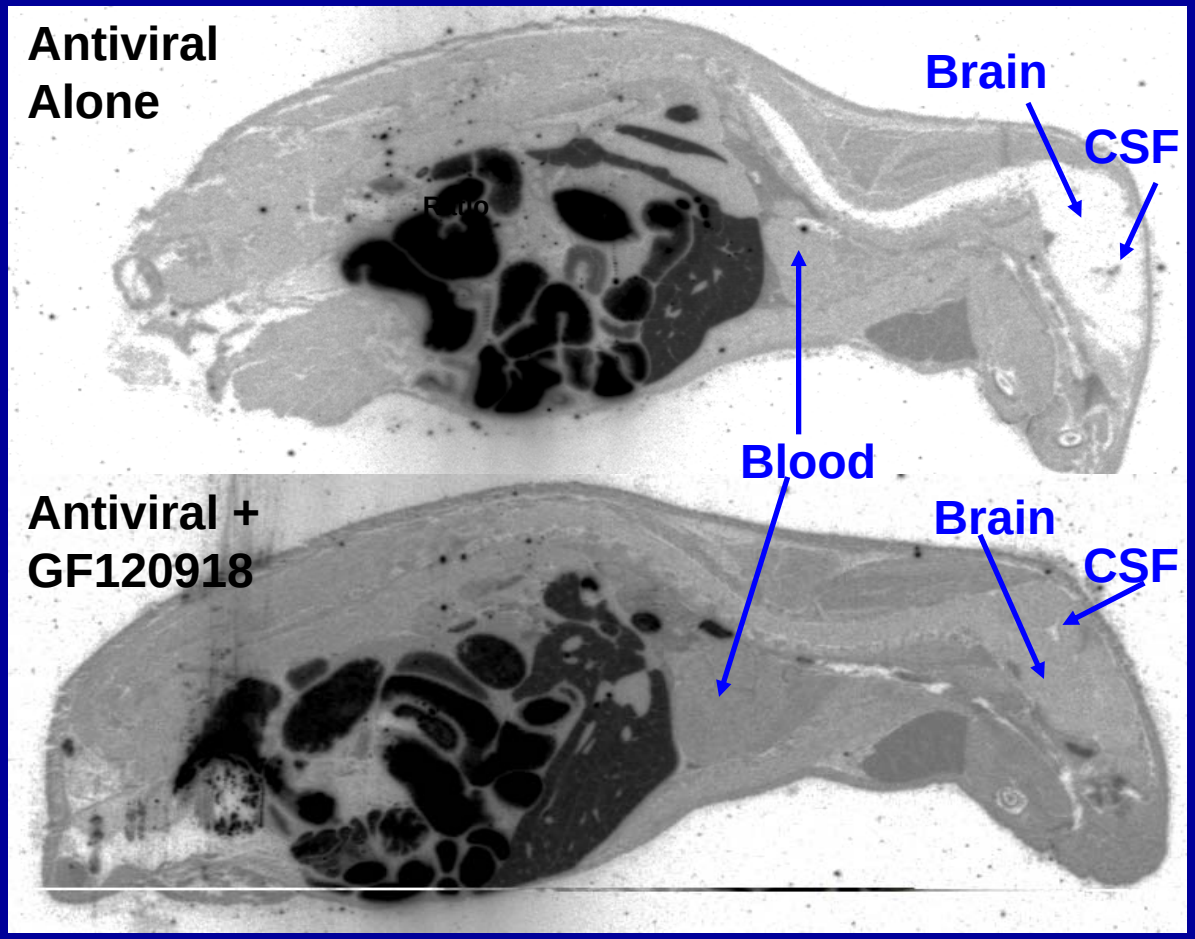
- **Utility**: Provides general view of distribution pattern of compound in tissues/organs, preliminary information on ADME properties of radiolabeled compound, correlation with observed toxicities, presence of biliary excretion or direct intestinal secretion
- **Requirements**: Radionuclides (^{3}H or ^{14}C; 50-100 μCi) administered i.v. or p.o. to preclinical species; animals frozen and mounted; 20 micron sections freeze dried, exposed to phosphor imaging plates, and quantified with imaging analysis or directly by LCMS
- **Advantages:** Provides information regarding organ distribution, unforeseen localization of radioactivity and new routes of elimination
- **Limitations**: Monitors distribution of total radioactivity and can't discriminate between parent and metabolites

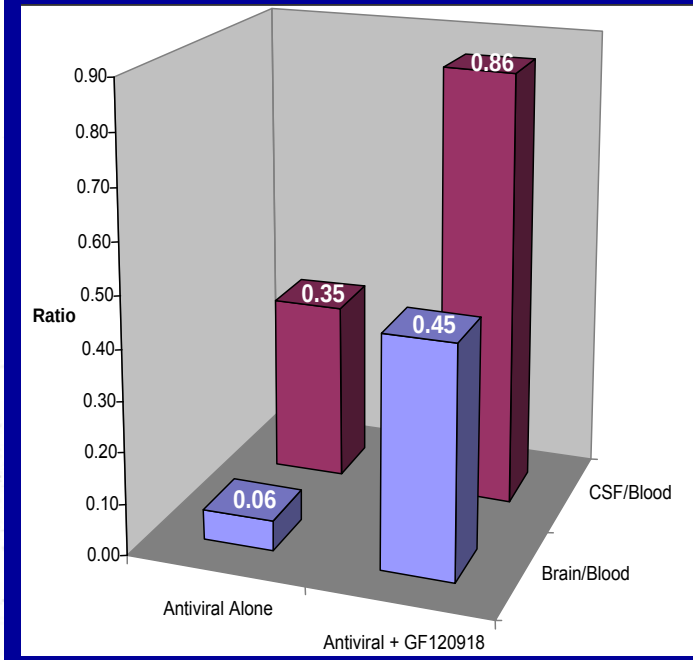
Use of WBA to Assess Pgp Inhibition of ^{3}H-Digoxin Distribution in Wild-Type and MDR 1a/1b $^{-/-}$ Mice



Courtesy of Glaxo, Inc.

Use of WBA to Assess Effect of GF120918 on Antiviral Distribution in Rats





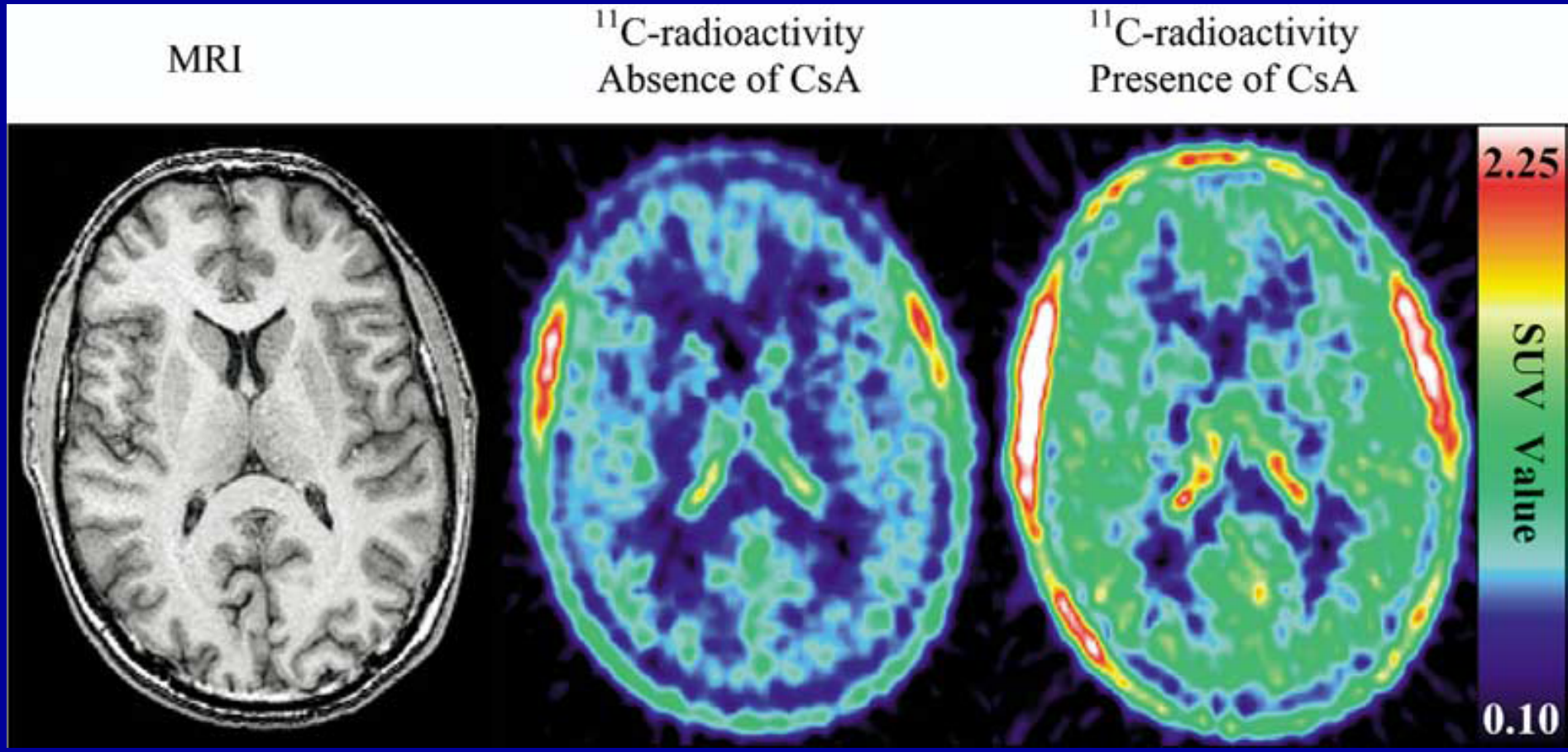
Courtesy of Glaxo, Inc.

Positron Emission Tomography (PET)

- **Utility**: Noninvasive imaging method to quantify activity of transport proteins in preclinical species or humans, or determine the functional impact of transport protein modulation associated with genetic polymorphisms, transport inhibition or induction
- **Requirements**: PET probes for specific transport proteins; typical isotopes: ^{11}C (~20 min); ^{13}N (~10 min); ^{18}F (~110 min)
- **Advantages**: Accurately measure amount of tracer accumulation in organ over time; direct measurement of transport protein activity in species of interest; noninvasive
- **Limitations**: Availability of specific, labeled probes; synthesis of radiolabeled compounds- due to short half-lives, cyclotron and radiochemistry lab must be in close proximity to PET imaging facility

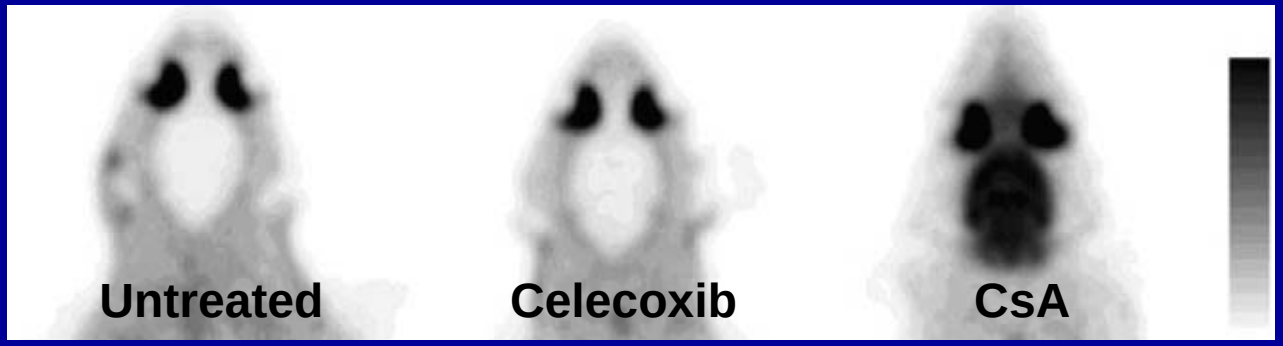
Use of PET to Evaluate Pgp Function at the BBB in Humans:

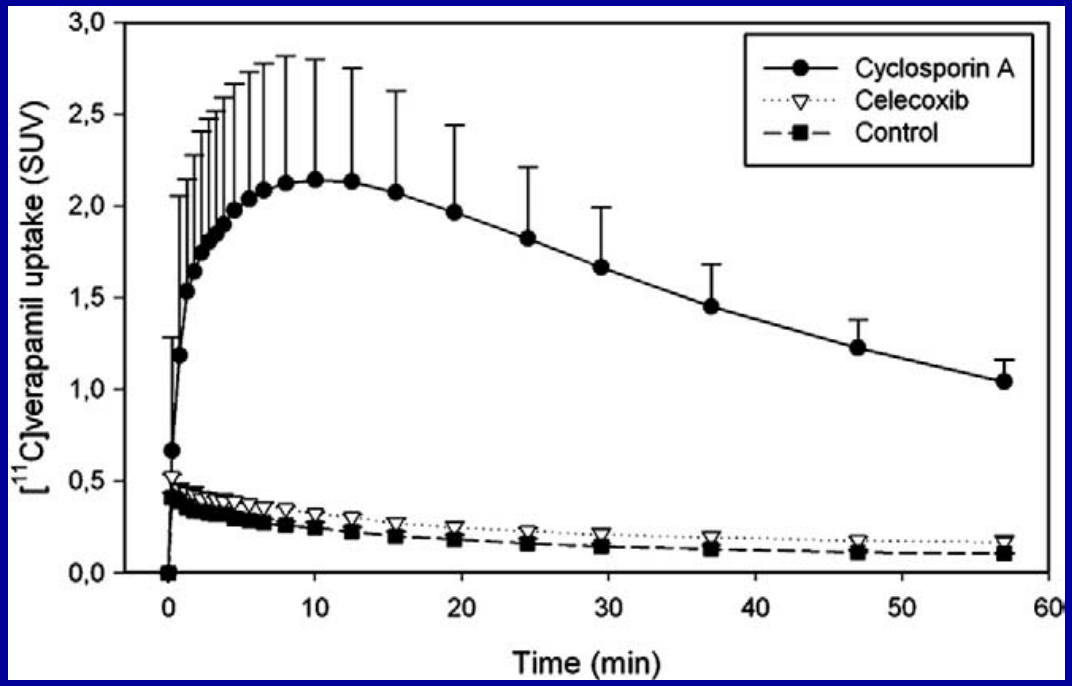
Inhibition of [^{11}C]Verapamil Transport by Cyclosporine



Sasongko et al., *Clin Pharmacol Ther* 77:503, 2005

Celecoxib Does Not Affect Pgp-Mediated Efflux of [^{11}C]Verapamil in Mice



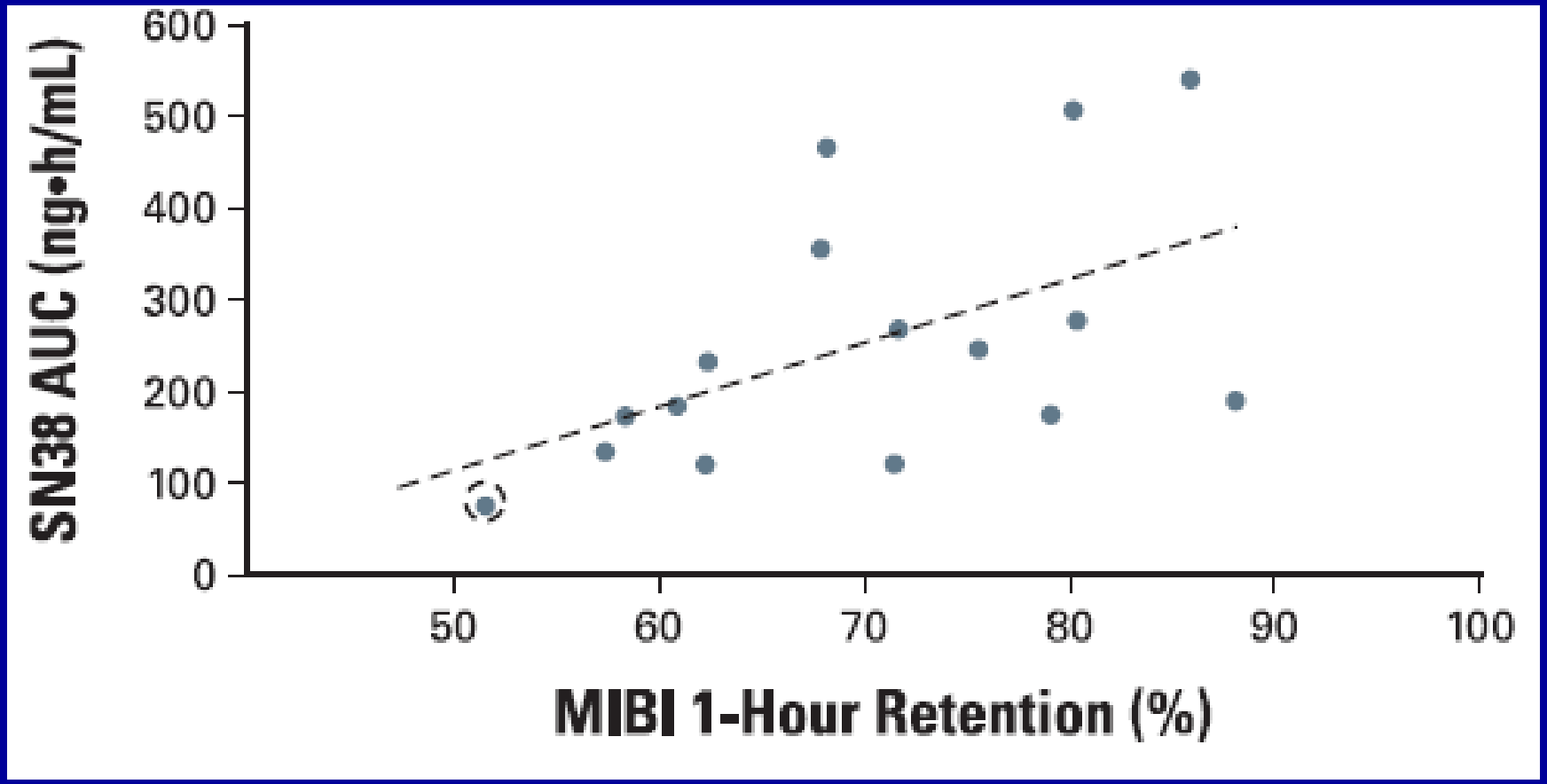


de Vries et al., *Nucl Med Biol* 35:459, 2008

Gamma Scintigraphy

- **Utility**: Noninvasive imaging method to quantify activity of transport proteins in preclinical species or humans, or determine the functional impact of transport protein modulation associated with genetic polymorphisms, transport inhibition or induction
- **Requirements**: Short-lived gamma emitting radioisotopes that are probes for specific transport proteins [*e.g.*, ^{99m}Tc-Mebrofenin, ^{99m}Tc-Sestamibi)]
- **Advantages**: Accurately measure amount of tracer accumulation in organ over time; direct measurement of transport protein activity in species of interest; noninvasive; $t_{1/2}$ more reasonable than PET probes
- **Limitations**: Availability of specific, labeled probes; quantitation issues

Relationship Between SN-38 AUC and ^{99m}Tc-Sestamibi (MIBI) Hepatic Retention



Michael *et al. J. Clin. Oncol. 24:4228, 2006*

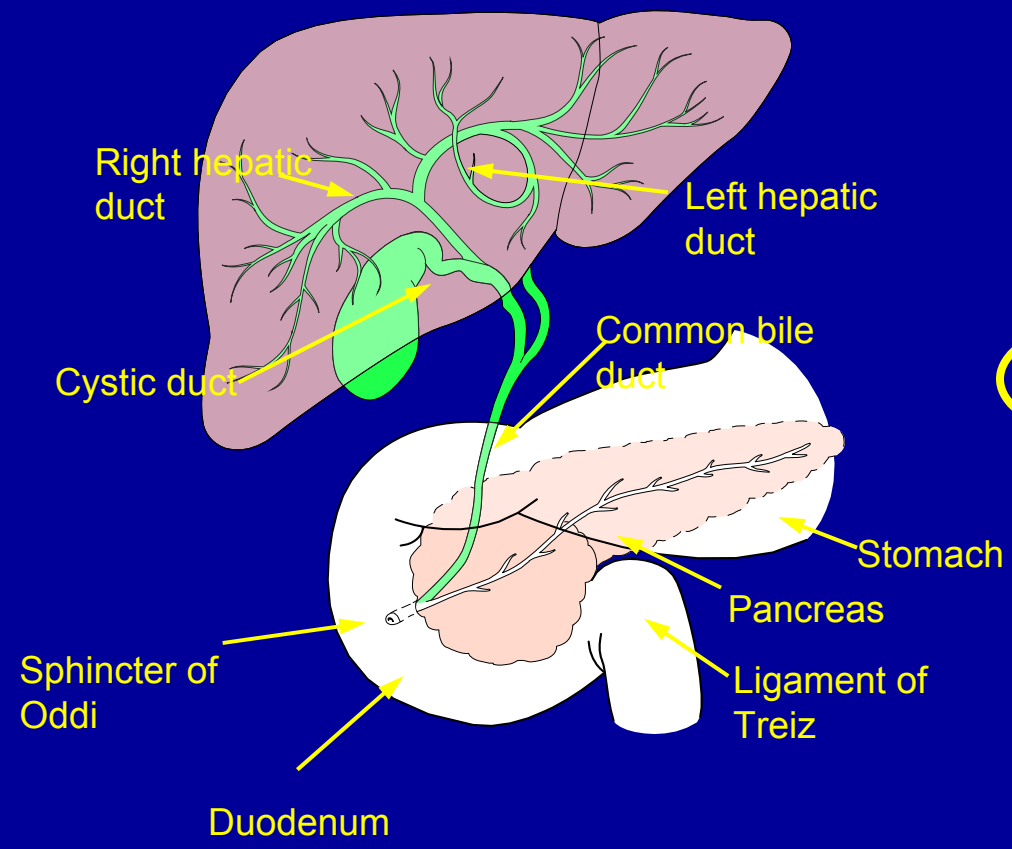
Relationship Between Hepatic Nuclear Imaging Parameters and Irinotecan Pharmacokinetics and Toxicity

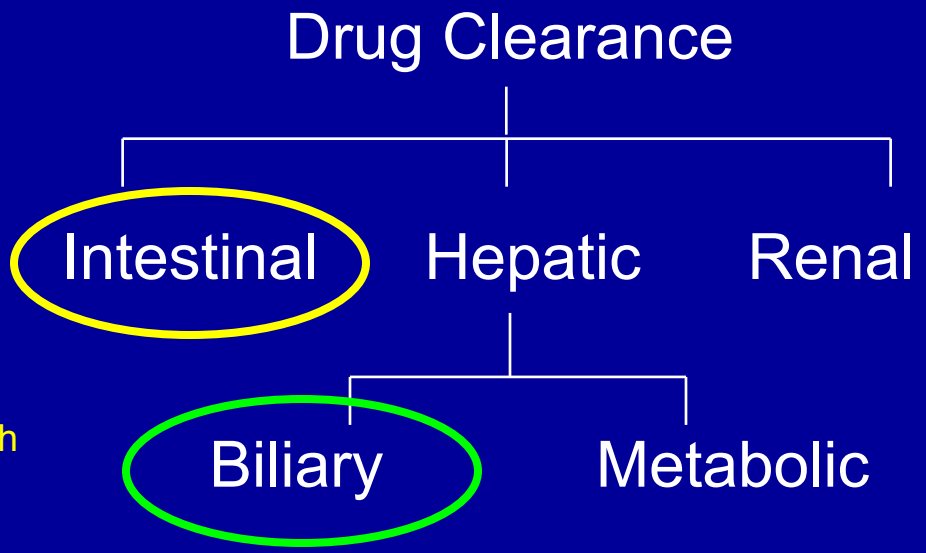
A significant, linear correlation was observed between SN-38 AUC and the percent retention at 1 hr of ^{99m}Tc-iminodiacetic acid and ^{99m}Tc- sestamibi. Data suggest that:

- A higher grade of neutropenia was associated with lower biliary excretion
  - Neutropenia has been positively correlated with SN-38 plasma concentrations
  - Reduced hepatic tracer clearance may be associated with increased SN-38 exposure
- Increased diarrhea was reported in patients with higher biliary transport function
  - Late onset diarrhea has been attributed to SN-38 intestinal secretion

Michael *et al. J. Clin. Oncol. 2006 24:4228*

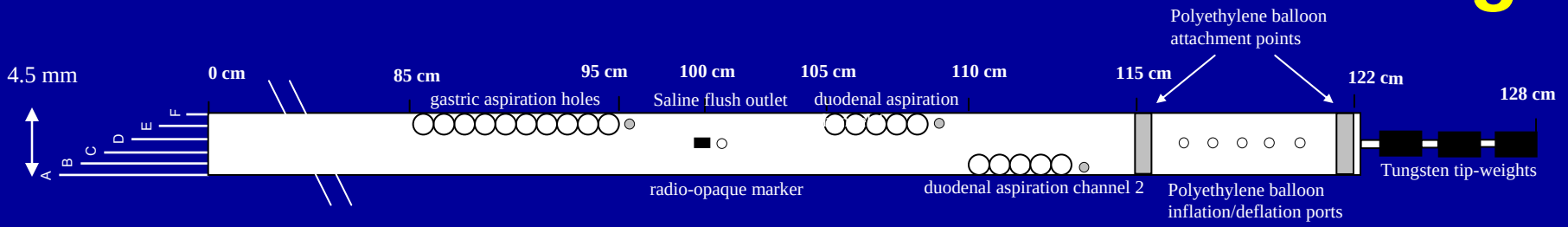
Application of Nuclear Imaging to Evaluate Biliary Clearance in Humans





- $Cl_{biliary}$ may contribute significantly to $Cl_{hepatic}$
- Altered $Cl_{biliary}$ due to genetics, disease states or drug interactions may affect pharmacological efficacy and/or toxicity (systemic, hepatic and/or intestinal)
- Species differences in hepatic transport proteins may limit the utility of animal models to predict human $Cl_{biliary}$

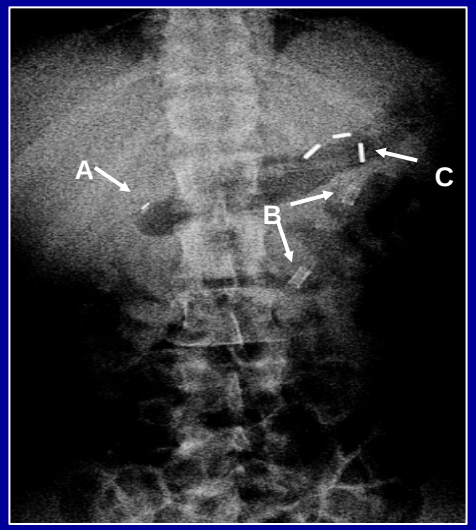
Oro-enteric Tube and Protocol Design



- Multi-lumen silicone extrusion
- Double duodenal aspiration channels
- Gastric aspiration channel
- Occlusive balloon (positioned in the distal duodenum by fluoroscopy)

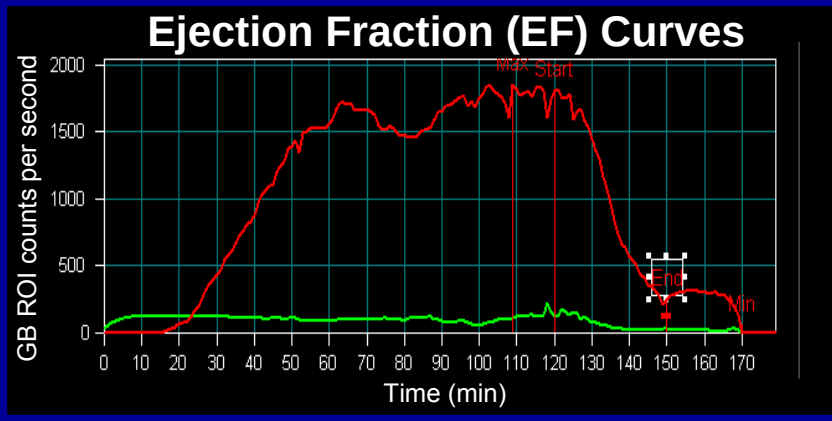
A = radio-opaque marker at pyloric sphincter
B = balloon attachment rings
C = tungsten tip weights at Ligament of Treitz

- Fatty meals to maintain gallbladder contractility
- IV administration of ^{99m}Tc radiotracers
- CCK8 IV infusion to contract gallbladder
- Gamma scintigraphy of abdominal area



Ghibellini G et al. *AAPS Journal,* 6 (4) Article 33, 2004

Calculation of Parameters and Corrected Biliary Clearance



$$Cl_{total} = \frac{Dose}{AUC_{0-\infty}}$$

$$Cl_{biliary} = \frac{X_{bile0-Tlast}}{AUC_{0-Tlast}}$$

$$\text{in vivo } Cl_{biliary} = \frac{X_{GB}}{AUC_{0-Tlast}}$$

$$X_{GB} = X_{bile-120\,min} + \left(X_{bile120\,min-y\,min} \Big/ EF \right)$$

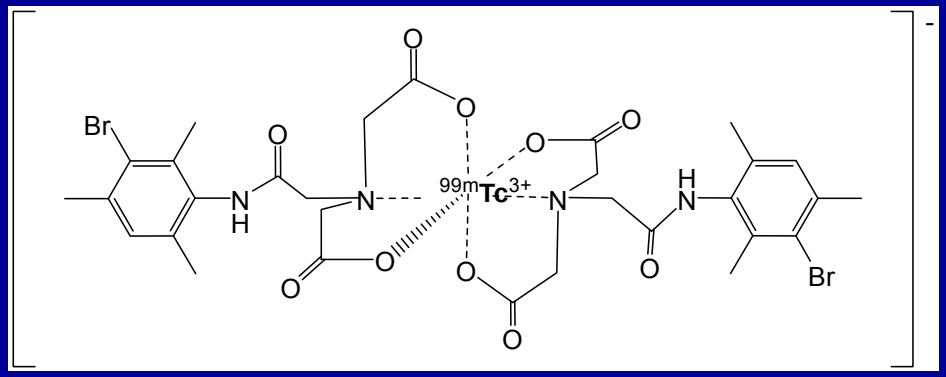
y → time over which the GB ejection fraction (EF) was calculated

$$EF = \frac{\left(GB_{preCCK} - GB_{postCCK}\right)}{GB_{preCCK}}$$

Based on counts from frontal planar image of GB
Normal EF > 35%

X_{GB} → Excreted Dose = corrected amount of drug collected from the duodenum when accounting for incomplete gallbladder (GB) contraction

Probe Compounds

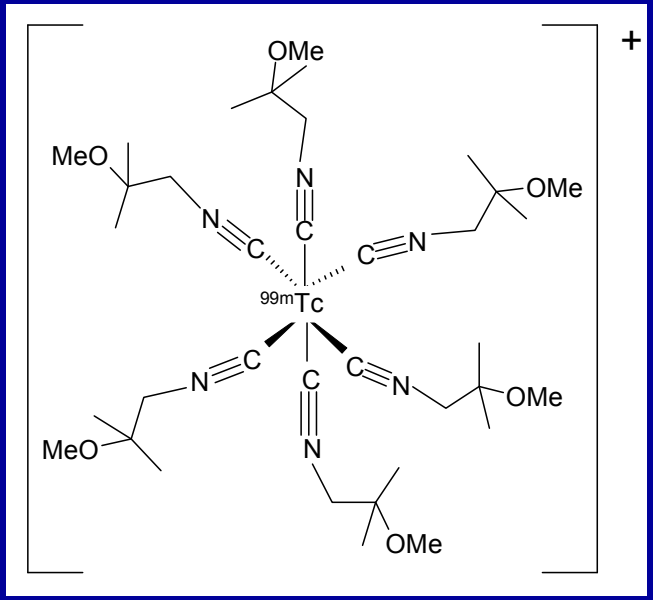


^{99m}Tc-Mebrofenin

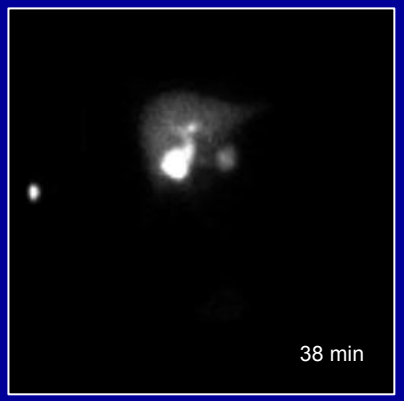
- Clinical Use: hepatobiliary imaging agent
- Liver uptake: 98%

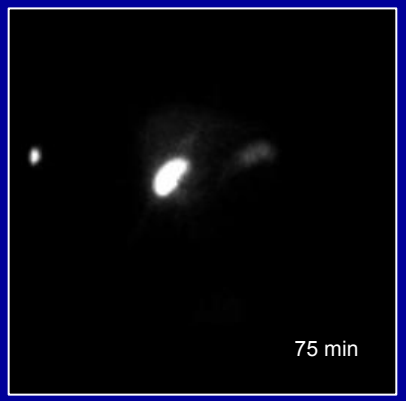
^{99m}Tc- Sestamibi

- Clinical Use: myocardial perfusion agent
- Substrate for P-gp, MRP1, BCRP and Mrp2
- 30-40% of dose excreted in feces; 27% in urine



Gamma Scintigraphic Images (0-180 min) of ^{99m}Tc-Mebrofenin Disposition





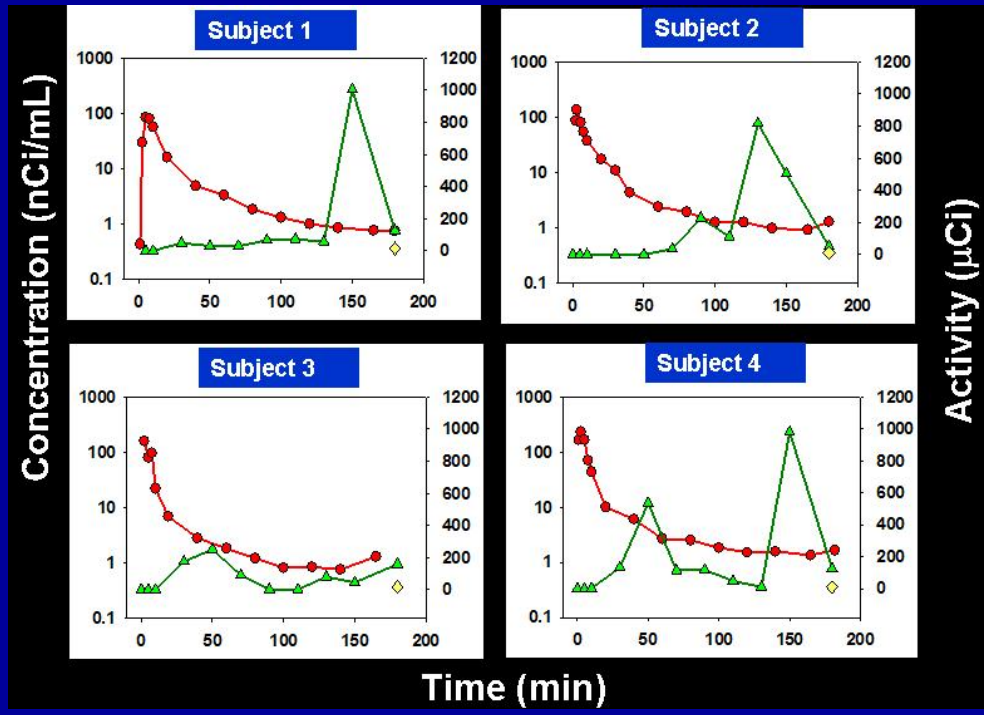
- Spontaneous and prolonged GB emptying into duodenum with successful aspiration of secretions
- Strong GB response to CCK8 and complete collection of bile
- Balloon positioned very close to Sphincter of Oddi

Ghibellini et al. *AAPS Journal* (2004); 6 (4) Article 33

^{99m}Tc-Mebrofenin Disposition

Parameter	Mean	S.D.
Dose (µCi)	2277	292
*Biliary Recovery	67.1	23.0
*Urinary Recovery	0.6	0.2
Ejection Fraction	0.64	0.4
*Recovery of Excreted Dose	84.2	9.3
Cl_{total}	17.3	1.7
$Cl_{biliary}$	12.5	3.6
in vivo $Cl_{biliary}$	16.1	3.2

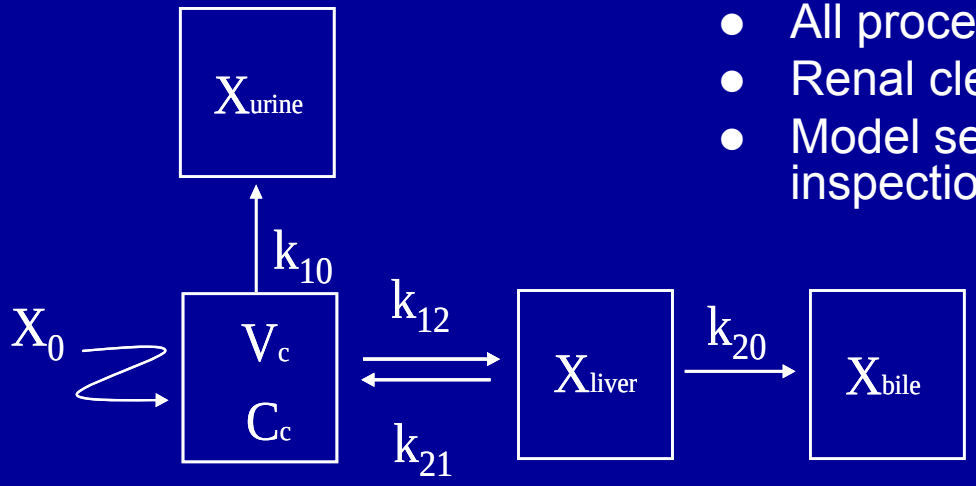
*Recovery expressed as % of dose
Cl values expressed as mL/min/kg



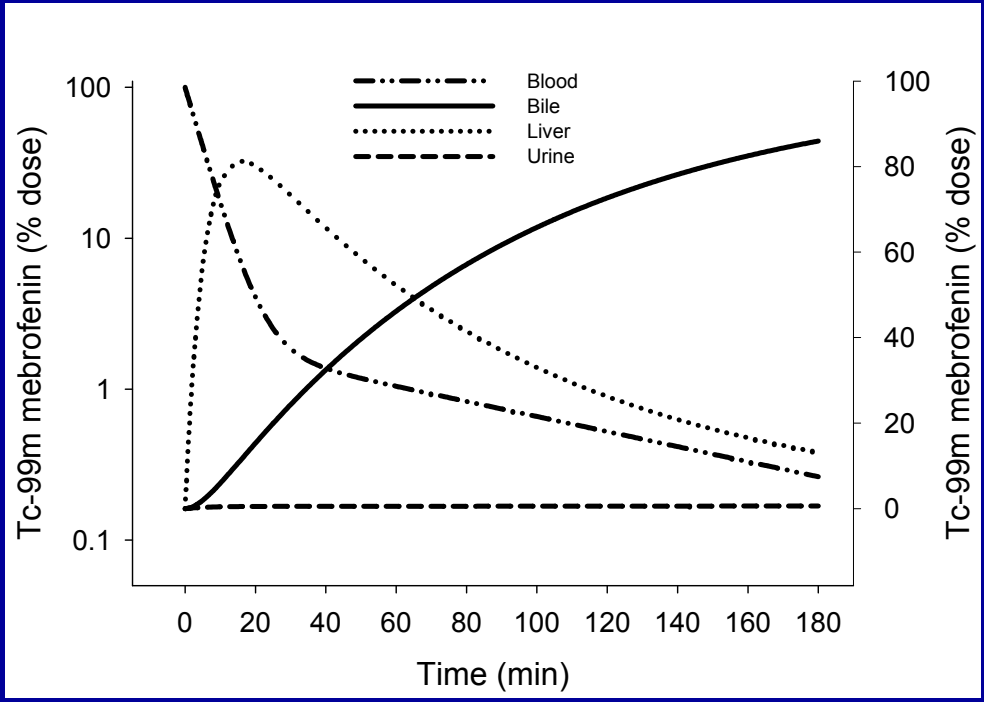
- Spontaneous and CCK8 stimulated GB emptying
- Intestinal occlusion and complete collection of bile were achieved
- *In vivo* $Cl_{biliary}$ similar to human liver blood flow (21 ml/min/kg)

Ghibellini et al. *AAPS Journal* (2004); 6 (4) Article 33

Pharmacokinetic Model to Describe ^{99m}Tc-Mebrofenin Disposition in Humans



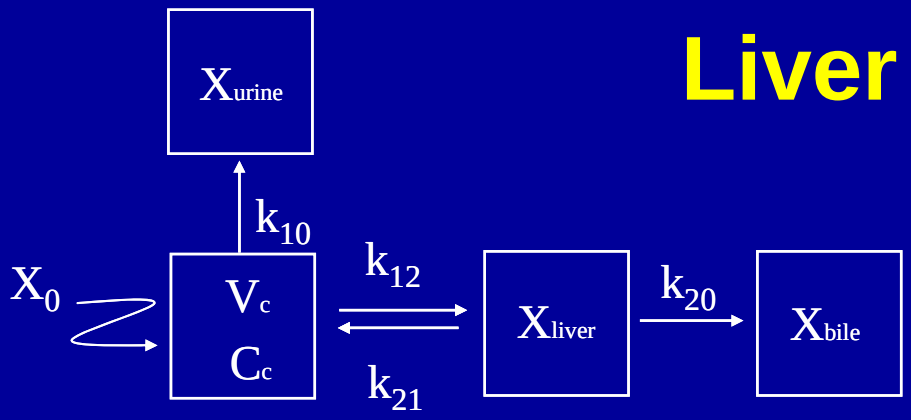
- All processes are linear and 1st -order
- Renal clearance solely via glomerular filtration
- Model selection based on: AIC, RSS, visual inspection of residual plots and of model fit to data



Parameter	Mean	S.D.
K_{12} (min^{-1})	0.18	0.06
K_{21} (min^{-1})	0.0034	0.0013
K_{20} (min^{-1})	0.012	0.003
K_{10} (min^{-1})	0.0009	0.0004
V_c (L)	9.56	3.25
Ratio K_{20}/K_{21}	3.5	0.8

Ghibellini *et al*. *Pharm Res*, 25:1851, 2008

Liver Disease

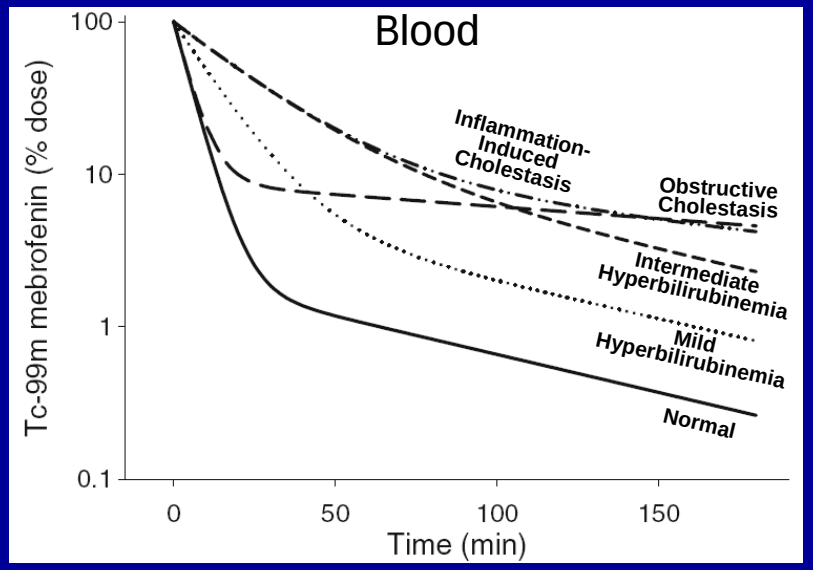


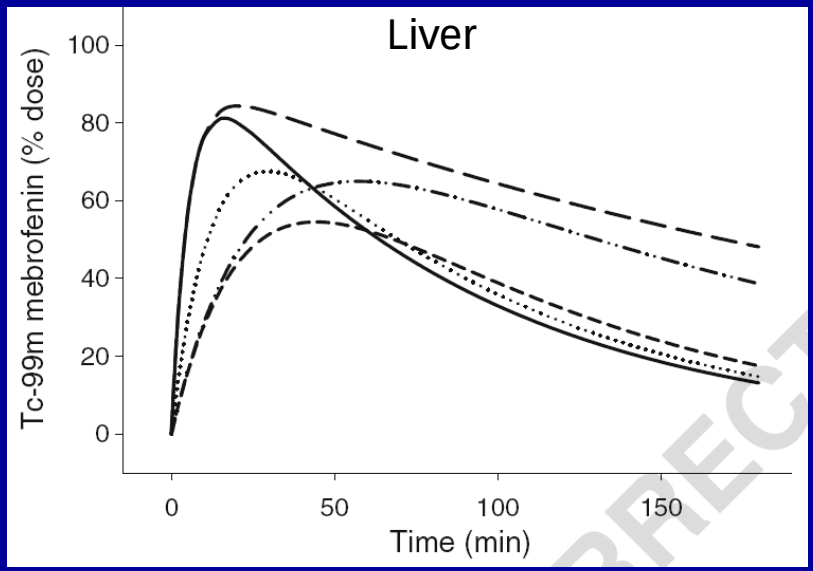
Liver Disease Simulations:

- Mild Hyperbilirubinemia:
  k_{12} ↓ 2.5-fold
- Intermediate Hyperbilirubinemia:
  k_{12} ↓ 5-fold
- Inflammation-induced Cholestasis:
  k_{12} ↓ 5-fold and k_{20} ↓ 2-fold
- Obstructive Cholestasis:
  k_{21} ↑5-fold and k_{20} ↓ 3-fold

Relevance

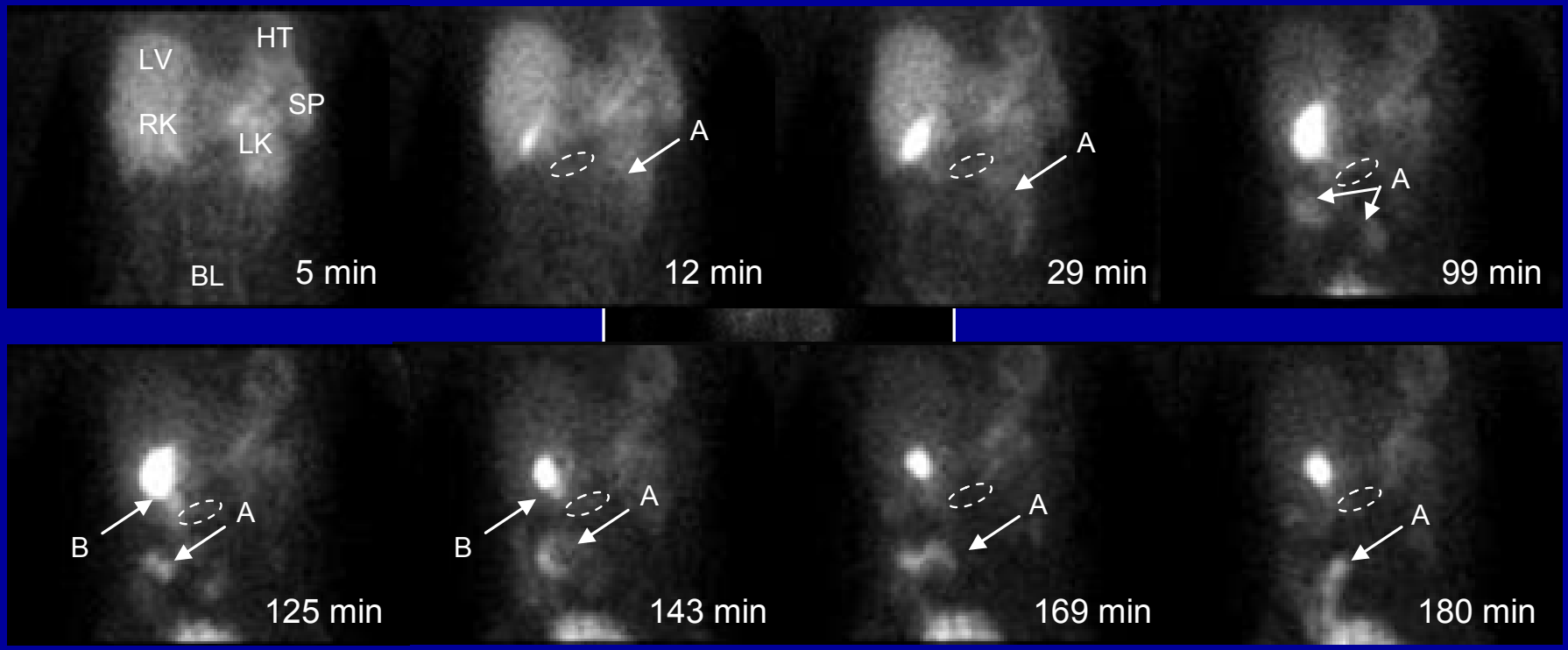
- Major differences in hepatic exposure are evident when biliary excretion is impaired





Ghibellini *et al*. *Pharm Res,* 25:1851, 2008

Gamma Scintigraphic Images (0-180 min) of ^{99m}Tc-Sestamibi Disposition



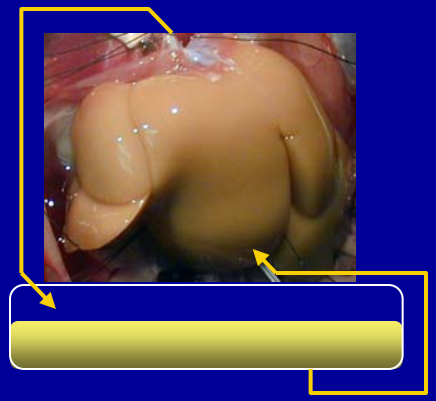
- Dotted oval outline: balloon
- Arrow A: intestinal secretion of ^{99m}Tc-sestamibi
- No visible contraction of GB nor radioactivity recovered from duodenal aspirates until CCK8 administration (120 min)
- No additional radioactivity in the GI tract below the balloon after CCK8 indicating successful occlusion of the intestine

Ghibellini et al., *Clin Pharmacol Ther*, 81:406, 2007

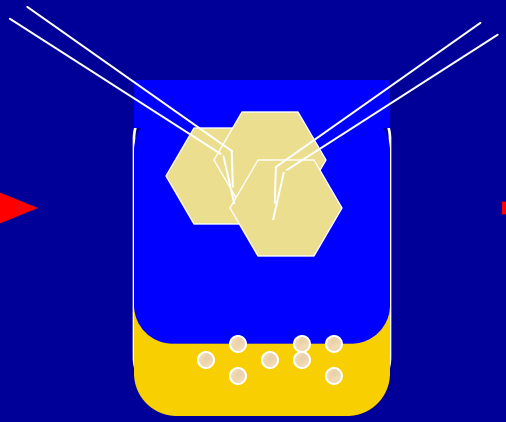
Sandwich-Cultured Hepatocytes

- **Utility**: Evaluation of mechanisms and extent of hepatic uptake, basolateral excretion and biliary excretion of drugs and derived metabolites in species of interest; evaluation of localization, trafficking and regulation of basolateral and canalicular transport proteins
- **Requirements**: Freshly isolated hepatocytes or transporter-competent cryopreserved hepatocytes
- **Advantages**: Hepatocytes cultured between 2 layers of gelled collagen establish intact canalicular networks, express relevant hepatic transport proteins, re-establish polarized excretion of compounds, and represent a useful model to investigate hepatobiliary disposition
- **Limitations**: Requires 3-4 days for proper localization of canalicular transport proteins

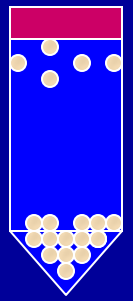
Sandwich-Cultured Hepatocytes: Experimental Procedures



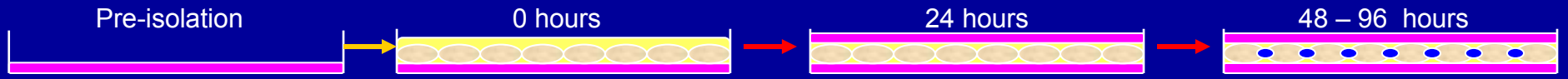
Liver Perfusion (~35 ml/min, 37ºC):
10 min Ca^{2+}-free with chelator
10 min collagenase digestion
Single pass or recirculating flow

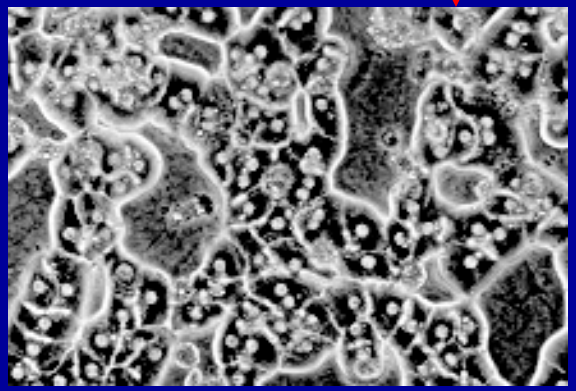


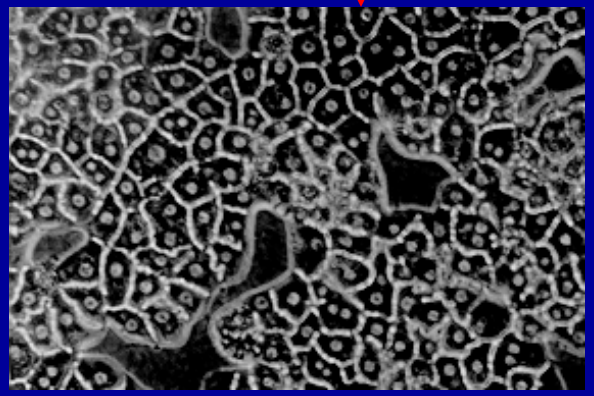
Hepatocyte isolation
Liver capsule gently torn



Percoll Gradient
85 – 95% Viability





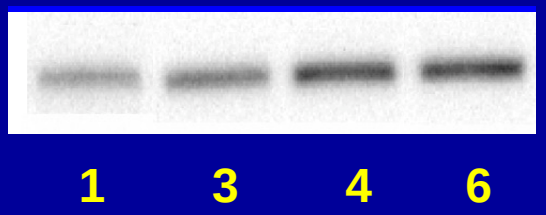


Liu *et al., Am J Physiol* 227:G12, 1999

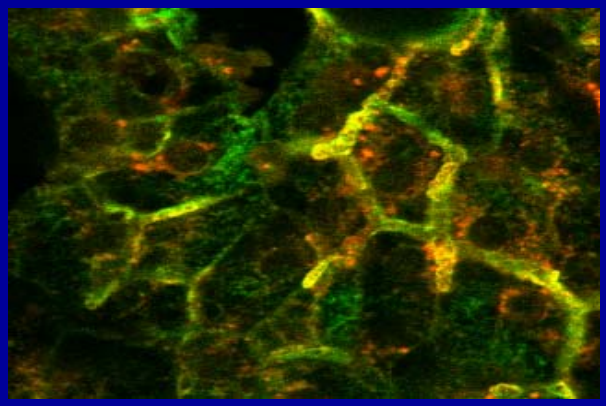
Zamek-Gliszczynski and Brouwer. In *Pharmaceutical Profiling in Drug Discovery for Lead Selection*. AAPS Press, 2004

MRP2 Expression, Localization and Function in Sandwich-Cultured (SC) Human Hepatocytes

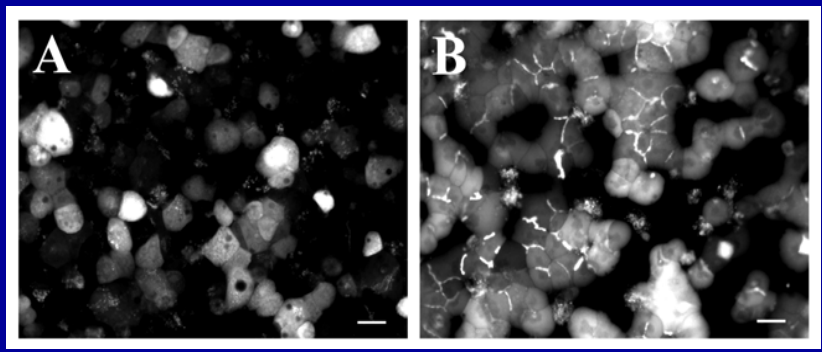
- Expression of MRP2 in SC human hepatocytes over days in culture



- Immunohistochemical co-localization of MRP2 (green) and MDR1 (red) in Day 6 SC human hepatocytes

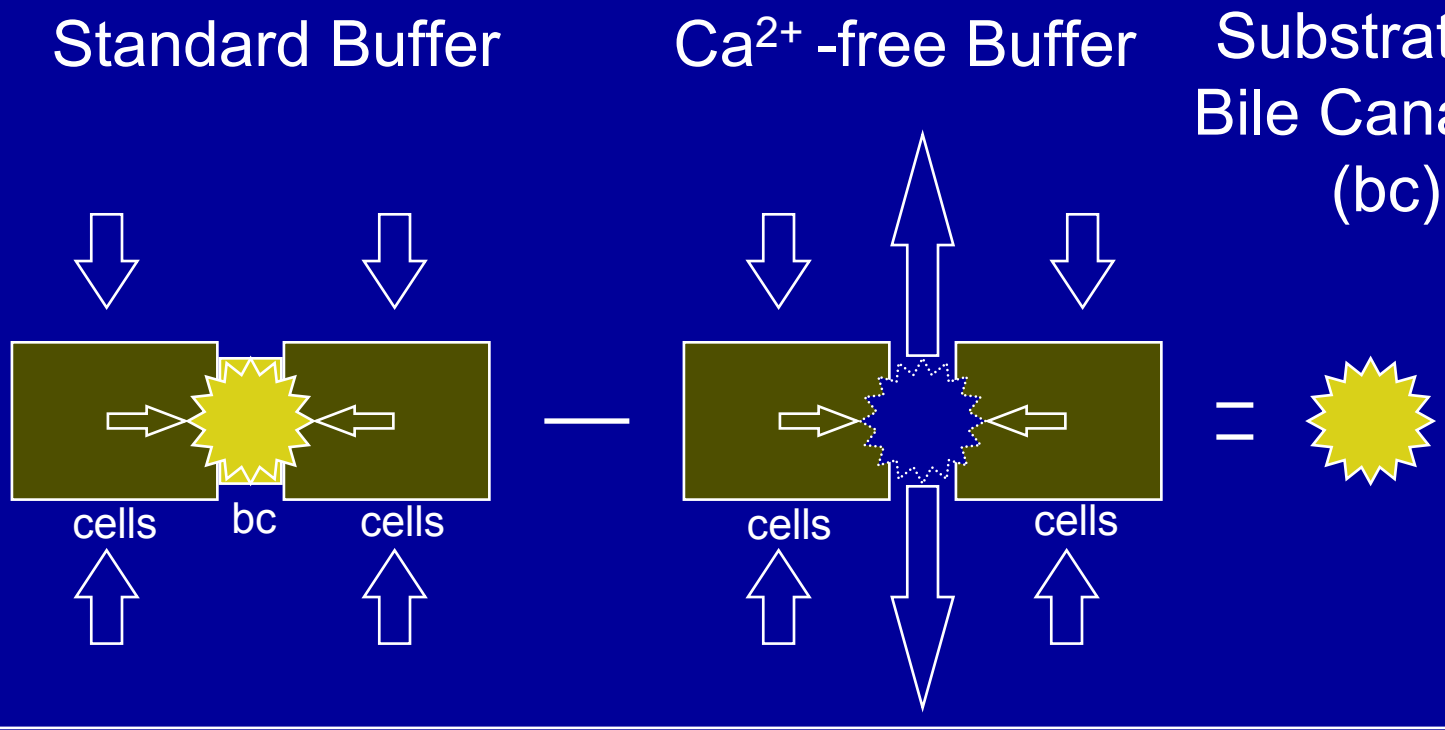


- Fluorescence of 5 (and 6)-carboxy-2,7-dichlorofluorescein in Day 1 and Day 6 SC human hepatocytes after incubation with 2 μM CDF diacetate for 10 min



Hoffmaster *et al*., *Pharm Res* 21:1294, 2004

Quantitation of Biliary Excretion in Sandwich-Cultured Hepatocytes (B-CLEAR®)



$$\text{Biliary Excretion Index (\%)} = \frac{\text{Accumulation}_{\text{cells + bc}} - \text{Accumulation}_{\text{cells}}}{\text{Accumulation}_{\text{cells + bc}}} \times 100$$

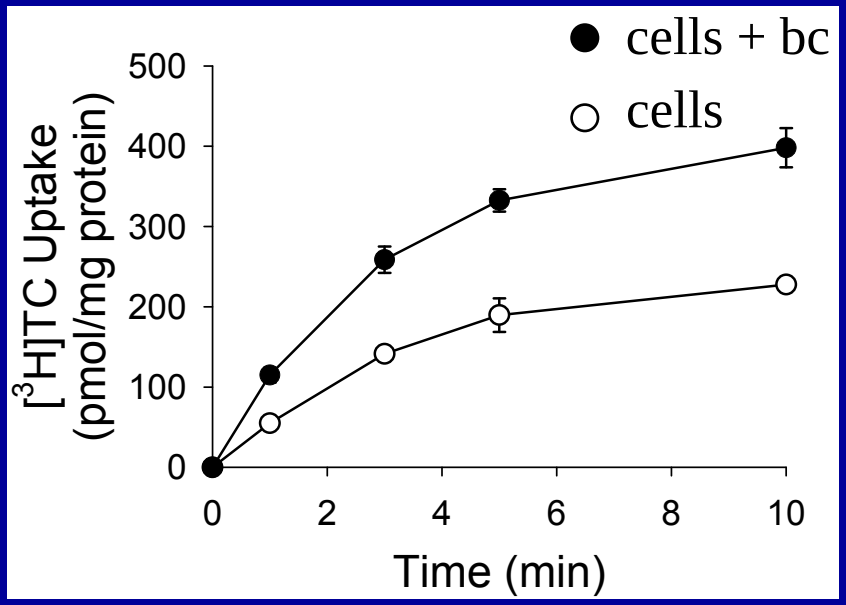
$$\textit{In Vitro}\ \text{Biliary Clearance} = \frac{\text{Accumulation}_{\text{cells + bc}} - \text{Accumulation}_{\text{cells}}}{\text{Time}_{\text{incubation}} \times \text{Concentration}_{\text{medium}}}$$

Qualyst

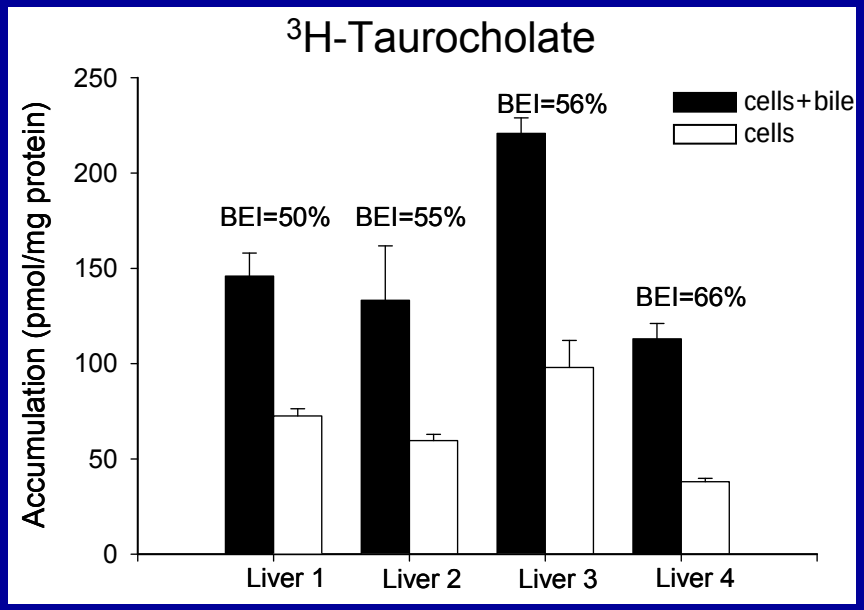
B-CLEAR® is covered by US Pat. No. 6,780,580 and other US and International patents both issued and pending.

Sandwich-Cultured Human Hepatocytes

- Human hepatocytes from 4 living donors cultured in sandwich configuration for 6 days
- On Day 6: taurocholate accumulation measured as marker of cell functionality
- If taurocholate BEI ≥ 50% then further experiments were conducted

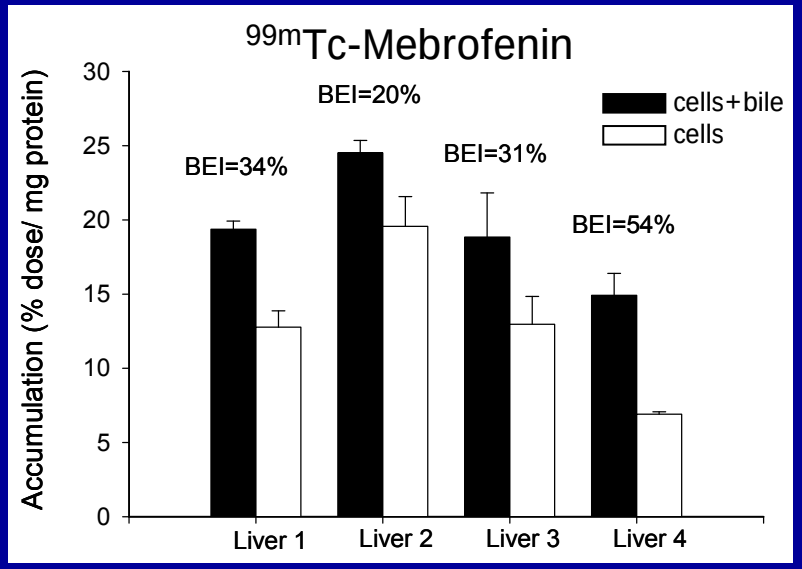


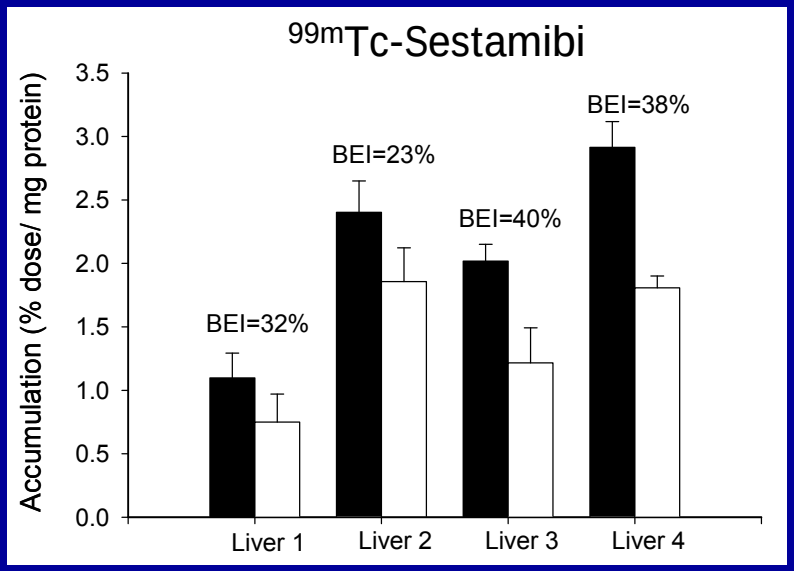
Taurocholate Accumulation in Sandwich-Cultured Human Hepatocytes

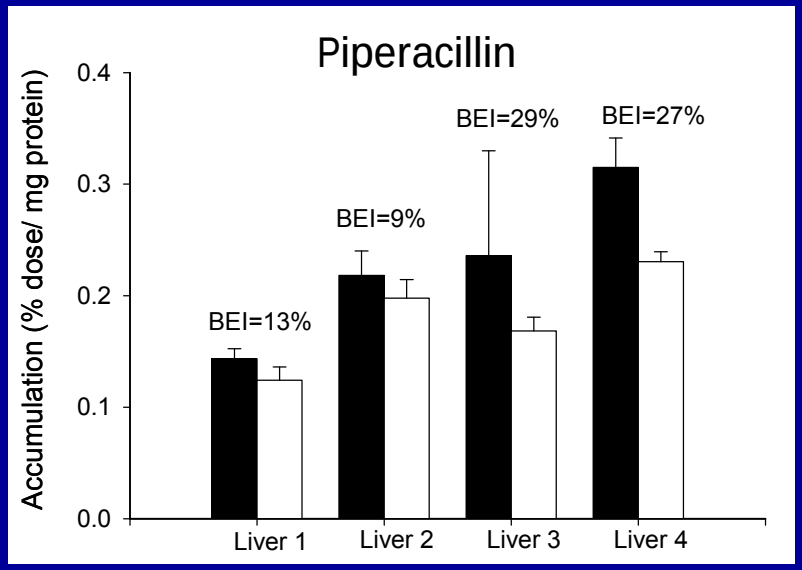


Ghibellini *et al.*, *Clin Pharmacol Ther*, 81:406, 2007

Day 6 Sandwich-Cultured Human Hepatoctyes



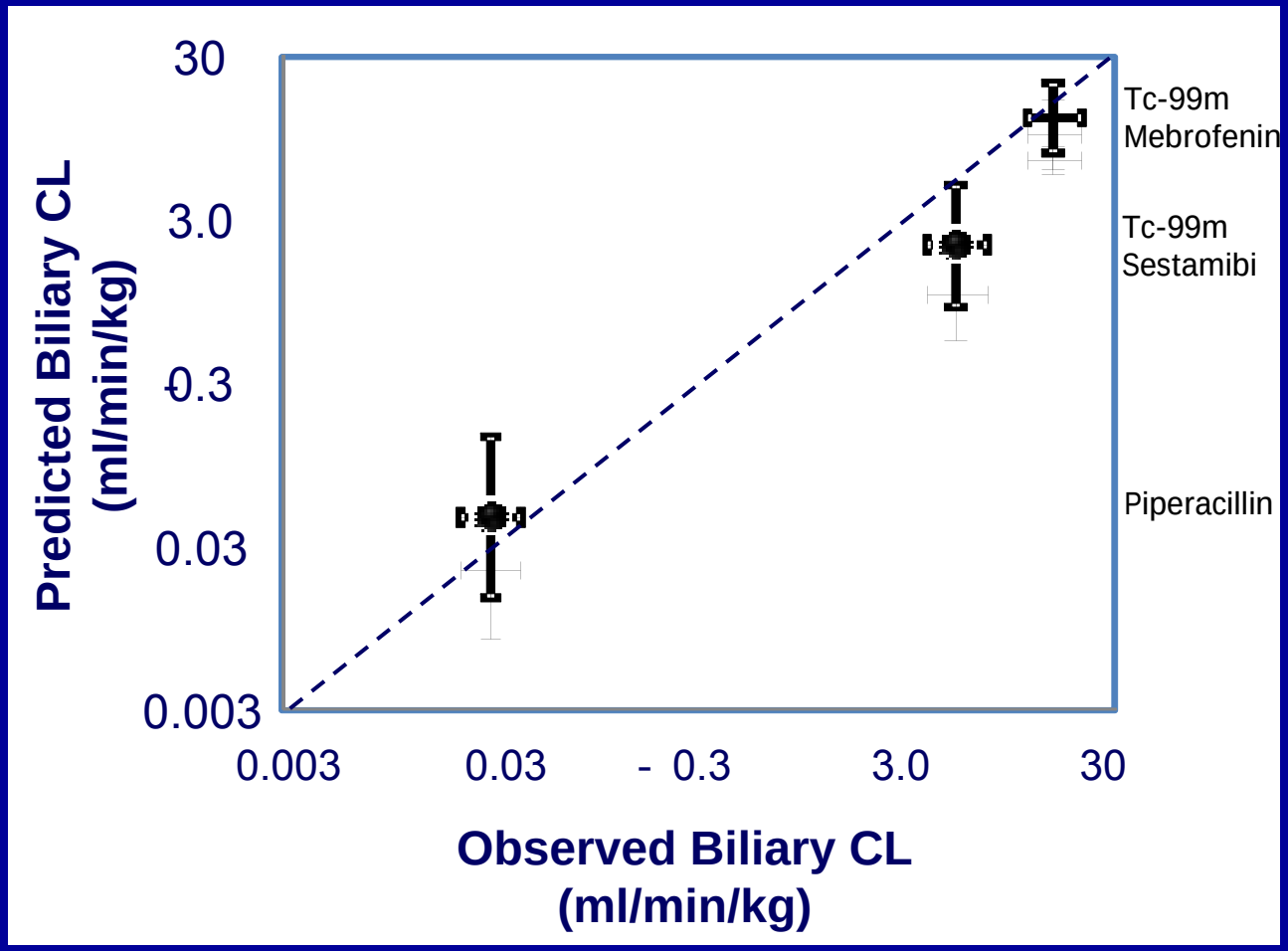




- ^{99m}Tc-mebrofenin accumulation over 10 min (0.5 µCi/mL)
- ^{99m}Tc-sestamibi accumulation over 10 min (0.5-5 µCi/mL)
- Piperacillin accumulation over 30 min (300 µM)

Ghibellini *et al., Clin Pharmacol Ther*, 81:406, 2007

In Vitro-In Vivo Correlation of $Cl_{biliary}$ Corrected for Hepatocellularity Per Gram Liver in Humans

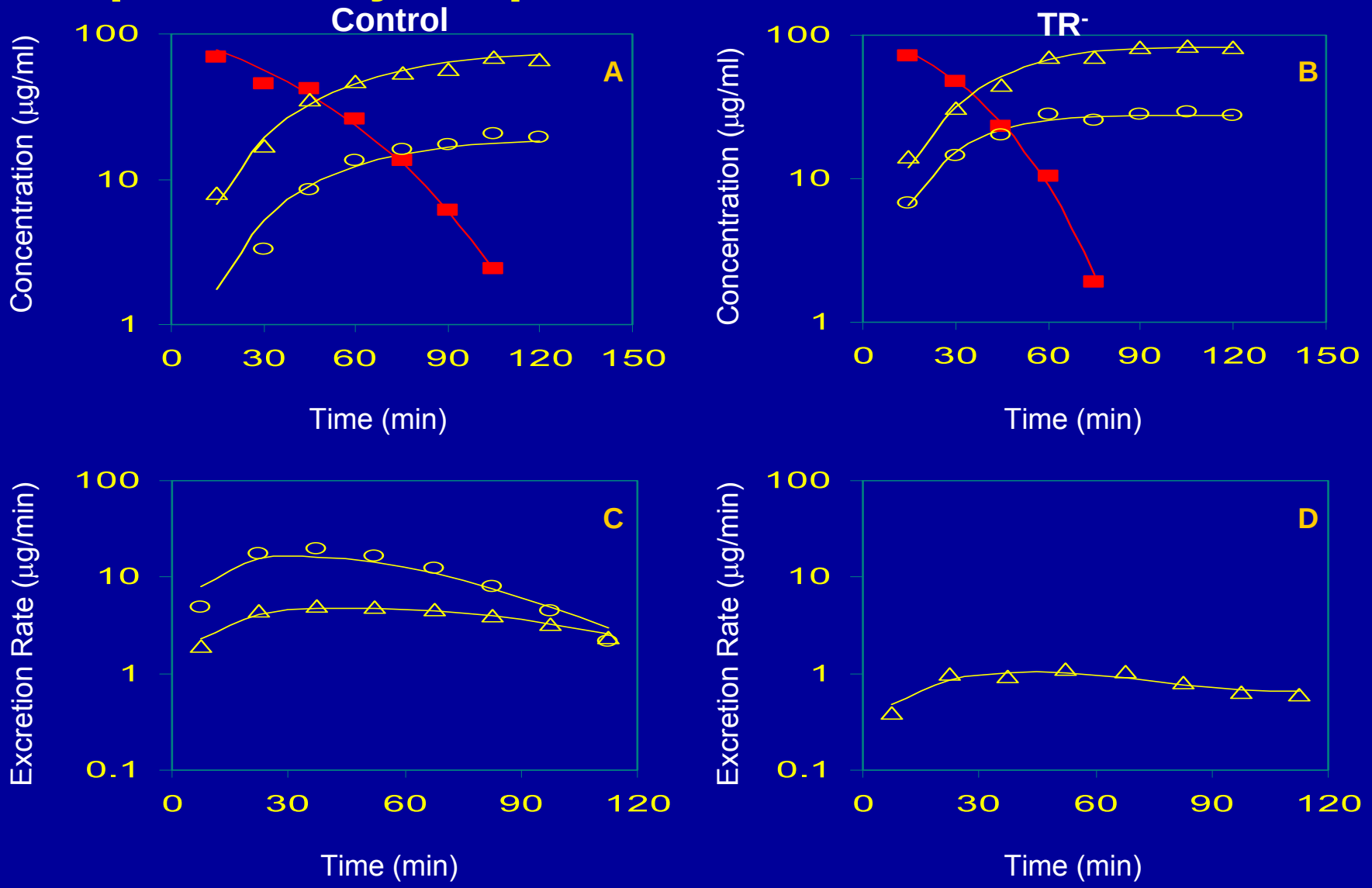


Ghibellini *et al., Clin Pharmacol Ther*, 81:406, 2007
Barter *et al., Curr Drug Metab*, 8:33, 2007

Imaging Techniques and Pharmacokinetic Modeling/Simulation

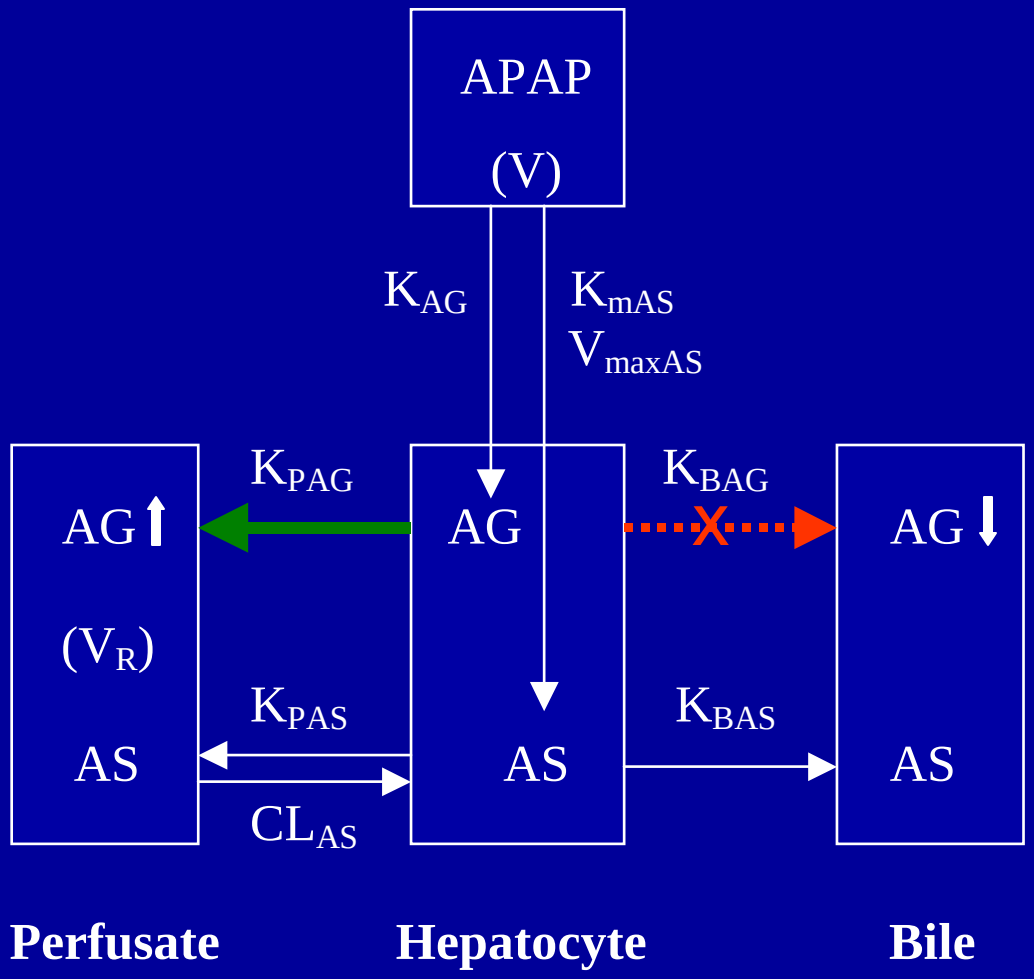
- Available Tools
  - Utility
  - Requirements
  - Advantages
  - Limitations
- When should these tools be used? What questions can be addressed?
- How can in vivo, in situ and in vitro drug transport data, coupled with modeling and simulation, be used to predict the impact of altered transport on drug disposition?

Use of Pharmacokinetic Modeling to Investigate the Hepatobiliary Disposition of APAP, AG and AS



Xiong, H. *et al., J Pharmacol Exp Ther 295:512-518*, 2000

Pharmacokinetic Model for Isolated Perfused TR$^-$ (Mrp2-deficient) Rat Livers



Xiong, H. *et al., J Pharmacol Exp Ther 295:512-518*, 2000

Pharmacokinetic Parameters Describing the Hepatobiliary Disposition of APAP, AG and AS

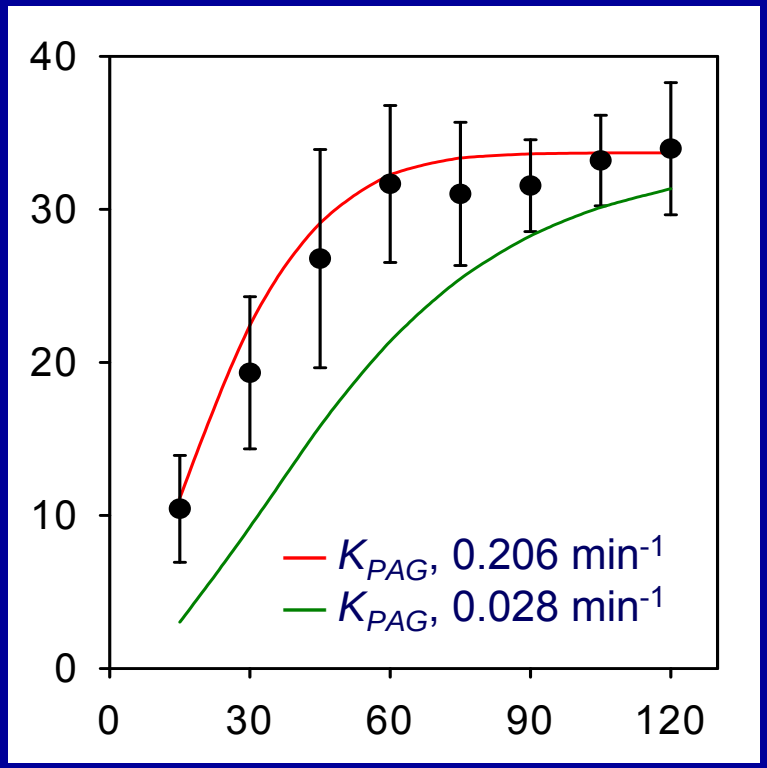
	CONTROL	TR^-
K_{OTHER} (min^{-1})	0.003 ± 0.001	N/A
K_{AG} (min^{-1})	0.007 ± 0.001	0.010 ± 0.003
V_{maxAS} (μg/ml/min/g liver)	0.106 ± 0.037	0.143 ± 0.036
K_{PAG} (min^{-1})	0.028 ± 0.004	0.206 ± 0.095*
K_{BAG} (min^{-1})	0.027 ± 0.016	N/A
K_{PAS} (min^{-1})	0.115 ± 0.051	0.108 ± 0.058
K_{BAS} (min^{-1})	0.007 ± 0.003	0.0005 ± 0.0002*
CL_{AS} (ml/min/g liver)	0.045 ± 0.013	0.132 ± 0.073
V (ml)	92.8 ± 5.7	95.3 ± 8.7

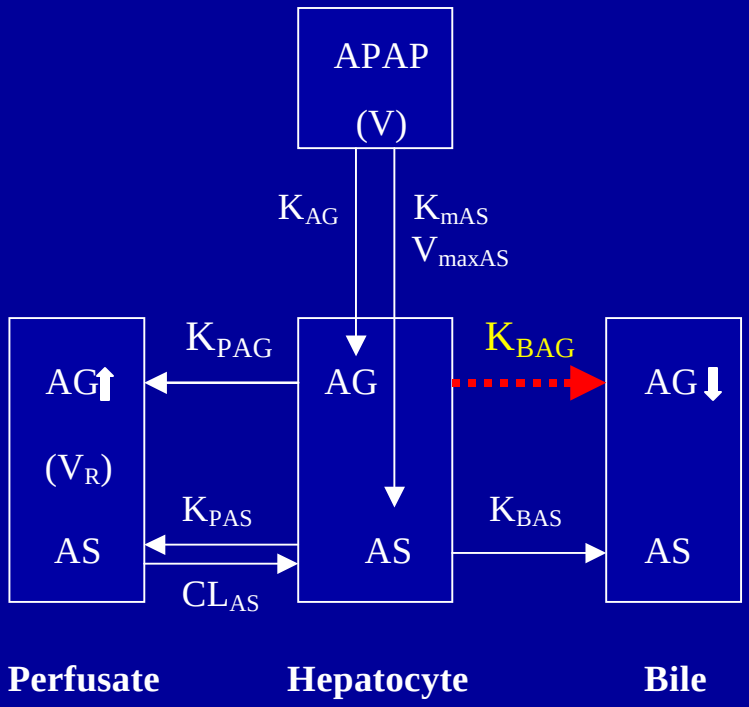
~7.4 fold ↑

[a] Mean ± SD, n = 4 for control group, n = 5 for TR^- group
[b] K_{mAS} was assumed to be 17 μg/ml (Tone *et al.*, 1990)
* $P < 0.05$

Xiong, H. *et al., J Pharmacol Exp Ther 295:512-518*, 2000

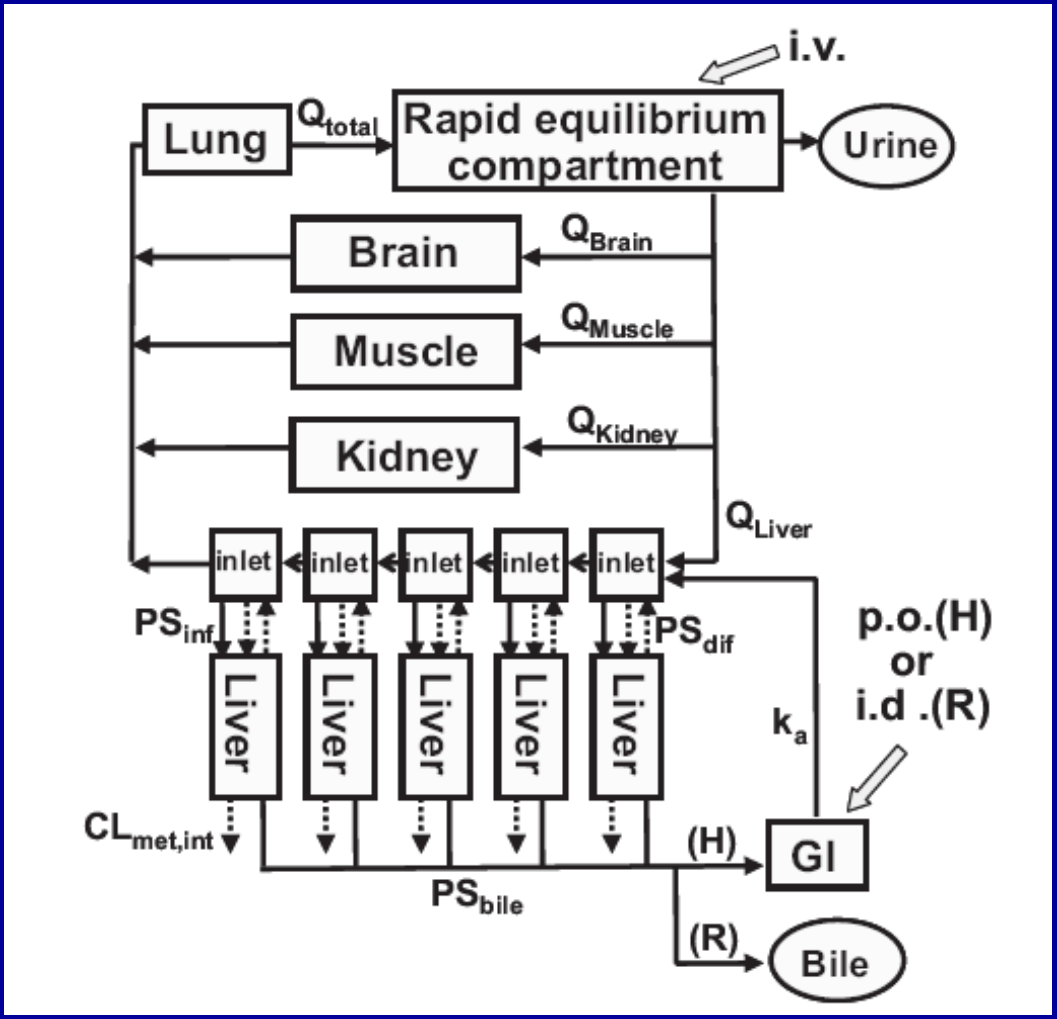
Increased Basolateral Egress of AG in TR$^-$ Rat Livers: Simulated AG Perfusate Concentration vs. Time Profile





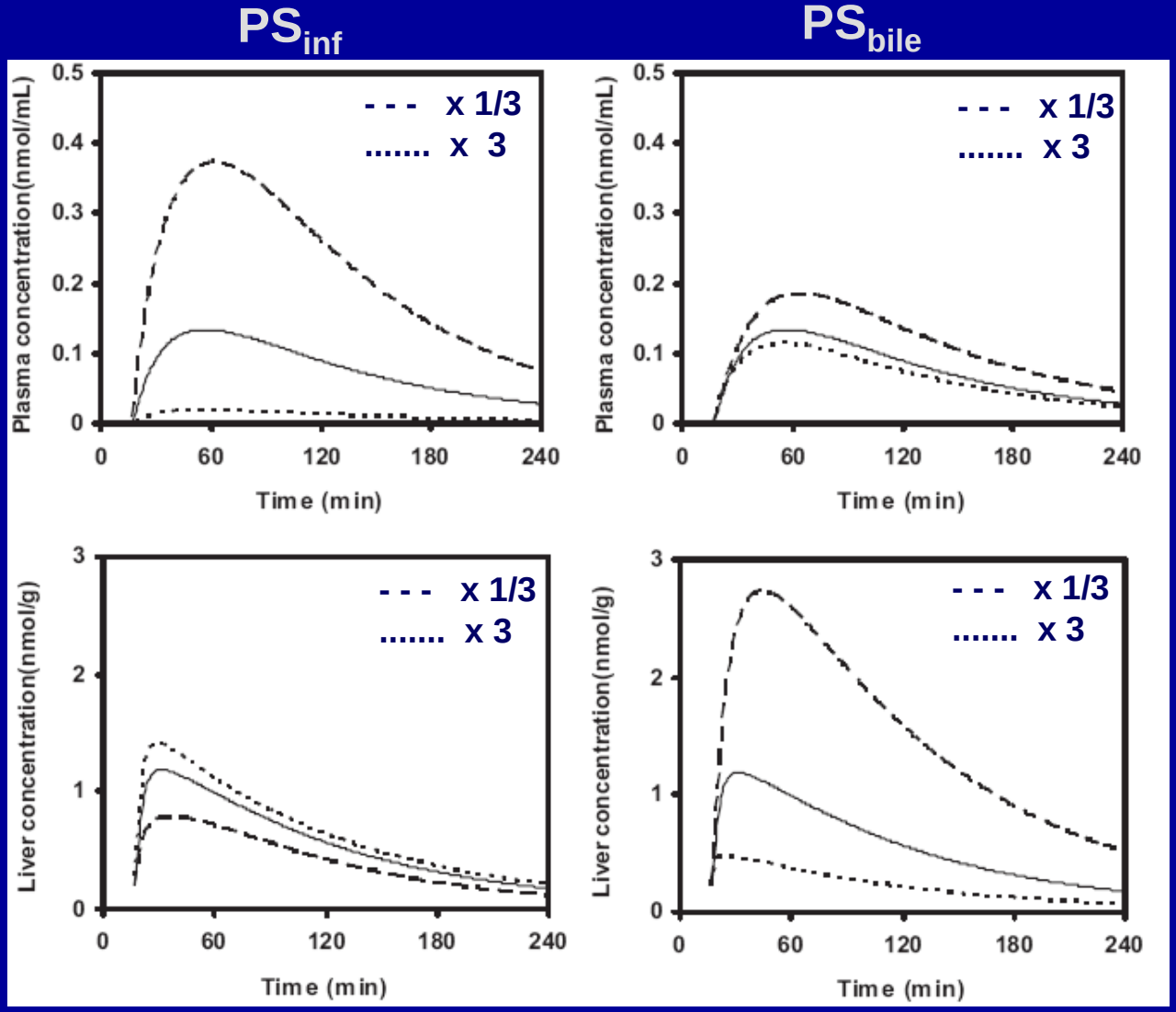
Xiong, H. *et al., J Pharmacol Exp Ther 295:512-518*, 2000

Schematic Diagram of the PBPK Model Predicting Pravastatin Disposition



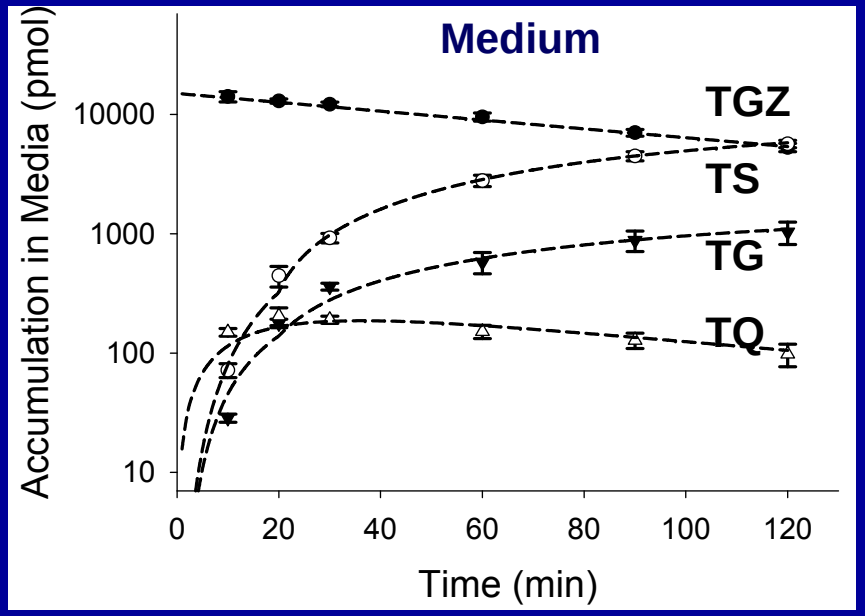
Watanabe *et al., J Pharmacol Exp Ther*, 328:652-662, 2009

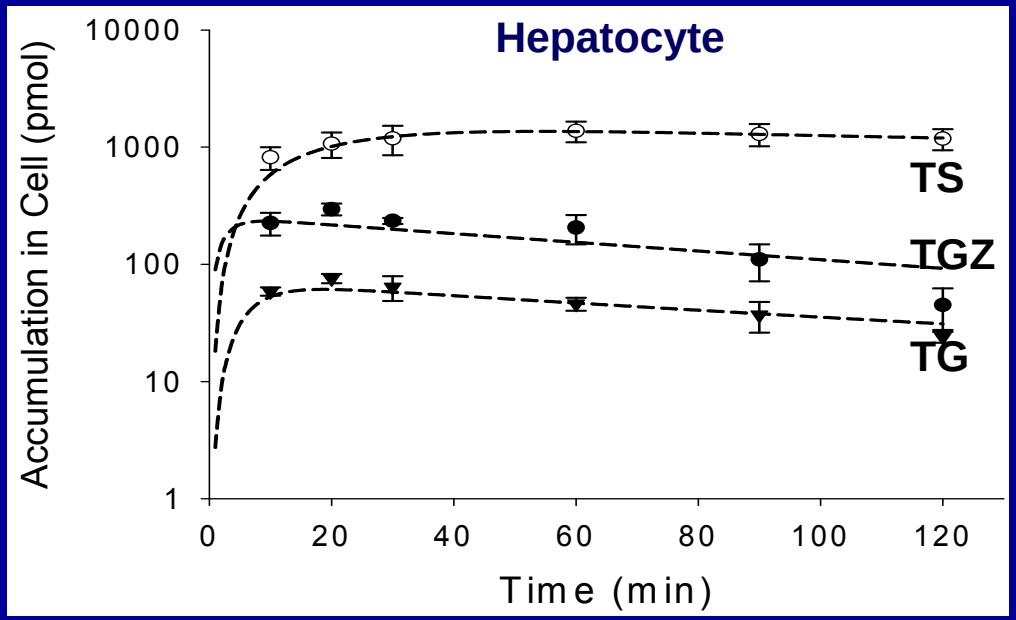
Effects of Changes in Transporter Activity on Plasma and Liver (Target Organ) Concentrations of Pravastatin in Humans

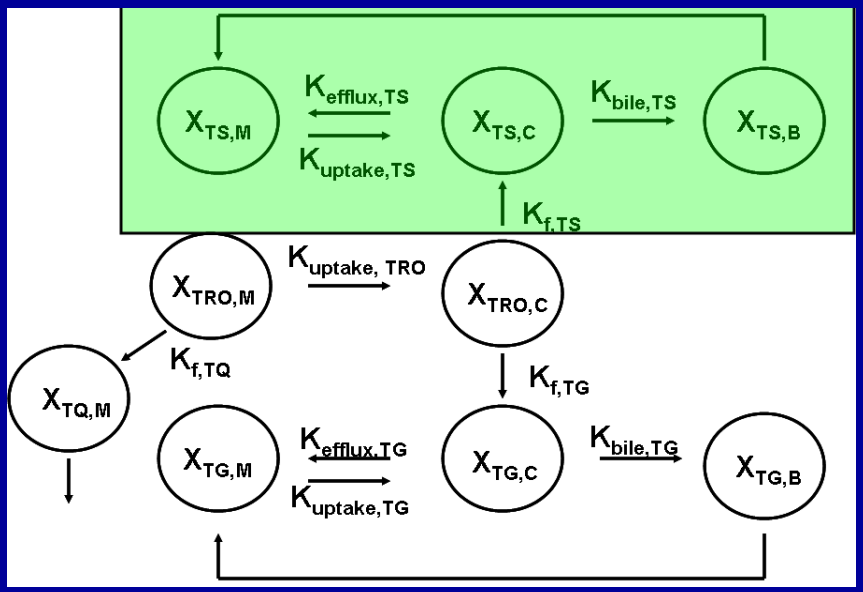


Watanabe *et al.*, *J Pharmacol Exp Ther*, 328:652-662, 2009

Disposition of Troglitazone and Metabolites in Sandwich-Cultured Rat Hepatocytes

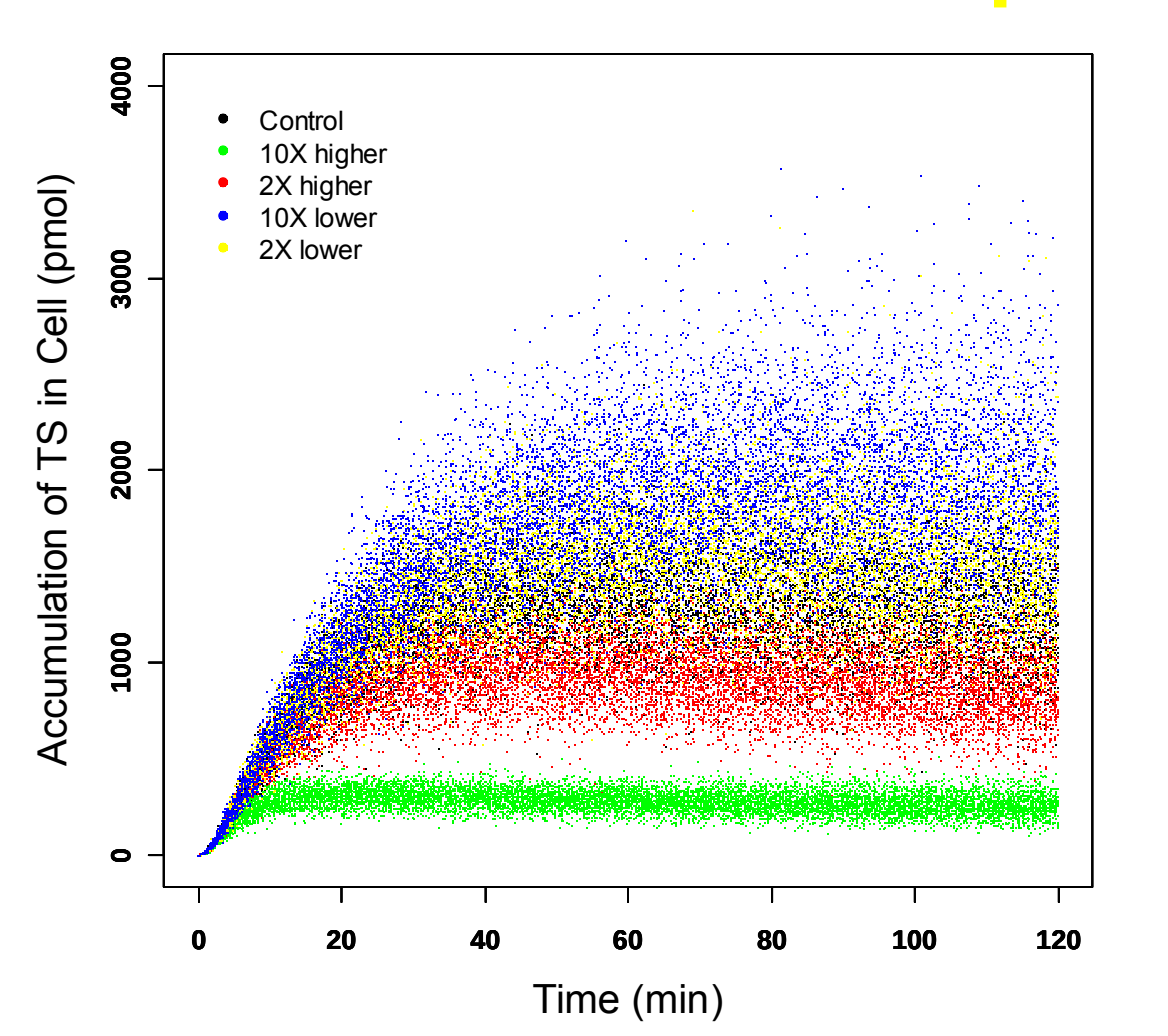






Lee *et al.*, *AAPS Transport Workshop, 2009*

Impact of $K_{bile,TS}$ Modulation on Troglitazone Sulfate (TS) Accumulation in Sandwich-Cultured Rat Hepatocytes



Lee *et al.*, *AAPS Transport Workshop, 2009*

Conclusions

- Numerous imaging methods (WBA, PET, Gamma Scintigraphy) may be used to examine drug distribution and quantify activity of transport proteins
- Nuclear imaging agents (^{99m}Tc-Mebrofenin; ^{99m}Tc-Sestamibi), coupled with use of a specialized oroenteric tube and clinical protocol, can be used to quantify $Cl_{biliary}$ of drugs *in vivo* in humans
- Application of gamma scintigraphy in drug development may provide insight regarding:
  - drug-drug interactions in hepatobiliary transport
  - intestinal secretion of drugs and/or metabolites
- *In vivo, in situ* and *in vitro* drug transport data, coupled with modeling and simulation, may be used to predict the impact of altered transport protein function, due to drug interactions, disease states or polymorphisms, on drug disposition

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