Nov 28, 2009 The 24th JSSX Lecture for Young Investigator's Award (奨励賞)

医薬品の肝胆系輸送を支配するトランスポーターの寄与率の解明および遺伝子多型、薬物間相互作用による体内動態の変動の定量的予測

(Quantitative prediction of the contribution of transporters to the hepatobiliary transport of drugs and the effects of their genetic polymorphisms and drug interactions on pharmacokinetics in humans)

東京大学 THE UNIVERSITY OF TOKYO

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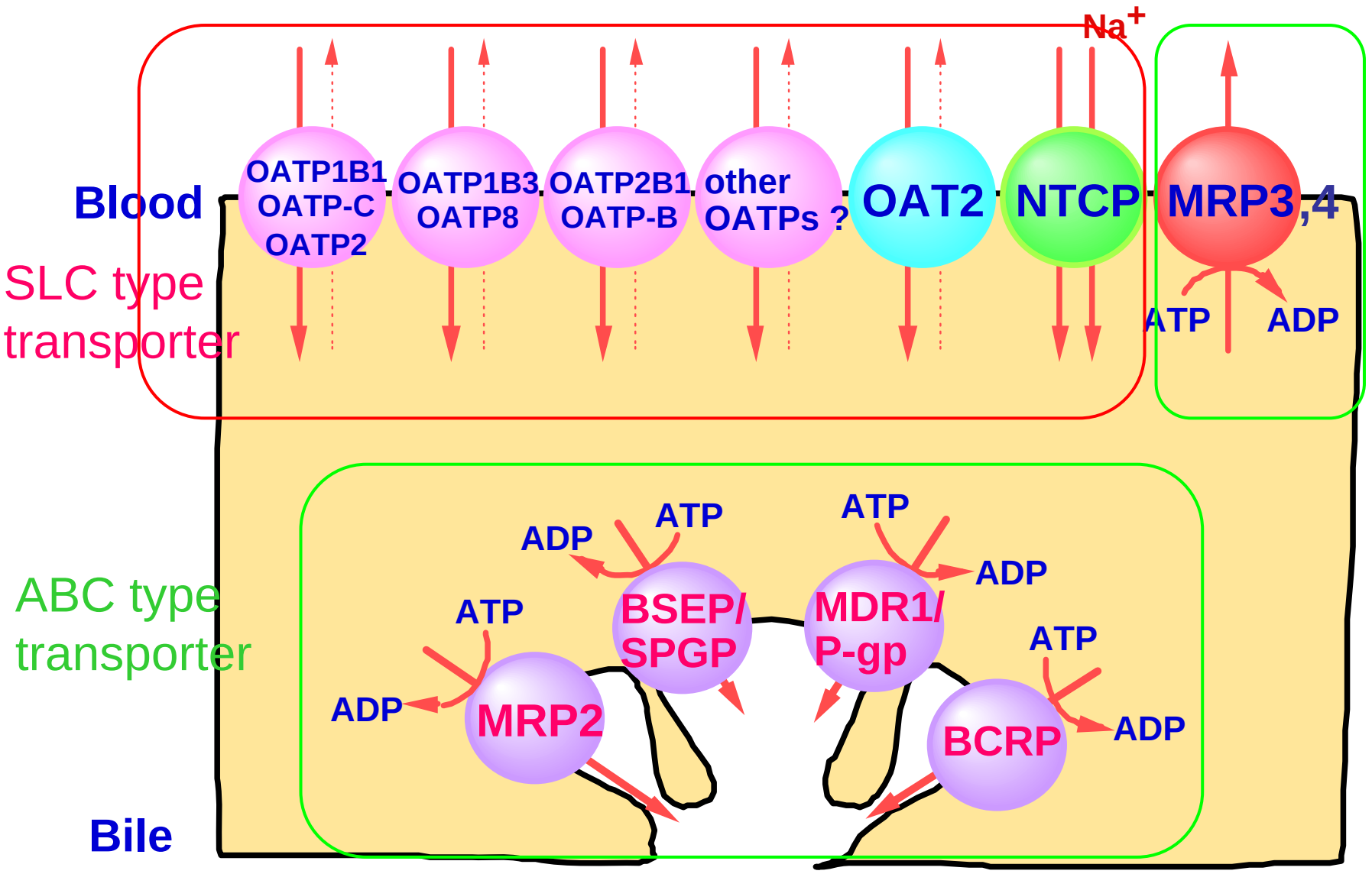
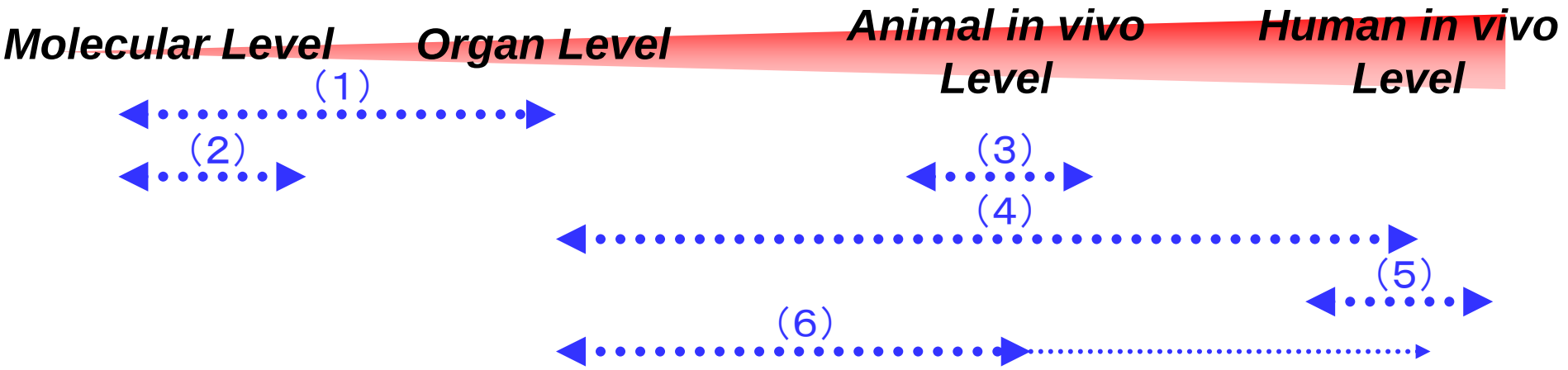
The University of Tokyo

Transporters responsible for the uptake and efflux of organic anions in human liver

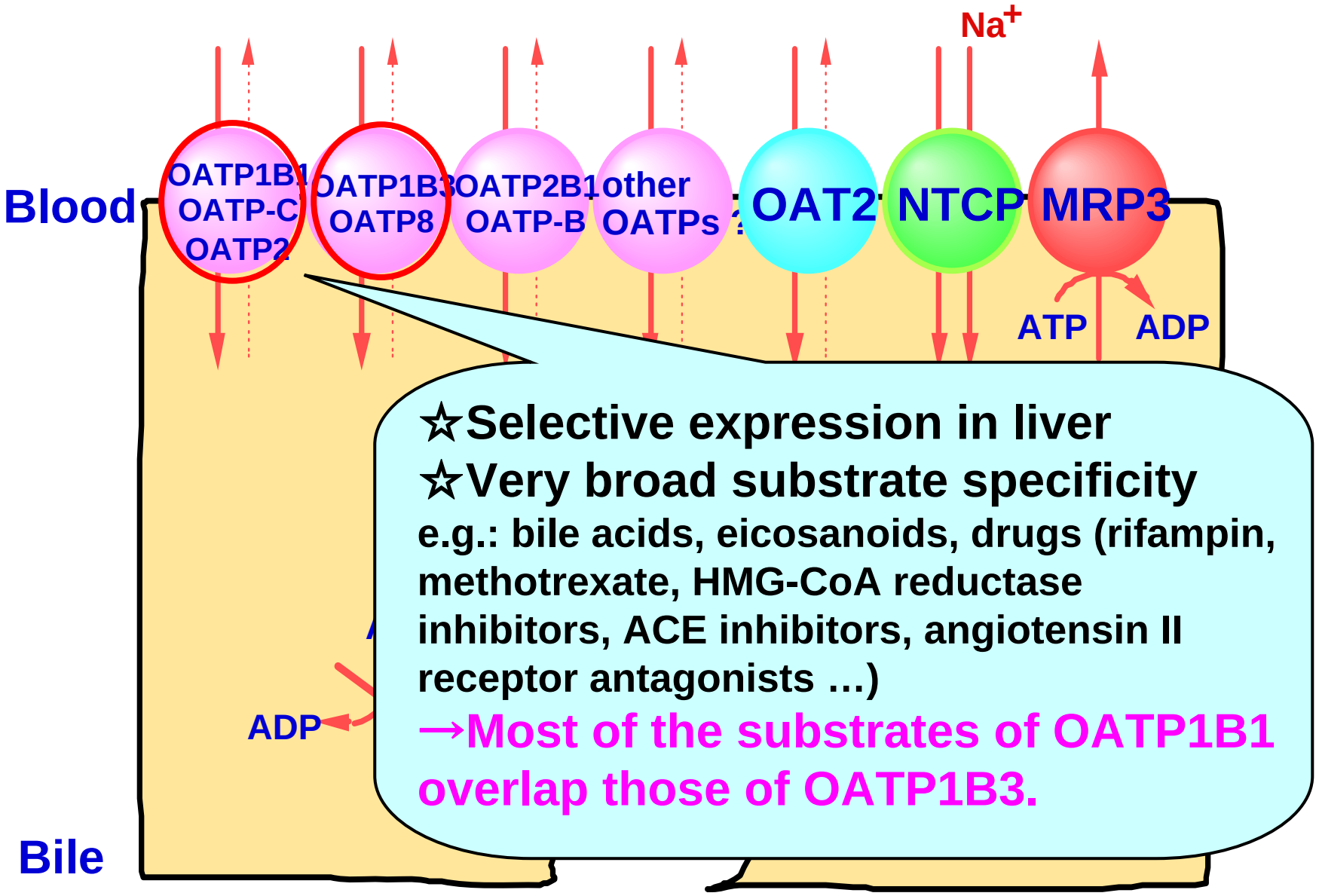


What I have done ….



（1）The contribution of OATP1B1 and OATP1B3 to the overall hepatic uptake of compounds in human hepatocytes
（2）A set of double transfectants for the easy evaluation of transporter-mediated hepatobiliary transport
（3）Role of efflux transporters in the PK of drugs by using knockout mice (statins)
（4）Prediction of in vivo clearance and fraction excreted into bile from in vitro data using hepatocytes and kidney slices
（5）Clinical study for investigating the relationship between genetic polymorphisms of transporters and PK of drugs
（6）Prediction of drug interaction from in vitro experiments

Transporters involved in the hepatic clearance of drugs in humans



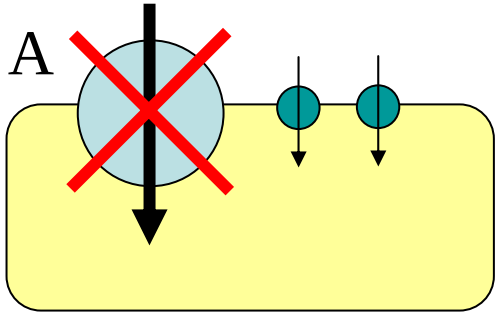
Why do we need to know the contribution of each transporter to the membrane transport ?

Assumption: ***In vitro*** **analysis revealed that the function of mutated uptake transporter A decreased to one-tenth compared with wild type.**

↓

Does this change affect the overall hepatic uptake ?

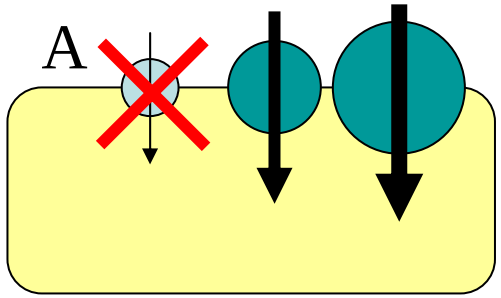
case 1: Contribution of A ＝ 90 %



Uptake clearance

=0.9 × 1/10 + 0.1 = 0.19

case 2: Contribution of A ＝10 %

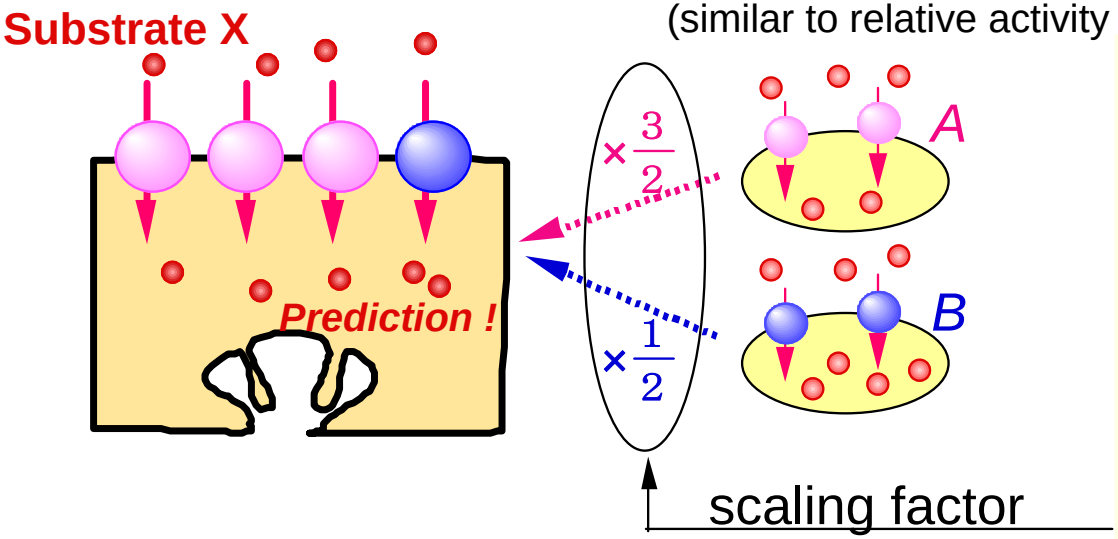


Uptake clearance

=0.1 × 1/10 + 0.9 = 0.91

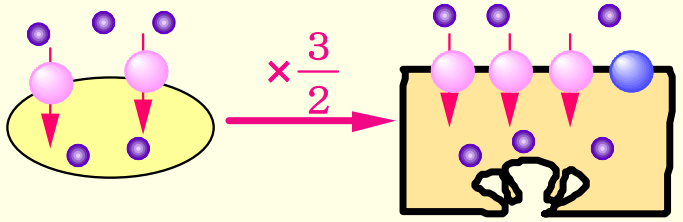
Strategy for determination of their contribution

(1)Using transporter-specific probes

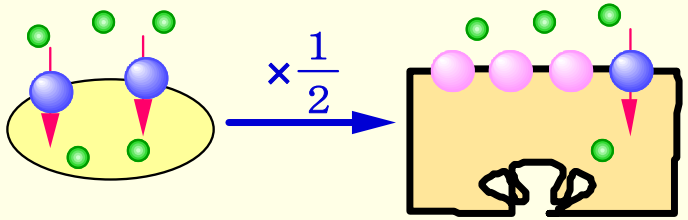


(similar to relative activity factor (RAF) method)

Probe Y
(transporter A-specific substrate)



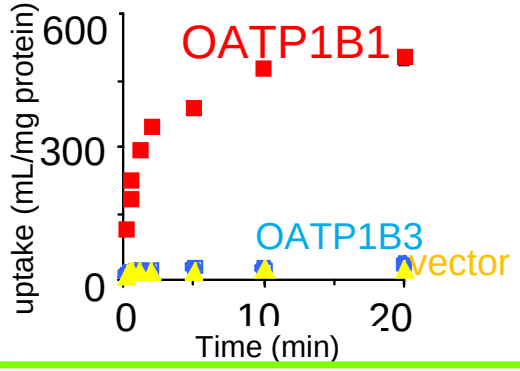
Probe Z
(transporter B-specific substrate)



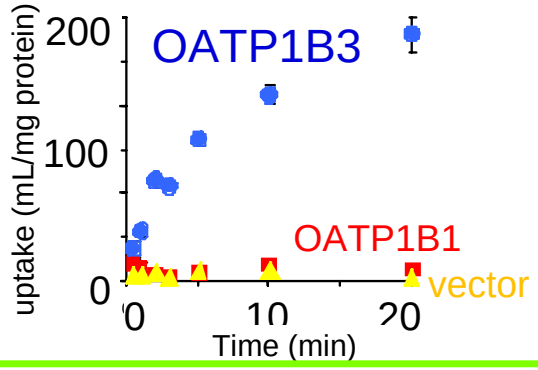
expression system

human hepatocyte

Estrone-3-sulfate (E-sul) → OATP1B1-selective



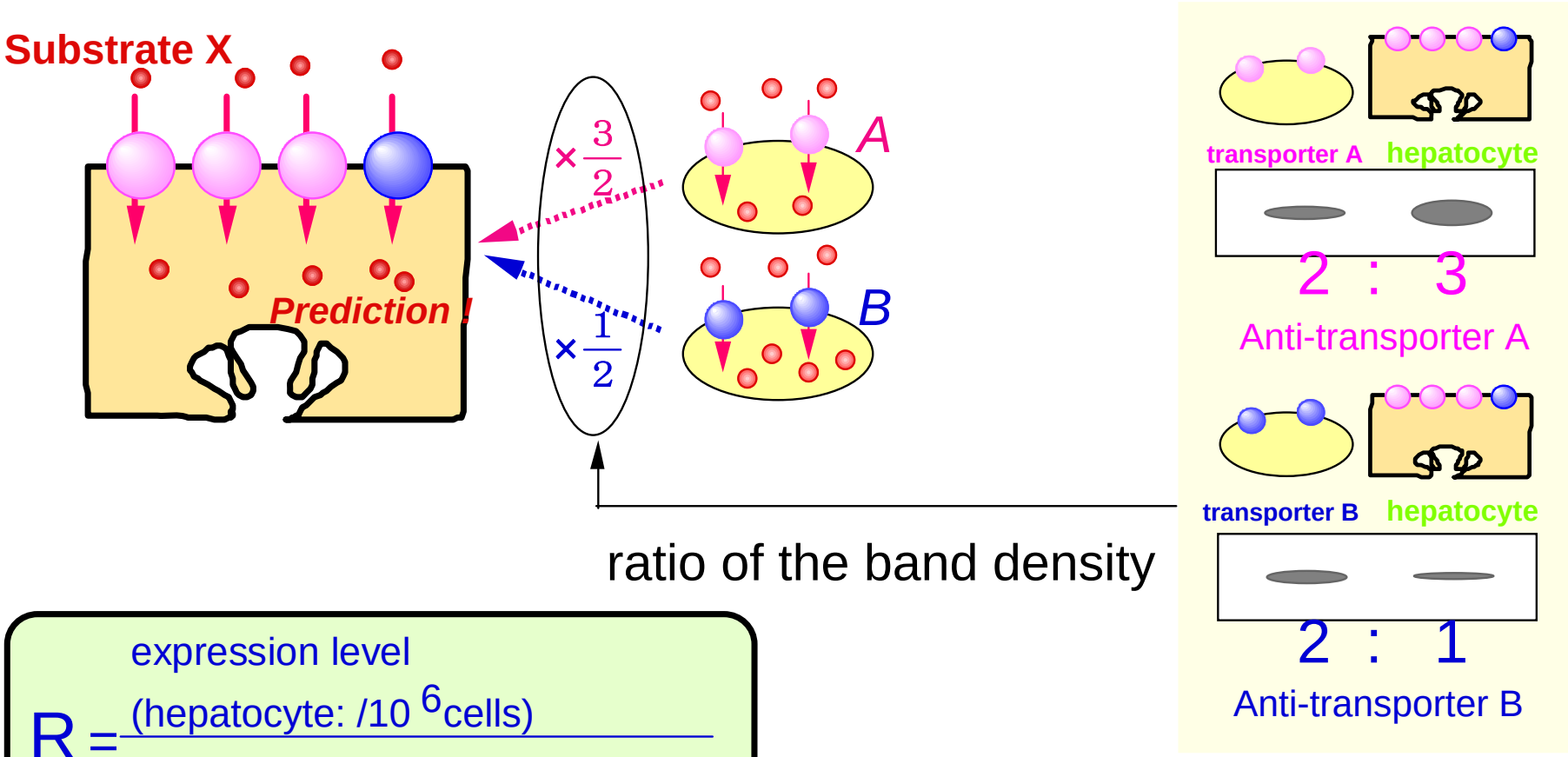
Cholecystokinin octapeptide (CCK-8) → OATP1B3-selective



(ref. Hirano M et al. J Pharmacol Exp Ther 311, 139 (2004))

Strategy for determination of their contribution

(2)Using Western blot analysis



$$R = \frac{\text{expression level (hepatocyte: /10}^{6}\text{cells)}}{\text{expression level (expression system: /mg protein)}}$$

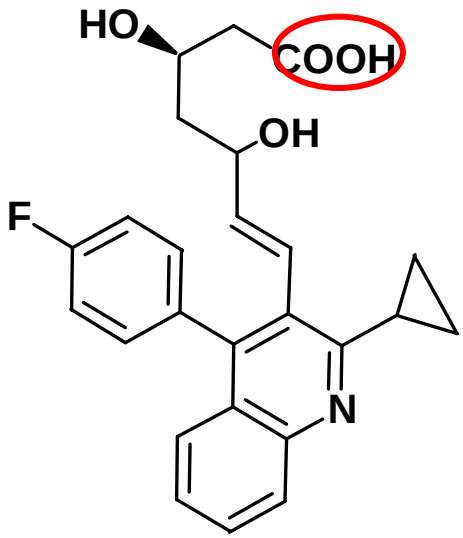
(ref. Hirano M et al. J Pharmacol Exp Ther 311, 139 (2004))

Characteristics of pitavastatin

★ Novel HMG-CoA reductase inhibitor

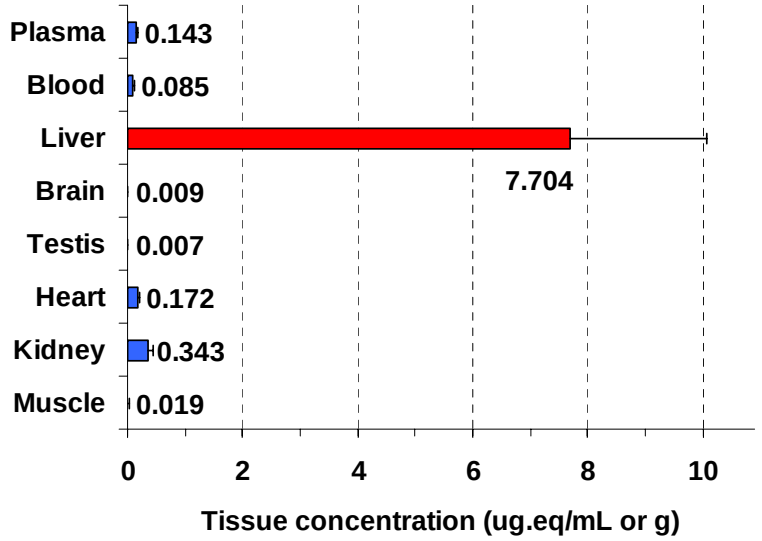
★ Pitavastatin is hardly metabolized in liver and excreted into bile in unchanged form.

★ Both OATP1B1 and OATP1B3 can accept pitavastatin.



pitavastatin (NK-104)
pKa = 4.40, 5.36
Log $D_{7.0}$ = 1.50

Radioactivity in tissues after oral administration (1mg/kg) of ^{14}C-pitavastatin in male rats (0.5 hr)



→Transporters may be involved in the concentrative accumulation of pitavastatin in liver.

○ Contribution of OATP1B1 and OATP1B3 to the hepatic uptake of $E_2$17βG and pitavastatin

Hepatocytes Lot	Ratio of transport activity $CL_{Hep}/CL_{transporter}$		Estimated activity (μL/min/10^6 cells) pitavastatin		E2 17βG	
	$R_{act, OATP1B1}$	$R_{act, OATP1B3}$	OATP1B1	OATP1B3	OATP1B1	OATP1B3
OCF	0.833	0.291	63.8 87.7 %	8.92 12.3 %	13.2 98.4 %	0.218 1.63 %
094	1.02	0.131	77.8 95.1 %	4.01 4.91 %	16.0 99.4 %	0.0979 0.607 %
ETR	0.437	0.0757	33.5 93.5 %	2.32 6.47 %	6.91 99.2 %	0.0565 0.812 %

→It is suggested that both $E_2$17βG and pitavastatin are taken up mainly by OATP1B1 in human hepatocytes.

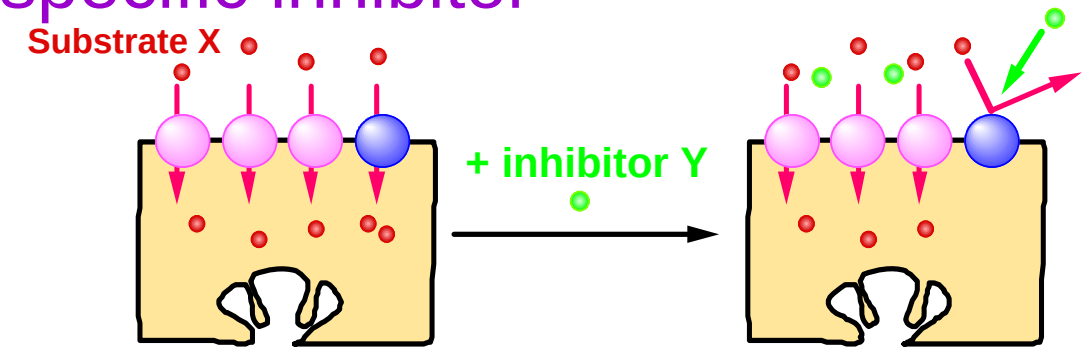
Substrate	Observed CL_{Hep} (μL/min/10^6 cells)			Estimated CL_{Hep} (μL/min/10^6 cells)		
	Lot. OCF	Lot. 094	Lot. ETR	Lot. OCF	Lot. 094	Lot. ETR
pitavastatin	61.3	113	39.2	72.7	81.8	35.8
E_2 17βG	13.5	17.0	5.49	13.4	16.1	6.97

→Observed uptake clearance is similar to the estimated clearance mediated by OATP1B1 and OATP1B3.

(ref. Hirano M et al. J Pharmacol Exp Ther 311, 139 (2004))

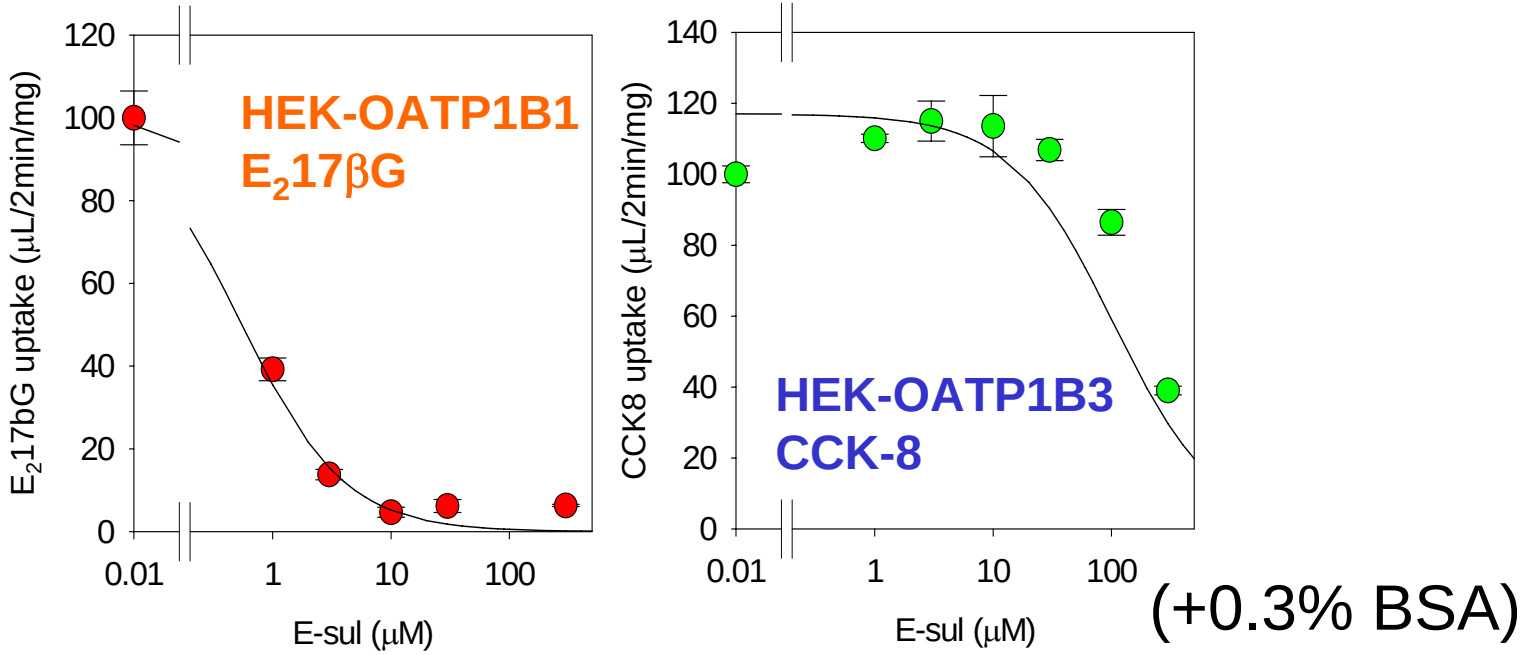
Strategy for determination of their contribution

(3)Using specific inhibitor

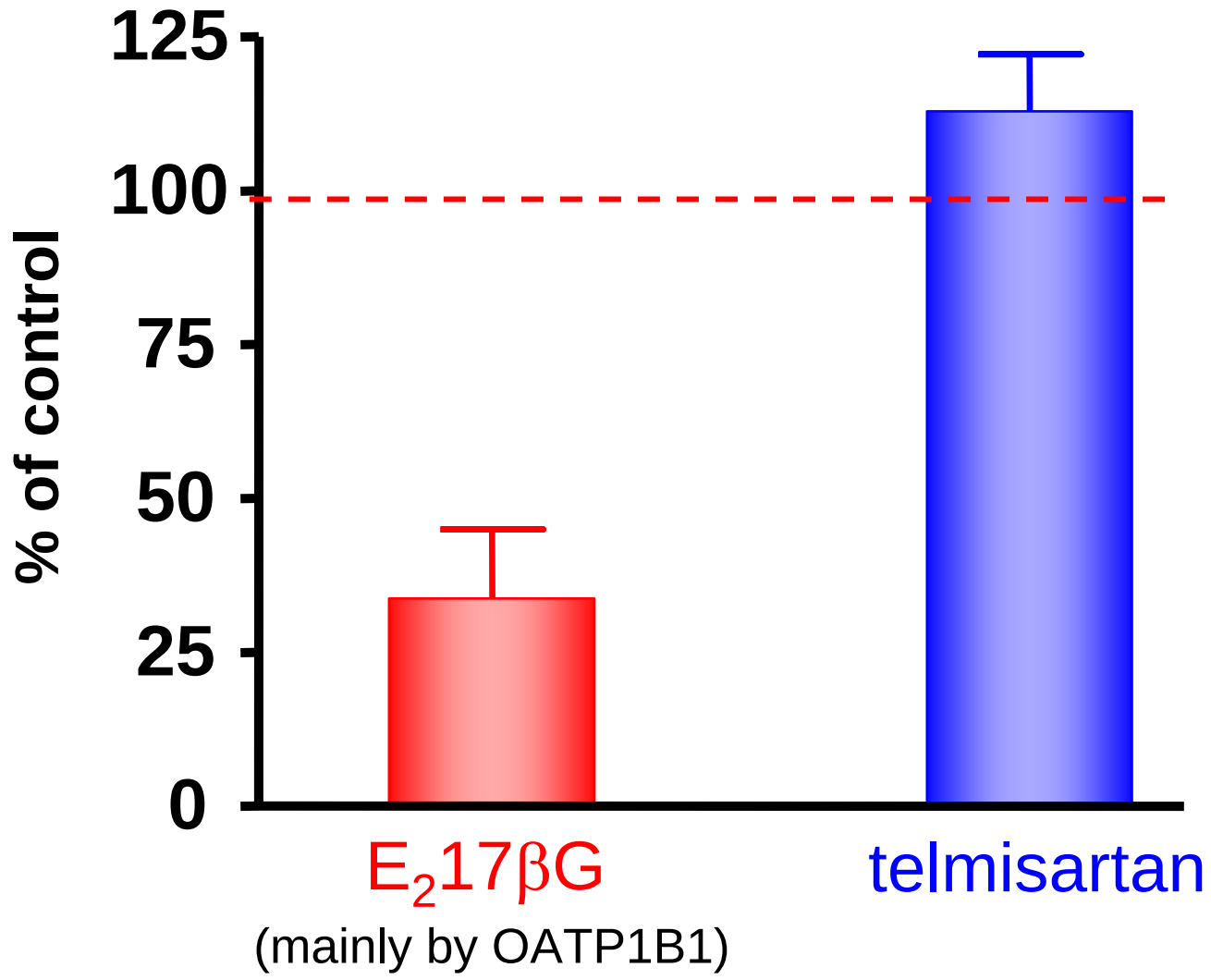


$$\textit{Contribution} = \frac{\textit{Uptake clearance (+inhibitor)}}{\textit{Uptake clearance (-inhibitor)}}$$

inhibitor: estrone-3-sulfate (E-sul) → OATP1B1-selective inhibitor



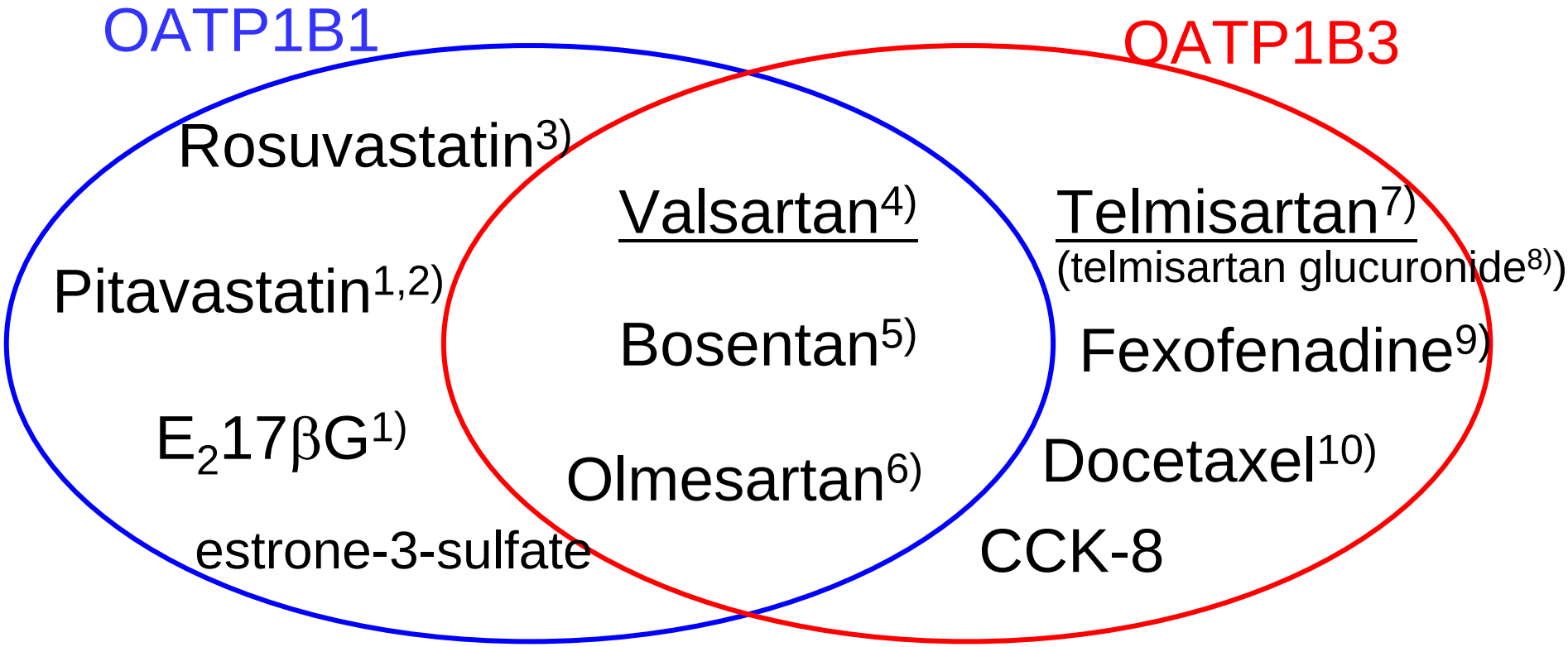
(+0.3% BSA)



Inhibitory effect of 30μM E-sul on the uptake of $E_2$17βG and telmisartan in human hepatocytes (+0.3% BSA)

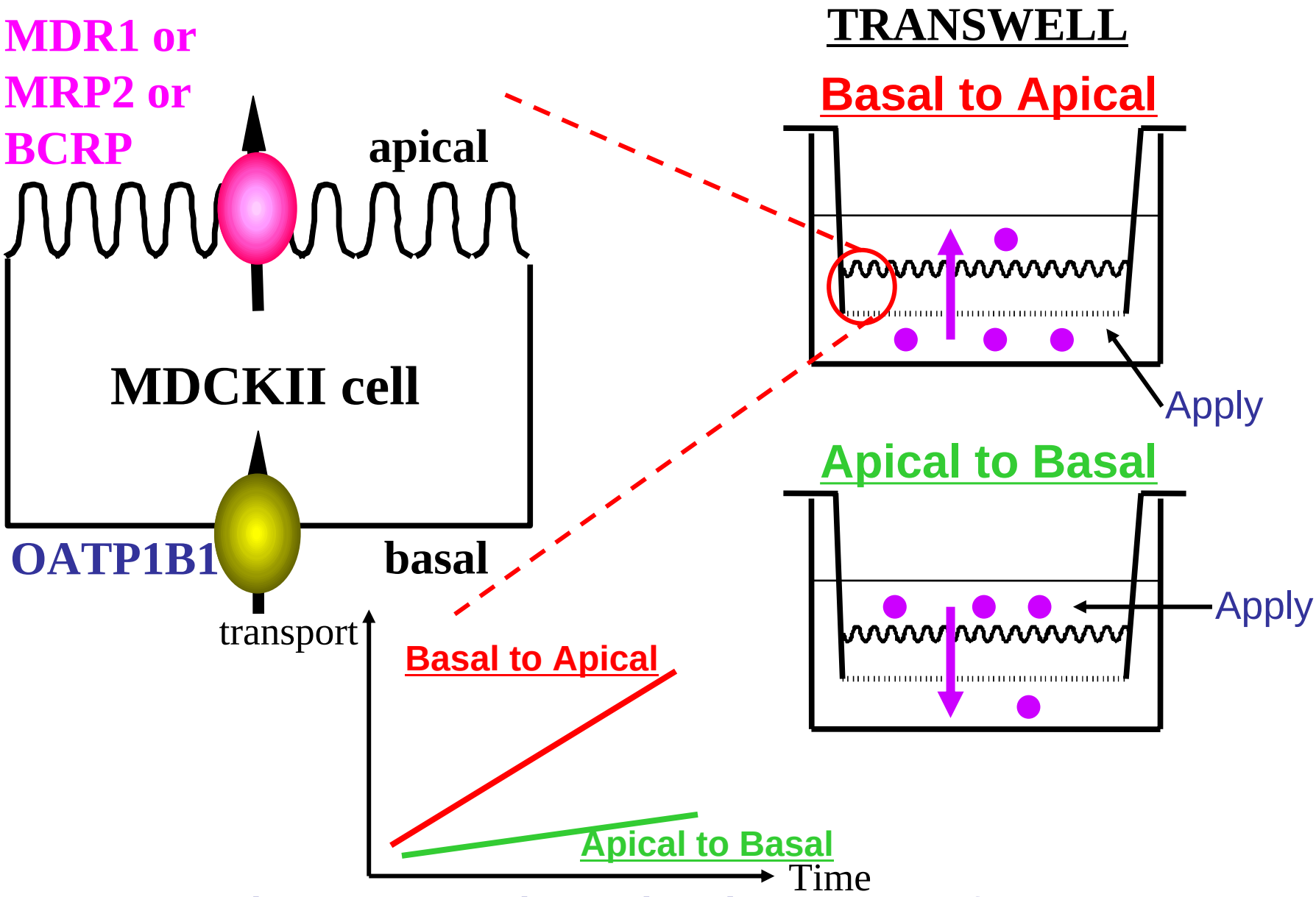
(Ishiguro N et al., DMD, 34, 1109-15 (2006))

Contribution of OATP1B1 and OATP1B3 to the hepatic uptake of drugs



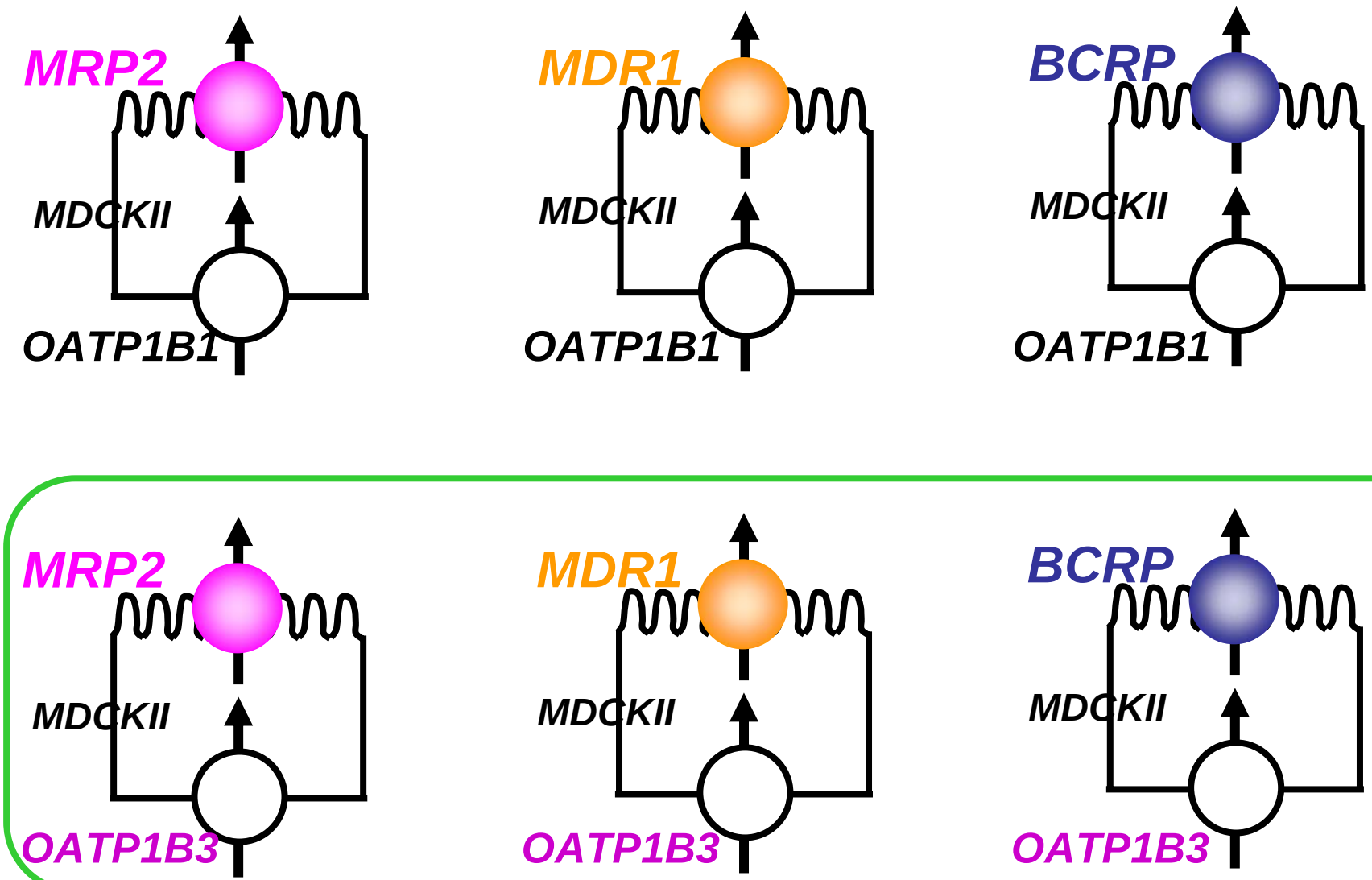
1) Hirano et al., JPET 311,139 (2004), 2) Hirano et al., DMD 34, 1229 (2006), 3) Kitamura et al., DMD, 36, 2014 (2008), 4) Yamashiro et al., DMD 34, 1247 (2006), 5) Kubo et al., unpublished, 6) Yamada et al., DMD, 35, 2166 (2007), 7) Ishiguro et al., DMD 34, 1109 (2006), 8) Ishiguro et al., DMD, 36, 796 (2008), 9) Shimizu et al., DMD, 33, 1477 (2005), 10) Yamada et al., 24th JSSX (2009)

→OATP1B3 as well as OATP1B1 is important for the hepatic uptake of drugs.



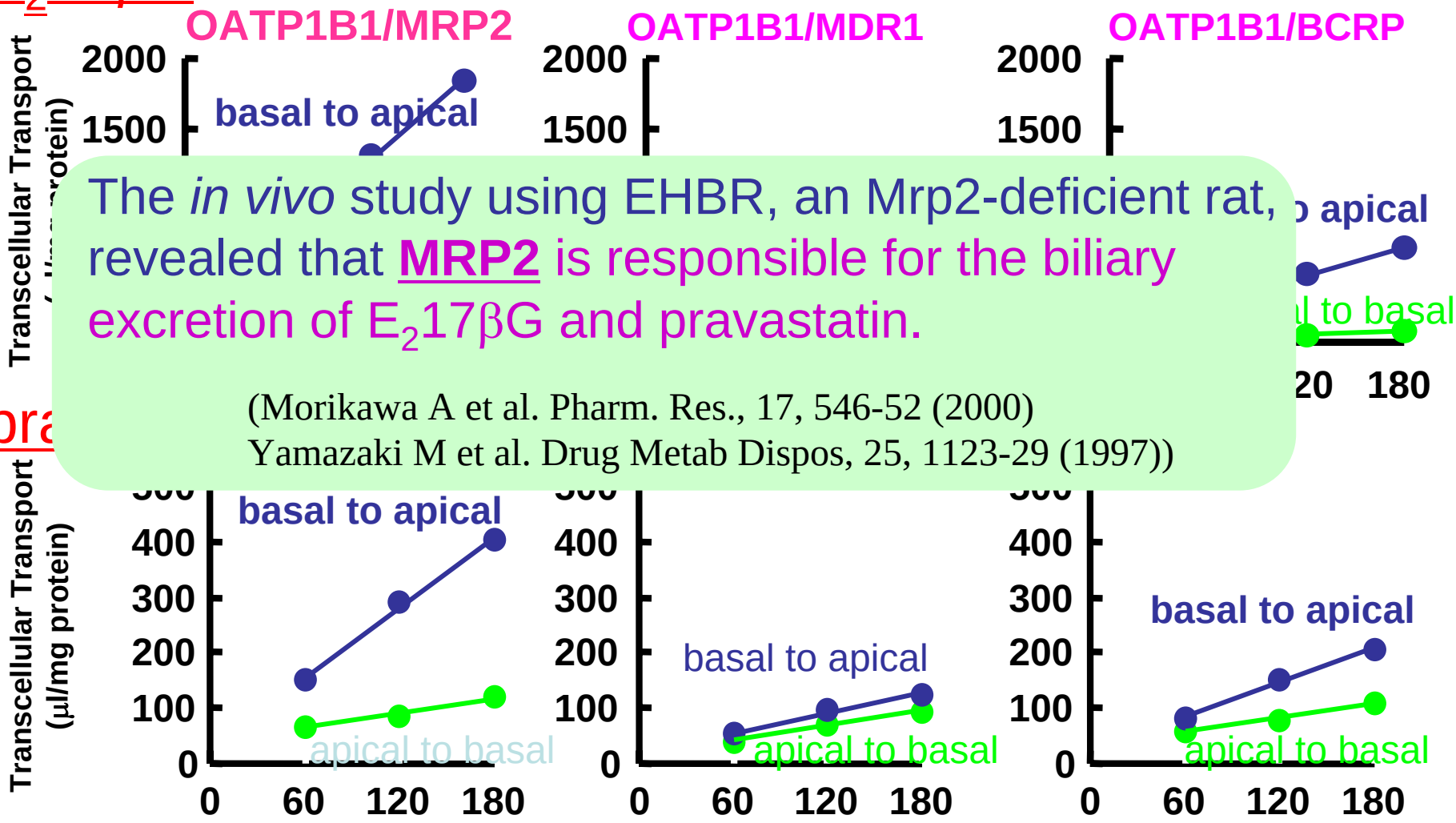
Experiments using double transfectants

(Sasaki M et al. J Biol Chem, 277, 6497-6503 (2001)
Matsushima S et al., J Pharmacol Exp Ther, 314, 1059-1067 (2005))



Construction of the double transfected cells expressing OATP1B3 and efflux transporters

$E_2$17βG



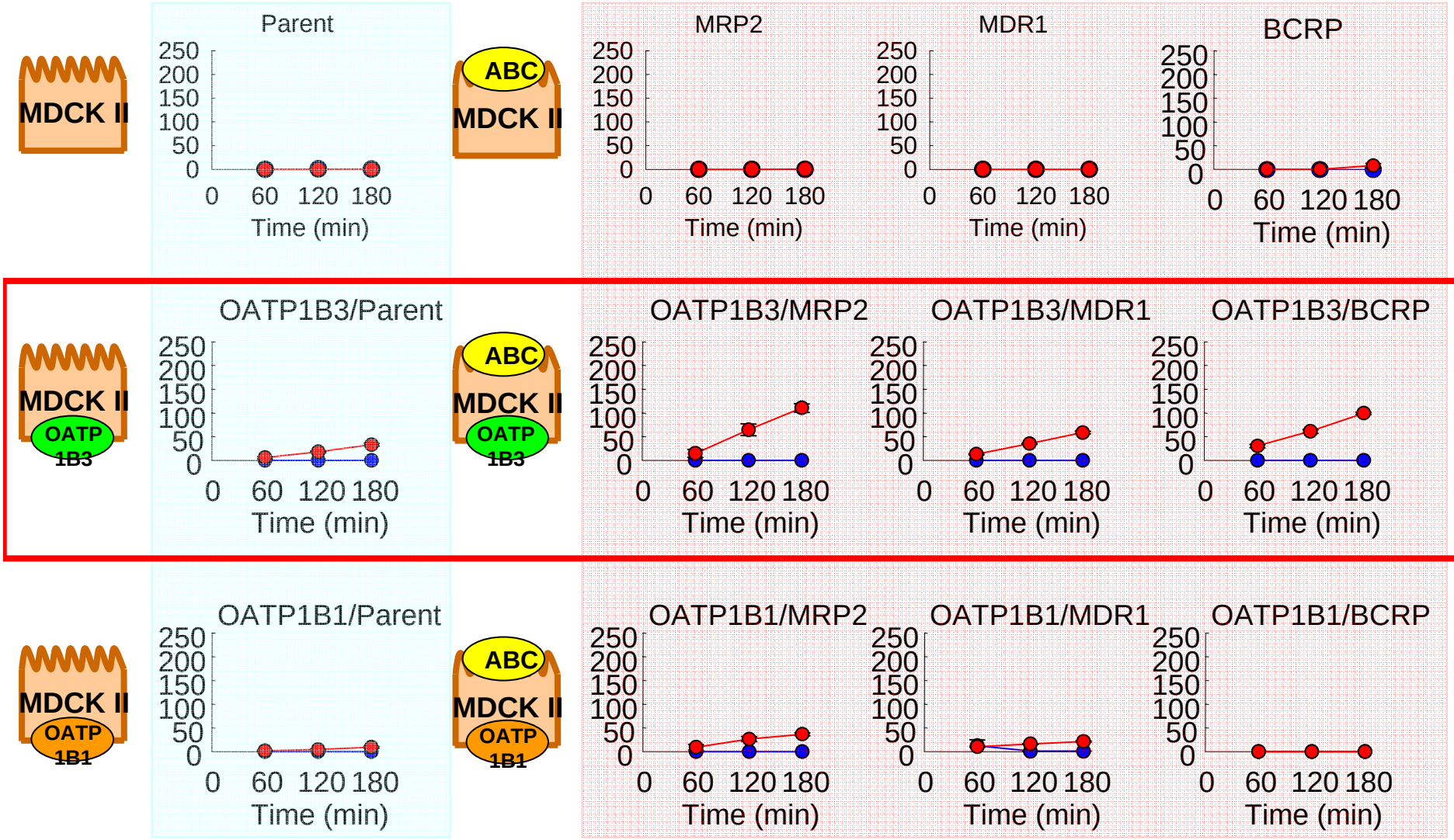
The *in vivo* study using EHBR, an Mrp2-deficient rat, revealed that **MRP2** is responsible for the biliary excretion of $E_2$17βG and pravastatin.

(Morikawa A et al. Pharm. Res., 17, 546-52 (2000)
Yamazaki M et al. Drug Metab Dispos, 25, 1123-29 (1997))

The transcellular transport of $E_2$17βG and pravastatin in OATP1B1/MRP2 double transfectant was the highest among these cells.

Matsushima S et al., J Pharmacol Exp Ther, 314, 1059-1067 (2005))

Transcellular transport of telmisartan glucuronide using double transfected cell lines



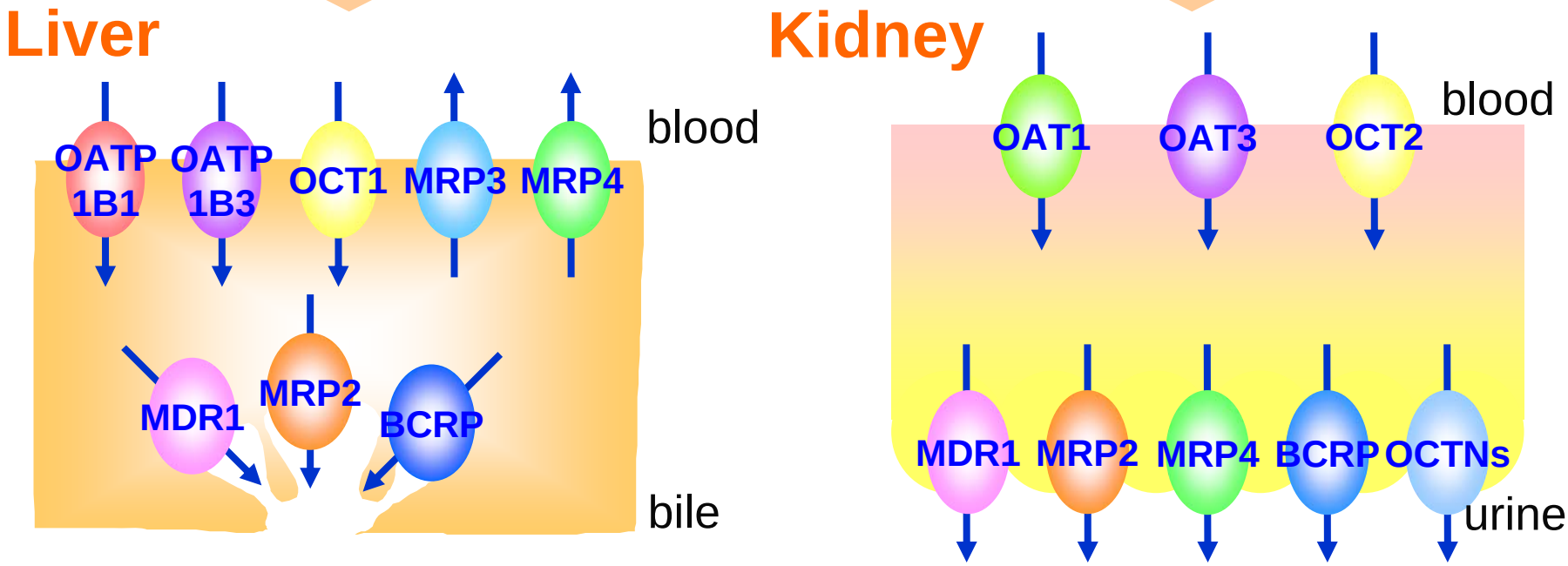
MRP2 **MDR1** **BCRP**

（Uptake of Tel-glu: mainly OATP1B3） (Ishiguro N et al., DMD, 36, 796 (2008)

Prediction of in vivo clearance and fraction excreted into bile from in vitro data using hepatocytes and kidney slices

Watanabe T et al., Drug Metab Dispos, 37, 1471-9 (2009)

Which way?



☆Multispecific substrate recognition of transporters

→a compound is often transported by multiple transporters !!

Prediction of in vivo clearance and fraction excreted into bile from in vitro data using hepatocytes and kidney slices

Watanabe T et al., Drug Metab Dispos, 37, 1471-9 (2009)

Intrinsic CL

Intrinsic transport and metabolic activity in each transporter and enzyme

Organ CL

Overall clearance in each elimination organ

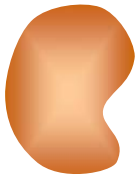
Total CL

Clearance from whole body

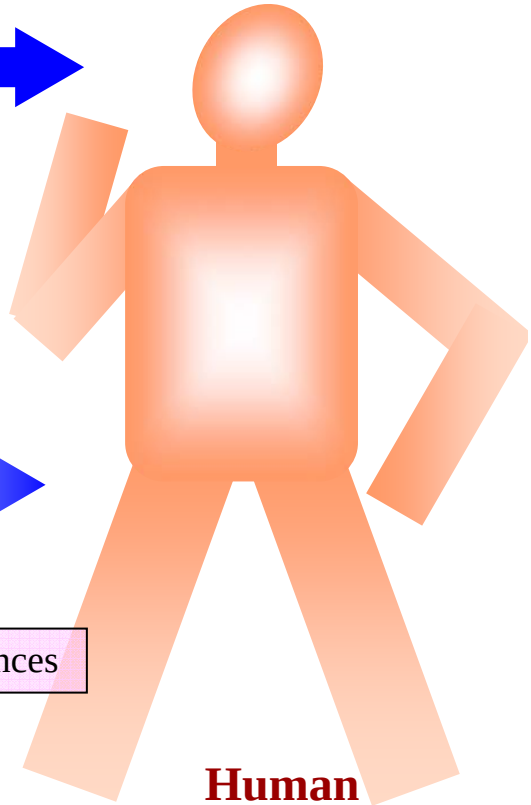
Cells

Uptake assay

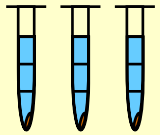
Liver



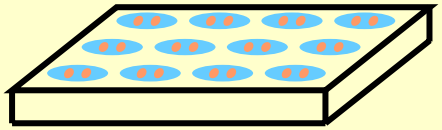
Kidney



Human



Uptake clearance in isolated hepatocytes ($\mu L/min/10^6$ cells)



Uptake clearance in kidney slices (mL/min/g kidney)

If CL_{int} is solely determined by uptake process…

CL_{int} ~ uptake intrinsic clearance

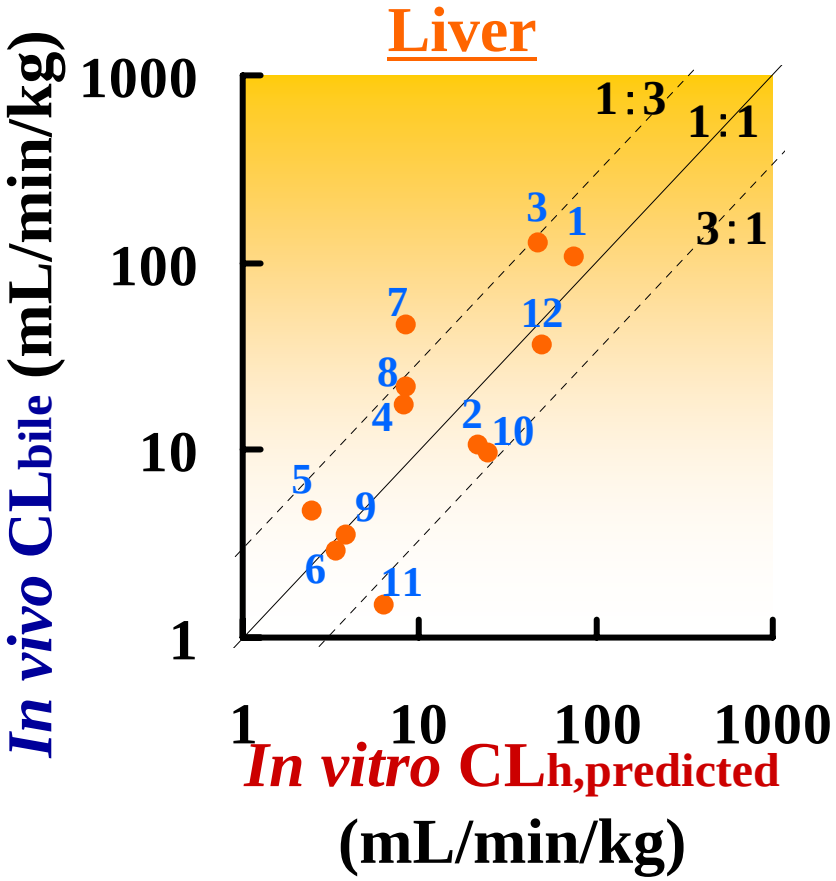
Hepatic clearance

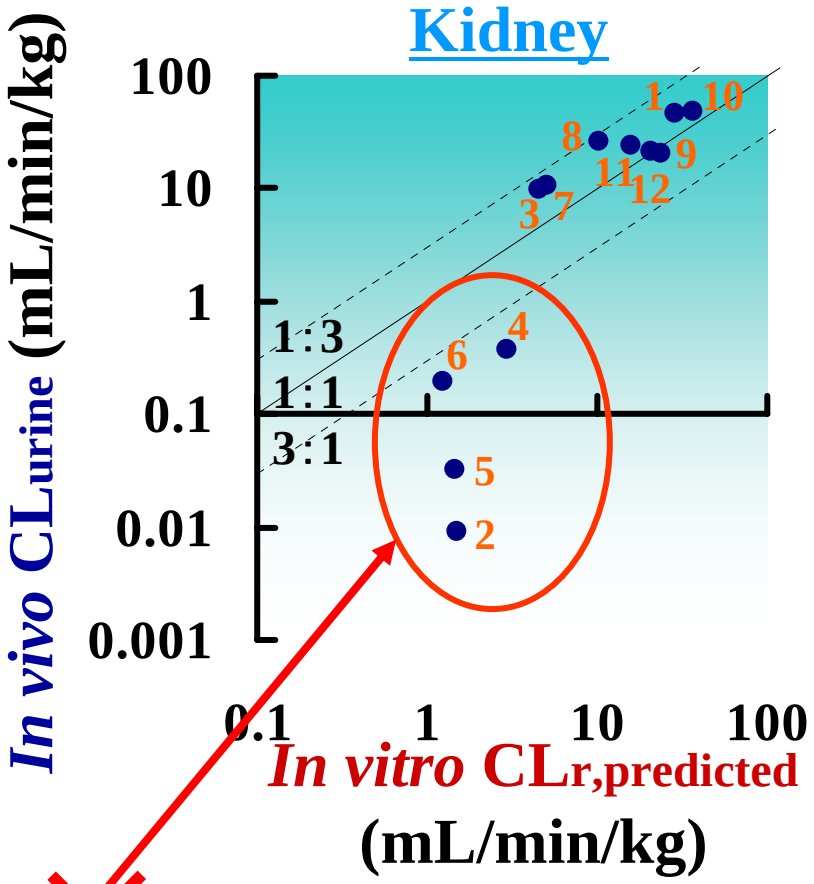
Renal clearance

Estimation based on the appropriate model including intrinsic CL, blood flow and unbound fraction

Sum of organ clearances

Can *in vivo* hepatic and renal clearance be predicted from *in vitro* uptake assay?





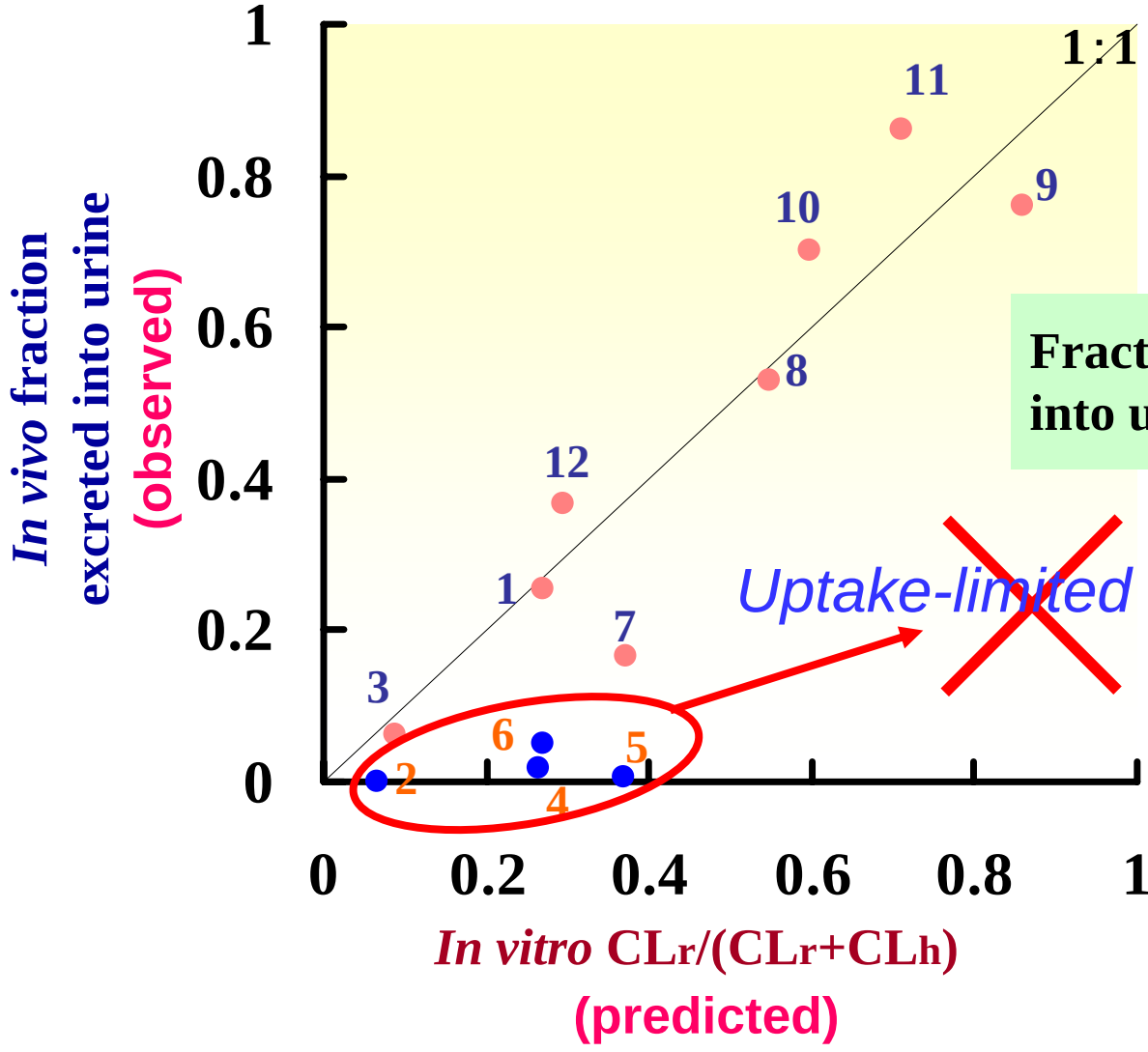
Uptake-limited clearance

→ Backflux from kidney to blood ?

→ Reabsorption from urine ?

☆ *In vivo* hepatic and renal clearance of drugs (except drugs whose renal clearance is small) can be predicted from uptake clearance obtained from *in vitro* assay.

Prediction of the elimination routes of drugs from *in vitro* uptake assay

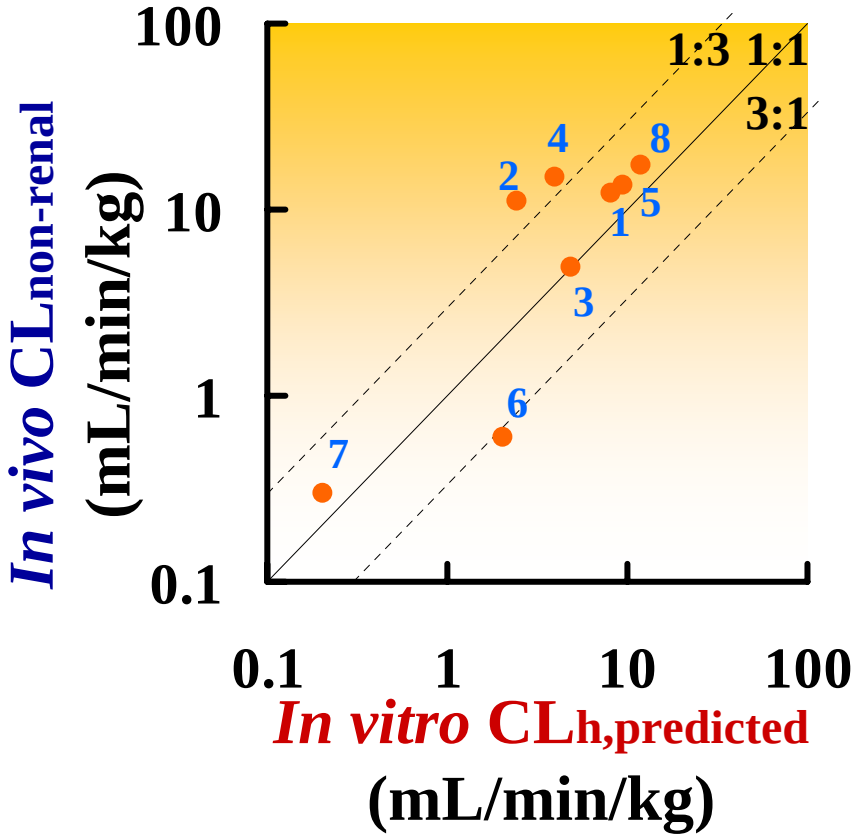


$$\text{Fraction excreted into urine } (f_{urine}) = \frac{CLr}{CLr+CLh}$$

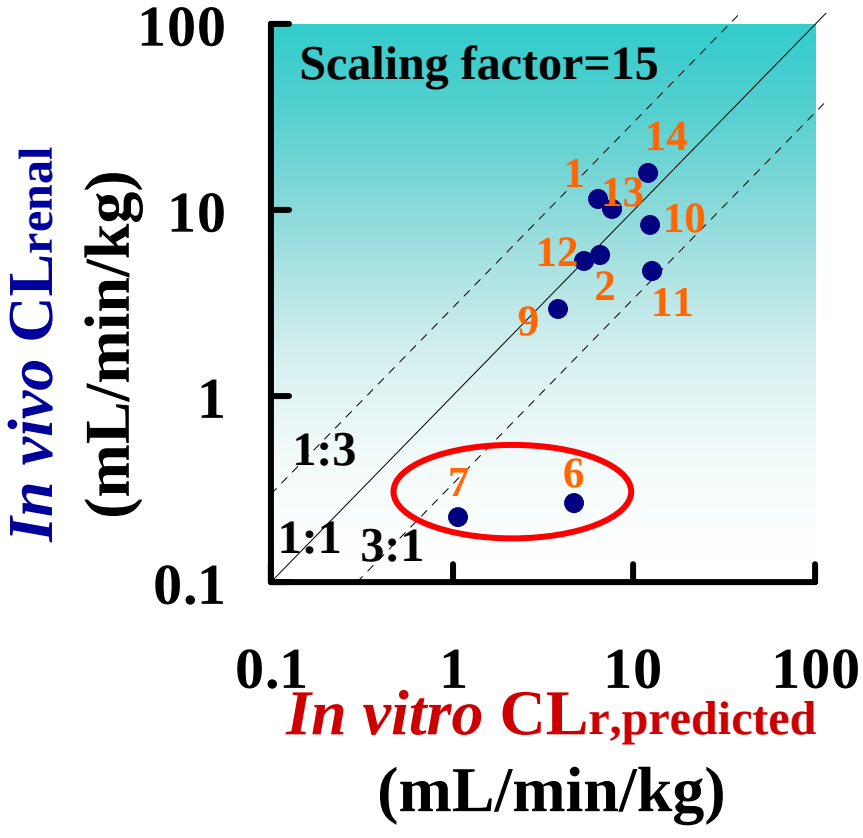
<u>The fraction excreted into bile and urine</u> at least among eight non-metabolized anions could be predicted.

Prediction of the organ clearance in humans from in vitro experiments

Liver



Kidney



1: pravastatin 2: rosuvastatin 3:cerivastatin 4: fluvastatin 5: atorvastatin 6:valsartan 7: olmesartan
8: telmisartan 9: MTX 10: adefovir 11:tenofovir 12: Trichlormethiazide 13: PCG 14: PAH

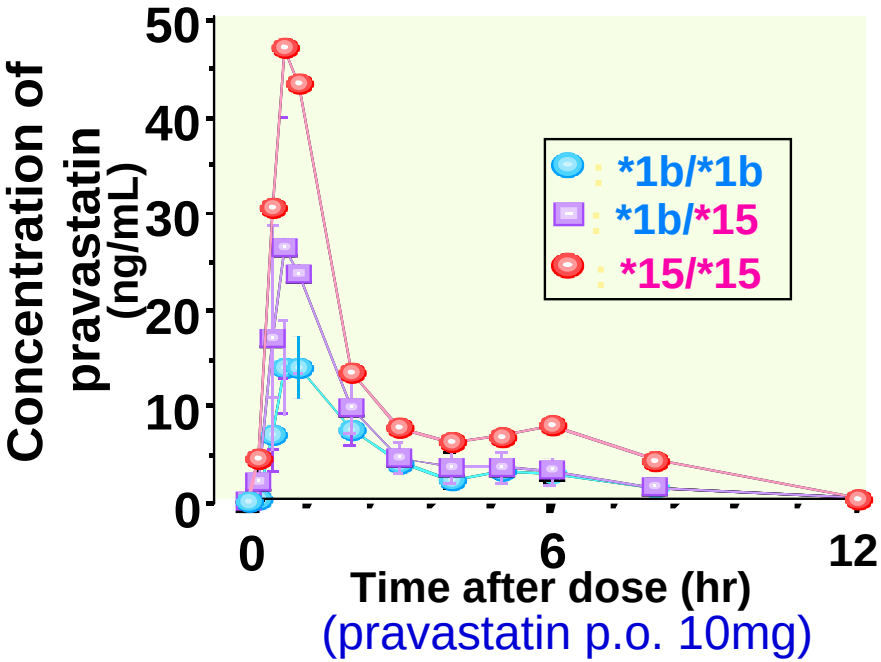
☆ *In vivo* hepatic and renal clearance of drugs (except drugs whose renal clearance is small) can be predicted also in humans.

Influence of OATP1B1 SNP(s) on the pharmacokinetics of pravastatin in Japanese Healthy Subjects

Nishizato Y *et.al.* Clin.Pharmacol.Ther.2003 ; 73(6) : 554-564

Haplotype of OATP1B1 SNPs

	130	174	Allele frequency (Japanese)
OATP1B1 *1a	N	V	(32.5%)
OATP1B1 *1b	D	V	(45.8%)
OATP1B1 *5	N	A	(0.0%)
OATP1B1 *15	D	A	(15.0%)



(pravastatin p.o. 10mg)

Pharmacokinetic Parameters of Pravastatin

genotype	N	CLnon-renal (L/kg/hr)	AUC (ng*hr/ml)
*1a/*1a	2	2.22	60.5
*1a/*1b	4	1.45 ± 0.72	47.2 ± 27.4
*1b/*1b	4	2.01 ± 0.42	44.2 ± 6.38
*1b/*15	9	1.11 ± 0.34	62.1 ± 21.8
*15/*15	1	0.28	111.8

Purpose & Protocol

- **Investigation of the effect of OATP1B1*1b on the pharmacokinetics of pravastatin, valsartan and temocapril (temocaprilat) in clinical study**

- **Clarification of the common factors which result in the inter-individual difference in the pharmacokinetics of three drugs**

Subjects

23 healthy volunteers

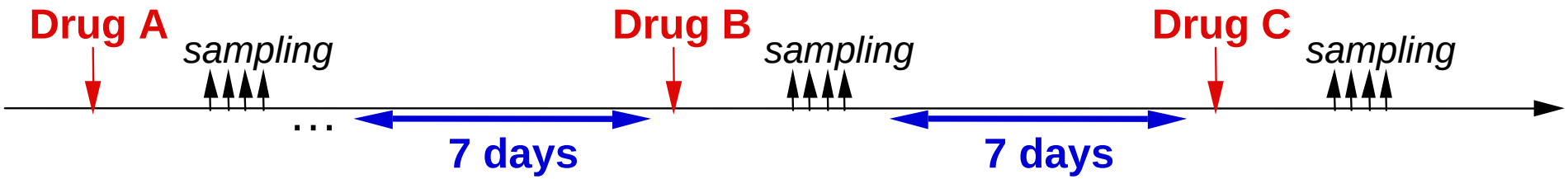
OATP1B1 diplotype

*1a/*1a	N=5
*1a/*15	6
*1b/*1b	7
*1b/*15	5

Trial

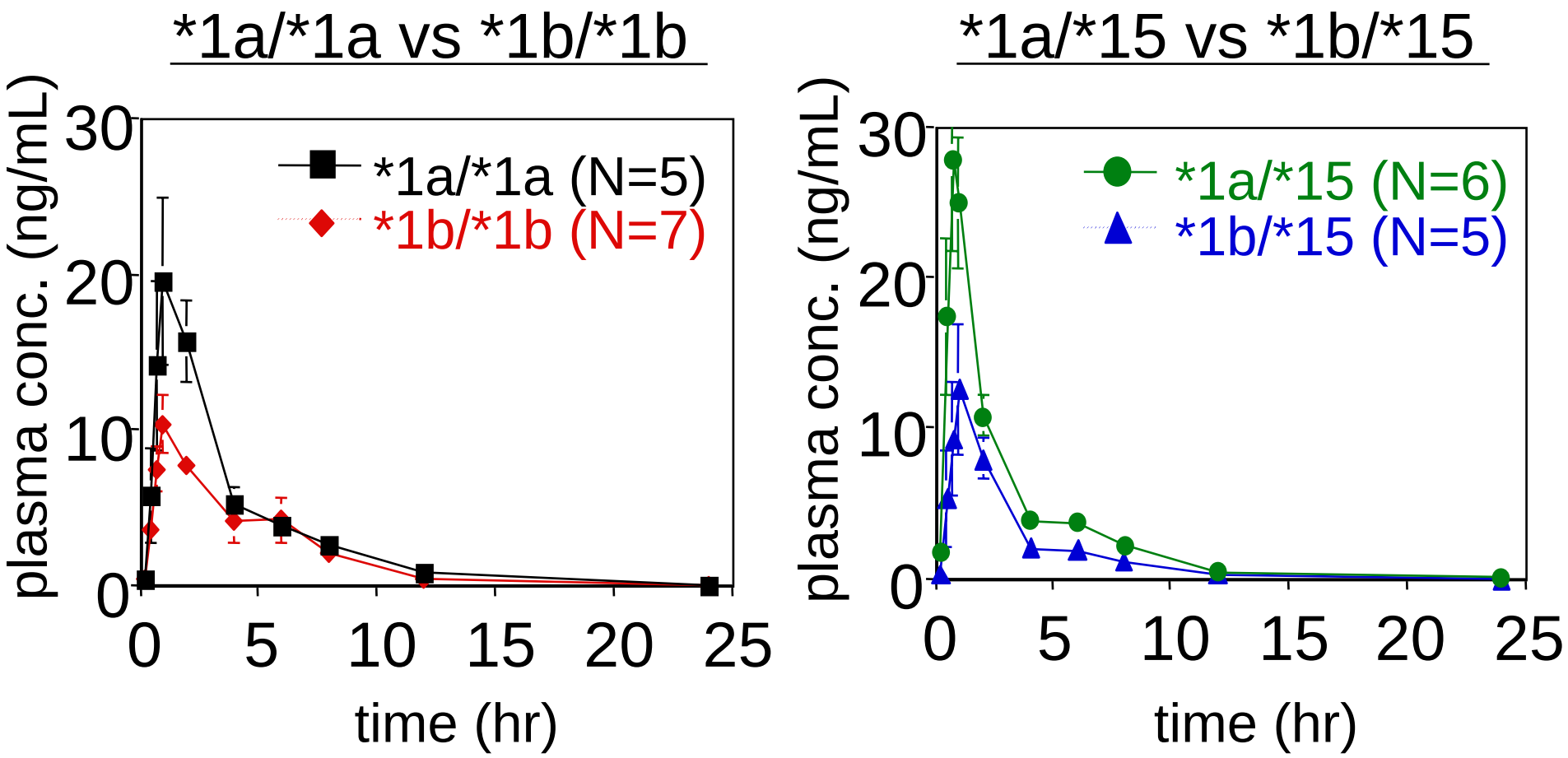
3-group crossover trial

pravastatin 10mg p.o.
temocapril 2mg p.o.
valsartan 40mg p.o.
→Blood (~24 hr), Urine (~24hr)



Maeda K et al. Clin Pharmacol Ther, 79, 427-36 (2006)

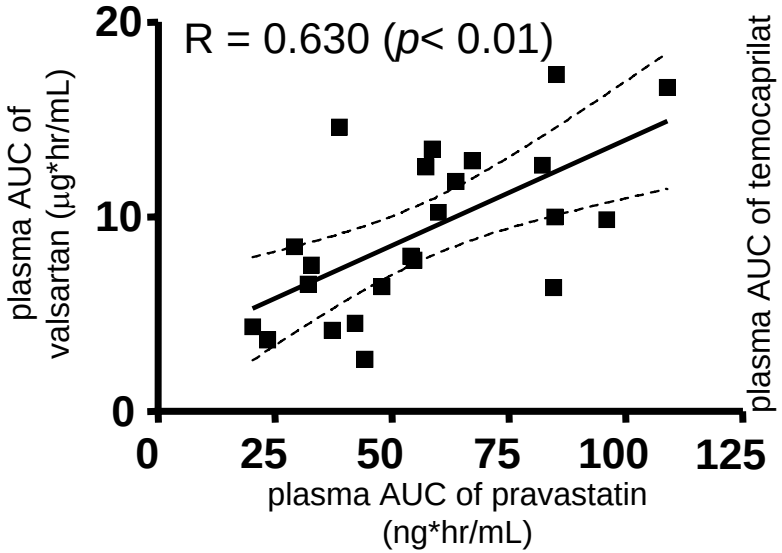
Effect of SNPs in OATP1B1 on the pharmacokinetics of pravastatin



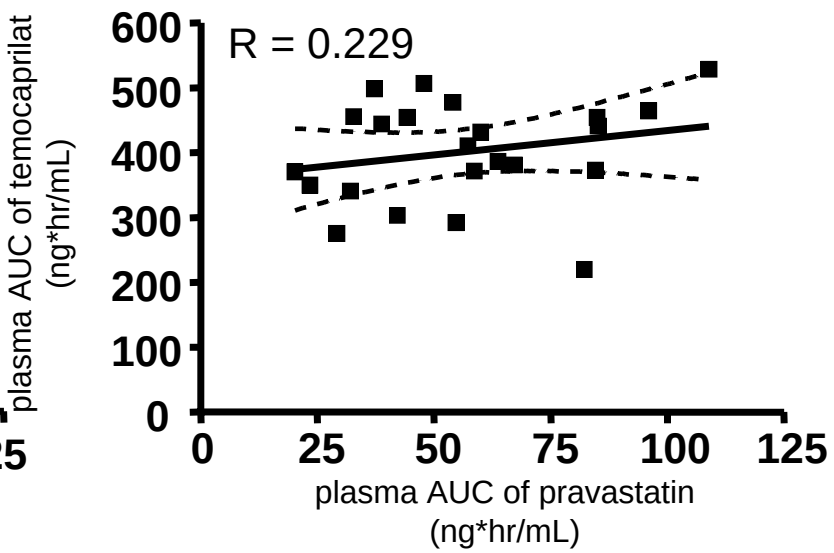
The subjects with OATP1B1*1b allele showed lower plasma concentration of pravastatin compared with those with OATP1B1*1a.

Correlation between the plasma AUC of two drugs in each subject

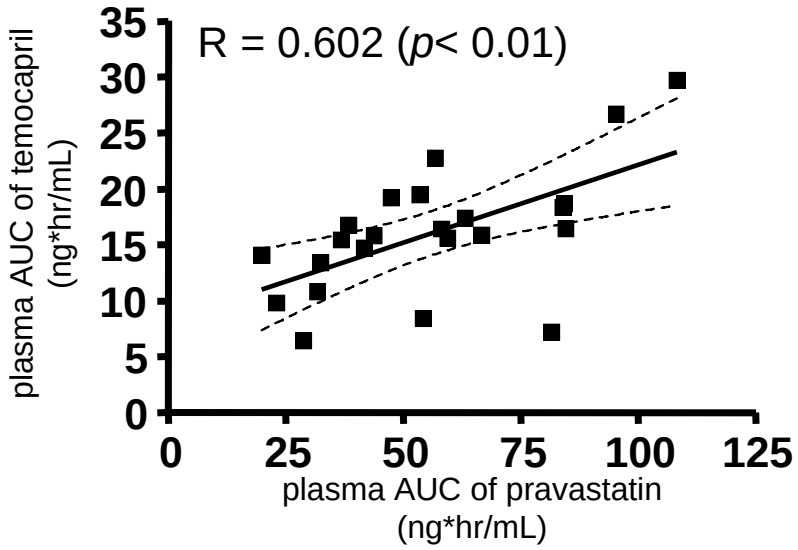
pravastatin vs valsartan



pravastatin vs temocaprilat

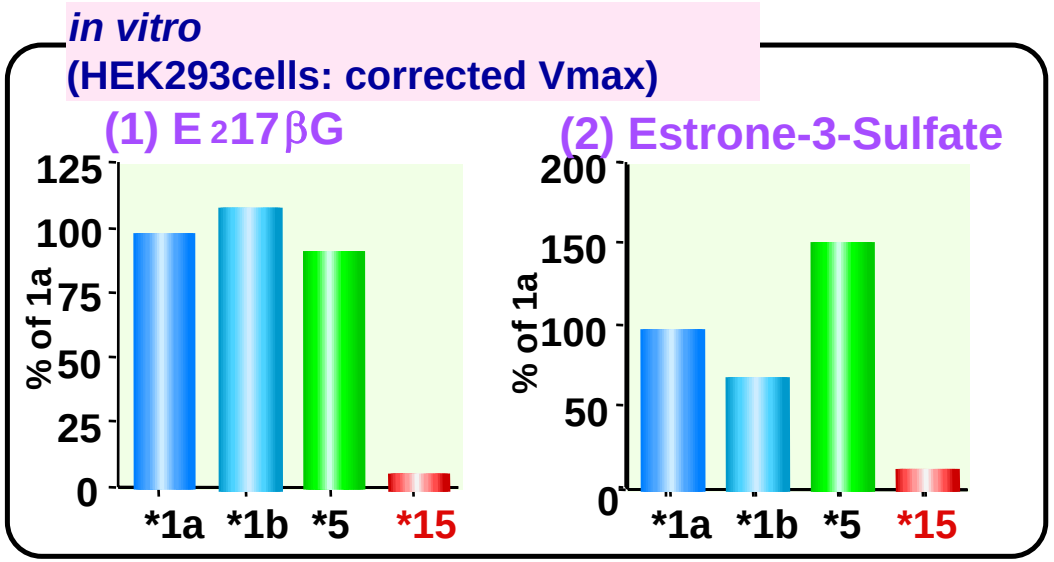


pravastatin vs temocapril



Summary

OATP1B1*1b may cause **the increase in the hepatic clearance** of pravastatin and possibly valsartan.



(Iwai M et al., Pharmacogenetics 14, 749 (2004))

WHY ?

1) Higher expression level of OATP1B1*1b in hepatocytes compared with *1a
2) Substrate dependence of the effect of *1b on the transport activity

Acknowledments

“I really want to express my great appreciation to …”

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Prof. Sugiyama teaches to me not only the science

But also how to entertain the audience…

(2000/ISRT Banquet)

(2003/ISDDC Banquet)

(1999/Lab X'mas)

Acknowledments

“I really want to express my great appreciation to …”

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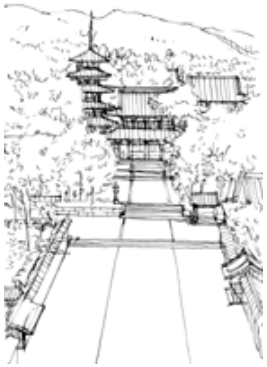
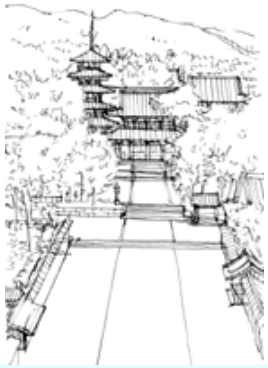
第一三共、日本ベーリンガーインゲルハイム、大日本住友製薬、ノバルティスファーマ、積水メディカル、興和、ファイザー、アストラゼネカ、サノフィアベンティス、シーエーシー……

Where I will need to go …

Current status



Future



We can see any biological phenomena quantitatively (time-dependent, concentration-dependent …).

→ Acceleration of drug discovery and development, Right prescription to the right patients.