Predicting DMPK of NMEs: What Do We Need in Terms of Science and Tools?

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New England Drug Metabolism Discussion Group

Gerald Miwa Retirement Symposium

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What are our limitations today that prevent us from predicting drug disposition for New Molecular Entities (NMEs)?

Science: **It is only during the last 7 years that we have recognized the importance of absorptive and efflux transporters to drug disposition and toxicology. Because of transporter-enzyme interplay our previous drug disposition theory is inadequate, which accounts for poor predictability.**

Tools: And most importantly, we have no simple, high-throughput preclinical tool to characterize transporter-enzyme interplay that allows human-animal comparisons.

Our group has carried out interaction studies in humans with cyclosporine, tacrolimus and sirolimus with and without ketoconazole, an inhibitor of CYP3A and P-gp, as well as with and without rifampin, an inducer of CYP3A and P-gp. These studies suggest that the major effect of the interaction is on bioavailability, as opposed to clearance, and that this interaction occurs primarily in the intestine.

Why does the CYP3A –P-glycoprotein Interaction Appear to Be More Important in the Intestine vs. the Liver?

Why do some CYP3A-Efflux Transporter Substrates Exhibit this Interplay and Others Do Not?

What about Drugs that are Not Metabolized? How Important is Transporter-Transporter Interplay?

If we can answer these questions, can this serve as the basis for predicting drug absorption and disposition for an NME?

Cellular and animal studies from our laboratory over the past six years examining transporter-enzyme interplay led us to make 22 predictions concerning drug absorption and disposition

Some of these predictions are the subject matter of this presentation but all may be found in our January 2005

paper

(d L.Z. Benet. Pharm. 5)

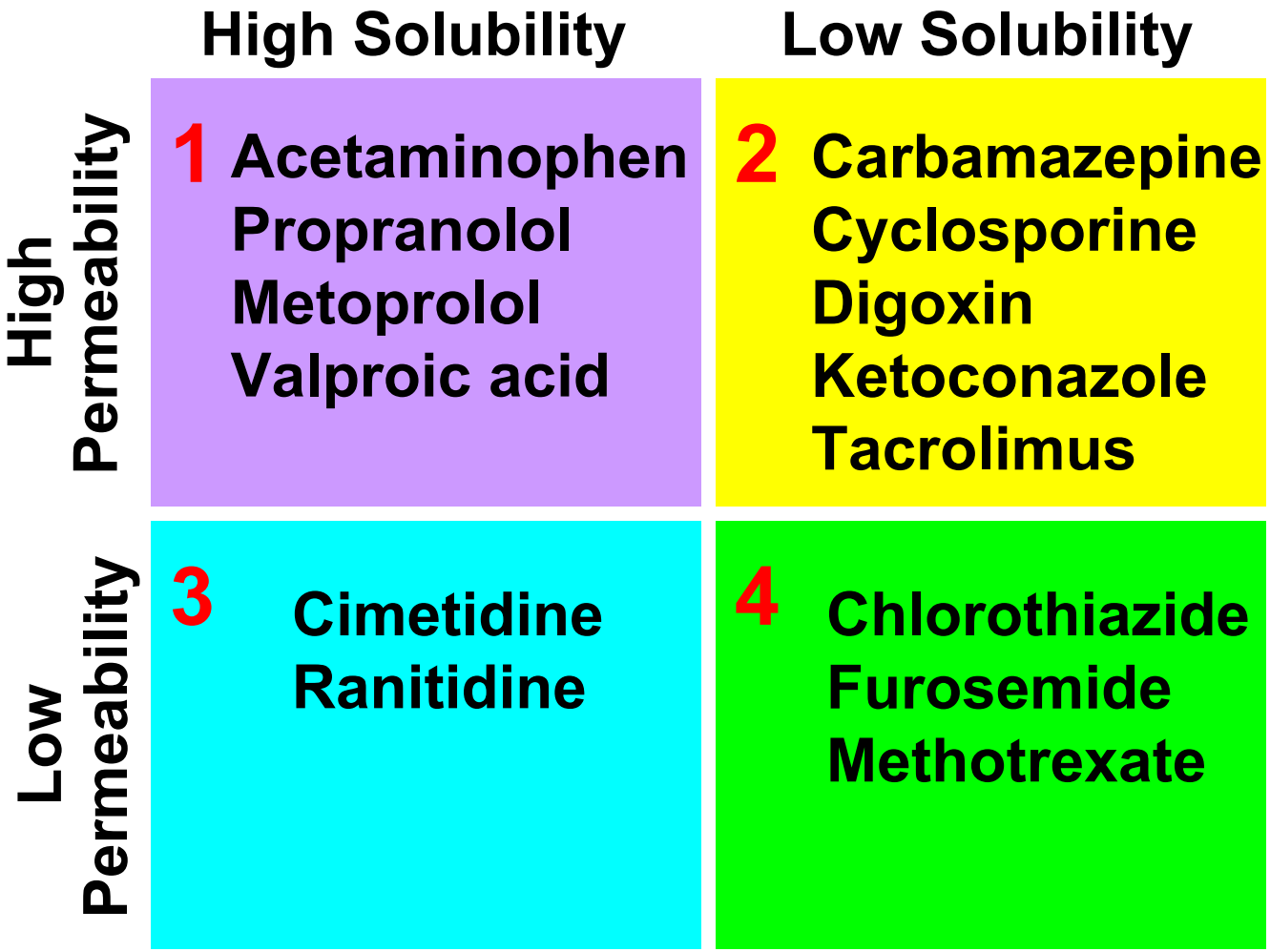
Most NEDMDG attendees will be familiar with the FDA's Biopharmaceutics Classification System (BCS)

The core idea in the BCS is that an *in vitro* transport model, centrally embracing permeability and solubility, with qualifications related to pH and dissolution, may qualify for a waiver of *in vivo* bioequivalence studies.

of the BCS is to: ***predict in vivo***
of drug products from in vitro measurements
ty and solubility.

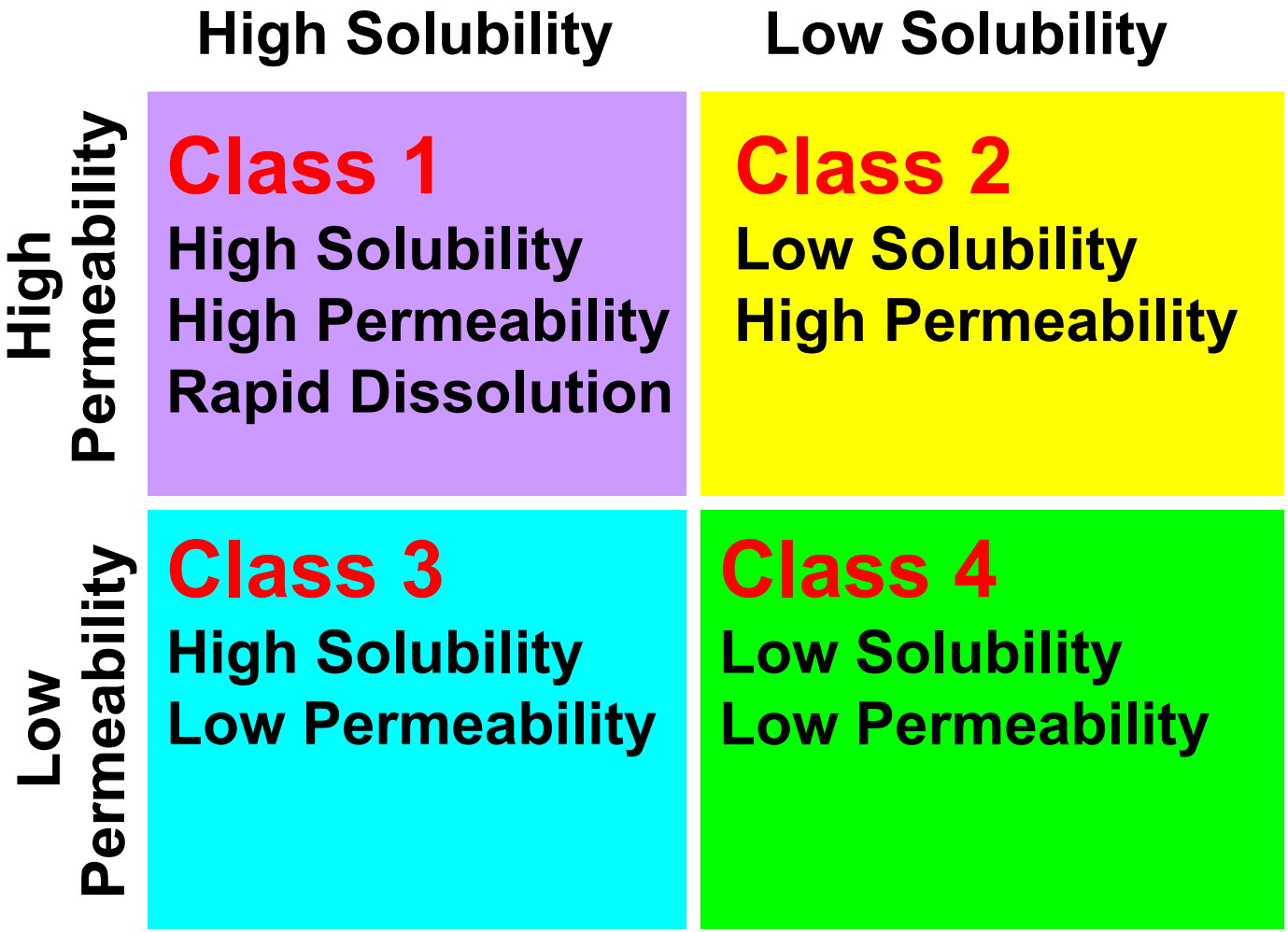
believe that the framework of the BCS can
ests of the earliest stages of discovery
edicting the absorption/disposition of NMEs.

Biopharmaceutical Classification



Amidon et al., Pharm Res 12: 413-420, 1995

Biopharmaceutical Classification



Amidon et al., Pharm Res 12: 413-420, 1995

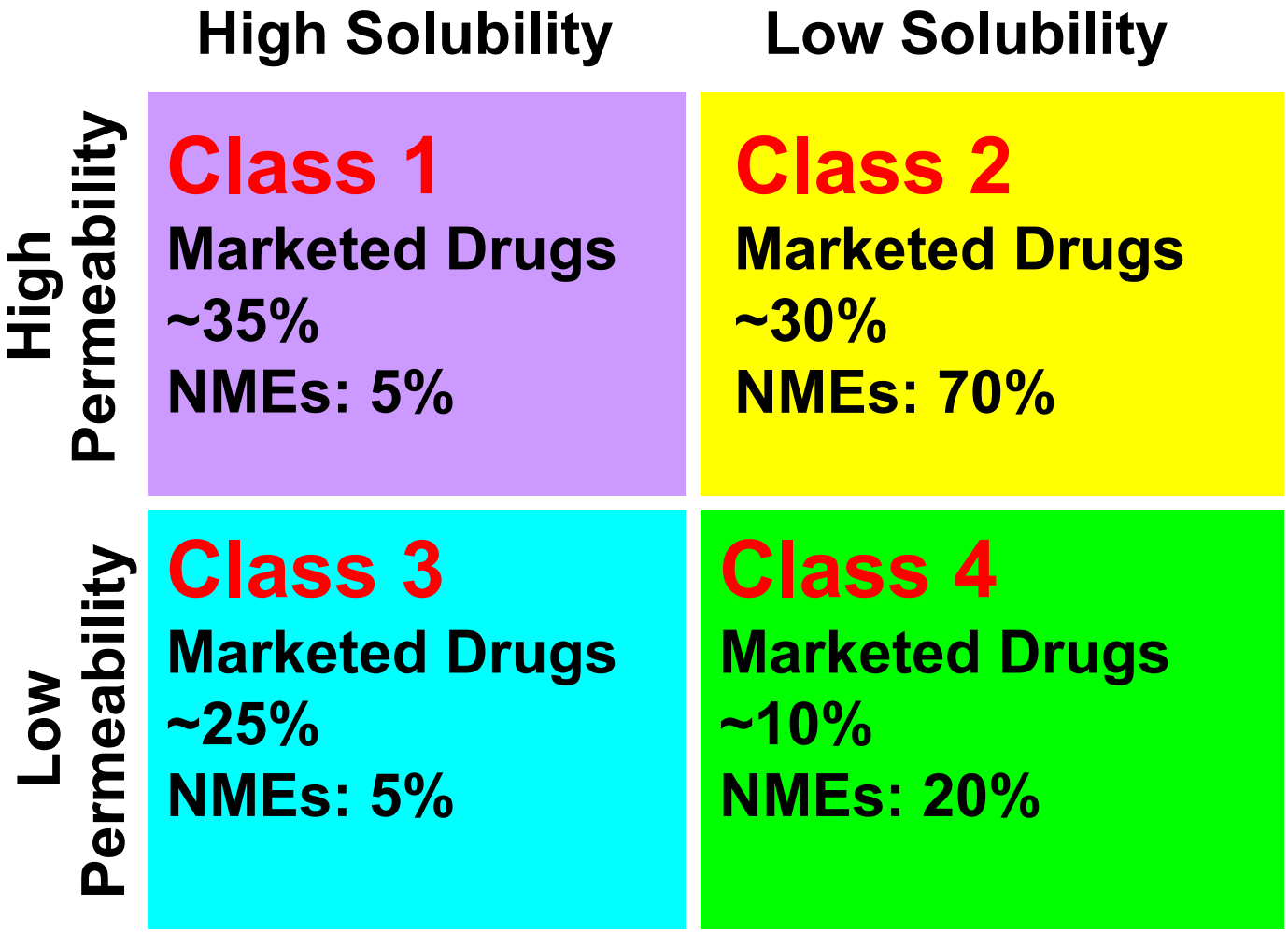
BCS High Solubility Criteria

A drug substance is considered "highly soluble" when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1-7.5 at 37°C.

BCS High Permeability Criteria

A drug substance is considered to be "highly permeable" when the extent of absorption in humans is determined to be ≥ 90% of an administered dose based on a mass balance determination or in comparison to an i.v. reference dose

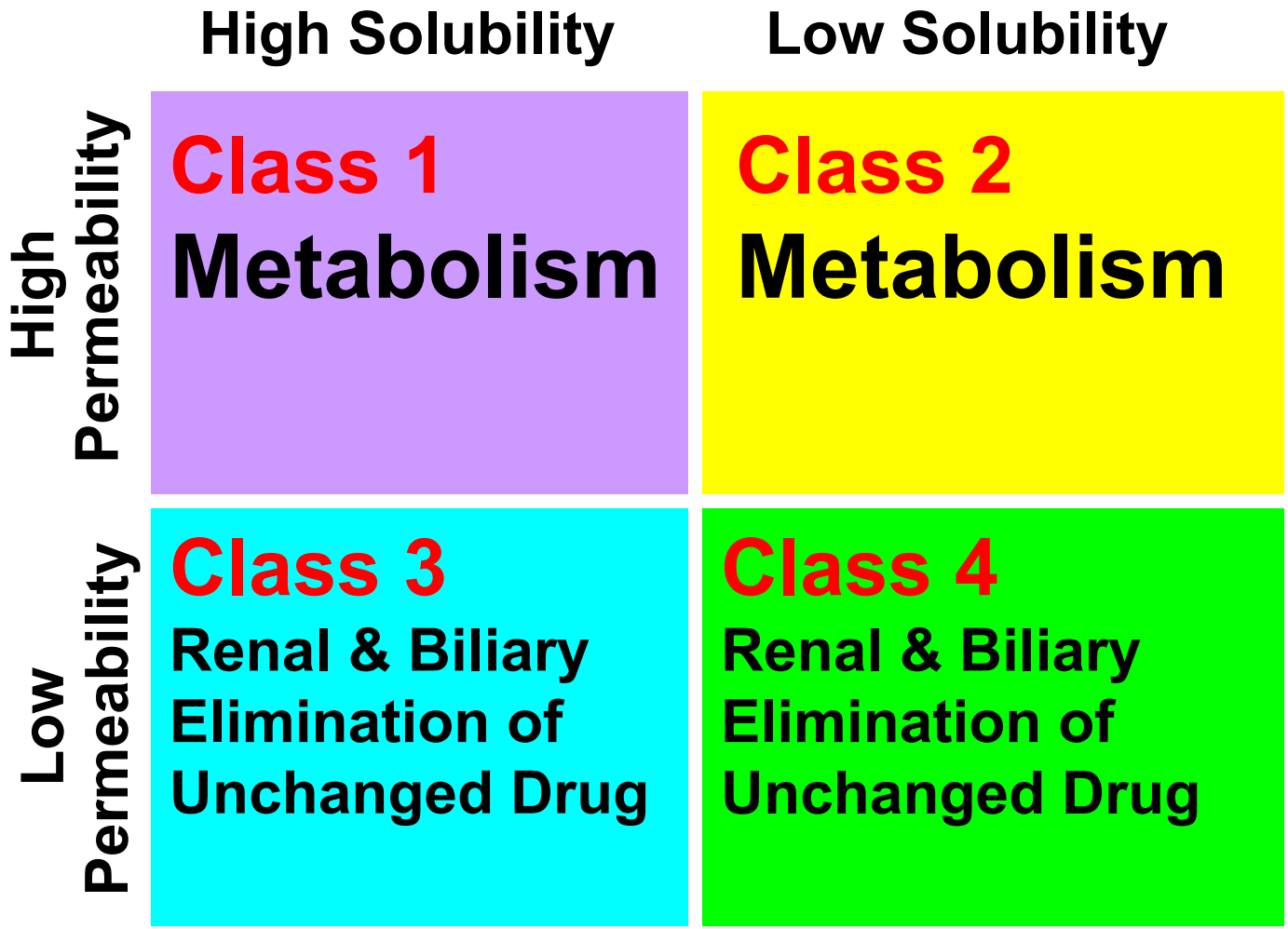
Distribution of Drugs on the Market vs. Small Molecule NMEs



	High Solubility	Low Solubility
High Permeability	**Class 1** Abacavir Acetaminophen *Acyclovir*[b] *Amiloride*[S,I] Amitryptyline[S,I] Antipyrine *Atropine* **Buspirone**[c] Caffeine *Captopril* Chloroquine[S,I] **Chlorpheniramine** Cyclophosphamide Desipramine **Diazepam** **Diltiazem**[S,I] **Diphenhydramine** Disopyramide **Doxepin** Doxycycline Enalapril Ephedrine Ergonovine Ethambutol Ethinyl Estradiol Fluoxetine[I] Glucose Imipramine[I] **Ketorolac** Ketoprofen Labetolol Levodopa[S] Levofloxacin[S] **Lidocaine**[I] Lomefloxacin **Meperidine** Metoprolol Metronidazole **Midazolam**[S,I] **Minocycline** Misoprostol ***Nifedipine***[S] Phenobarbital Phenylalanine Prednisolone **Primaquine**[S] Promazine Propranolol[I] **Quinidine**[S,I] Rosiglitazone Salicylic acid Theophylline Valproic acid **Verapamil**[I] Zidovudine	**Class 2** **Amiodarone**[I] **Atorvastatin**[S, I] **Azithromycin**[S ,I] **Carbamazepine**[S,I] **Carvedilol** Chlorpromazine[I] **Cisapride**[S] *Ciprofloxacin*[S] **Cyclosporine**[S, I] **Danazol** **Dapsone** Diclofenac Diflunisal Digoxin[S] *Erythromycin*[S,I] Flurbiprofen **Glipizide** **Glyburide**[S,I] Griseofulvin Ibuprofen **Indinavir**[S] Indomethacin **Itraconazole**[S,I] **Ketoconazole**[I] **Lansoprazole**[I] **Lovastatin**[S,I] *Mebendazole* Naproxen Nelfinavir[S,I] ***Nifedipine***[S] Ofloxacin Oxaprozin Phenazopyridine Phenytoin[S] Piroxicam Raloxifene[S] **Ritonavir**[S,I] **Saquinavir**[S,I] **Sirolimus**[S] Spironolactone[I] **Tacrolimus**[S,I] Talinolol[S] **Tamoxifen**[I] **Terfenadine**[I] Warfarin

	High Solubility	Low Solubility
Low Permeability	**Class 3** *Acyclovir* *Amiloride* [S,I] Amoxicillin [S,I] Atenolol *Atropine* Bisphosphonates Bidisomide *Captopril* Cefazolin Cetirizine Cimetidine [S] *Ciprofloxacin* [S] Cloxacillin Dicloxacillin [S] *Erythromycin* [S,I] Famotidine Fexofenadine [S] Folinic acid *Furosemide* Ganciclovir *Hydrochlorothiazide* Lisinopril Metformin *Methotrexate* Nadolol Pravastatin [S] Penicillins Ranitidine [S] Tetracycline Trimethoprim [S] Valsartan Zalcitabine	**Class 4** Amphotericin B Chlorthalidone Chlorothiazide Colistin *Ciprofloxacin* [S] *Furosemide* *Hydrochlorothiazide* *Mebendazole* *Methotrexate* Neomycin

Major Routes of Drug Elimination



What are the Implications?

- If you know the intestinal absorption (or more likely a surrogate as Caco-2 permeability) of an NME, you can predict whether the major route of elimination of the NME will be metabolism.
- Note that the permeability parameter does not predict the ability for the NME to enter the liver/ hepatocytes (since a number of non-metabolized Classes 3 & 4 compounds will be excreted in the bile), but rather the access to the metabolic enzymes within the hepatocytes.

Biopharmaceutics Drug Disposition Classification System

BDDCS

	High Solubility	Low Solubility
Extensive Metabolism	**Class 1** High Solubility Extensive Metabolism (Rapid Dissolution and ≥70% Metabolism for Biowaiver)	**Class 2** Low Solubility Extensive Metabolism
Poor Metabolism	**Class 3** High Solubility Poor Metabolism	**Class 4** Low Solubility Poor Metabolism

Profs. Gordon Amidon and Hans Lennernas have carried out extensive and expensive human intestinal intubation studies to determine the absorption/permeability of a group of ~30 drugs that served as a basis for using metoprolol as the cut-off marker for absorption greater than 90%

In a recently published paper (Takagi et al., Mol. Pharm., **3**:631-643, 2006) the human permeability numbers for 29 reference "drugs" are compiled in a Journal publication, giving all of us the opportunity to test various permeability surrogates against the experimental human values.

Reference "Drugs"

- α-Methyldopa
- Amoxicillin
- Antipyrine
- Atenolol
- Carbamazapine
- Cephalexin
- Cimetidine
- Creatinine
- Desipramine
- D-Glucose
- Enalapril
- Enalaprilat
- Fluvastatin
- Furosemide
- Hydrochlorothiazide
- Ketoprofen
- Levodopa
- Lisinopril
- L-Leucine
- Losartan
- Metoprolol
- Naproxen
- Phenylalanine
- Piroxicam
- Propranolol
- Ranitidine
- Terbutaline
- Valacyclovir
- Verapamil

Ability to Correctly Classify BCS Permeability for Estimated CLog P and Log P vs. Metabolism as Compared to Human Jejunal Permeability Measures

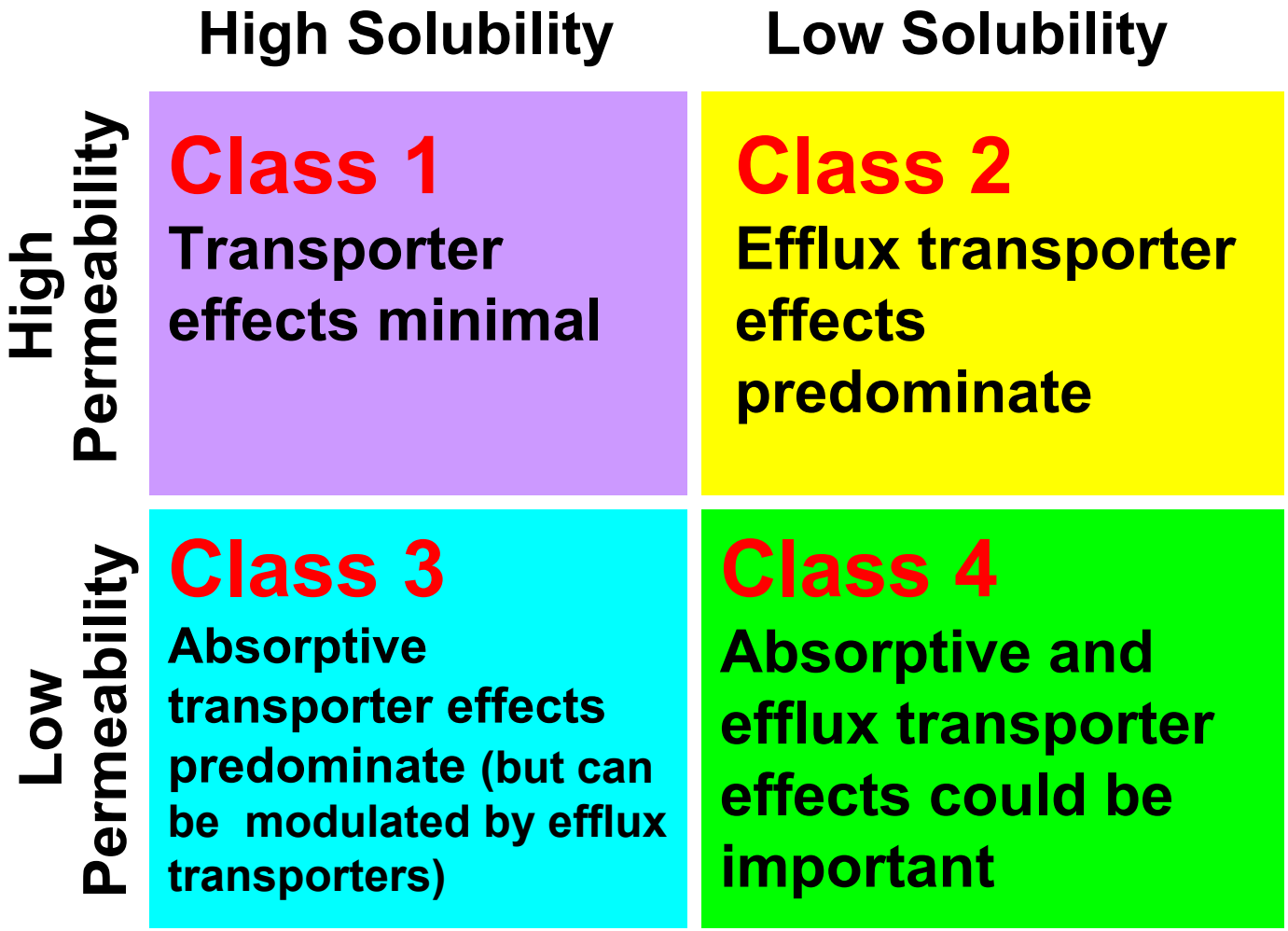
CLog P	Log P	Extensive vs Poor Metabolism
19 of 29	19 of 27	27 of 29
65.5%	70.4%	93.1%

A major advantage of BDDCS is that drugs can generally be correctly classified without running expensive and time consuming permeability studies in humans.

At this time, BDDCS may not be sufficient for the regulatory agencies, but it gives scientists a roadmap for predicting drug disposition and drug-drug interaction characteristics very early and with little additional expense.

Let's see further predictions

Oral Dosing: Transporter Effects



Transporter effects will be minimal for Class 1 compounds. The intestine is sufficiently leaky that small molecular weight, soluble, nonpolar compounds readily pass the membrane, or alternatively the high permeability-solubility of such compounds allows large concentrations in the gut to saturate any transporter, both efflux and absorptive. That is, Class 1 compounds may be substrates for both uptake and efflux in cellular systems under the right conditions, but transporter effects will not be important clinically.

Efflux transporter effects will predominate for Class 2 compounds. The high permeability of these compounds will allow ready access into the gut membranes, but the low solubility will limit the concentrations coming into the enterocytes, thereby preventing saturation of the efflux transporters.

Transporter-enzyme interplay will be primarily important for Class 2 compounds that are substrates for CYP 3A and Phase 2 gut enzymes (e.g. glucuronosyltransferases) where efflux transporter effects can control the access of the drug to the gut enzymes. Absorption of Class 2 compounds is primarily passive and a function of lipophilicity.

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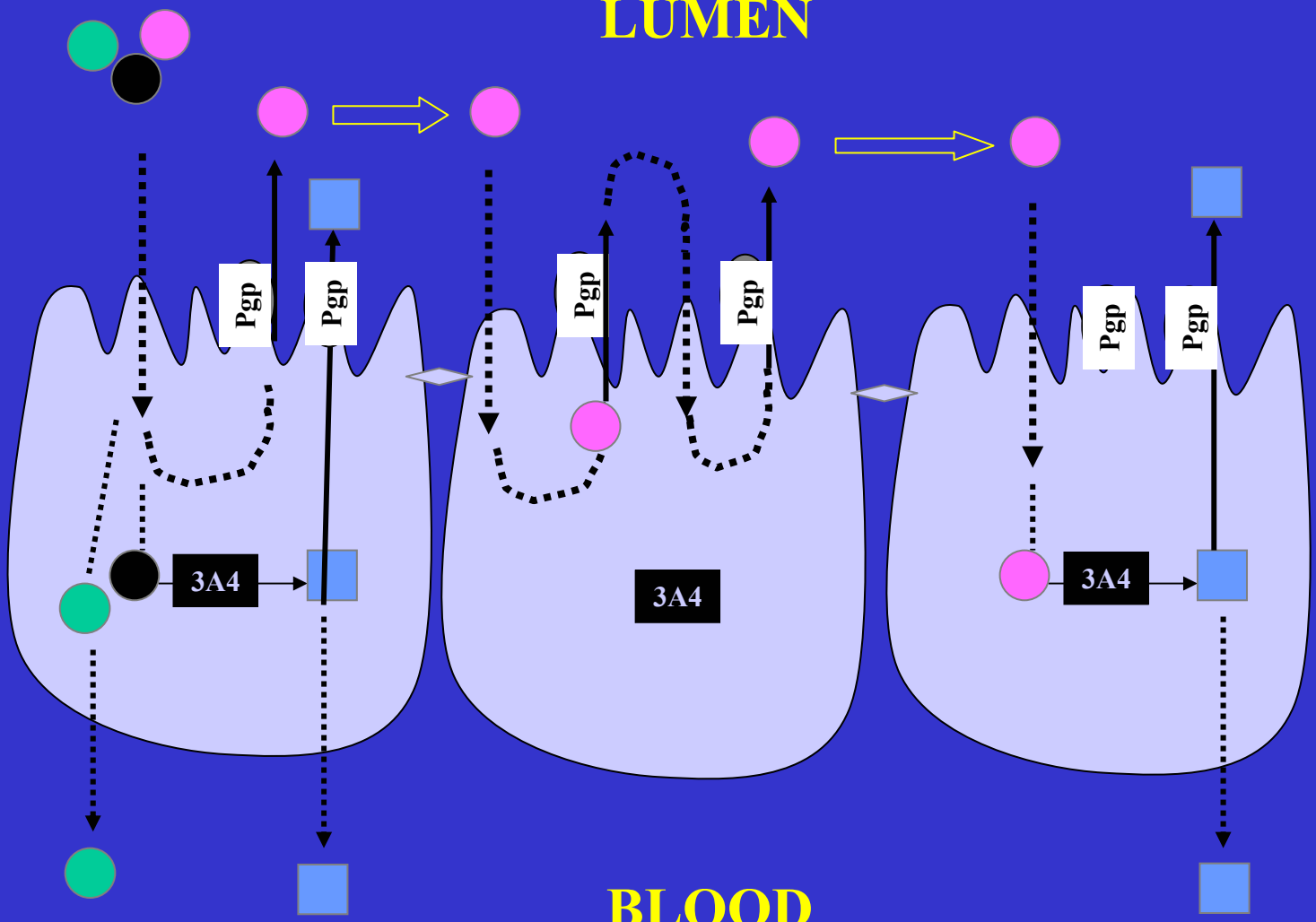
P-gp and CYP3A4 Interplay in the Enterocytes

Drug not metabolized or transported in the gut

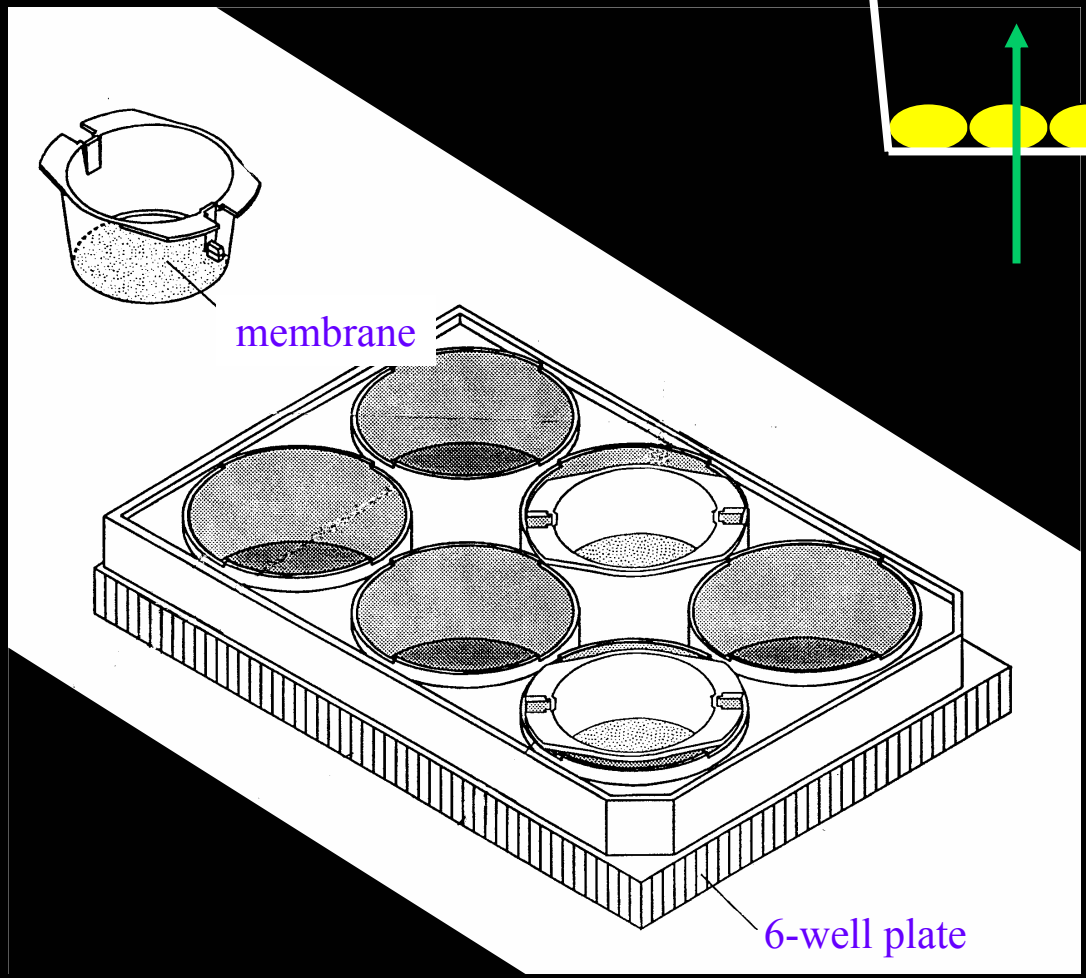
Drug metabolized on first entrance

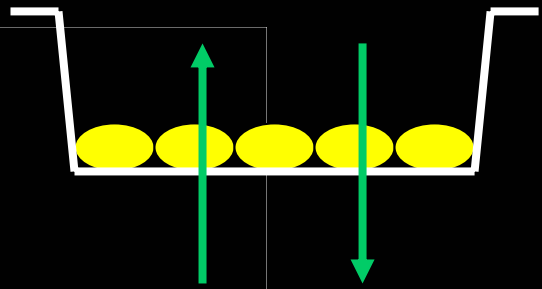
Drug cycled 4 times before metabolized

Drug metabolites



The *In Vitro* Model that Led to These Hypotheses





-P- A4
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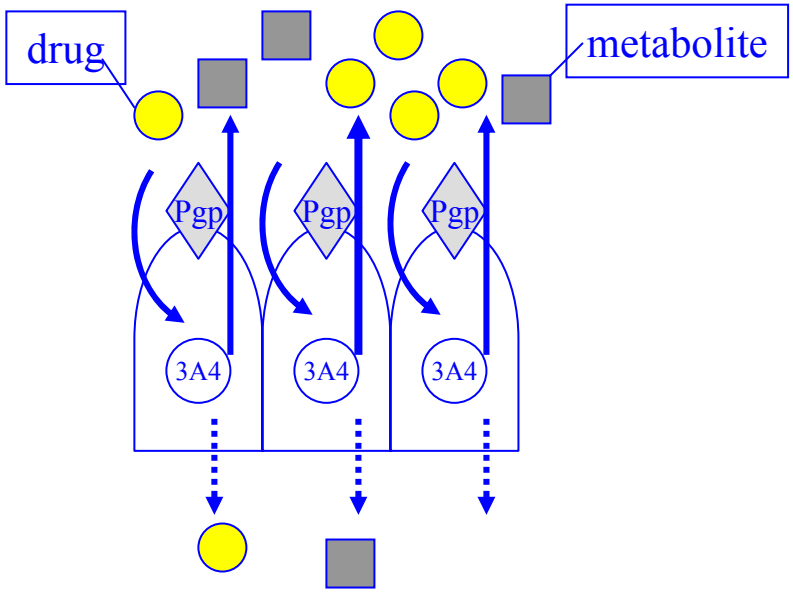
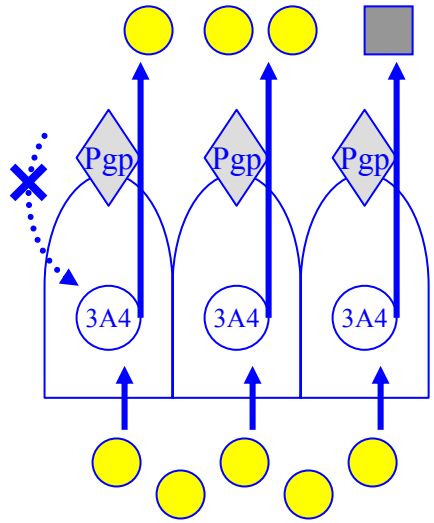
Studies to Characterize Transporter-Enzyme Interplay in CYP3A4 Transfected Caco-2 Cellular Systems

- Cummins et al., Pharm. Res., **18**: 1102-1109 (2001)
- Benet et al., Adv. Drug Deliv. Rev., **50**: S3-S11 (2001)
- Cummins et al., J. Pharmacol. Exp. Ther., 300: 1036-1045 (2002)
- Cummins et al., J. Pharmacol. Exp. Ther., 308: 143-155 (2004)

Apical Dose



Substrates diffusing into cells will be pumped out by P-gp and have another opportunity to diffuse in:
- more metabolites formed
- less parent traversing membrane

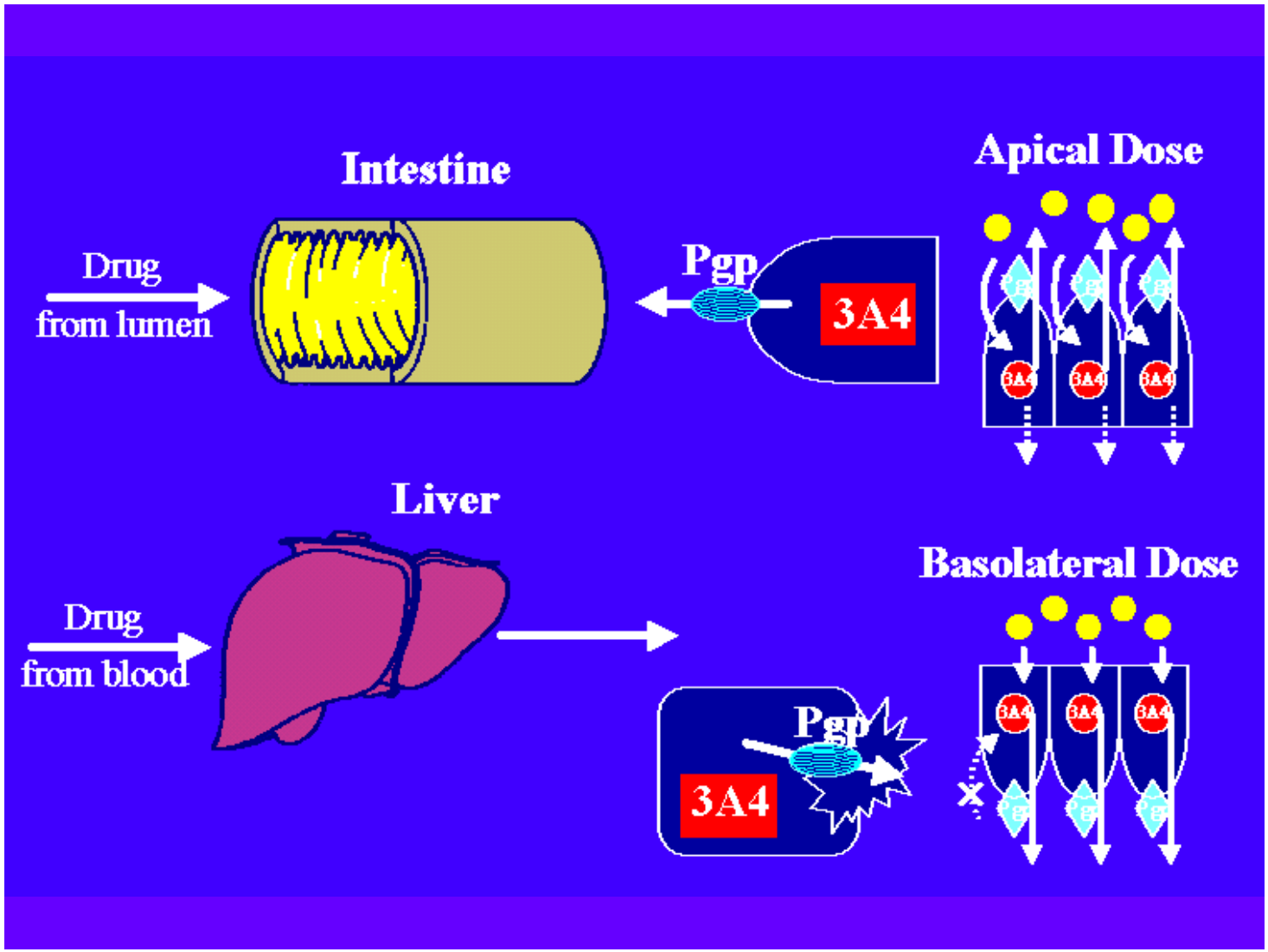


Basolateral Dose

Substrates diffusing into cells will be pumped out by P-gp but not diffuse back in because it is against the concentration gradient:
- less metabolites formed
- more parent traversing membrane

Current Drug Metabolism

Cover October 2003 Issue



DISPOSITION OF TACROLIMUS IN ISOLATED PERFUSED RAT LIVER: INFLUENCE OF TROLEANDOMYCIN, CYCLOSPORINE AND GG918

C-Y. Wu and L.Z. Benet

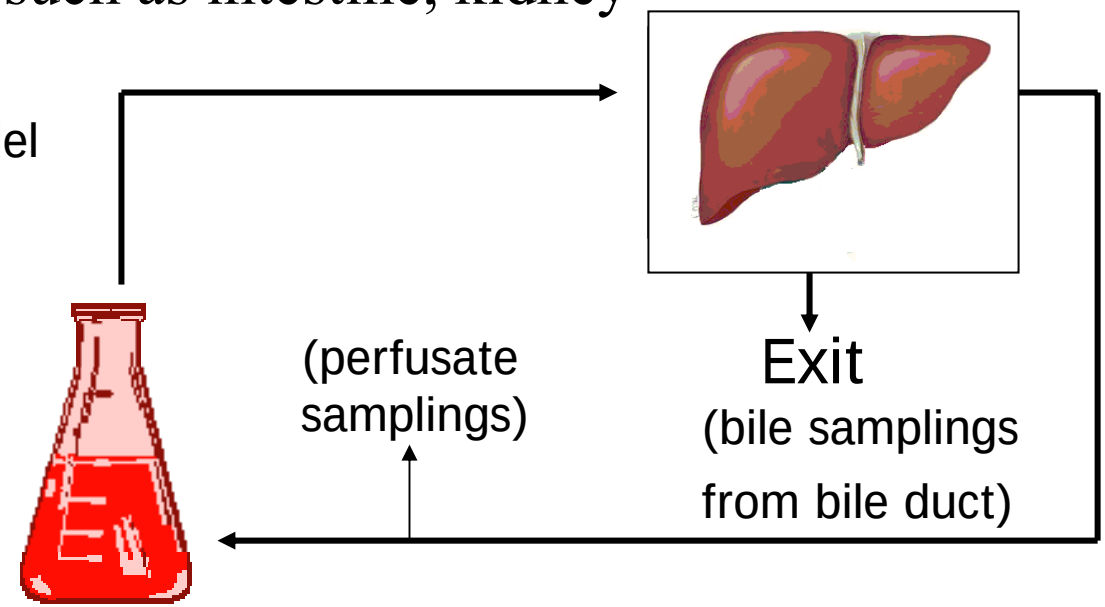
Drug Metab. Dispos. 31(11): *1292-1295, 2003*

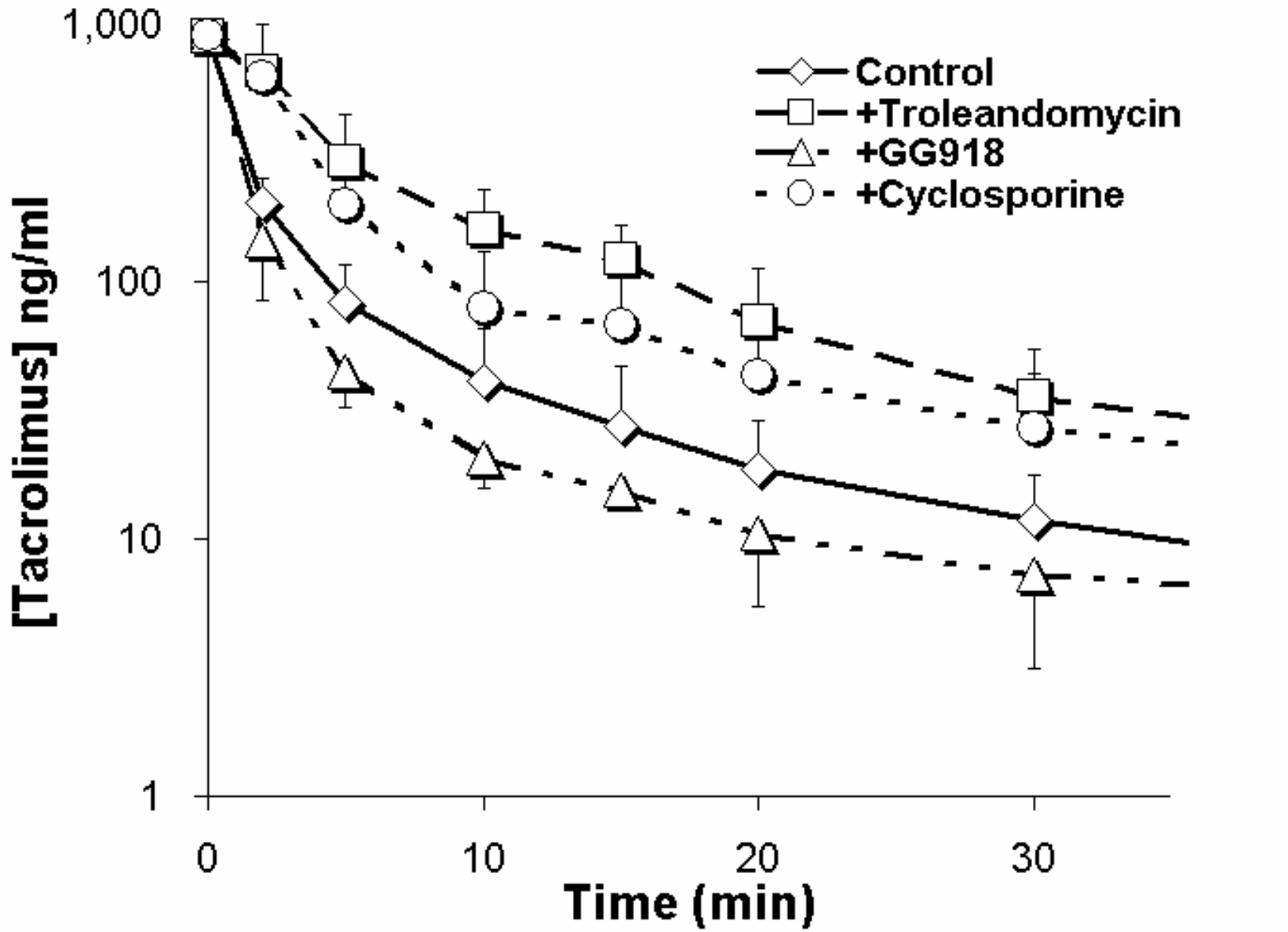
IPRL System

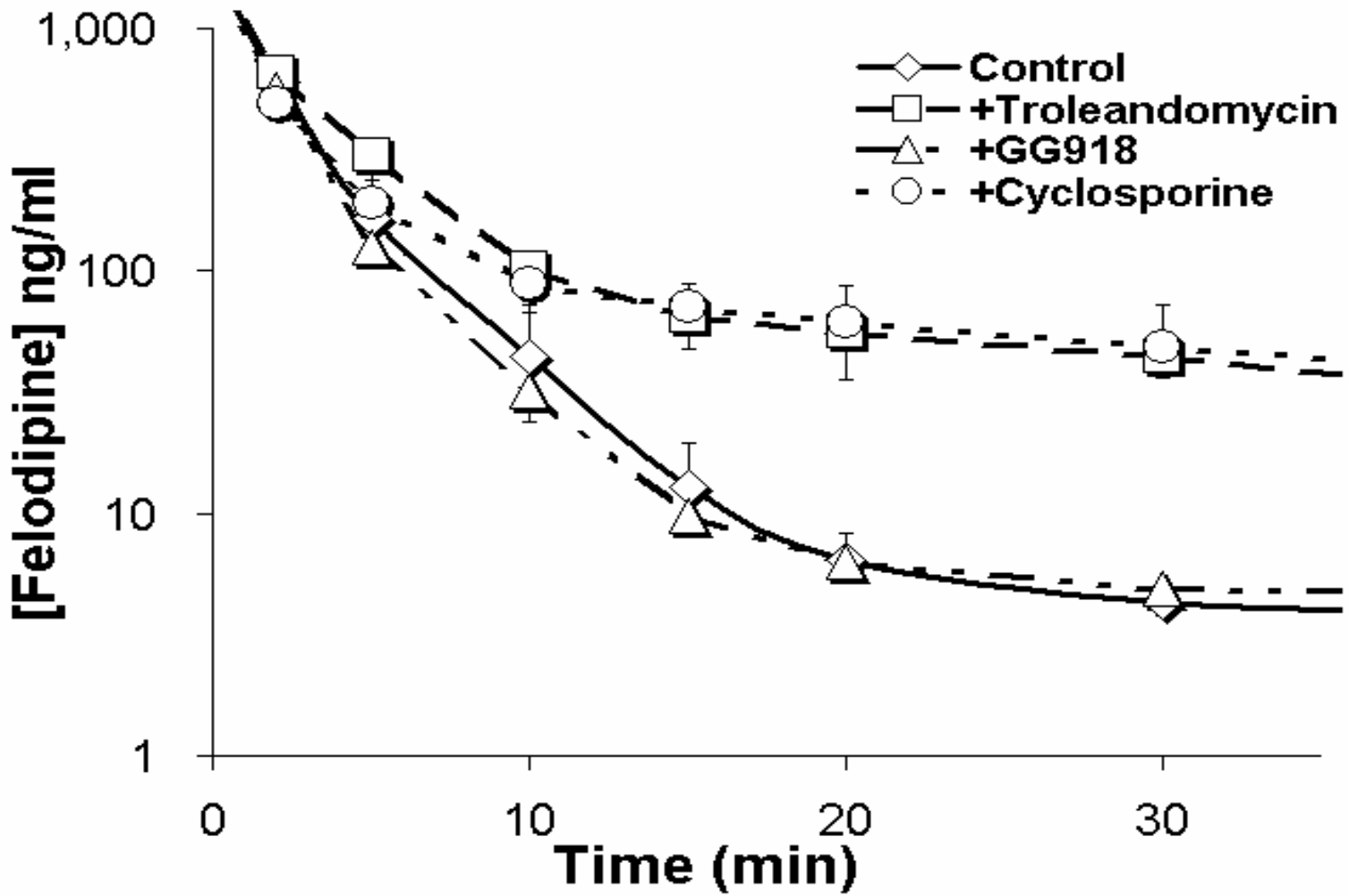
ISOLATED PERFUSED RAT LIVER

- Purpose:
  - Ideal model for examining alterations in the hepatobiliary disposition of substrates without the influence of metabolism/excretion by other organ systems such as intestine, kidney

2-Compartmental Model

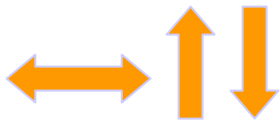



1,000
100
10
1
[Tacrolimus] ng/ml
0
10
20
30
Time (min)
Control
+Troleandomycin
+GG918
+Cyclosporine


1,000
100
10
1
[Felodipine] ng/ml
0
10
20
30
Time (min)
Control
+Troleandomycin
+GG918
+Cyclosporine

Inhibition of the efflux pump, P-glycoprotein, with no effect on CYP 3A enzyme, will cause decreased extraction ratio in the intestine resulting in increased AUC, but increased extraction ratio in the liver resulting in decreased AUC.

Predicted AUC Changes for In Vivo - In Situ Studies

	Gut	Liver
Inhibit P-gp		
Inhibit 3A		
Inhibit P-gp+3A		

Following oral dosing, major significant interactions will occur for Class 2 drugs that are substrates for intestinal enzymes (e.g. CYP3A, UGTs) and efflux transporters (e.g. P-gp, MRP2, BCRP) since concomitant inhibition of the intestinal enzyme and the efflux transporter both lead to less gut metabolism that synergistically increase systemic AUC. It is not surprising that drugs removed from the market due to drug-drug interactions predominate for orally dosed drugs that are substrates for CYP3A and P-gp.

What about Class 3 and 4 drugs?

Absorptive transporter effects will predominate for Class 3 compounds. Sufficient drug will be available in the gut lumen due to good solubility, but an absorptive transporter will be necessary to overcome the poor permeability characteristics of these compounds.

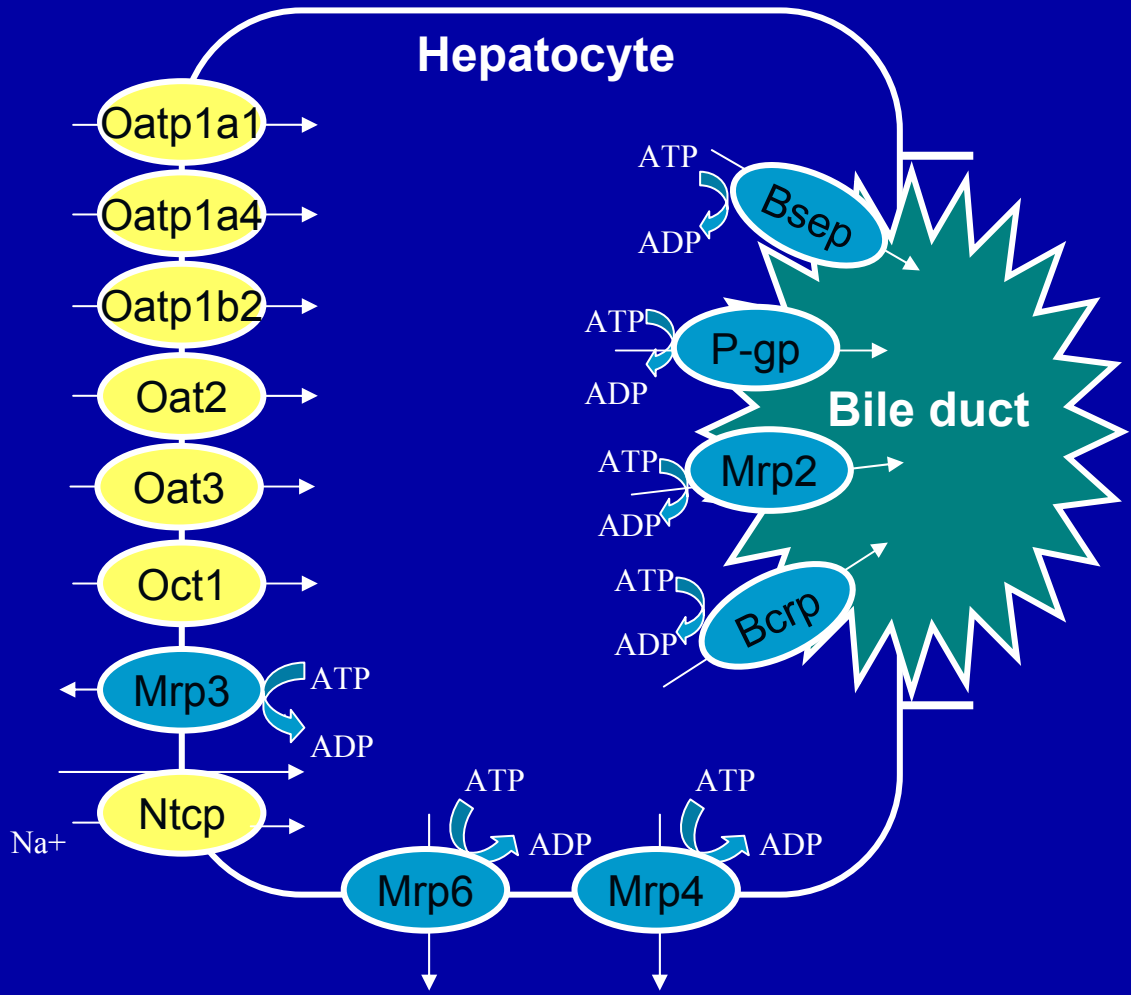
Reflect on the drugs that Profs. Richard Kim and Yuichi Sugiyama study in double transfected cellular systems, i.e., both uptake and efflux transporters added.

Reflect on the compounds for which companies such as Xenoport attempt to improve absorption through a transporter prodrug approach.

All of the substrates upon which they work are nonmetabolized Class 3 and Class 4 drugs.

However, since influx of Class 3 (and Class 4) compounds will be rate limited by an absorptive transporter, the counter effects of efflux transporters will not be saturated and can also be important

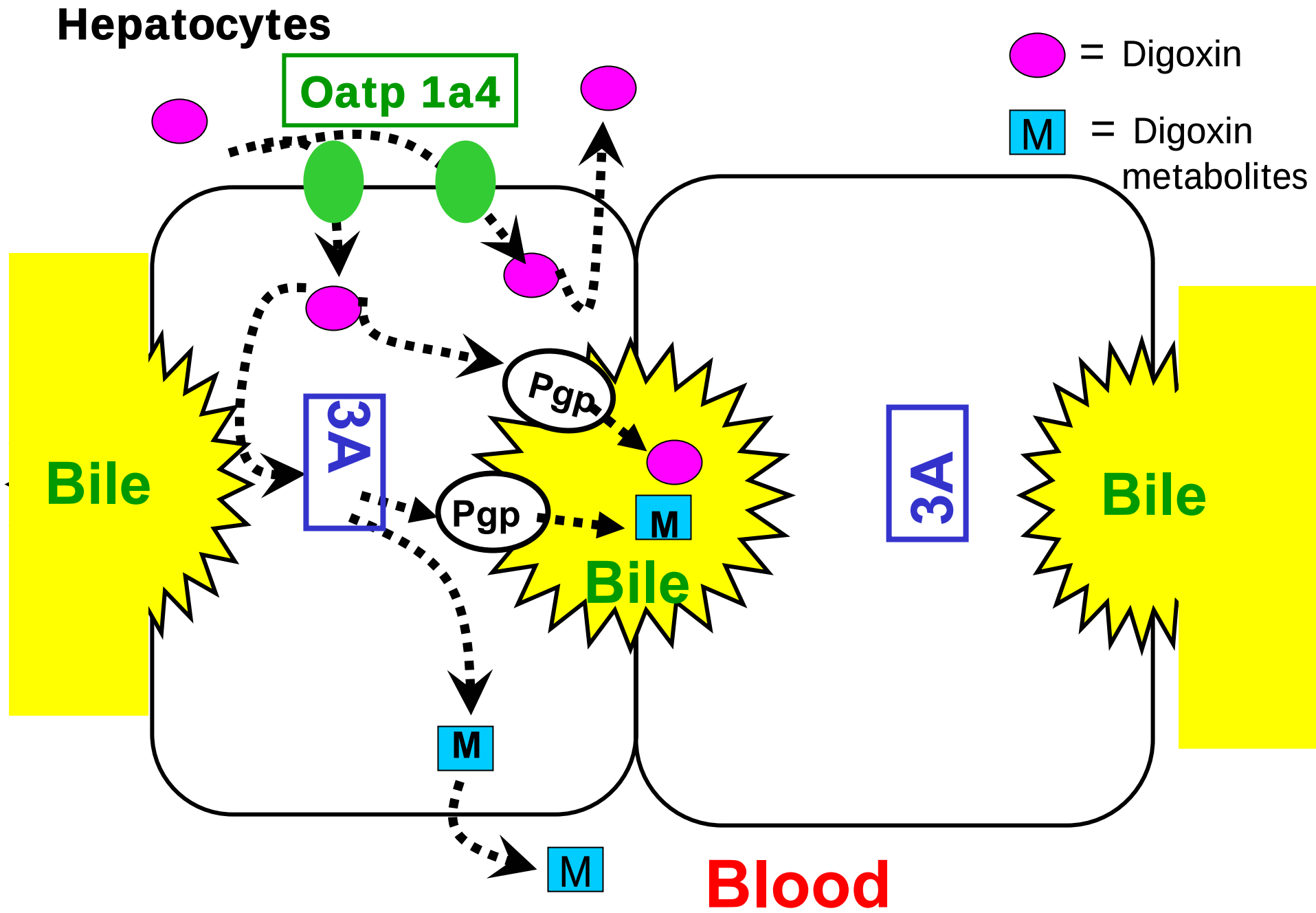
Hepatic Uptake and Efflux Transporters in Rat Liver



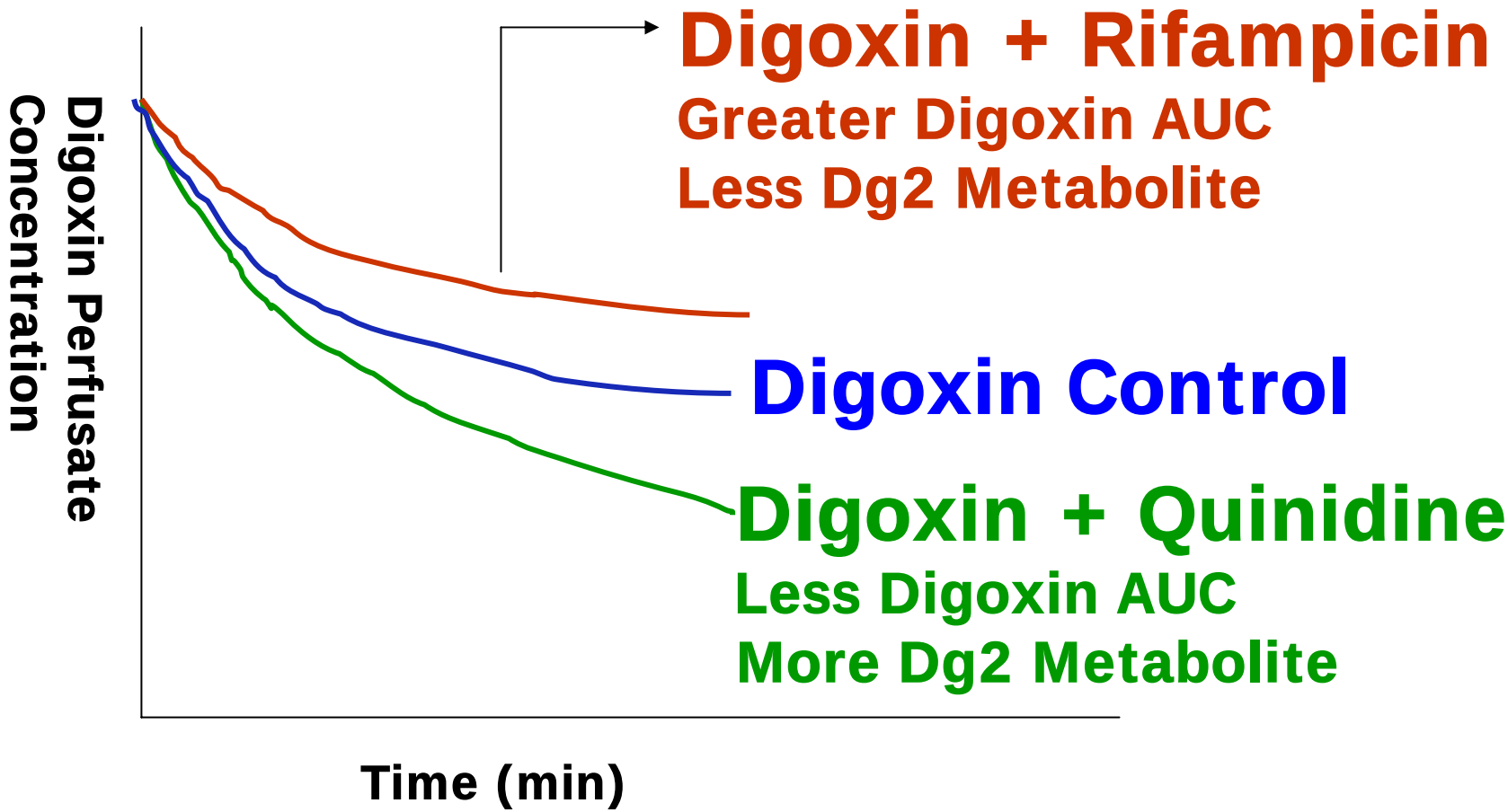
Ex situ Inhibition of Hepatic Uptake and Efflux Significantly Changes Metabolism: Hepatic Enzyme-Transporter Interplay

Y.Y. Lau, C-Y. Wu, H. Okochi & L.Z. Benet

J. Pharmacol. Exp. Ther. 308: 1040-1045, 2004


Hepatocytes
Oatp 1a4
= Digoxin
M = Digoxin metabolites
Bile
3A
Pgp
Pgp
M
Bile
3A
Bile
M
M
Blood

Hypothesis


Digoxin + Rifampicin
Greater Digoxin AUC
Less Dg2 Metabolite
Digoxin Control
Digoxin + Quinidine
Less Digoxin AUC
More Dg2 Metabolite
Digoxin Perfusate Concentration
Time (min)

Control livers perfused with digoxin only *vs.* livers co-perfused with quinidine or rifampicin

	Treatment group		
Parameter	**Control**	**Quinidine**	**Rifampicin**
Digoxin AUC (nM·min)	3880 ± 210	3220 ± 340 **	5200 ± 240*
Dg2 AUC (nM·min)	1480 ± 90	1690 ± 120**	1130 ± 200**
Dg2 AUC/digoxin AUC	0.382 ± 0.029	0.530 ± 0.076*	0.217 ± 0.037**
Dg2/digoxin in liver	0.131 ± 0.023	0.488 ± 0.192*	0.136 ± 0.045
Liver/Perfusate (inhibitor)	N/A	8.08 ± 0.72	13.3 ± 3.0

*** Significantly different from control, $p < 0.005$.**
**** Significantly different from control, $p < 0.05$.**

Multiple transporters affect the disposition of atorvastatin and its two active hydroxy metabolites: Application of in vitro and ex situ systems

Y.Y. Lau, H. Okochi, Y. Huang & L.Z. Benet

J. Pharmacol. Exp. Ther., **316**:762-771 (2006)

Disposition of atorvastatin and its two hydroxy metabolites in rats: Application of isolated perfused liver and in vivo studies following oral and intravenous administration

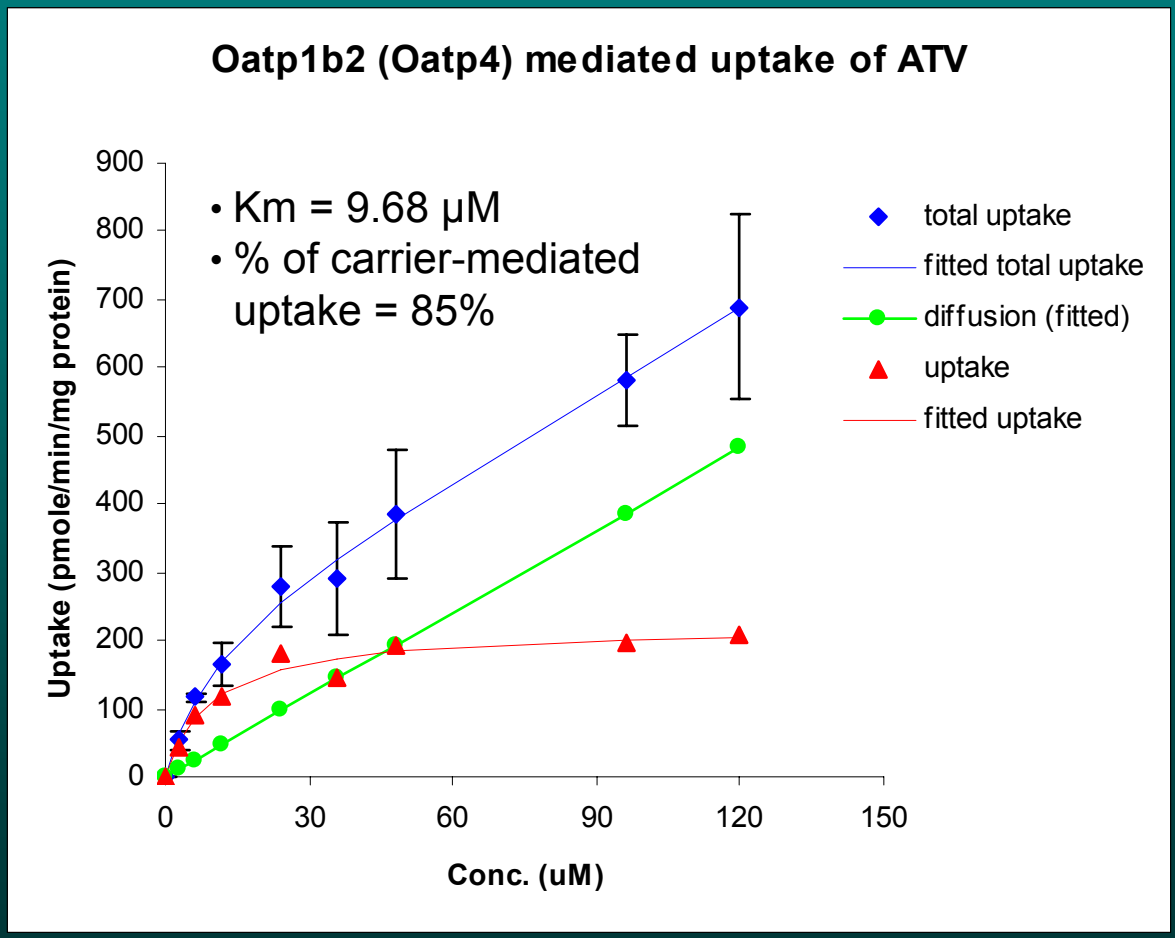
Y.Y. Lau, H. Okochi, Y. Huang & L.Z. Benet

Drug Metab. Dispos., **34**:1175-1181 (2006)

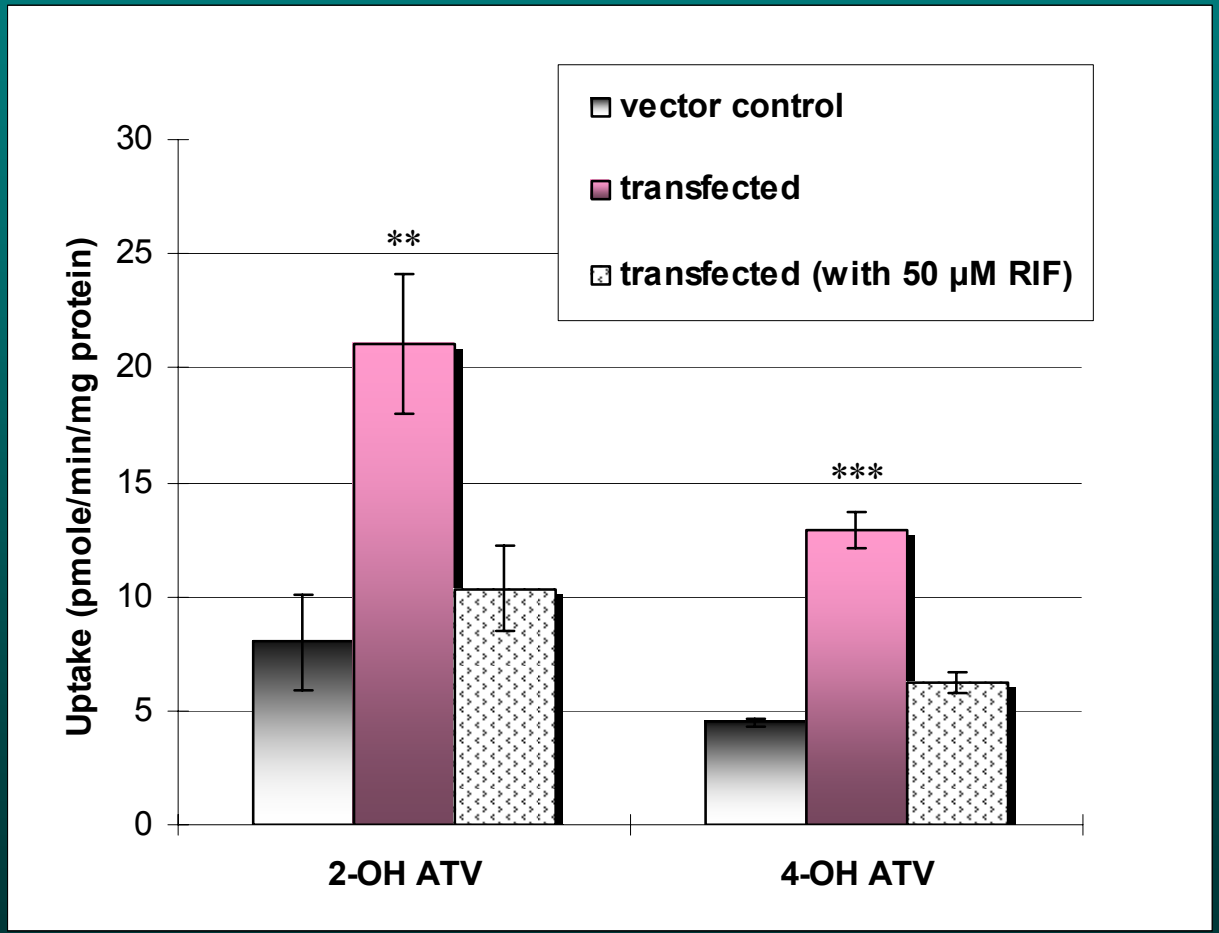
Effect of OATPB1 transporter inhibition on the pharmacokinetics of atorvastatin in healthy volunteers

Y.Y Lau, Y. Huang, L. Frassetto & L.Z. Benet

Clin. Pharmacol. Ther., **81**:194-204 (2007)


Oatp1b2 (Oatp4) mediated uptake of ATV
• Km = 9.68 μM
• % of carrier-mediated uptake = 85%
Uptake (pmole/min/mg protein)
900
800
700
600
500
400
300
200
100
0
0
30
60
90
120
150
Conc. (uM)
total uptake
fitted total uptake
diffusion (fitted)
uptake
fitted uptake

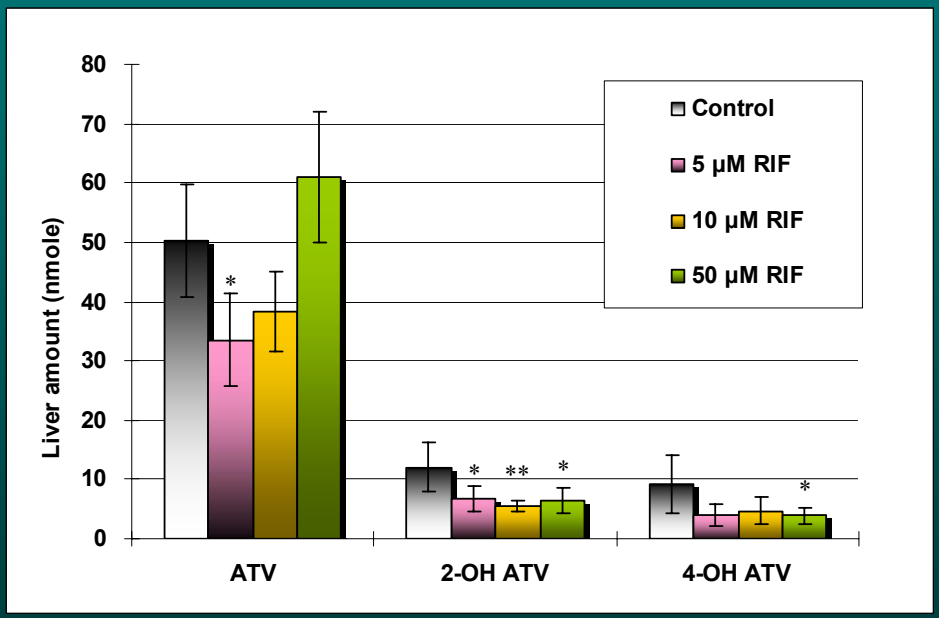
ATV metabolites uptake by Oatp1b2

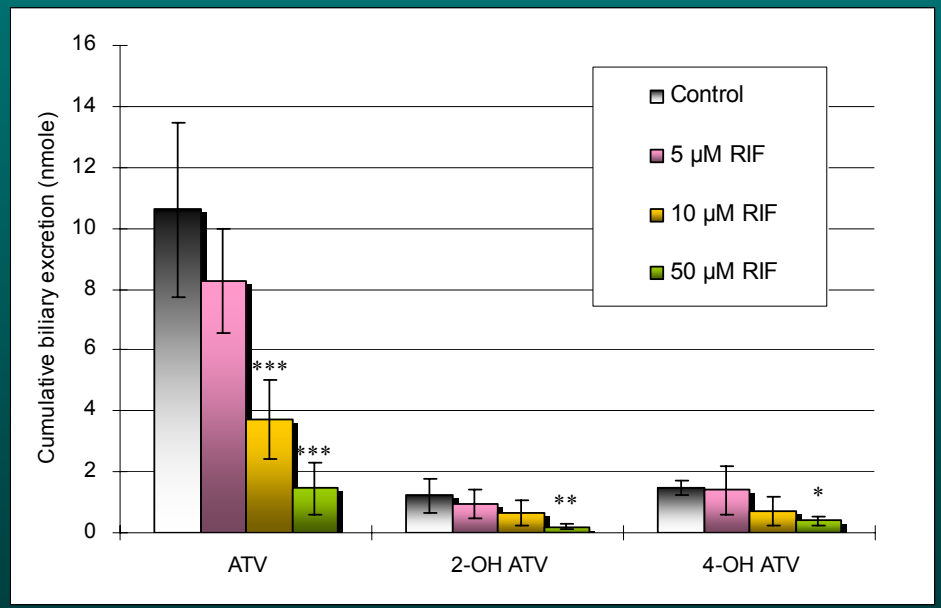


PK Parameters -- IPRL

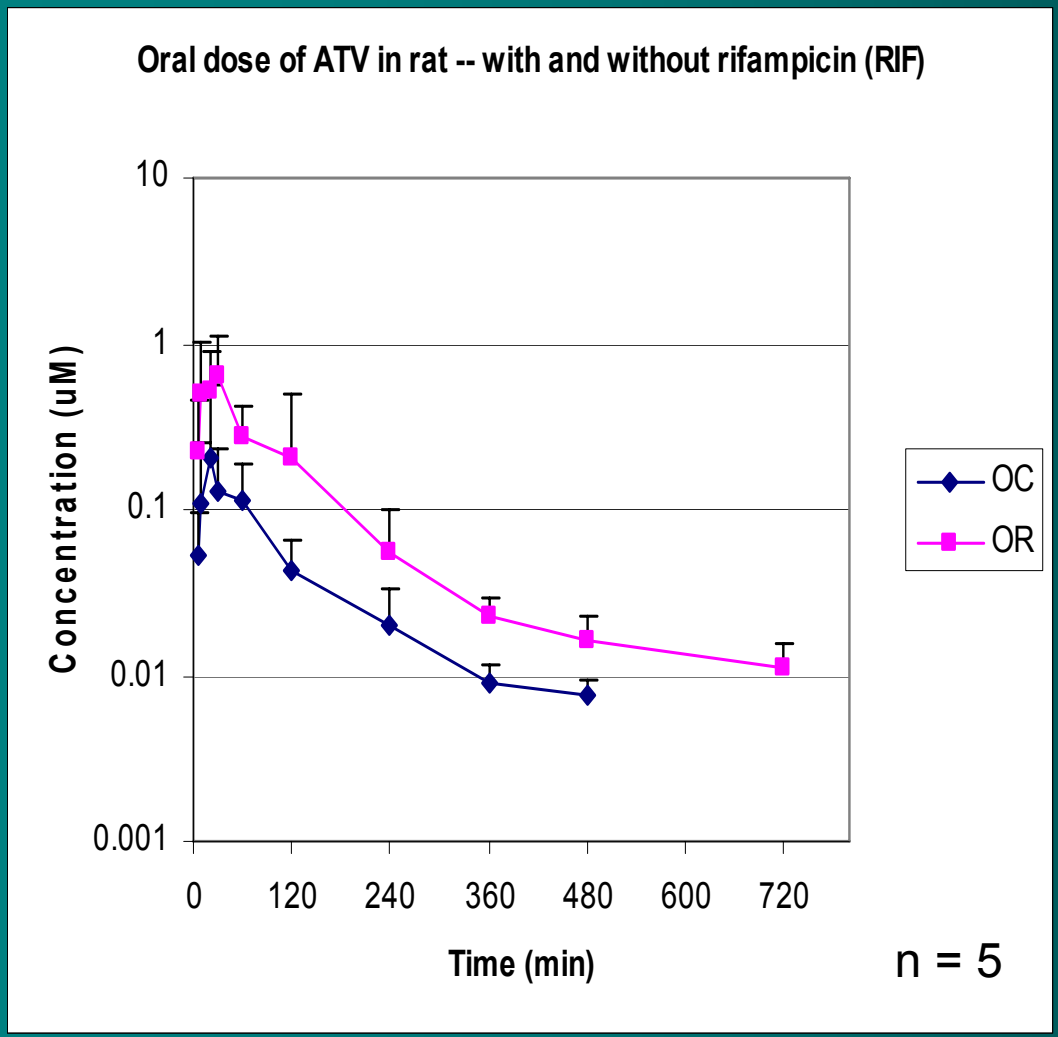
	Treatment group			
Parameter	**Control**	**RIF 5 μM**	**RIF 10 μM**	**RIF 50 μM**
ATV AUC (μM ·min)	8.55 ± 1.89	13.3 ± 1.7*	17.8 ± 3.1***	21.4 ± 3.0***
2-OH ATV AUC (μM ·min)	2.93 ± 0.48	3.51 ± 0.75	5.33 ± 0.64 **	5.84 ± 0.71***
4-OH ATV AUC (μM ·min)	2.44 ± 0.36	2.90 ± 0.92	4.44 ± 0.06**	5.23 ± 0.08***
OH ATV AUC/ATV AUC	0.66 ± 0.18	0.48 ± 0.08	0.56 ± 0.09	0.53 ± 0.08
OH ATV/ATV in liver	0.43 ± 0.16	0.35 ± 0.15	0.28 ± 0.11	0.17 ± 0.05*
bile/liver (ATV)	0.21 ± 0.04	0.26 ± 0.10	0.10 ± 0.05	0.026 ± 0.019**
bile/liver (OH ATV)	0.14 ± 0.06	0.13 ± 0.08	0.13 ± 0.08	0.059 ± 0.017*
RIF in liver (μM)	N/A	11.2 ± 2.1	18.4 ± 3.6	121.0 ± 19.3

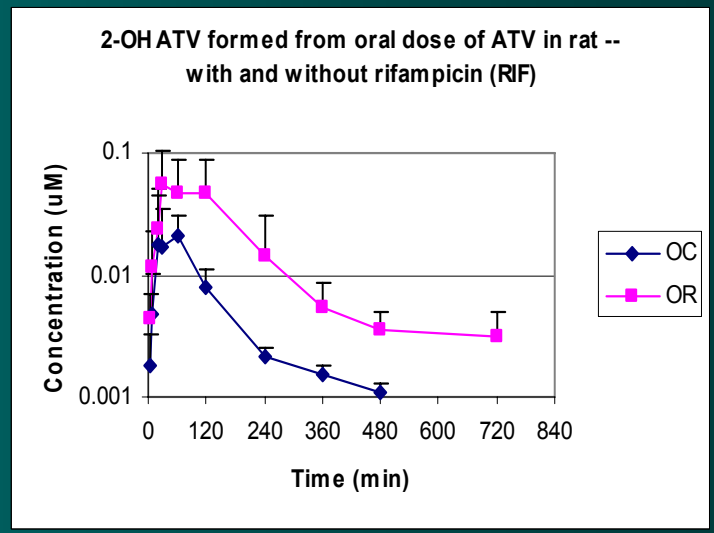
Amounts of Atorvastatin (ATV) and metabolites in rat liver and bile as affected by Rifampicin (RIF)

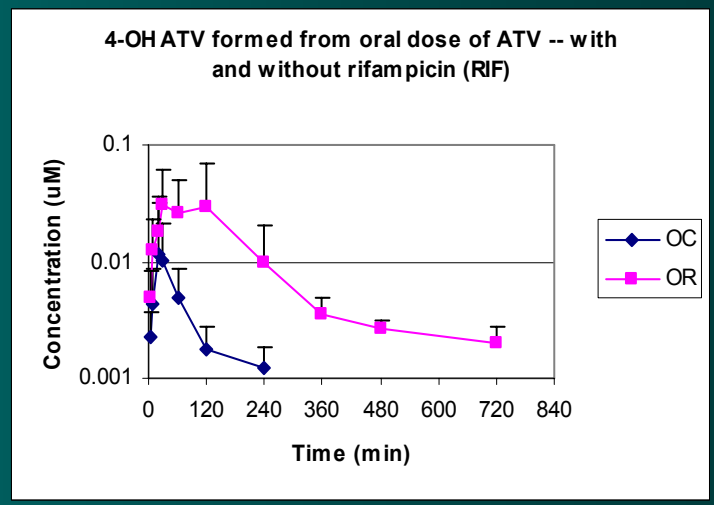




Oral dose of ATV with and without RIF







Elucidating the Effect of Final Day Dosing of Rifampin in Induction Studies on Hepatic Drug Disposition and Metabolism

Justine L. Lam, Sarah B. Shugarts, Hideaki Okochi, and Leslie Z. Benet

J. Pharmacol. Exp. Ther. 319, 864-870 (November 2006).

Area under the curve (AUC) of Digoxin (Dg3) and Dg2 in rat hepatocyte incubations were determined for three treatment sets

(Each value represents mean ± S.D., n = 6)

	Uninduced Control	**Induced Alone**	**Induced + Rifampin**
AUC Digoxin (Dg3) (μM • min/mg)	**26.9 ± 1.3**	**13.7 ± 0.9 ***	**27.3 ± 0.9**
AUC Dg2 (μM • min/mg)	0.463 ± 0.009	6.06 ± 0.21 *	2.18 ± 0.87 *
Mass Balance (%)	89.3 ± 5.7	43.3 ± 0.6 *	93.3 ± 9.7

Effects of Uptake and Efflux Transporter Inhibition on Erythromycin Breath Test Results

L.A. Frassetto, S. Poon, C. Tsourounis, C. Valera and L.Z. Benet

Clin. Pharmacol. Ther., in press, May 2007

March 14 [Epub ahead of print]

Sixteen healthy volunteers (8 M, 8 F) randomized to receive EBT on 3 occasions: at baseline, after a 30 min iv infusion of rifampin 600mg (OATP inhibitor) and after a 30 min iv infusion of lansoprazole 30 mg (MDR1 inhibitor)

EBT at Baseline, and after Rifampin and Lansoprazole (^{14}C met/hr)

	All	Males	Females	ΔM vs F	P-value
Baseline	2.63±0.70	2.21±0.61	3.04±0.53	0.83±0.72	**0.014**
EBT + Rifampin	2.18±0.65	1.73±0.45	2.64±0.52	0.92±0.38	**0.0003**
ΔRifampin from Baseline	-0.44±0.40	-0.49±0.45	-0.40±0.36		**0.0005** 0.077 **0.018**
EBT + Lansopra	2.88±0.92	2.24 ±0.49	3.51 ±0.80	1.27±0.81	**0.0003**
ΔLansopra from Baseline	+0.25±0.51	+0.03 ±0.51	+0.47		0.071 0.89 **0.018**

My reaction to the many studies that use midazolam, diazepam and verapamil as model substrates?

The science is great and the correlations are excellent, but so much of the work is carried out with Class 1 compounds, where we are able to ignore transporter effects. Will the methodology be useful and reliable when we investigate NMEs?

Use of microdosing to predict pharmacokinetics at the therapeutic dose: Experience with 5 drugs

Lappin et al. Clin. Pharmacol. Ther. 80:203-215(Sept. 2006)

⌘ Warfarin CL/F 65%; V/F 380%, $t_{1/2}$ 560%

oral 5mg/oral 100 µg

⌘ ZK253 F = 0.0016; F_{micro} <1

oral 50mg/iv 100 µg (oral 100 µg below limit of detection)

⌘ Diazepam CL 106%, V 137%, $t_{1/2}$ 79%

iv 10 mg/iv 100 µg

⌘ Midazolam CL/F 99%, V 52%, $t_{1/2}$ 84%, F 97%

oral 7.5 mg/oral 100 µg

⌘ Erythromycin $t_{1/2}$ 99%

oral suspension 250 mg/iv 100 µg (oral 100 µg below limit of detection)

Authors conclude that "microdose data from 3 of the 5 drug candidates tested would have predicted the therapeutic dose PK well"

Conclusions-Science

Understanding transporter-enzyme interactions in terms of the permeability and solubility of drug compounds offers the potential for predicting:

a. **Major routes of elimination**
b. **Transporter effects of drug absorption**
c. **Food (High Fat Meal) effects**
d. **Transporter effects on post absorption systemic levels and after i.v. dosing**
e. **Enzyme transporter interplay**
f. **Drug-drug interaction potential and its relationship to enzyme-transporter interplay**

Conclusions-Science Continued

g. Previously unexplained effects of renal disease on hepatic metabolism that can result from accumulation of substances (toxins) in renal failure that modify hepatic uptake and efflux transporters. (Sun et al., Effect of Uremic Toxins on Hepatic Uptake and Metabolism of Erythromycin. Drug Metab. Dispos. 32(10): 1239-1246, 2004)

h. The translation of pharmacogenetic differences in metabolic enzymes (genetic polymorphisms) that do not always result in the expected differences *in vivo*. Phenotype-genotype discordance (as well as changes in the relationship as a function of disease states) may be explained by the effects of transporters on metabolic clearance.

Conclusions-Science Continued

i. Obtaining a realistic perspective of new techniques (e.g., predictive ADME , QSAR, microdosing, systems biology). If validation of the technique primarily uses Class 1 drugs, there is no assurance that the technique will work for NMEs.

Conclusions-Science Continued

Understanding transporter-enzyme interactions in terms of the permeability and solubility of drug compounds offers the potential for predicting:

j. How animal disposition kinetics may predict human metabolic and elimination pharmacokinetics (allometric scaling)

In our rush to get NME's into humans we seem to have given up on using animal models to select the appropriate compound within a series, with good reason, since predictability for metabolized compounds is poor.

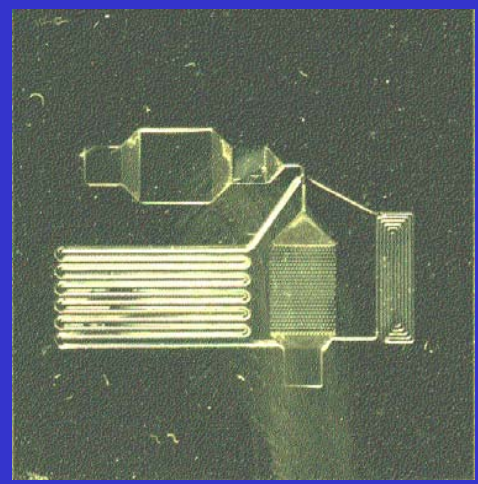
What is needed ?

A simple, rapid, flow-through preclinical tool to mimic the *in vivo* interplay of enzymes and transporters that allows drug molecules to encounter hepatocytes in a system that maintains the viability of the enzymatic processes, the basolateral uptake processes and the apical efflux processes for which integrity is difficult to maintain in isolated hepatocytes. Such a system can then be expanded to include, in series, flow-through enterocytes prior to the hepatocytes. The system must be high through-put and amenable to incorporating hepatocytes and enterocytes from animal species and humans.

Such a novel preclinical tool would provide great insights into the ADME of NMEs and expose the reasons for the discordance often found between ADME characteristics of drug molecules across animal species versus humans. Such a flow through cellular system could also include target tissue samples that would be useful in defining the toxicologic potential of NMEs and their metabolic by-products.

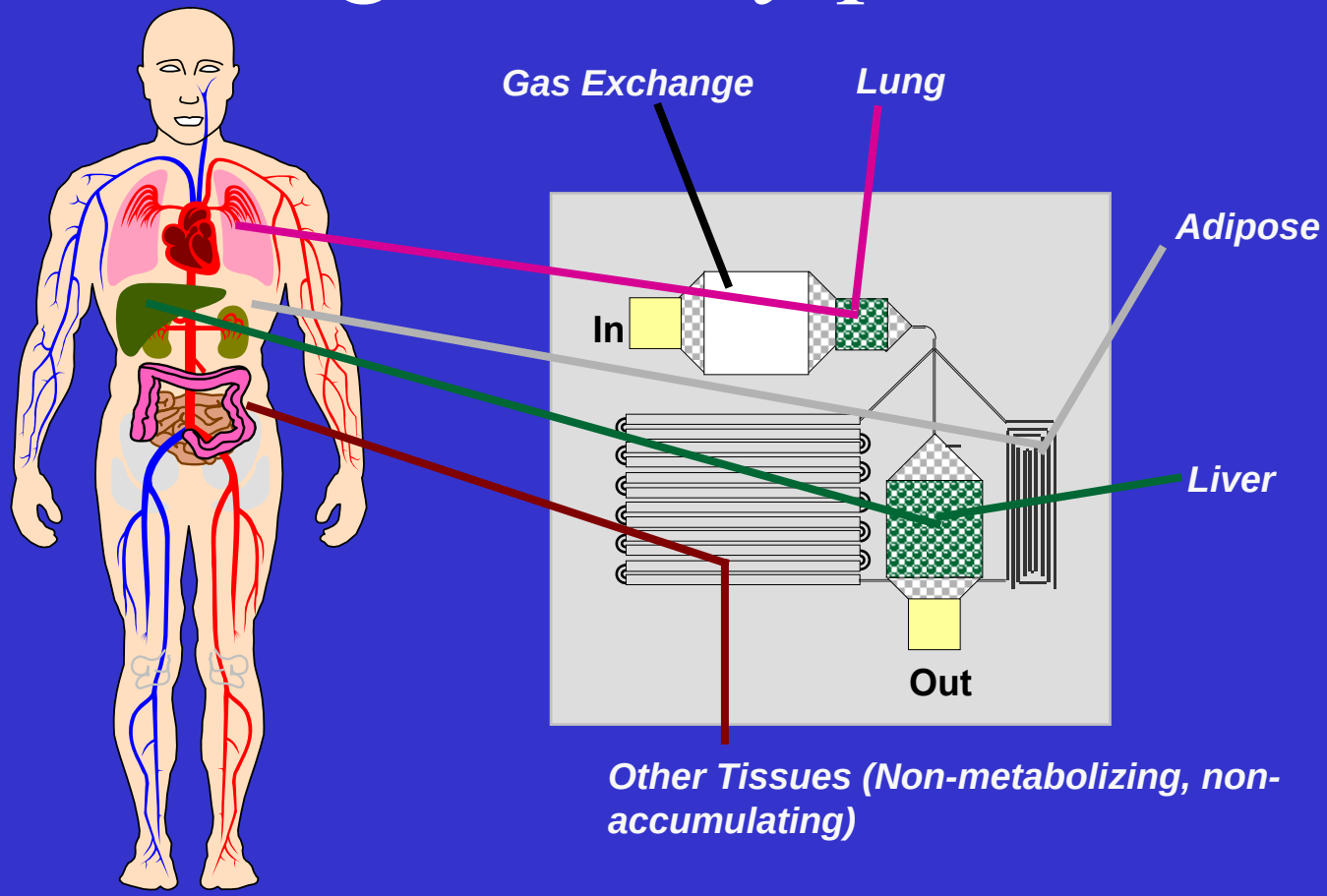
Dr. Michael Shuler, Professor and Chair Department of Bioengineering, Cornell University, Dr. Martin Yarmush, Professor of Surgery and Bioengineering, Harvard Medical School and Dr. Gregory Baxter, Hμrel Corp., have developed microfluidic, cell-based biochips

- ⌘ Individual compartments contain cultures of living cells of different organs
- ⌘ Heterogeneous cell types mimic different organs or tissues of an animal (and humans)
- ⌘ Compartments fluidically interconnected
- ⌘ Fluid and compounds recirculate as in a living system

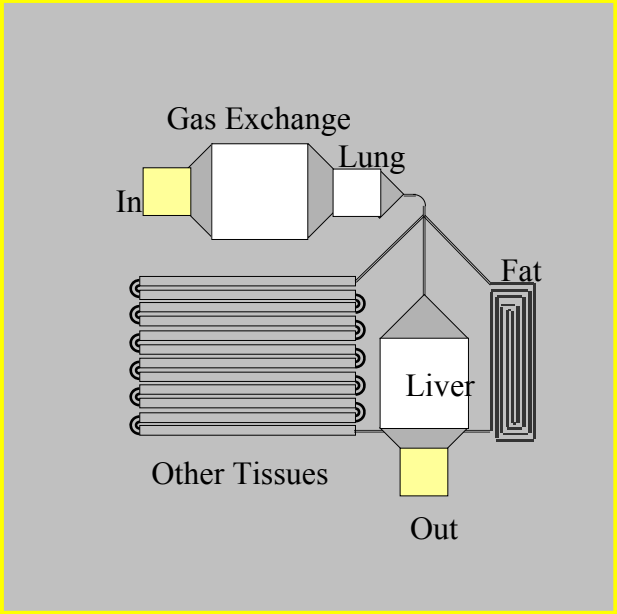


(See ***Nature***, **435**: 12-13, May 5, 2005;
Forbes, August 15, 2005, pp. 53-54;
The Observer, September 25, 2005, p.7;
Newsweek, October 10, 2005, p.59)

HμREL™: cell-based, *in vivo*-surrogate assay platform



Underlying science



- **Physiologically based, pharmacokinetic ("PBPK") model**
- **Cell Culture Analog ("CCA") concept: cells or cellular products, cultured in separate but fluidically connected sub-compartments, functionally represent various organs**
- **PBPK-derived values embodied in geometry of compartments and circulatory channels mimic key physiological parameters (cell-to-blood volume ratio, circulatory transit time, residence time, etc.)**

(see: www.hurelcorp.com)

HμREL™ instrument prototype

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- **Yvonne Lau, PhD**
- **Hideaki Okochi, PhD**
- **Chi-Yuan Wu, PhD**

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