Membrane Transporters in Drug Development

Dr Raymond Evers
Merck & Co
Drug Metabolism and Pharmacokinetics
P.O. Box 2000
Rahway, NJ 08816
Raymond_Evers@merck.com

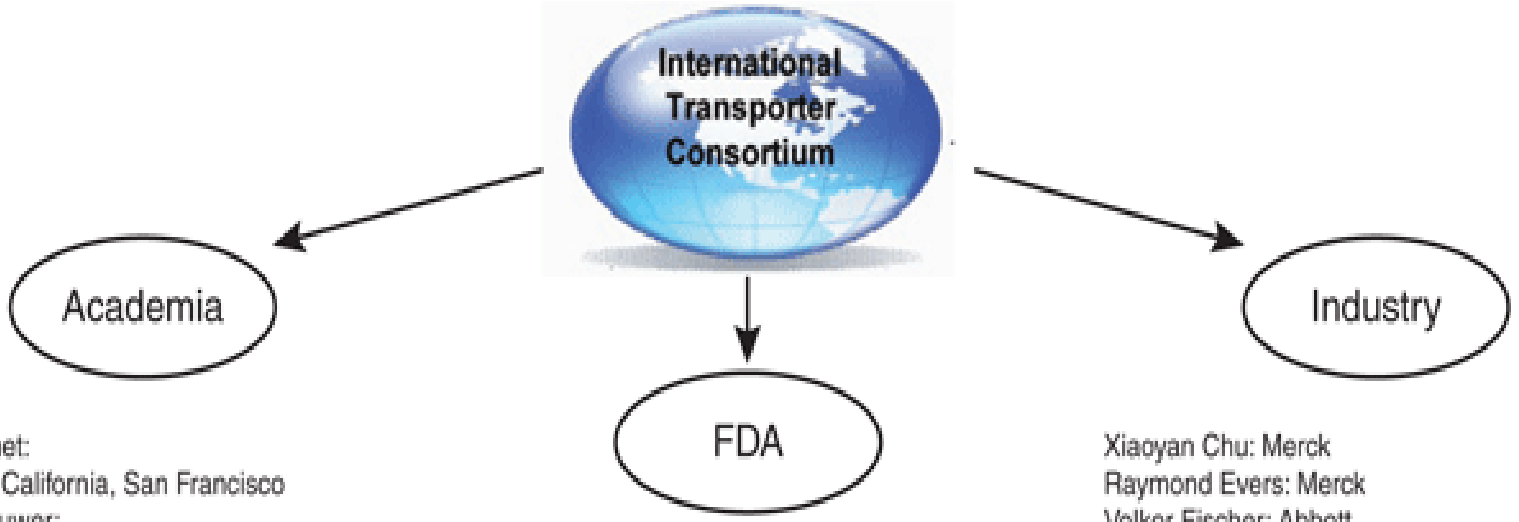
MERCK

Outline

- Part 1
  - Overview of the ITC
  - Transporters covered by the ITC
  - Decision trees

- Part 2
  - Case Studies
    - OATP-mediated DDIs
    - Digoxin-Rifampin DDI

Transporters and the FDA (Critical Path Initiative)



Leslie Z. Benet:
University of California, San Francisco
Kim L.R. Brouwer:
University of North Carolina at Chapel Hill
Kathleen M. Giacomini:
University of California, San Francisco*
Toshihisa Ishikawa: RIKEN Yokohama Institute*
Dietrich Keppler: German Cancer Research Center
Richard B. Kim: University of Western Ontario
Mikko Niemi: University of Helsinki
Yuichi Sugiyama: University of Tokyo
Peter. W. Swaan: University of Maryland
Stephen H. Wright: University of Arizona

Shiew Mei Huang:
Food and Drug Administration*
Lei Zhuang:
Food and Drug Administration

Xiaoyan Chu: Merck
Raymond Evers: Merck
Volker Fischer: Abbott
Katheen M. Hillgren: Lilly Research Laboratories
Keith A. Hoffmaster: Novartis
Caroline A. Lee: Pfizer
Joseph W. Polli: GlaxoSmithKline
Donald J. Tweedie:
Boehringer Ingelheim Pharmaceuticals*
Joseph A. Ware: Genentech
Maciej Zamek-Gliszczynski:
Lilly Research Laboratories

Goals of the International Transporter Consortium

- Provide an update on the current thinking on transporters
- For *in vitro* studies, provide a focus on studies that can have a translational clinical interpretation
  - Limit raising red flags with *in vitro* studies that cannot be addressed *in vivo* in the clinic
- Explore gaps and suggest ways forward
- Provide a coordinated approach: academia, industry and regulatory
- Help to move the science forward
  - Decision trees to assist drug development and regulatory agencies
  - Consensus on current scientific status
  - Gather support to move the ADME transport area forward

International Transporter Consortium

Workshop
Bethesda North Marriott
October 2nd and 3rd, 2008

- Sponsored by FDA Critical Path
- Workshop organized by Drug Information Association (DIA)
- Co-sponsorship by AAPS, ISSX, PhRMA
- Provide a focus to initiate a White Paper for completion in 2009

White Paper: Nature Reviews Drug Discovery 2010 Vol 9, p. 215-236

Membrane Transporters in Drug Development
The International Transporter Consortium, ITC
Corresponding authors: K. Giacomini, S-M. Huang and D. Tweedie

- Basic Introduction and Summary of Transporter
  - Highlights what we know
- Methods for Studying Transporters
  - Current solutions and future prospects
- Drug Development Issues
  - Decision trees

White Paper – What It Is and What It Is Not

What it is…..

- A **consensus view** on the current thinking
  - What is known about the relative importance of transporters?
  - Where should one put effort?
- The known unknowns
  - What facts are known to be untrue (dispelling myths)?
  - Where are our gaps in knowledge (where should we increase our knowledge)?
- **A guideline** (not a guidance/rules) towards what should be considered during development.
  - Whitepaper biased toward **NDA submission**

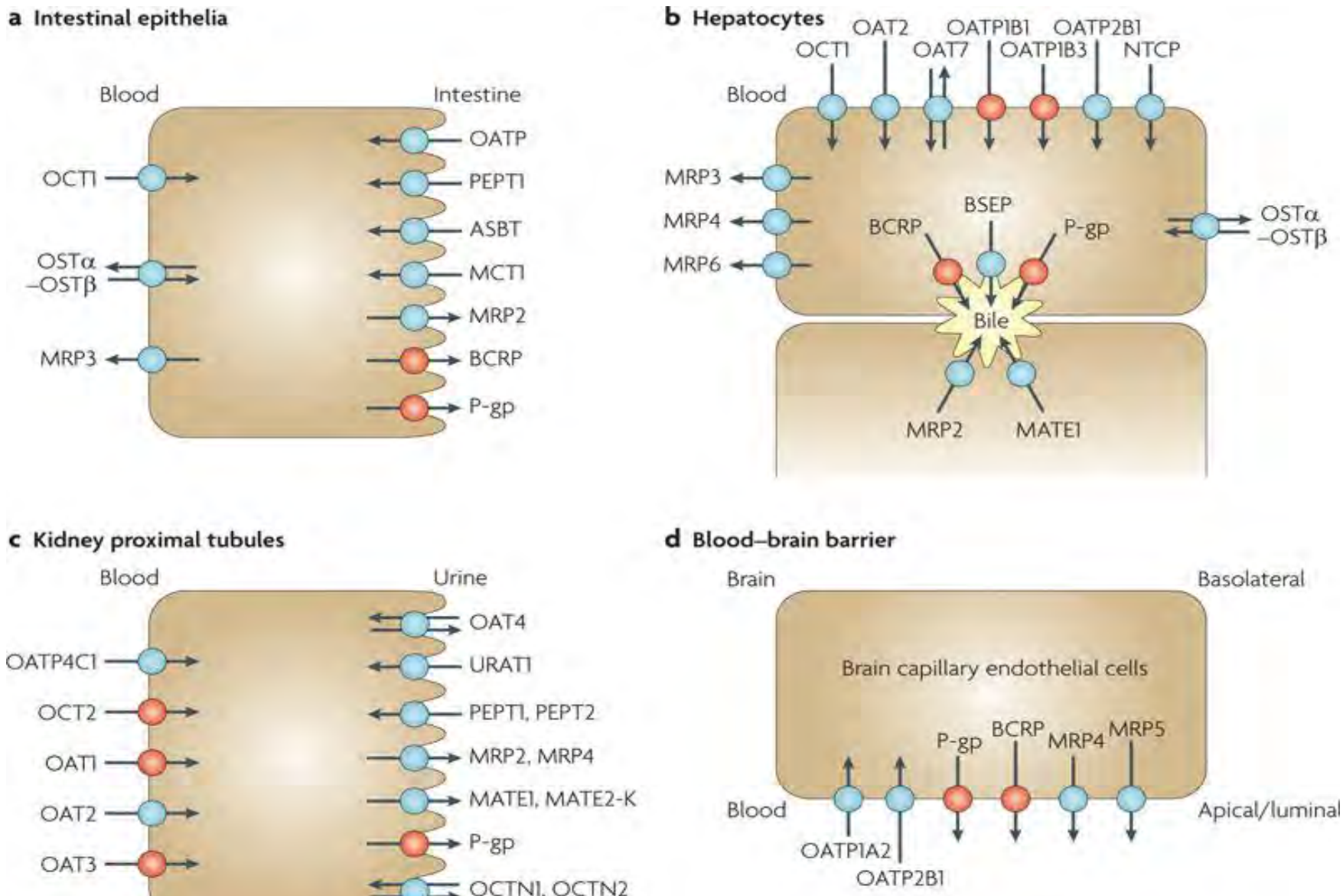
and what it is not…..

- A complete literature review.
- A **prescriptive guidance on what to do and how to do it**, with a clear description of what it will mean.
- A consensus document that everyone agrees to.
- A description of all of the exceptions.
  - Your experience is important and we would certainly appreciate you sharing that with the scientific community.
- **Decision trees are not definitive**.
  - Included to help move the science forward by acting as templates for discussion
  - Not must do's

Transporters

- Two Families of Transporters (400+ members)
  - 30 Contribute to the efficacy and safety of drugs
- ABC Transporters
  - ATP-binding cassette
  - Present in tissue barriers and excretory organs, can move compounds against a concentration gradient
    - P-glycoprotein (P-gp, ABCB1)
    - Breast cancer resistance protein (BCRP, ABCG2)
    - Multidrug resistance proteins (MRP Family)
- SLC transporters
  - Organic Solute Carrier Transporters
  - Found throughout the body, play a role in cellular homeostasis and distribution of nutrients.
    - OATs (OAT1 - SLC22A6), OAT3 - SLC22A8)
    - OCT/OCTNs (OCT2 –SLC22A2)
    - OATPs (OATP1B1- SLCO1B1, OATP1B3-SLCO1B3)

Expression of Transporters in Major Human Organs



Nature Reviews Drug Discovery, 2010

Transporters Selected for Evaluation in Drug Development

Transporter	Gene name	Examples of clinical substrates and inhibitors[a]	Tissue
OATP1B1	*SLCO1B1*	Statins, repaglinide, valsartan, olmesartan, *ritonavir, lopinavir, rifampin, cyclosporine*	Hepatocyte (sinusoidal)
OATP1B3	*SLCO1B3*	Statins, telmisartan, *rifampin, cyclosporine, lopinavir*	Hepatocyte (sinusoidal)
OAT1	*SLC22A6*	Zidovudine, lamivudine, zalcitabine, acyclovir, tenofovir, ciprofloxacin, methotrexate, *probenecid*	Kidney proximal tubule, placenta
OAT3	*SLC22A8*	Furosemide, bumetanide, *probenecid*	Kidney proximal tubule, choroid plexus, brain endothelia
OCT2	*SLC22A2*	Metformin, pindolol, procainamide, ranitidine, varenicline, *cimetidine*	Kidney proximal tubule
MDR1	*ABCB1*	Digoxin, loperamide, fexofenadine, *itraconazole, cyclosporine, quinidine, elacridar*	Intestinal enterocyte, kidney proximal tubule, hepatocyte (canalicular), brain endothelia
BCRP	*ABCG2*	Topotecan, statins (e.g., rosuvastatin), *elacridar*	Intestinal enterocyte, hepatocyte (canalicular), kidney proximal tubule, brain endothelia, placenta, stem cells, mammary gland

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MDR, multidrug resistance, OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter.

[a]Substrates and inhibitors (shown in italics) may not be selective for a transporter and may interact with multiple transporters.

Data from refs. 3–13.

Transporter Information in Drug Labeling

P-gp	Aliskiren, ambrisentan, [aprepitant], *clarithromycin*, colchicine, [dexvenafaxine], *dronedarone*, [eltrombopag], *everolimus*, fexofenadine, [fosaprepitant], [ixabepilone], *lapatinib*, *maraviroc*, *nilotinib*, *paliperidone*, posaconazole, [prasugrel], [[propafenone]], propranolol, *ranolazine*, saxagliptin, silodosin, sirolimus, sitagliptin, *tipranavir***, *tolvaptan*, topotecan, [vorinostat]
OATP1B1	Atorvastatin, *cyclosporine*, *eltrombopag****, *lapatinib*, valsartan
OATP	Ambrisentan
OAT	Sitagliptin (OAT3)
OCT	Metformin, pramipexole, [saxagliptin], [sitagliptin], varenicline (OCT2)
BCRP	Lapatinib, topotecan
MRP	Mycophenolate (MRP2), [ixabepilone] (MRP1),valsartan (MRP2)

*Not an extensive list: data based on a preliminary survey of electronic PDR and Drugs@FDA on September 18, 2009. They are substrates, *inhibitors*, ***both substrates and inhibitors***, [not a substrate or an inhibitor], or [[not studies as a substrate or an inhibitor]]; **:Tipranavir is also a P-gp inducer *** an inhibitor; its labeling contains a list of OATP1B1 substrates

<Huang, SM, Zhang L, Giacomini KM, Clin Pharmacol Ther January 2010>

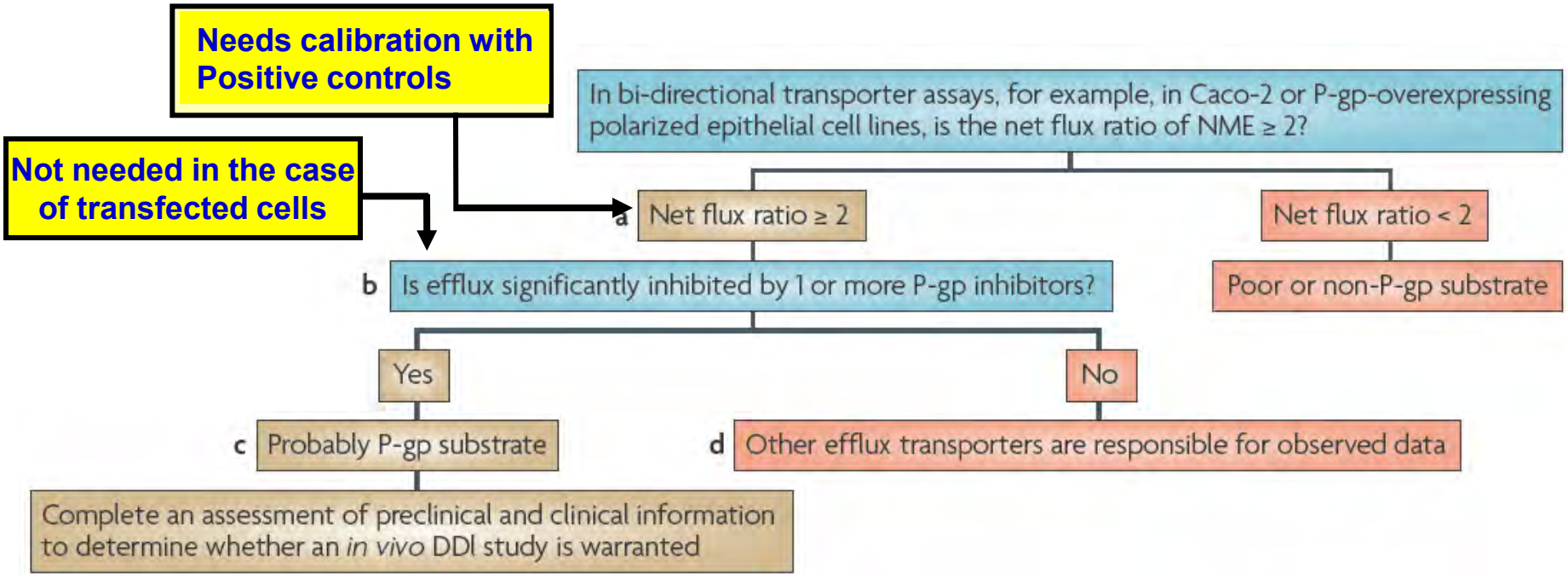
Use of Decision Trees

- Pros
  - Evolution of concept
  - Generate discussion points
  - Offers flexibility

- Cons
  - Rigid interpretation: prescriptive and overly cautious
  - Insufficient knowledge to populate the decision points
  - Lack of selective substrates and inhibitors
  - Not fully vetted

"The evolution and appropriate application of the decision trees will require constant monitoring"

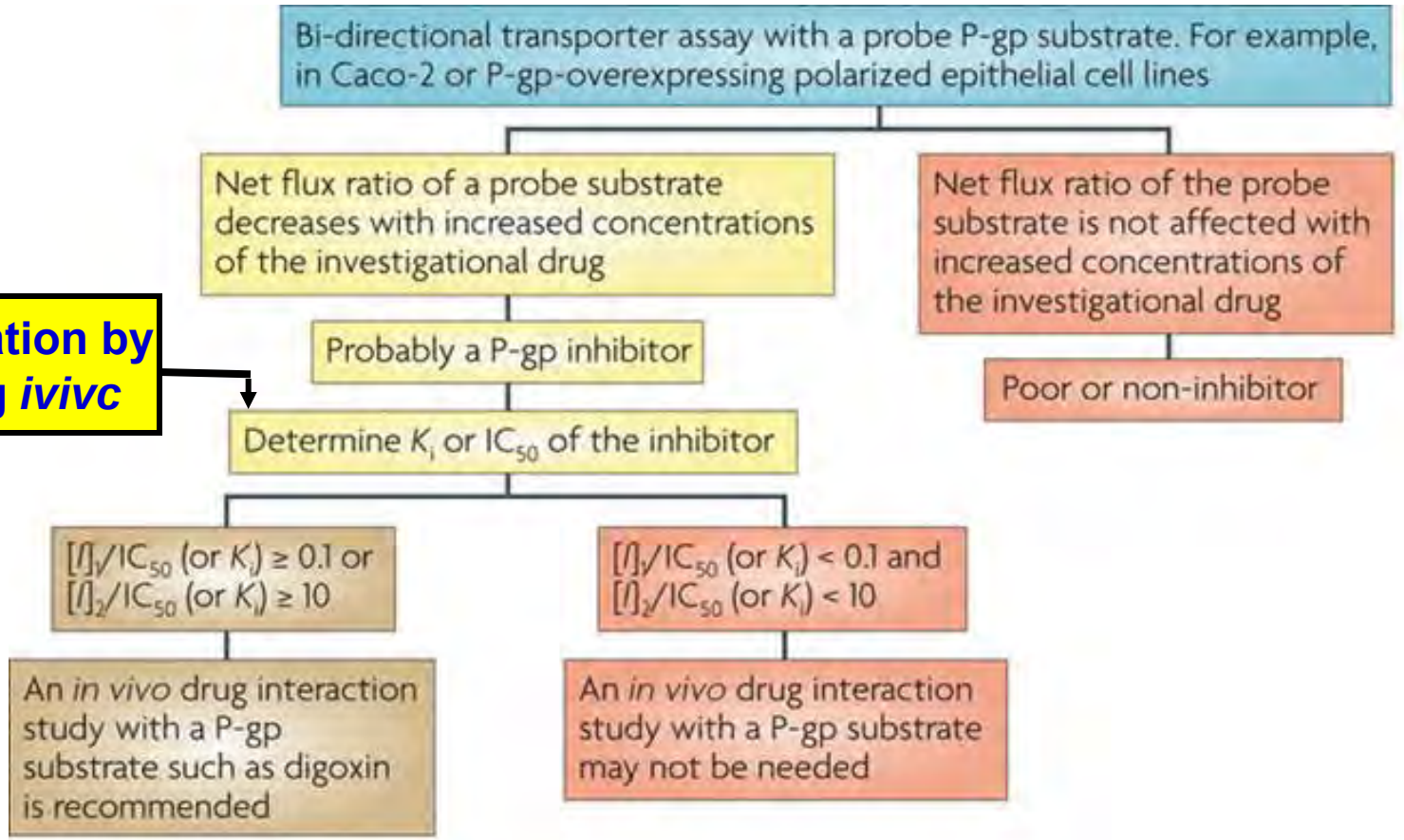
Pgp/BCRP Substrate Decision Tree



Many drugs that are efflux substrates are extensively absorbed

- Factors contributing to efflux limited absorption are:
  - high Km, Vmax
  - low solubility
  - low permeability
  - metabolic stability
  - low dose.

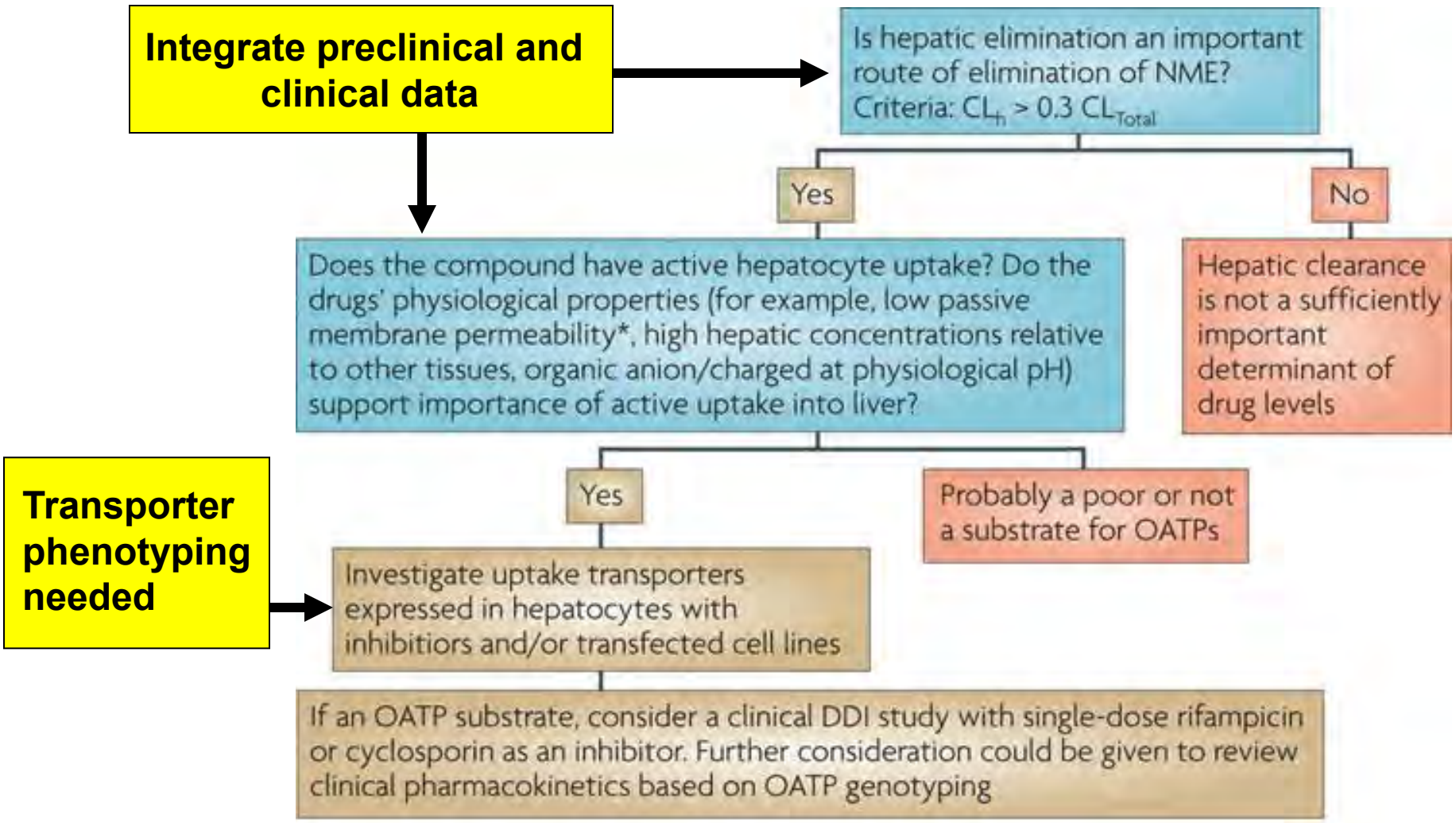
Decision Tree for Pgp Inhibitor Interactions



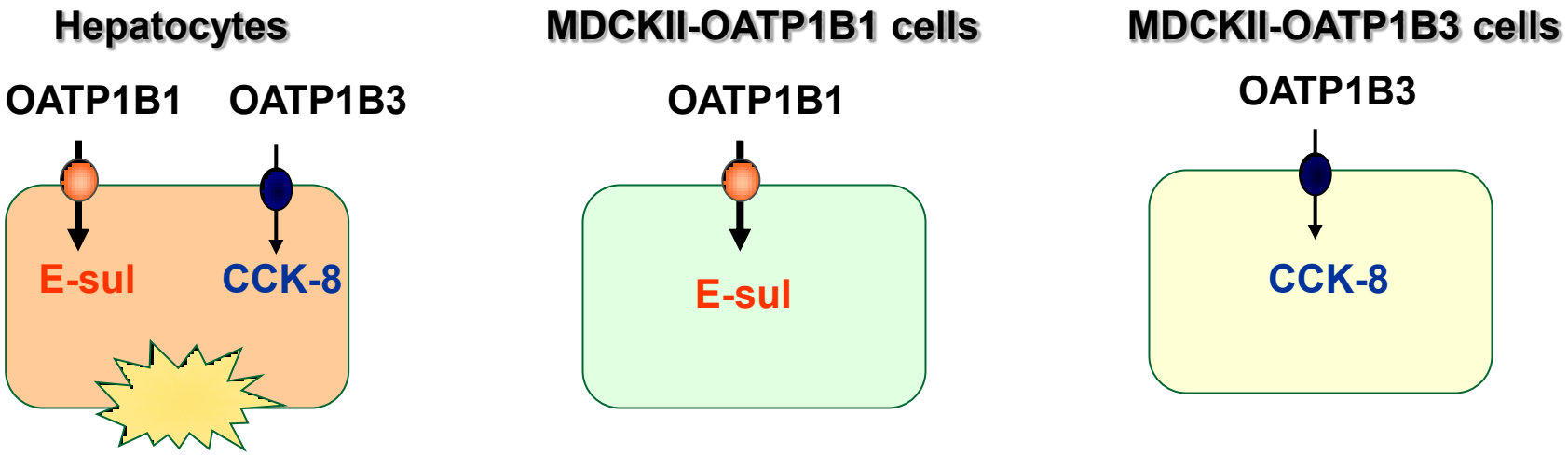
Needs calibration by establishing *ivivc*

- $[I_1]$ is steady-state total C_{max} at the highest clinical dose
- $[I_2]$ is the GI concentration calculated at dose (mg)/250 mL

OATP Substrate Decision Tree



Relative Expression and Activity Factors



Relative Expression Factor (REF)

$$REF_{\mathrm{OATP1B1}} = \frac{Exp_{\mathrm{Hep,\ OATP1B1}}}{Exp_{\mathrm{OATP1B1,\ OATP1B1}}}$$

$$REF_{\mathrm{OATP1B3}} = \frac{Exp_{\mathrm{Hep,\ OATP1B3}}}{Exp_{\mathrm{OATP1B3,\ OATP1B3}}}$$

Relative Activity Factor (RAF)

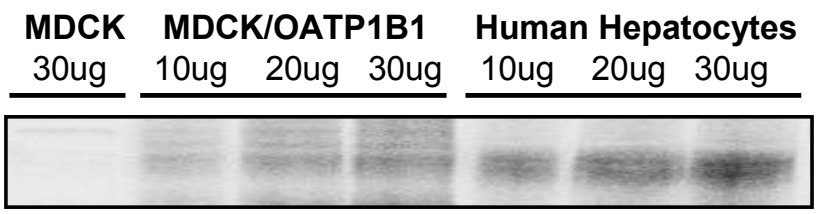
$$RAF_{\mathrm{OATP1B1}} = \frac{CL_{\mathrm{Hep,\ ESul}}}{CL_{\mathrm{OATP1B1,\ ESul}}}$$

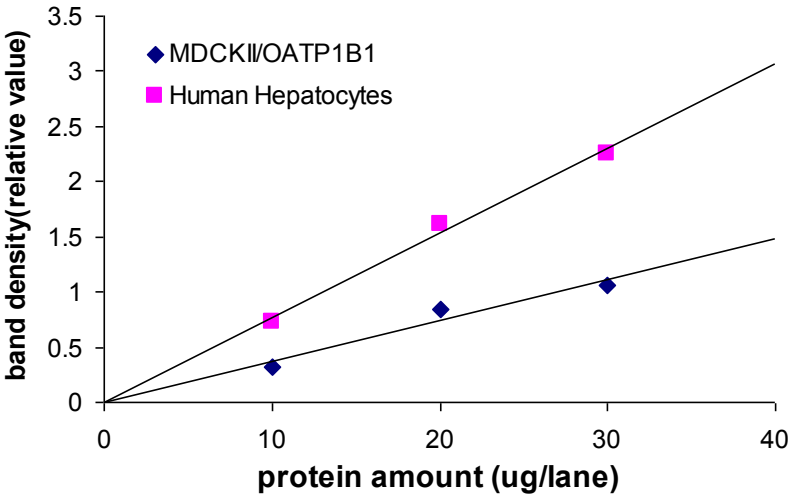
$$RAF_{\mathrm{OATP1B3}} = \frac{CL_{\mathrm{Hep,\ CCK8}}}{CL_{\mathrm{OATP1B3,\ CCK8}}}$$

Shitara et al., 2006

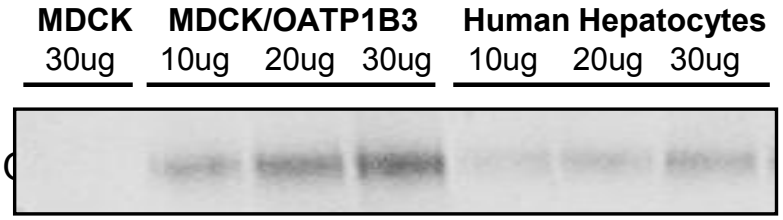
REF for OATP1B1 and OATP1B3

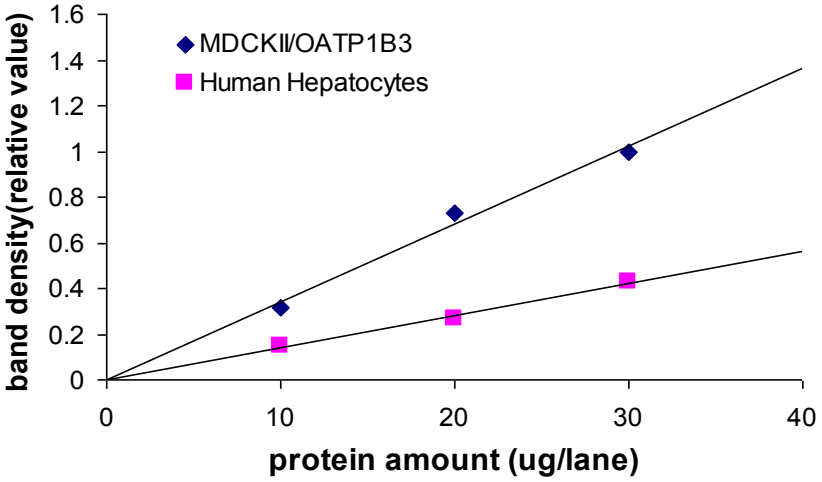
OATP1B1





OATP1B3



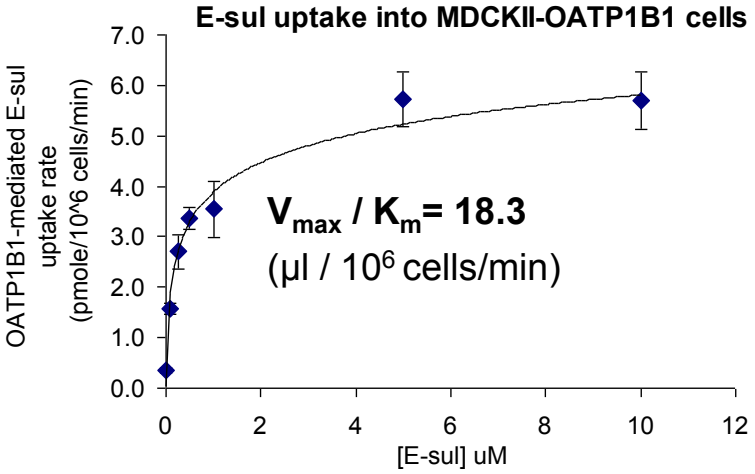


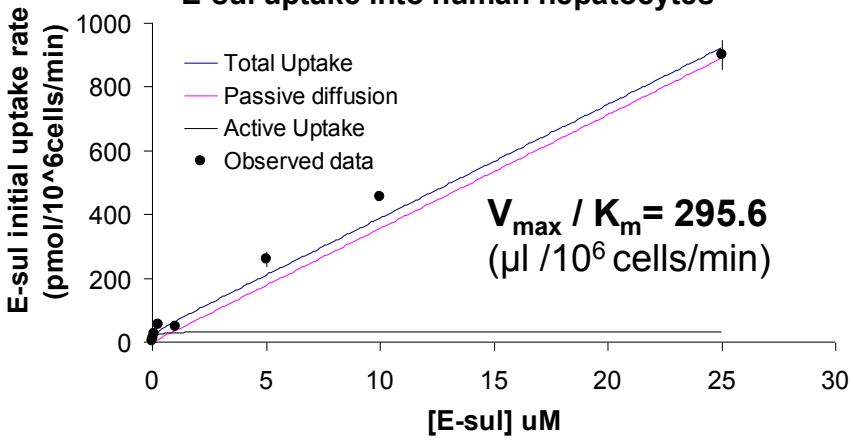
$$REF_{OATP1B1} = Exp_{Hep,OATP1B1} / Exp_{OATP1B1,\ OATP1B1} = 16.9$$

$$REF_{OATP1B3} = Exp_{Hep,OATP1B3} / Exp_{OATP1B3,\ OATP1B3} = 2.8$$

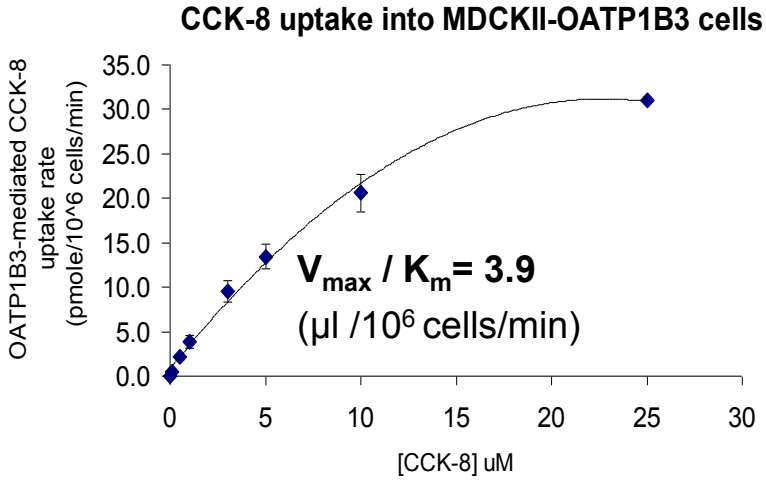
RAF for OATP1B1 and OATP1B3

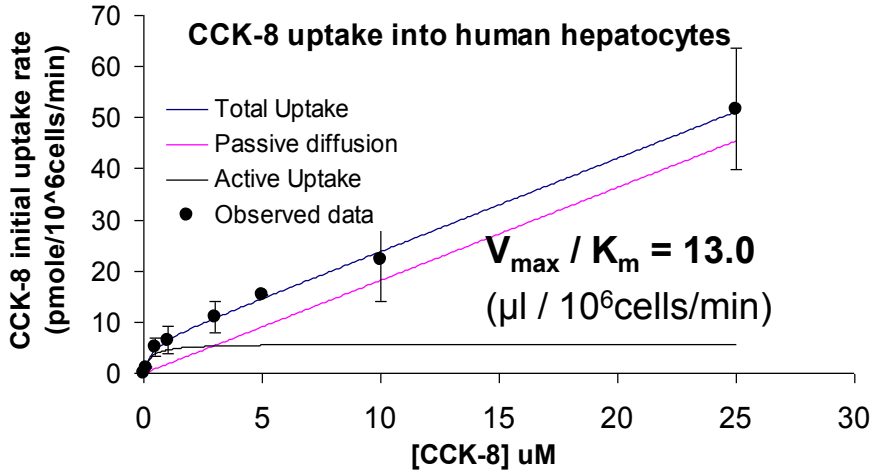
$$RAF_{OATP1B1} = CL_{Hep,E\text{-}sul} / CL_{OATP1B1,\ E\text{-}sul} = 16.2$$





$$RAF_{OATP1B3} = CL_{Hep,CCK\text{-}8} / CL_{OATP1B3,\ CCK\text{-}8} = 3.4$$





Relative Contribution of OATPs to Pitavastatin Uptake Clearance

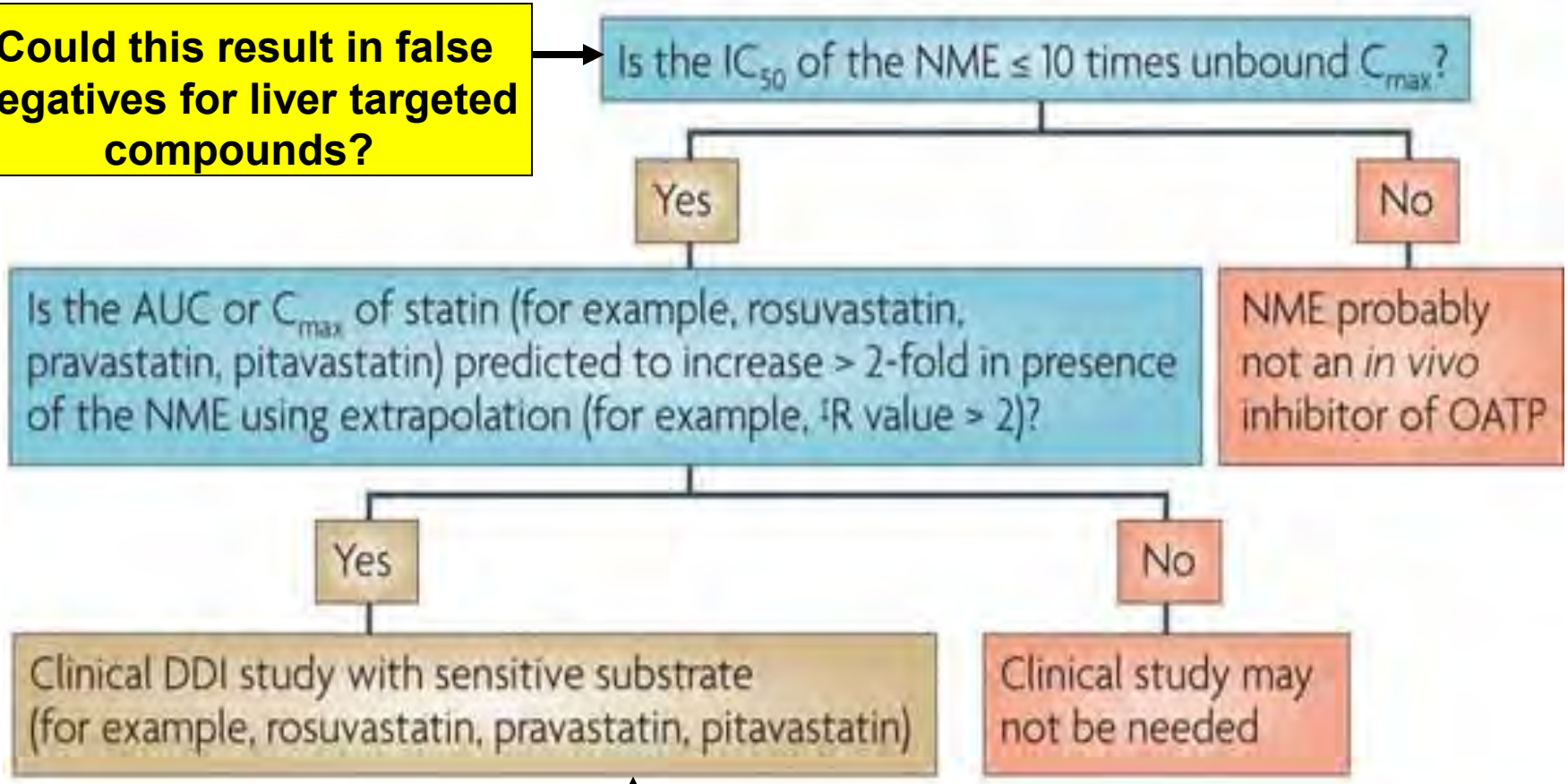
Transporter	Km (uM)	Vmax (pmole/min/10^6cells)	CL_{int} (ul/min/10^6cells)
OATP1B1	4.5±1.2	18.8±1.3	4.2
OATP1B3	6.5±3.2	9.3±1.7	1.4

Transporter	CI_{int} (ul/min/10^6cells)	RAF	Estimated CL_{int} from RAF	Relative contribution (%)	REF	Estimated CL_{int} from REF	Relative contribution (%)
OATP1B1	4.2	16.2	67.5	93.4	16.9	70.8	94.7
OATP1B3	1.4	3.4	4.8	6.6	2.8	4.0	5.3

- OATP1B1 is the major transporter for the hepatic uptake of pitavastatin in human hepatocytes
- Data obtained by RAF and REF methods are comparable

OATP Inhibition Decision Tree

Could this result in false negatives for liver targeted compounds?



Most sensitive probe needs to be established

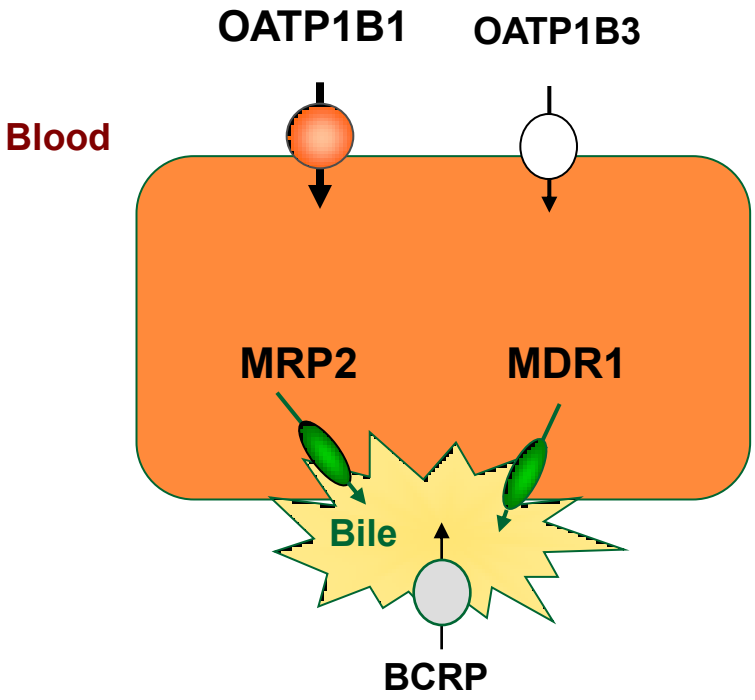
Examples of OATP-Mediated Clinical DDIs

Perpetrator	Victim	Effect Interaction	Possible Mechanism
Cyclosporin A	Pravastatin Pitavastatin Rosuvastatin	Pravastatin AUC↑890% Pitavastatin AUC ↑360% Rosuvastatin AUC↑610%	OATP1B1 OATP1B1/NTCP?
Cyclosporin A	Atorvastatin	Atorvastatin AUC ↑(~ 7.4 fold)	OATP1B1/CYP3A4
Rifampicin (single dose)	Bosentan Atorvastatin	Bosentan trough conc ↑500% Atorvastatin AUC ↑833%	OATP1B1/1B3, CYP3A4? OATP1B1/Pgp?
Lopinavir / ritonavir	Rosuvastatin	AUC ↑107%	OATP1B1

The International Transporter Consortium et al., 2010; He et al., 2009

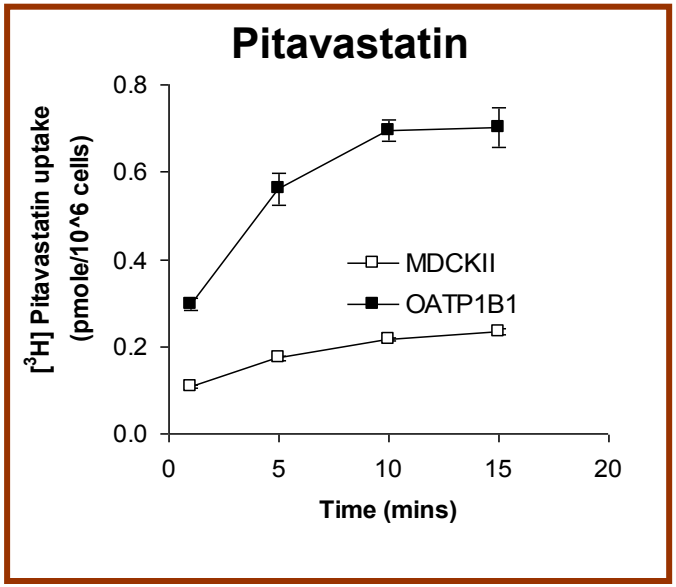
Selection of Probe Substrates for *In Vitro* Assays

Pitavastatin



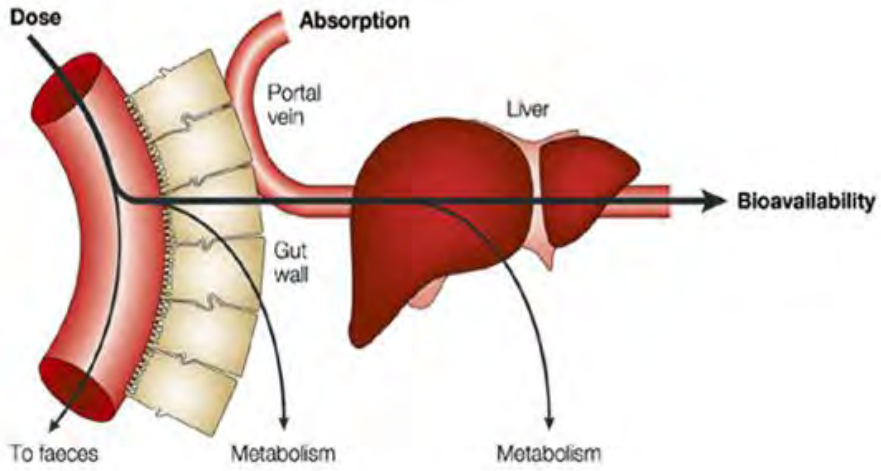
➢ OATP1B1-mediated uptake is the rate-determining step in the hepatic elimination of pitavastatin in rats and likely humans (Watanabe et al., 2010)

In Vitro Model for OATP1B1-Mediated DDIs



- **Cellular uptake**
  - OATP1B1 transfected MDCKII cells
- **Probe substrates**
  - Pitavastatin
    - Higher *in vitro* transport activity compared to other statins (e.g., pravastatin, rosuvastatin, and simvastatin acid)

Prediction of OATP-mediated DDIs based on *In vitro* data



•To estimate hepatic DDIs, the maximal free plasma concentration at the inlet to the liver ($I_{in, max}$) needs to be considered

$$I_{in, max} = I_{max} + (Fa * Dose * ka/Qh)$$

***I*max:** the reported value for the maximum plasma concentration of the inhibitor in the systemic circulation in clinical situation
Fa: the absorbed fraction of inhibitor
ka: the absorption rate constant in the intestine (0.1 min-1, minimum gastric emptying time is 10 mins)
Qh: the hepatic portal blood flow rate in humans (1150 ml/min)

$$R = 1 + (fu * I_{in, max} / Ki)$$

Ki: in vitro data obtained using OATP1B1-expressing cell line
fu: the blood unbound fraction of the inhibitor

- **R-value represents the ratio of uptake clearance in the absence of the inhibitor to that in its presence**

Hirano et al., (2006) DMD 34, 1229-1236

R-values and DDI Potential

Perpetrator	IC_{50} (μM) OATP1B1 Probe: Pitavastatin	$Cmax_u/IC_{50}$	Victim (*in vivo*)	R-Value	Clinical DDI[a] (fold AUC↑)
CsA (100mg oral)	**0.3**	**0.2**	**Pitavastatin**	**1.8**	**Yes (4.5 x)**
Rifampicin (600mg oral)	**1.4**	**0.6**	**Atorvastatin**	**2.8**	**Yes (8 x)**
Lopinavir [b] (400mg oral)	**0.4**	**0.8**	**Rosuvastatin**	**2.4**	**Yes (2 x)**
Amprenavir [c] (600mg oral)	**10**	**0.1**	**Rosuvastatin**	**1.4**	**No**
Gemfibrozil (600mg oral bid)	**89.5**	**0.03**	**Pitavastatin**	**1.1**	**No (1.3 x)**
Ritonavir (100mg oral bid)	**0.8**	**0.04**	**Rosuvastatin**	**1.1**	**No**

R-values correlate with clinical DDIs qualitatively

[a]: *Lau et al., 2007; Hasunuma et al., 2003; Mathew et al., 2004; Busti et al., 2008, He et al., 2009*

OATP1B1 Inhibitors *In Vivo*: Lack of Specificity

- **Cyclosporine A**
  - Inhibits also OATP1B3, OATP2B1, NTCP, Pgp, MRP2, and CYP3A4
- **Rifampicin** (single dose)
  - Inhibits also OATP1B3, and weakly inhibits CYP3A4
- **Gemfribrozil and –O-glucuronide**
  - Inhibits also OATP1B3, OATP2B1, NTCP, and CYP2C8
- **Clarithromycin, erythromycin, roxithromycin, telithromycin**
  - Inhibit also OATP1B3, Pgp, and CYP3A4
- **Indinavir, ritonavir, saquinavir**
  - Inhibit also OATP1B3, OATP2B1, Pgp, and CYP3A4

(Modified from Niemi: FDA Critical Path Transporter Workshop, 2008)

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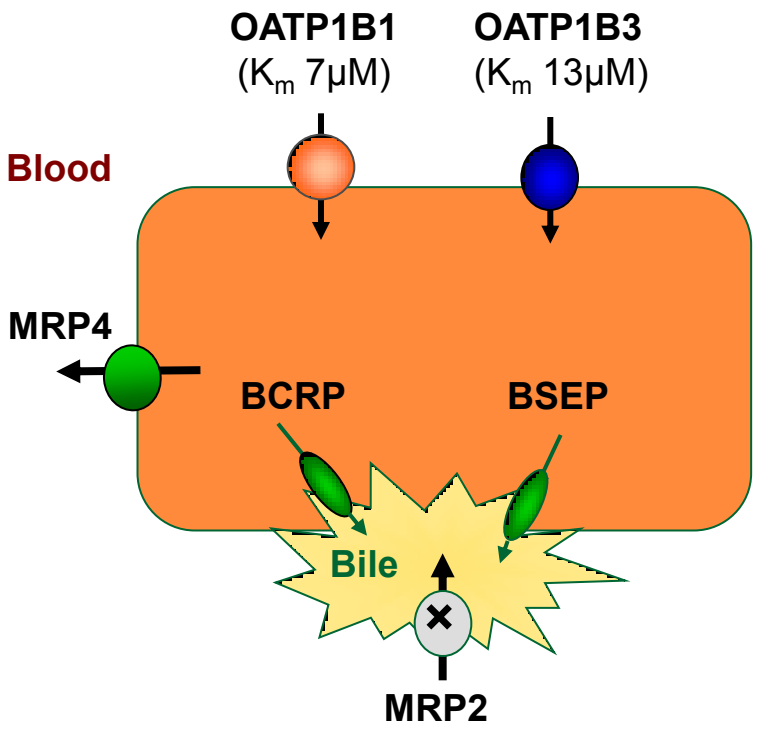
MRL-A: A Perpetrator for OATP1B1 *In Vivo*?

Background of MRL-A

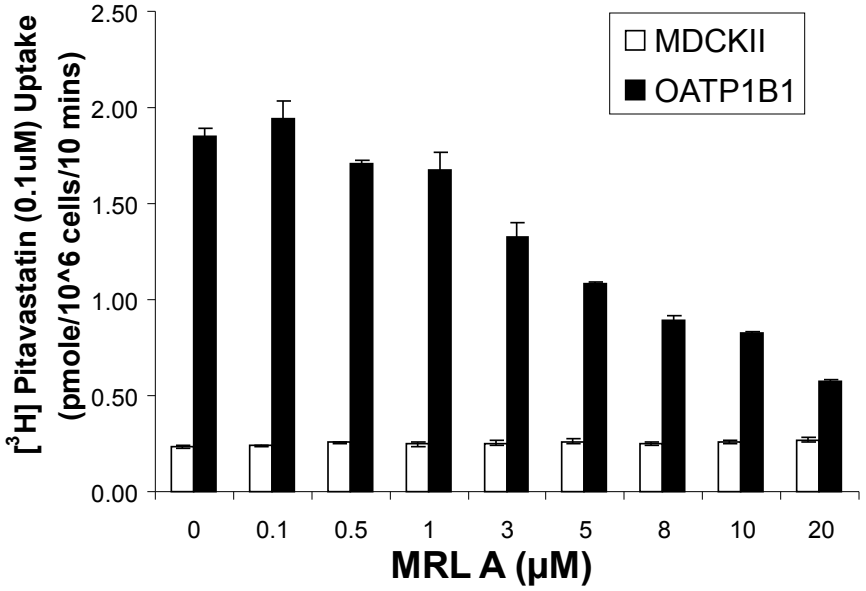
Transport of A by human hepatic transporters

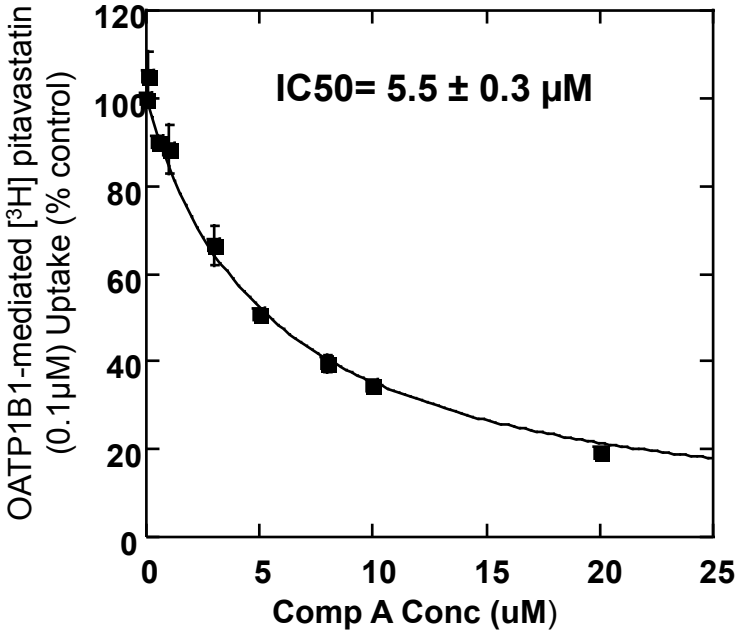


- Transported into liver through hepatic uptake transporters OATP1B1 and OATP1B3
- Low apparent permeability
- Minimal metabolism in the liver
- Eliminated into bile by the hepatic efflux transporters BSEP and BCRP
- ***Potential for OATP-mediated DDIs?***

MRL-A: OATP1B1 *In Vitro* Inhibition

Effect of MRL-A on OATP1B1-Mediated Pitavastatin Uptake in MDCKII-OATP1B1 Cells





- MRL-A showed concentration-dependent inhibition of OATP1B1-mediated pitavastatin uptake with an IC_{50} of 5.5 μM

Potential of MRL-A to Act as a Perpetrator of OATP1B1-Mediated DDIs *in Vivo*

- OATP1B1 primarily responsible for uptake of pitavastatin

- R value (Hirano et al., 2006, DMD 34, 1229-1236)
  - $R = 1 + (f_u * I_{in,max}/IC50)$
  - Imax = 1.45µM at 50mg oral dose
  - Assume Fa=1, Ka=0.03 min^{-1} as the worst case scenario

  - If $I_{in,max}$ is 3.6 µM and f_u is 0.01
  - **R = 1.01**

Propensity of MRL-A to cause a DDI with pitavastatin is low: No DDI study conducted

Case Study: A Perpetrator for OATP1B1 *In Vivo*?

Background of MRL-B

- An inhibitor for OATP1B1 and OATP1B3
- Substrate for OATP1B1 and 1B3, and efflux transporter MRP2
- High plasma protein binding (99%); expected Cmax 2 μM and 3.5 μM at 200mg and 600mg oral dose

Probe substrates	IC_{50} (μM)	
	OATP1B1	OATP1B3
Pitavastatin	0.4	-
Atorvastatin	0.2	-
Simvastatin Acid	0.1	-
BSP	-	0.3

Prediction of OATP1B1-Mediated DDIs of MRL-B Based on *In Vitro* Data

Perpetrator	IC_{50} (μM) OATP1B1 Probe: Pitavastatin	R-Value	Victim in clinical DDIs	Clinical DDI[a] (fold AUC↑)
CsA (100mg oral)	0.3 ± 0.13	1.8	Pitavastatin Atorvastatin	4.5 x 7.4 x
Rifampicin (600mg oral)	1.4 ± 0.19	2.8	Atorvastatin	8 x
Gemfibrozil (600mg oral bid)	89.5 ± 17.5	1.1	Pitavastatin Atorvastatin	No (1.3 x) No (1.24 x)
Ritonavir (100mg oral bid)	0.84 ± 0.19	1.1	Rosuvastatin	No
MRL-B (200mg, oral)	**0.44 ± 0.09**	**1.2**	?	NA
MRL-B (600mg, oral)	**0.44 ± 0.09**	**1.54**	?	NA

***In vitro* data suggest that MRL-B has a DDI potential with statins at the dose of 600 mg; clinical DDI study is recommended**

Lau et al., 2007; Hasunuma et al., 2003; Mathew et al., 2004; Busti et al., 2008

Considerations OATP1B1-Mediated DDIs

- If the R-value is close to 1, no potential for OATP-mediated DDIs

- In vitro considerations
  - Pitavastatin is a good probe substrate for OATP1B1
  - Calculations of R-values as a "worst case scenario" approach
    - Assumptions: rapid gastric emptying and complete absorption
    - Model inhibitors are not specific for OATP1B1
  - For victim drugs, the relative contribution of OATP1B1 to liver uptake clearance needs to be measured for quantitative predictions

- Clinical considerations
  - Victim drugs
    - OATP1B1: statins (which is the most sensitive OATP1B1 substrate?)
    - OATP1B3: telmisartan
  - Perpetrator drugs
    - CsA: inhibits several transporters and CYP3A4
    - Rifampin (single dose)

Outline

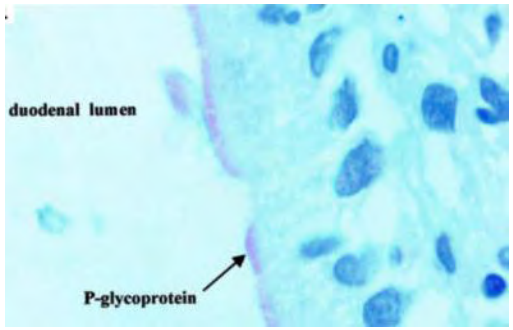
- Part 1
  - Overview of the ITC
  - Transporters covered by the ITC
  - Decision trees

- Part 2
  - Case Studies
    - OATP-mediated DDIs
    - Digoxin-Rifampin DDI

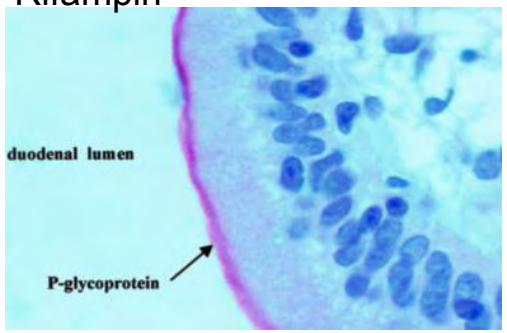
Digoxin as a Probe for Intestinal P-glycoprotein: Rifampin Induction Reduces Digoxin Exposure

- Rifampin 600mg QD x 10d
- 3.5-fold increase in duodenal P-gp immunoreactivity

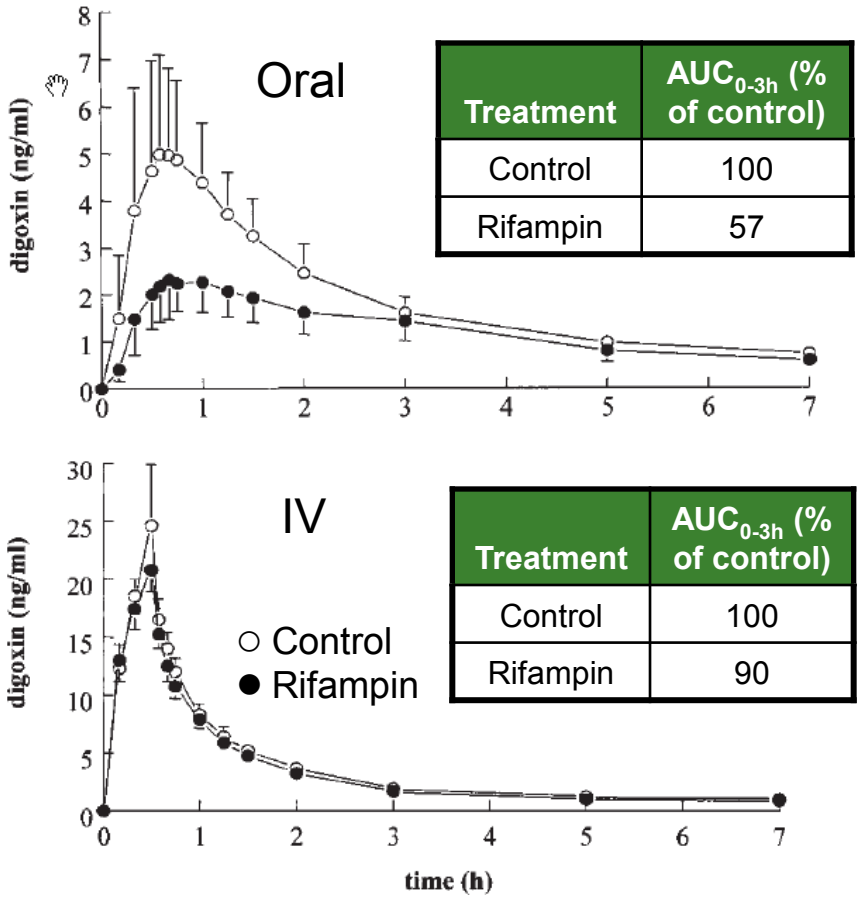
Control



Rifampin



- Digoxin
  - $AUC_{0\text{-}3h}$ and C_{max} reduced
- Bioavailability reduced from 63% to 44%
- No change in renal clearance
- No change in $t_{1/2}$ (~55h)

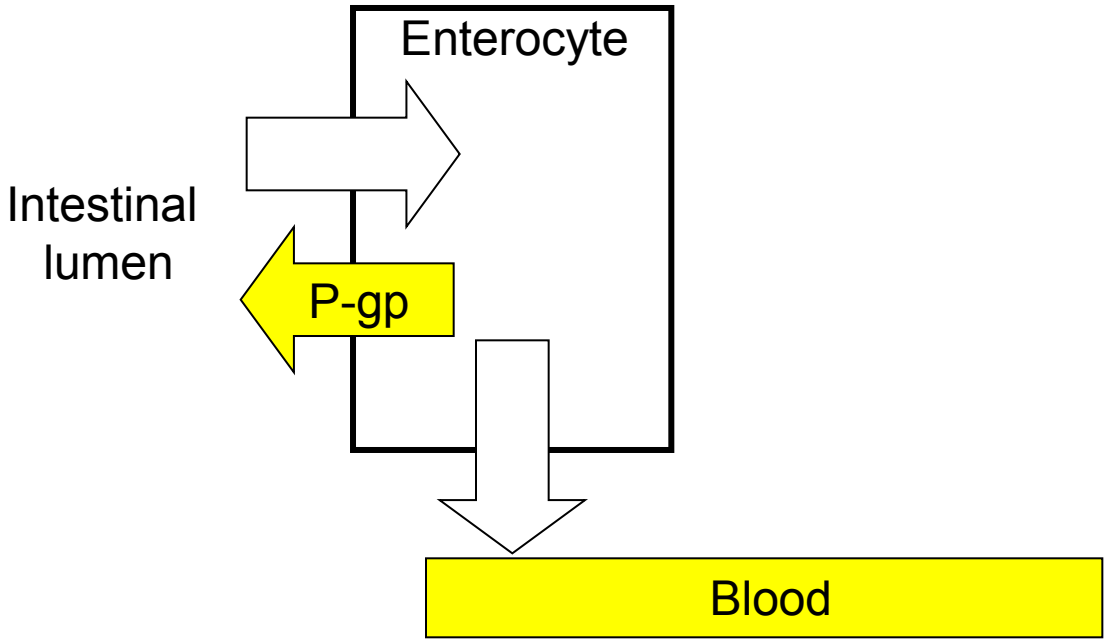


Treatment	$AUC_{0\text{-}3h}$ (% of control)
Control	100
Rifampin	57

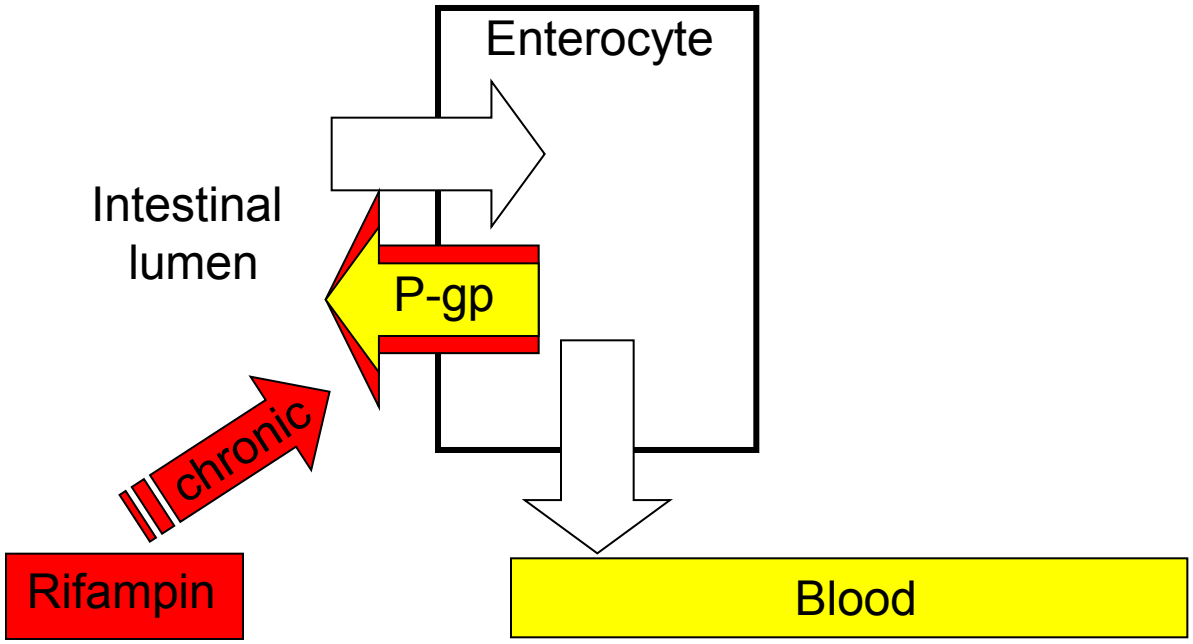
Treatment	$AUC_{0\text{-}3h}$ (% of control)
Control	100
Rifampin	90

Greiner et al., 1999 JCI 104: 147-153

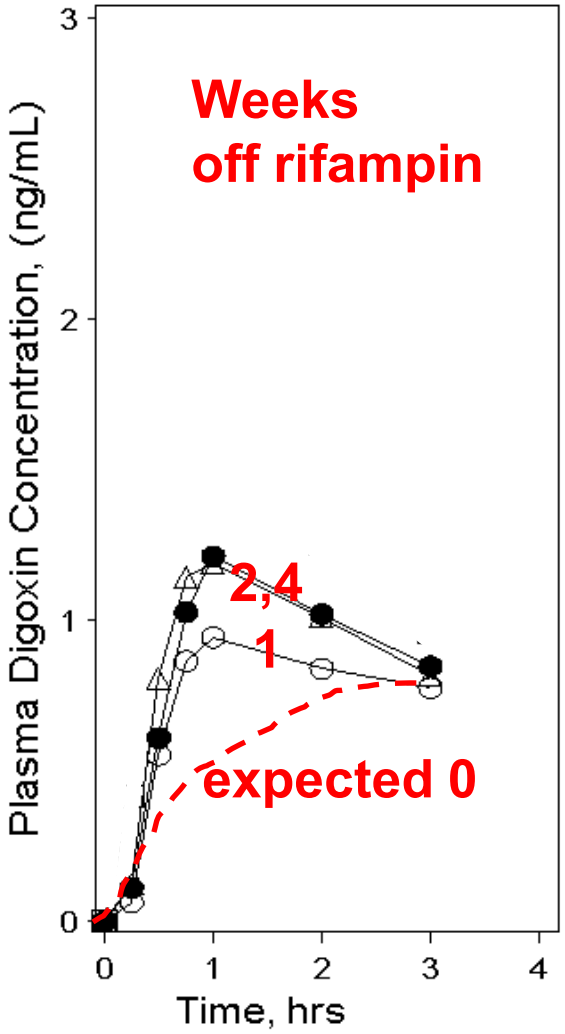
Digoxin Human Pharmacokinetics (0-3h)



Digoxin Human Pharmacokinetics (0-3h)

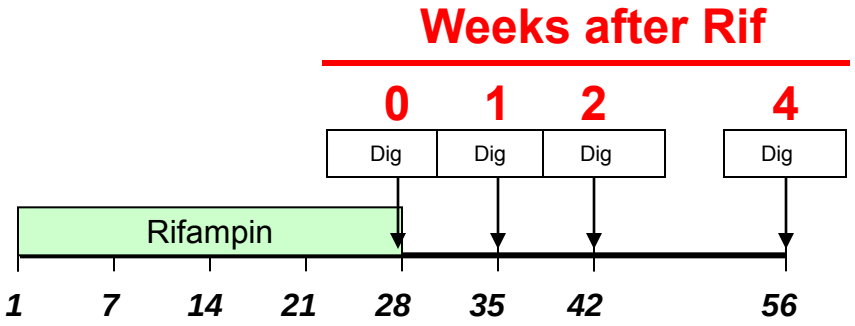


Merck Study: Rifampin Effect on Digoxin PK



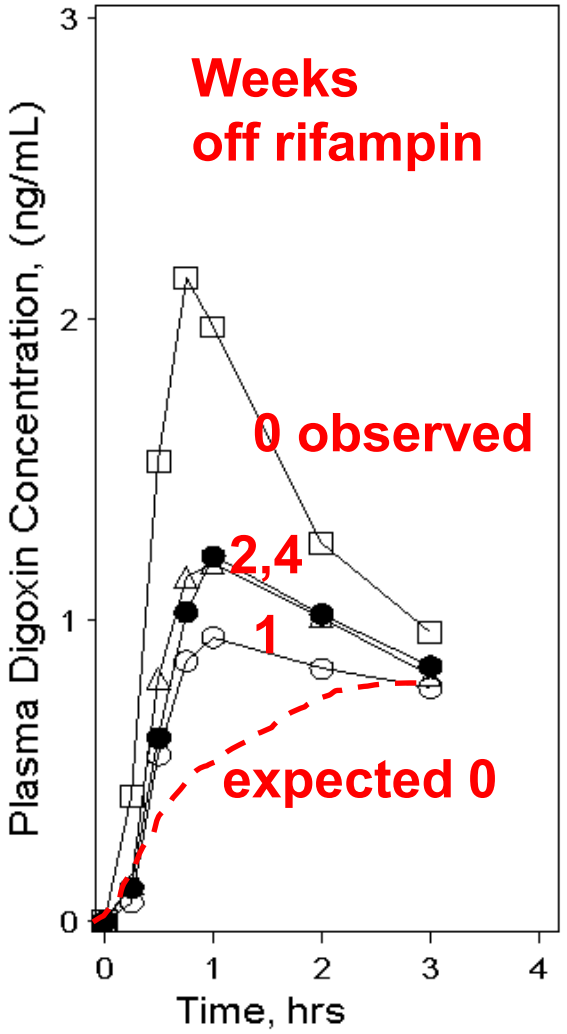
Wks after rifampin	AUC (% of 4wk)	Cmax (% of 4wk)
expected 0	~57	~48
1	82	74
2	98	89
4	100	100

Expected is based on Greiner et al.

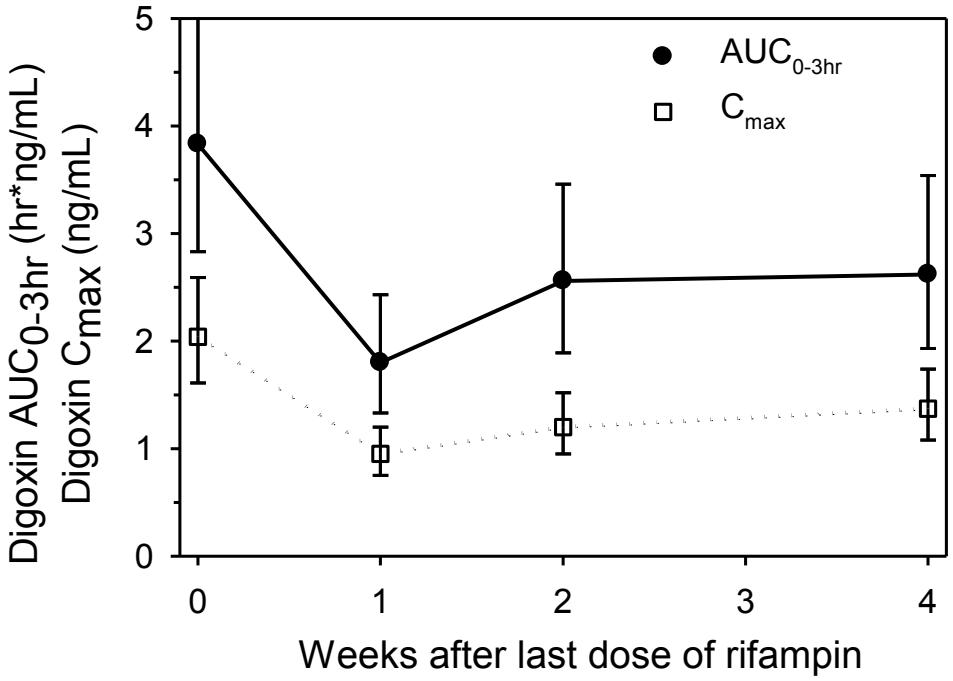


Reitman et al., 2010, CPT, in press

Rifampin Effect on Digoxin PK



Wks after rifampin	AUC (% of 4wk)	Cmax (% of 4wk)
expected 0	~57	~48
0	148	154
1	82	74
2	98	89
4	100	100



DDI Study Design: Timing of Dosing

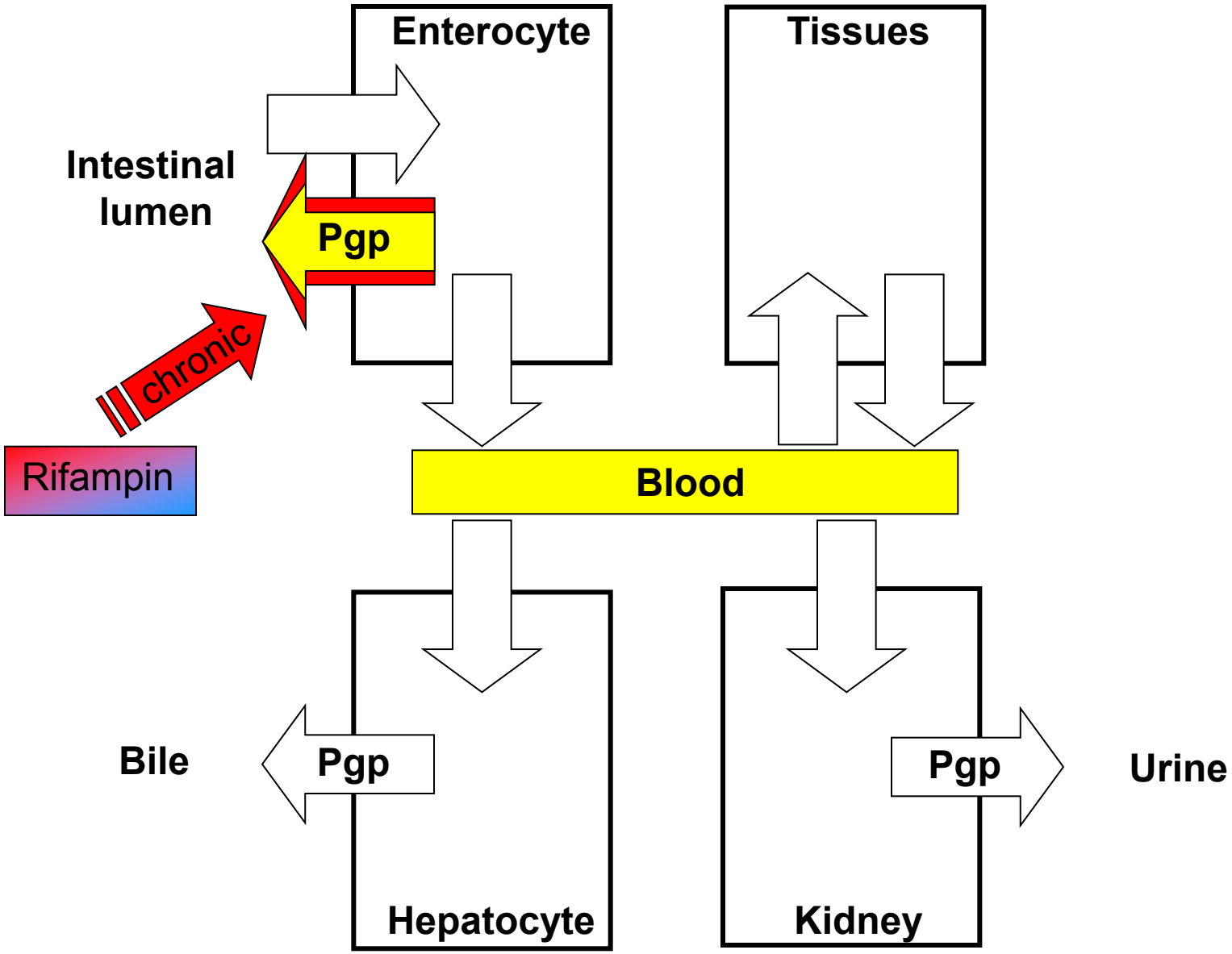
Difference:

Dosing of digoxin was 1h after rifampin

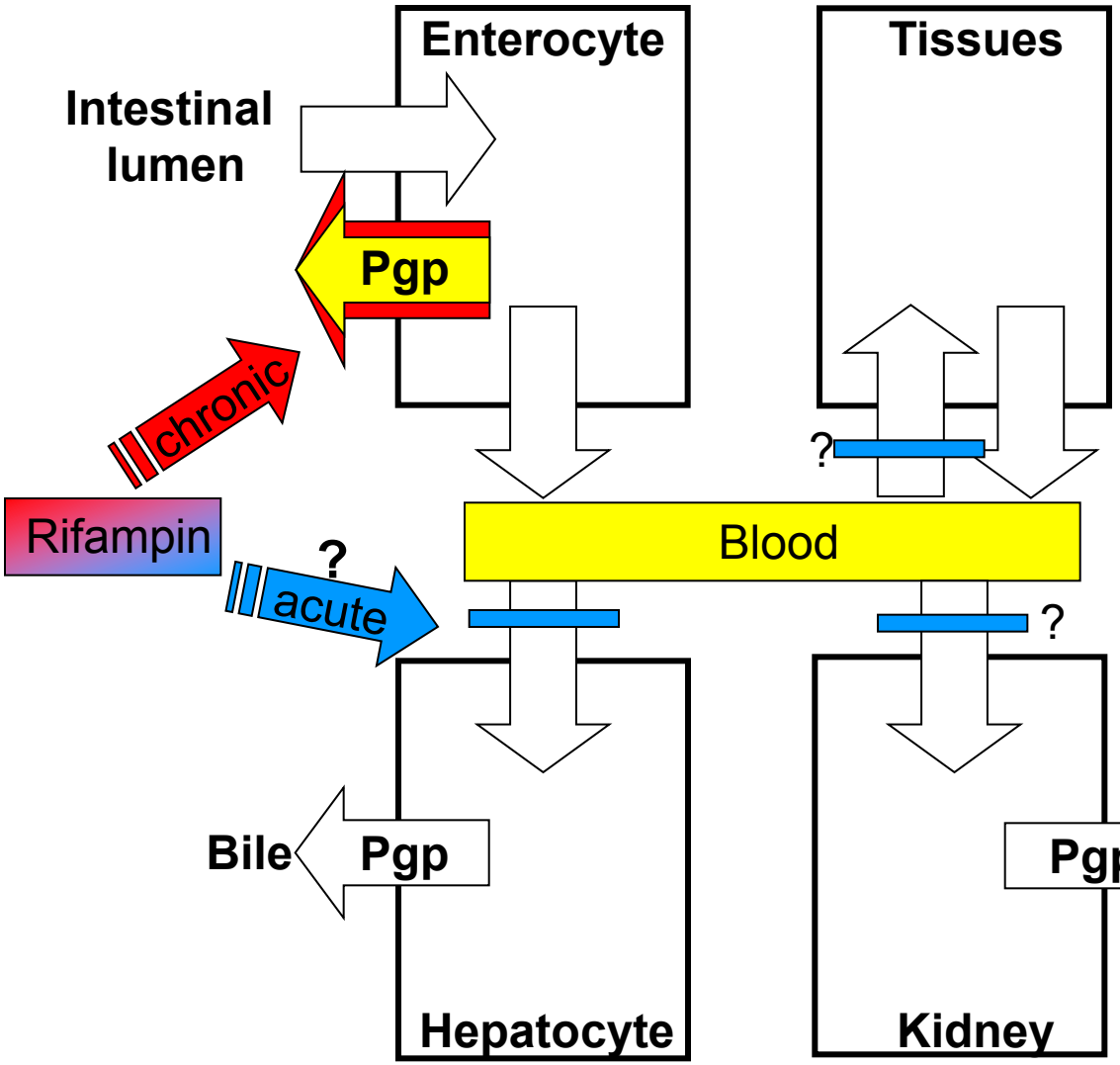
versus

8h apart in Greiner et al. (M. Eichelbaum, personal communication)

Digoxin Human Pharmacokinetics



Digoxin Human Pharmacokinetics: Rifampin Effect *via* Inhibition of Tissue Uptake

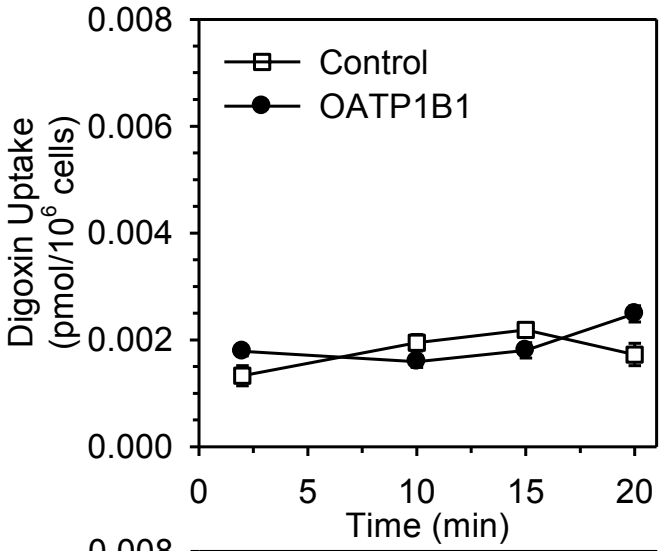


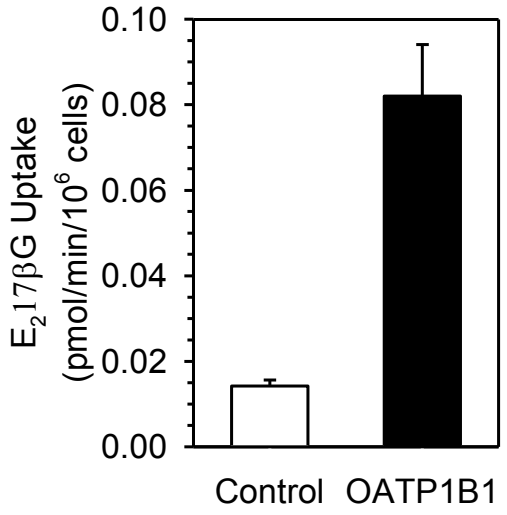
Rifampin:

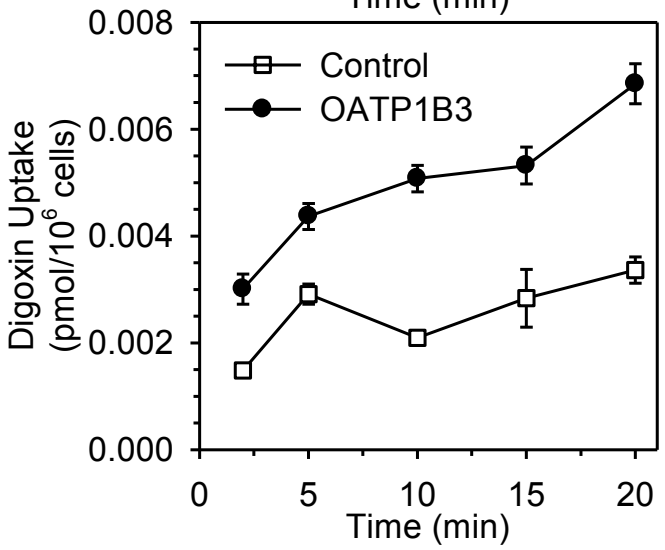
- Inhibits digoxin uptake into tissues
- *Eg.*, *via* rifampin inhibition of digoxin transport by OATP1B3

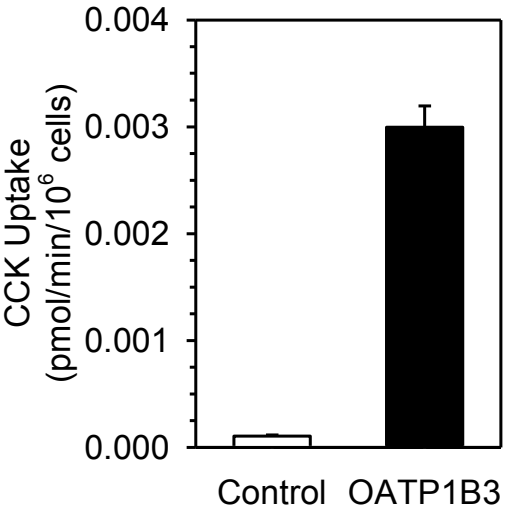
Digoxin: Weak OATP1B3 Substrate, Not OATP1B1

- No detectable digoxin transport in MDCKII-OATP1B1 cells
- Weak digoxin transport in MDCKII-OATP1B3 cells (at 25 ^{o}C)

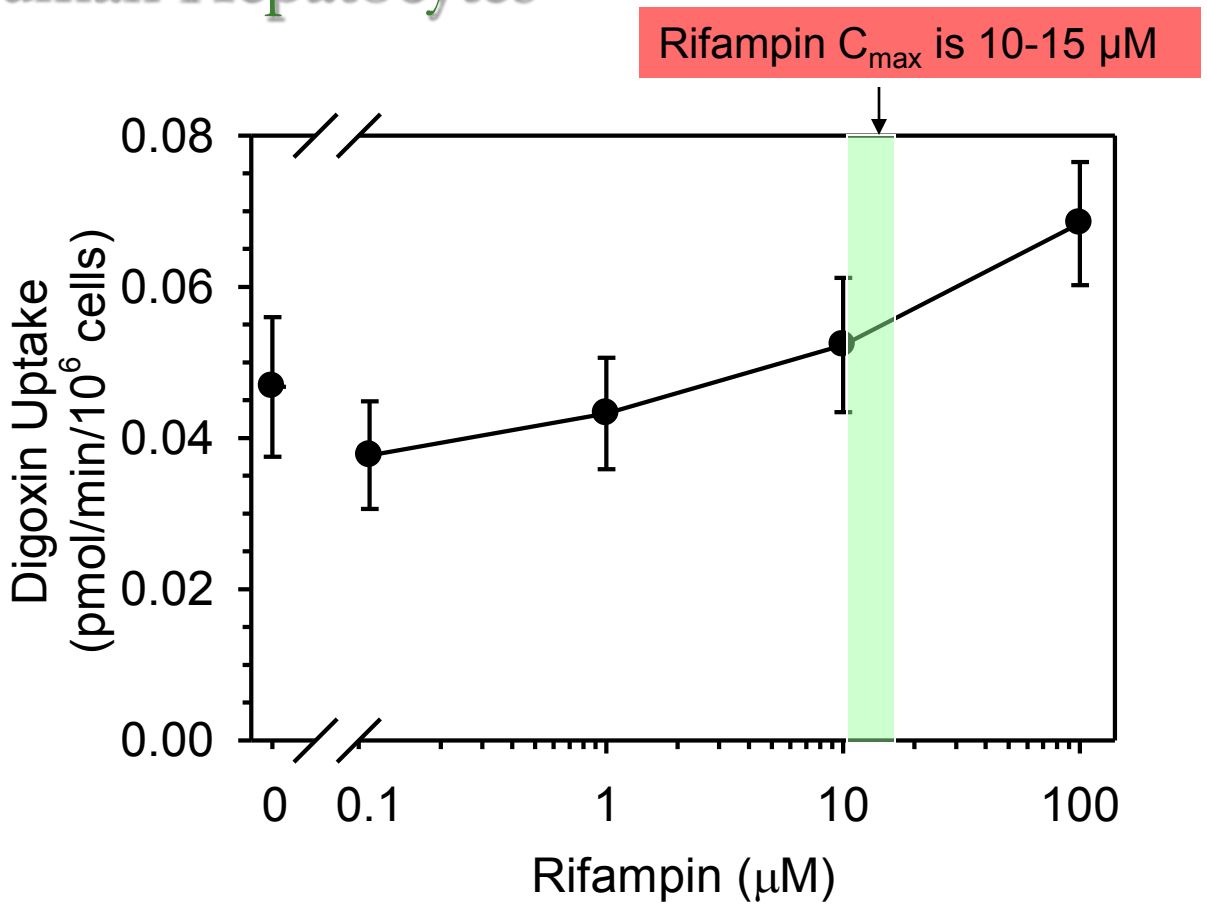








In vitro, Rifampin Does Not Inhibit Digoxin Uptake into Human Hepatocytes

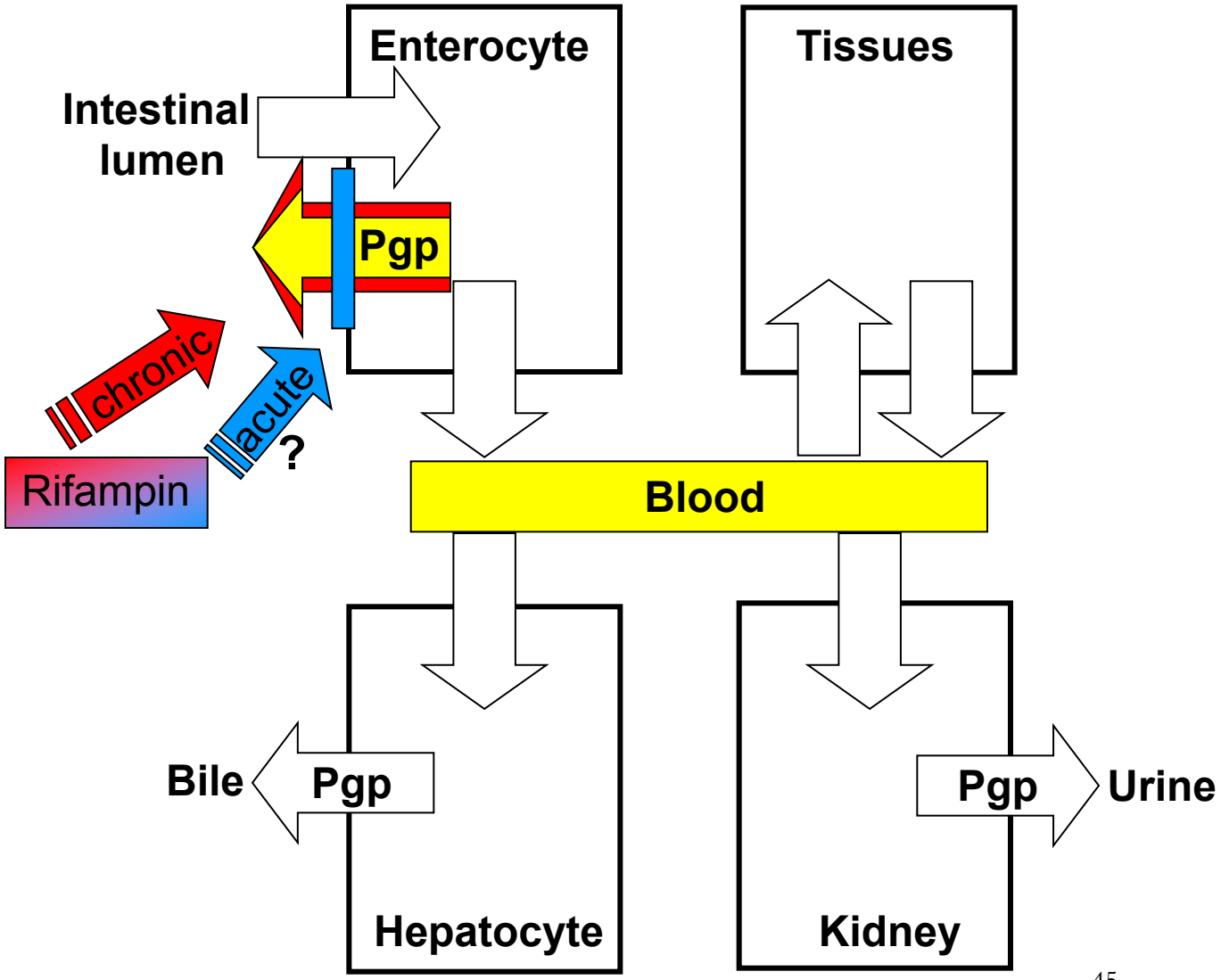


Rifampin effect unlikely to be *via* inhibition of liver (tissue) uptake

Digoxin Human Pharmacokinetics: Rifampin Effect via Inhibition of Efflux From Enterocyte?

Rifampin:

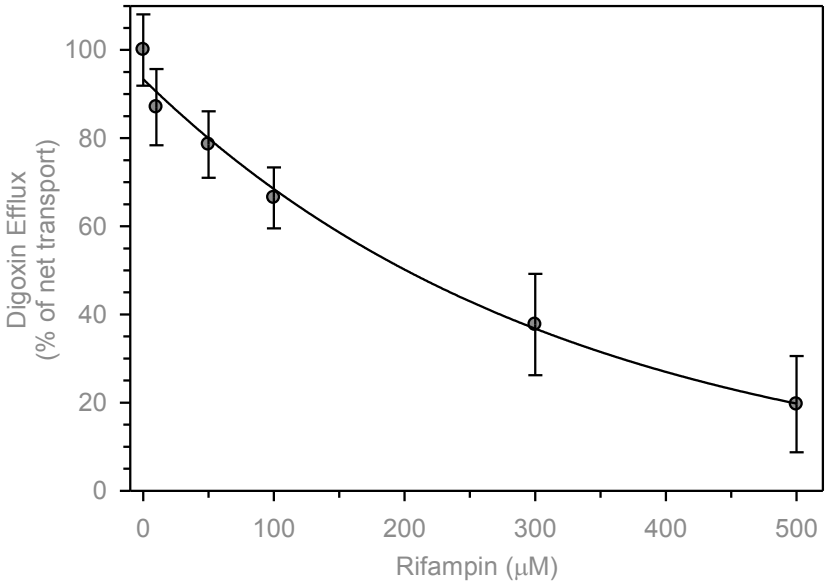
- Inhibits digoxin efflux from enterocyte
- Via inhibition of P-gp (or other transporter)?



In vitro, Digoxin Transport by P-gp is Inhibited by Rifampin

- Rifampin inhibits digoxin transport:

  IC_{50} = 169 ±18 μM
- Literature data for rifampin inhibition of P-gp transport of other substrates gives similar IC_{50}s (70-220 μM)
- 600mg rifampin in 250 ml is 2920 μM
- $[I_2]/IC_{50}$ = 17



Hypothesis: acute rifampin effect is *via* inhibition of P-gp-mediated efflux of digoxin from enterocyte

General Conclusions

- Applications of the ITC decision trees does provide general guidance to development programs
  - But more experience and sharing of data is needed
- Standardization and calibration of *in vitro* assay systems is important for consistent and meaningful data interpretation
- Experience with probe drugs that can be used in the clinic as victims or perpetrators of transporter-mediated DDIs is needed
  - Drugs specific or selective for one transporter may not exist

Questions and Comments

The ITC considers the NRDD paper as a work in progress, and is interested in obtaining feedback, including areas that have not been included in this report but should be considered in the next version as well as controversial concepts.

Please send any comments to the corresponding authors via the AAPS Drug Transporter Focus Group's website.

http://www.aaps.org/inside/focus_groups/drugTrans/ITCwhitepaper.asp

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