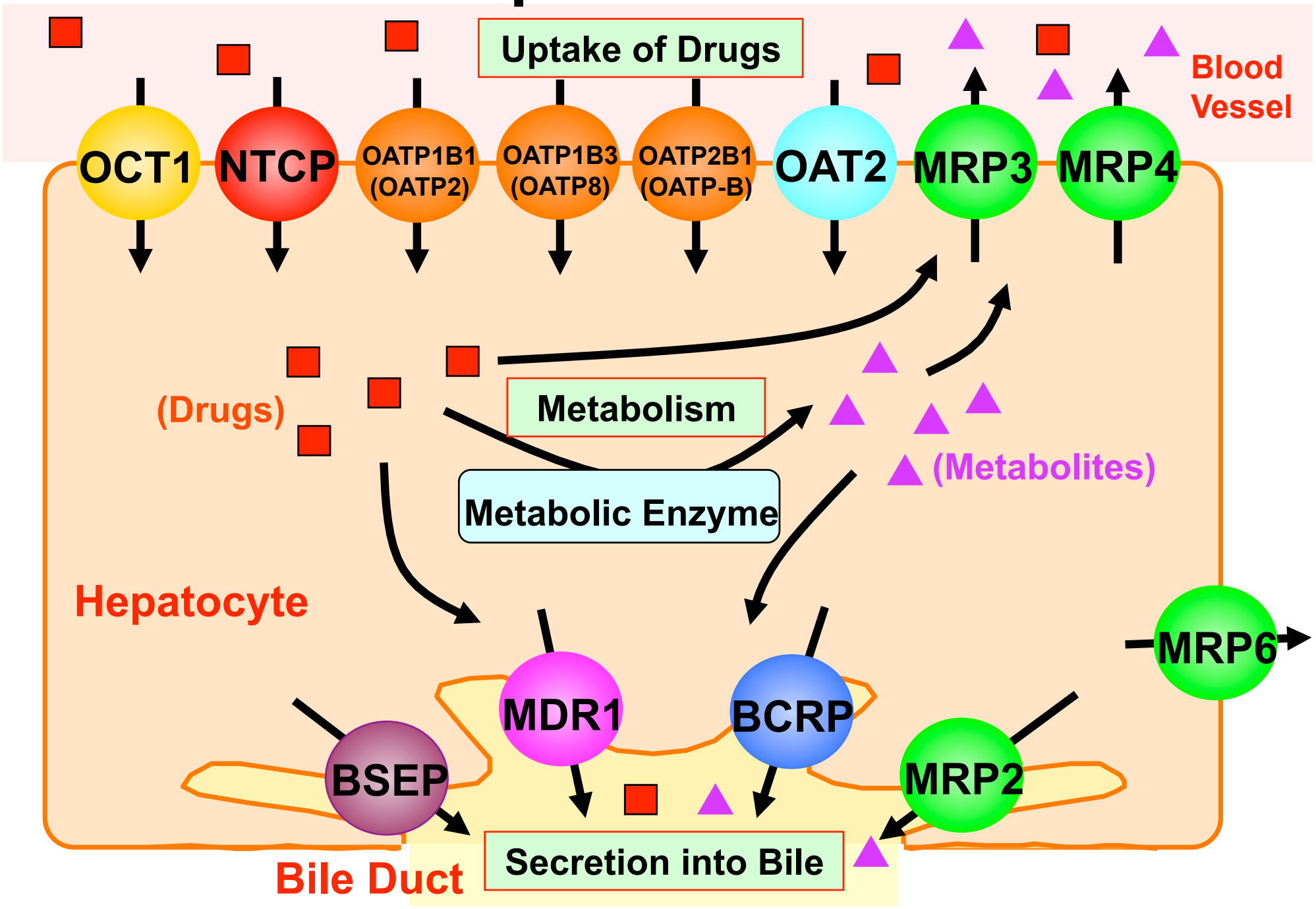
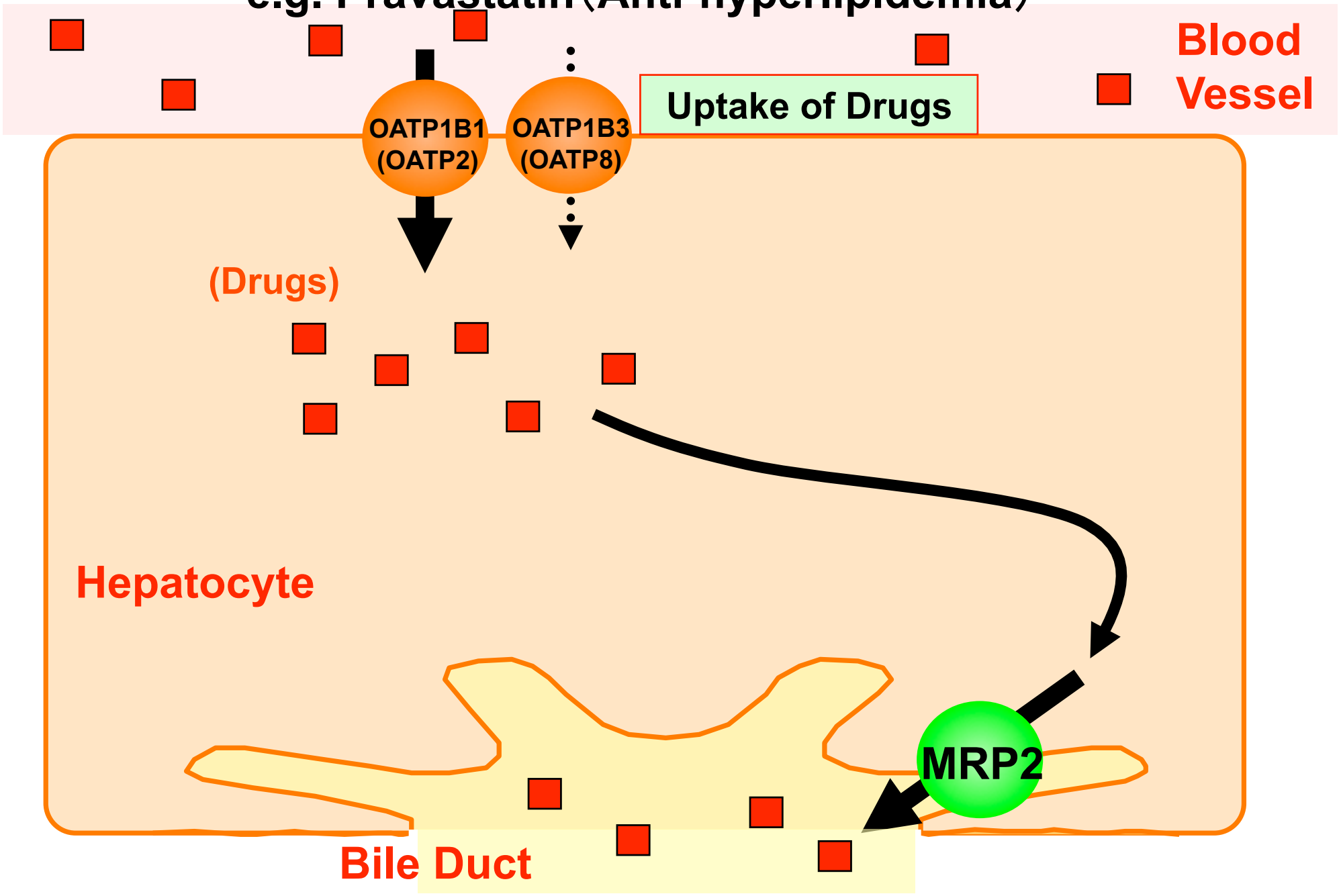
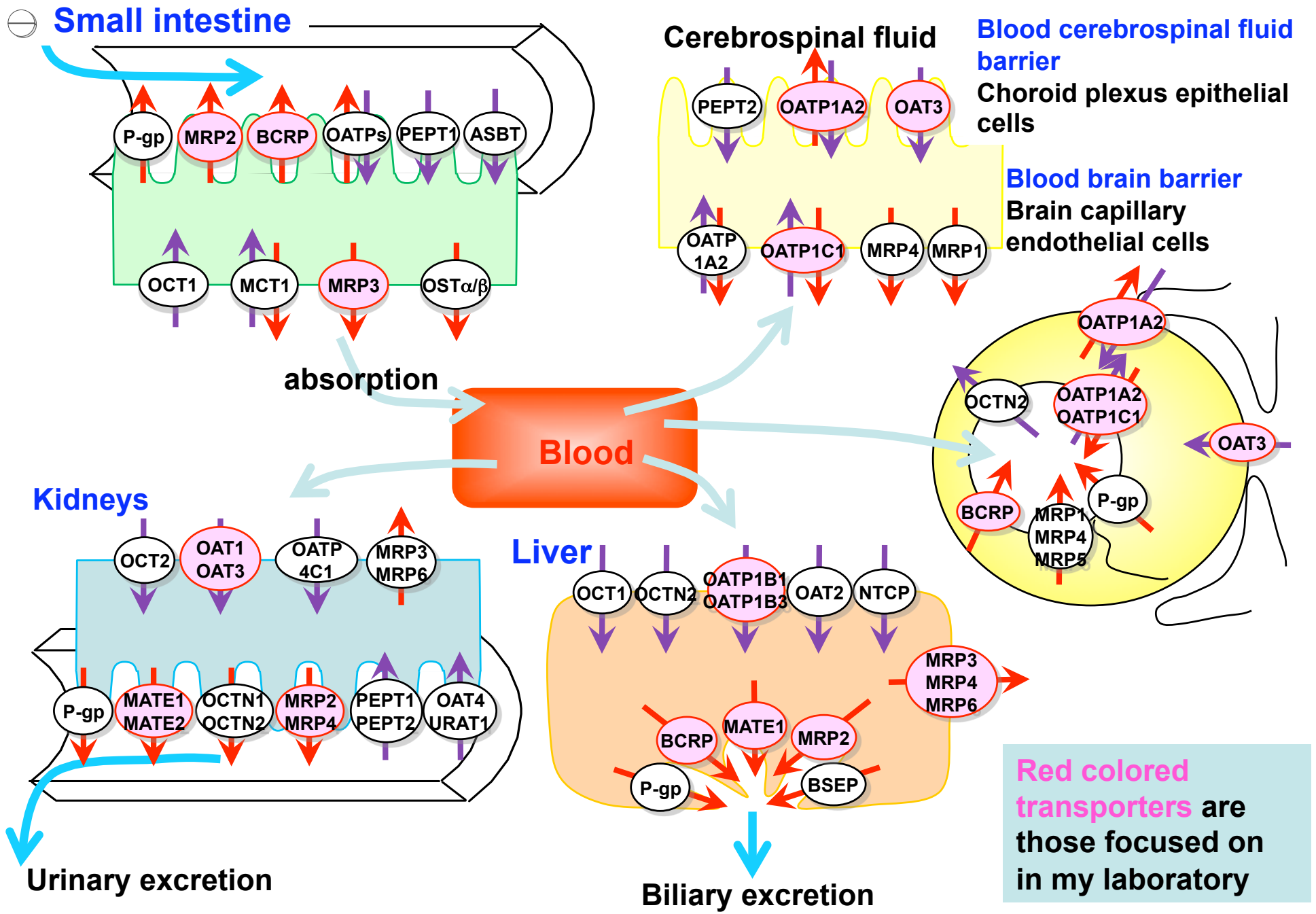
Transporters in Liver



Drug Metabolism in Liver

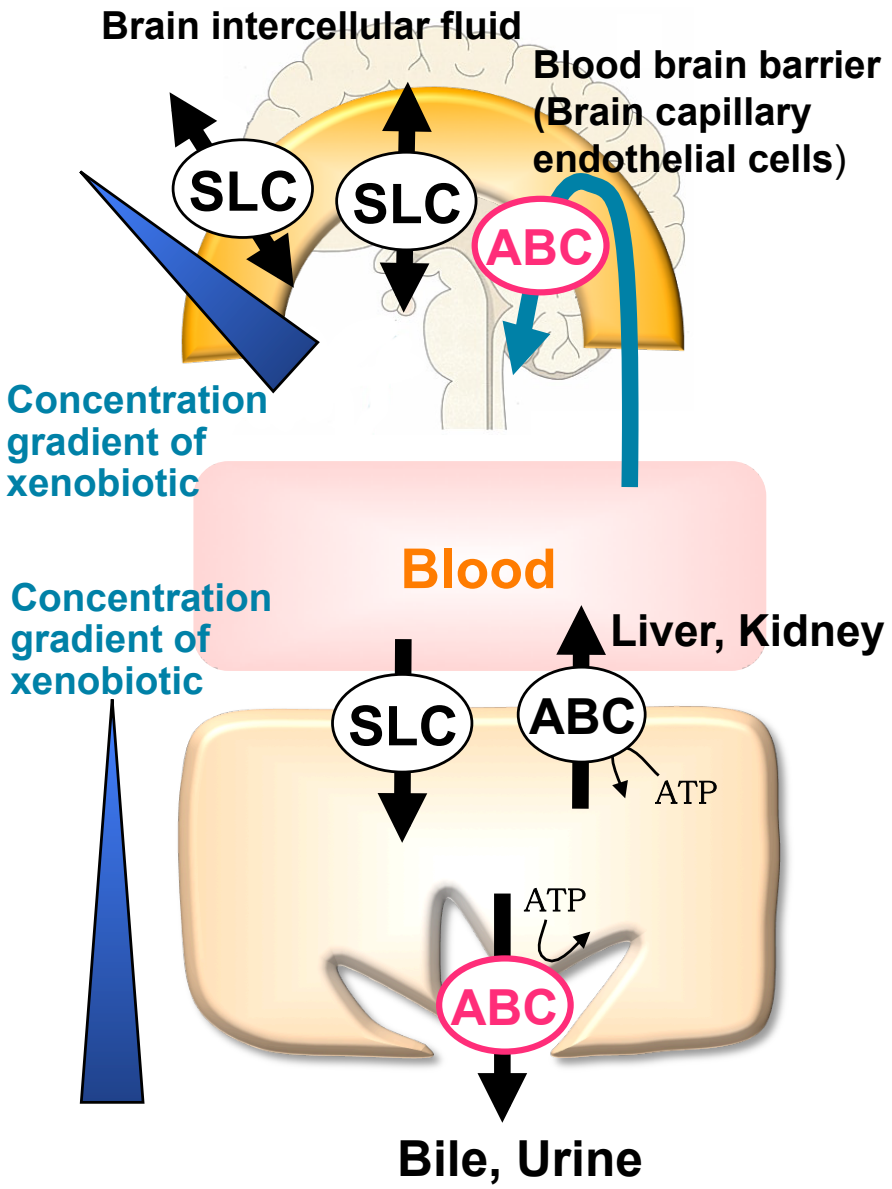
e.g. Pravastatin（Anti-hyperlipidemia）



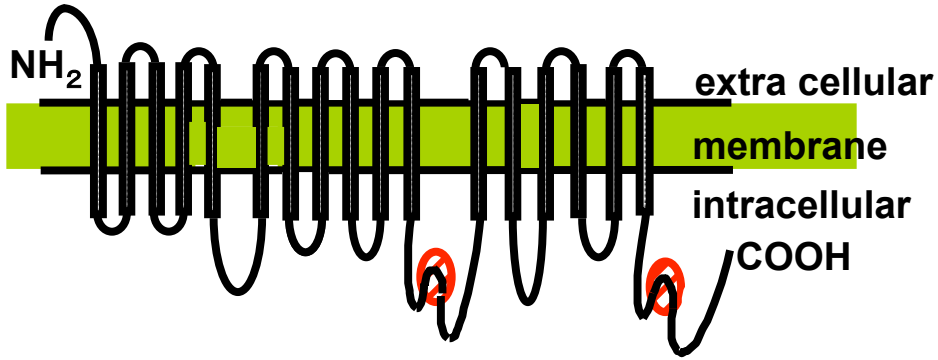


Main Transporters Expressed in Liver, Kidneys, Small Intestine and Brain

Role of Transporters in Xenobiotic Detoxification



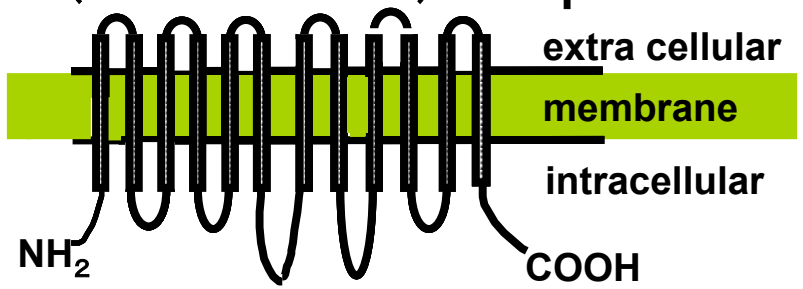
ABC（ATP binding cassette ）transporter



ATP Binding Cassette (ABC)

e.g. MRP2 MDR1 BCRP BSEP MRP3 MRP6

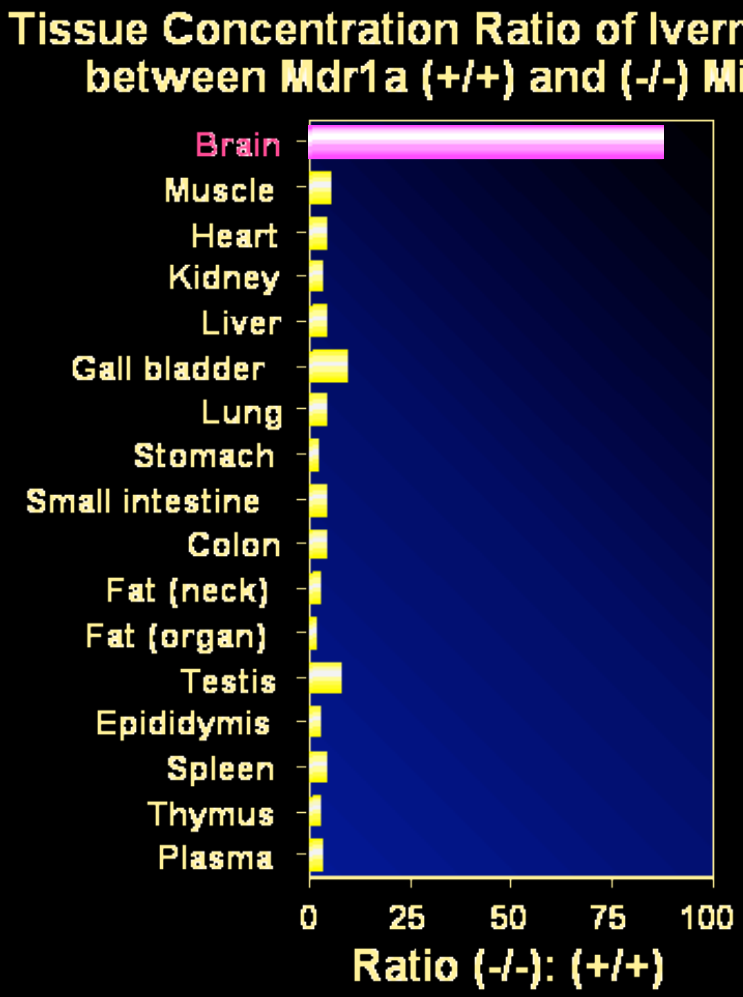
SLC（solute carrier ）transporter



e.g. OATP NTCP OAT OCT

Giacomini KM and Sugiyama Y, Membrane transporters and drug response, Chapter 2 in "Goodman & Gilman's The Pharmacological Basis of Therapeutics 11th edition", 2005

Increased Sensitivity to the Neurotoxic Pesticide Ivermectin in Mdr1a (-/-) Mice



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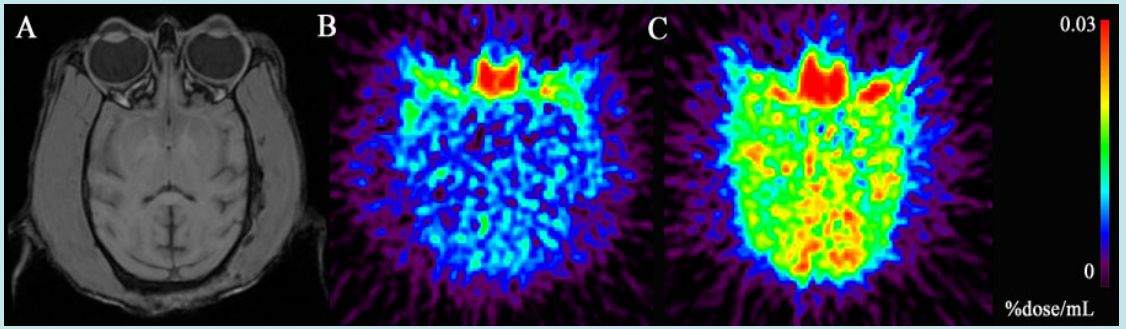
Results with a PET study in monkeys

Control state P-gp inhibition condition



Inhibition of P-gp significantly increased the brain concentration of drug.

‡ Lee YJ, et al J Pharmacology Exp Ther 316:647-53 (2006)

In duodenum, P-gp expression levels altered depending on SNPs. Similar correlation between P-gp expression and SNPs in brain could be expected to lead to altering intracerebral drug concentration.

A clinical study in healthy volunteers
[^{11}C]verapamil - Non-invasive measurement of intracerebral drug concentration with PET scans.
(in collaboration with Dr. T. Suhara, National Institute of Radiological Science)

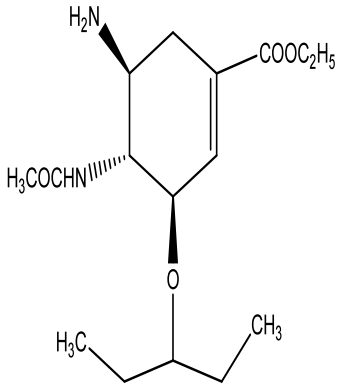
Takano A, et al J Nucl Med. 47:1427–33, 2006.

Individual Difference in P-gp Gene Polymorphism, Xenobiotic Excretion in Brain Capillary, and Drug Penetration into Brain

Background 1. Physical Properties of Oseltamivir and its Active Metabolite

Oseltamivir (TAMIFLU):Anti-influenza drug

Parent form



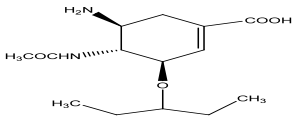
M.W. : 312.40
Log D (at pH7) : -0.30
pKa : 14.68 (Acidic)
8.81 (Basic)

hydrolysis

Carboxylesterase

(CES)

Ro 64-0802 (pharmacologically-active form)



M.W. : 284.35
Log D (at pH7) : -2.05
pKa : 4.13 (Acidic)
9.26 (Basic)

- Oseltamivir is the prodrug form of Ro64-0802, a selective inhibiter of influenza virus neuraminidase.
- In Japan, prescriptions for oseltamivir in 2006 exceeded 10 million.
- Abnormal behavior in young patients with influenza being treated by oseltamivir have been reported.

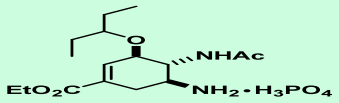
Background 2. Abnormal Behavior after Oseltamivir Treatment

- Since being launched in 2001, abnormal behaviors including jumping and falling from balconies have occurred in 211 cases of oseltamivir treatment in Japan (as of June 2007).
- Incidence of the abnormal behavior by age was 33.6% in less than 10 years, 44.5% in 10-19, reaching 78.1% in total. The adverse effects of abnormal behavior was reported mostly by the patients aged 19 and younger (from YAKUJI NIPPO).
- Although causal relationship between oseltamivir exposure and abnormal behavior remains unclear, in March 2007 Japan's Ministry of Health and Welfare directed that the drug be not prescribed to teenagers.

- Precise scientific epidemiologic analysis of clinical data should be required before drawing conclusion on the causal relationship.
- Given that a cause of abnormal behavior is the oseltamivir exposure', 'what mechanism could be plausible' is discussed in this study by examining the following pharmacokinetic mechanism prior to the results of epidemiological analysis.

1. Pharmacokinetic regulatory mechanism of oseltamivir and its acid form in peripheral tissues
2. Brain penetration of both compounds and relevant molecular mechanism

Oseltamivira (Tamiflu)



GI tract (absorption)

CNS Adverse Effects ?

Central nervous System (CNS)

ester form → acid form

esterase

OAT3

P-gp

MRP4

Brain endothelial cells (BBB)

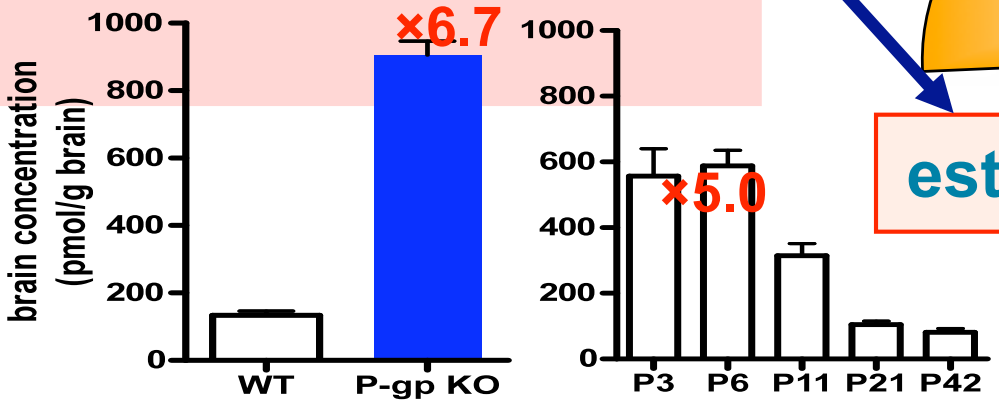
Blood

ester form → acid form

esterase

Liver

P-gp KO largely increases the brain concentration of tamiflul.



Decreased brain P-gp concentration in young rats results in increase of brain Tamiflu concentration.

Brain Distribution Mechanism in Anti-influenza Oseltamivir (Tamiflu)

Probability of Adverse Effects
(1 in 10,000)

- F(Tox) = Fmdr1 x Fother trans x Fces
- 0.001 0.1 0.1 0.1
- 0.00001 0.05 0.01 0.02
- 0.000001 0.01 0.01 0.01

Virtual Clinical Trials

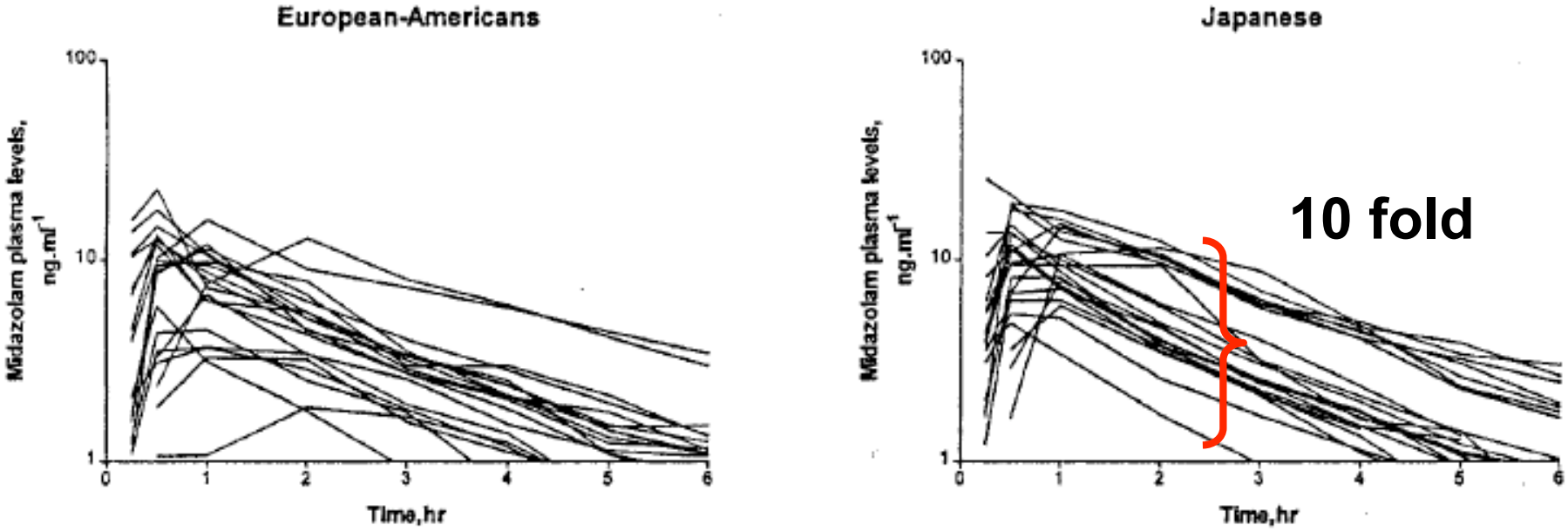
Collection of individual variability data on physiological and biochemical parameters

A variety of virtual persons on computer

Real person

Virtual person

Time Course of Plasma Concentration of Midazolam after 2 mg Oral Administration



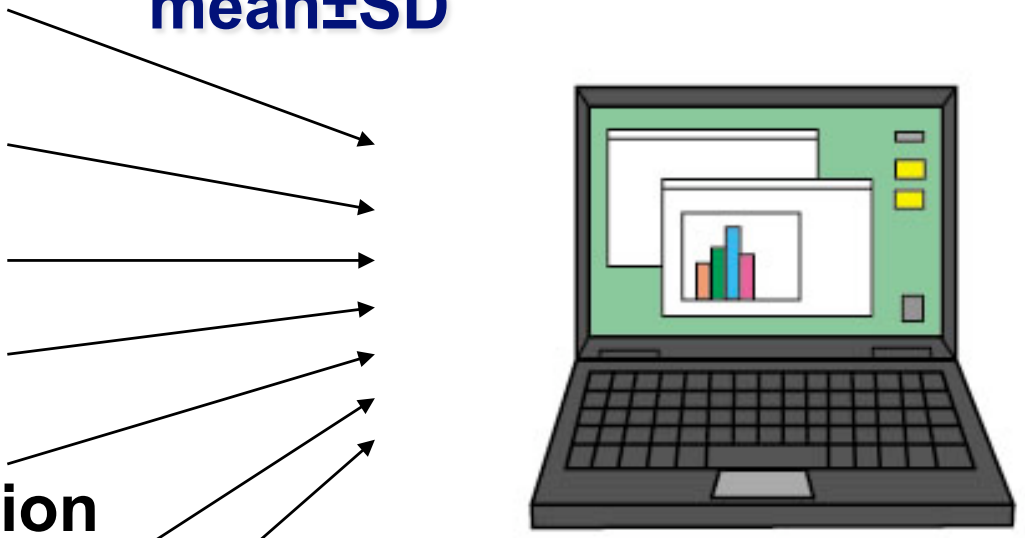
Clin Pharmacol Ther. 69, 333, 2001

Approx. 10-fold individual differences are shown in the CYP3A4 substrate without definite gene polymorphisms.

‡ Provided by Dr M. Kato (Chugai Pharmaceutical)

Factors responsible for individual variation (Dominant factors of pharmacokinetics)

- Body weight
- Liver weight
- Enzyme level
- Transporter level
- Serum protein concentration
- Hepatic blood flow rate
- Glomerular filtration rate



Virtual Person with various conditions

Parameters generated from random numbers

- **Body weight**
- **Liver weight**
- **Enzyme level**
- **Transporter level**
- **Serum protein concentration**
- **Hepatic blood flow rate**
- **Glomerular filtration rate**

Pharmacokinetics of Midazolam

Japanese
63.6 ±7.4 kg

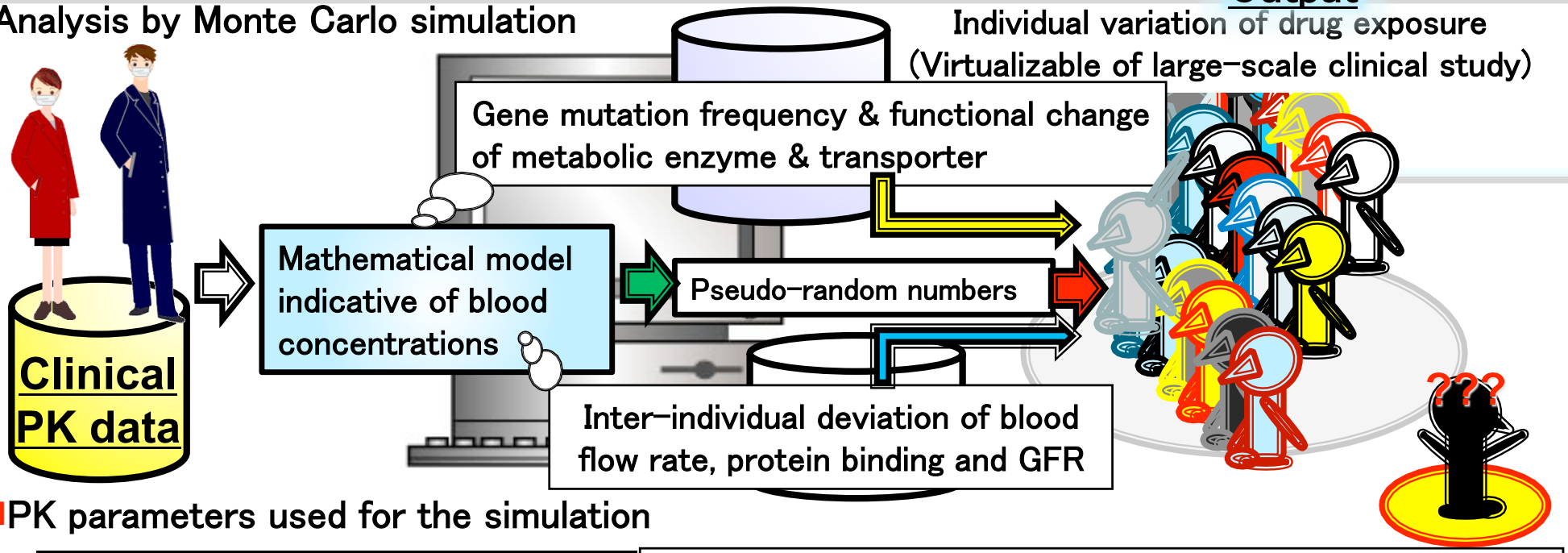
Europeans & North Americans
83.2 ±10.6 kg

Common parameters
CLint,h: 16.3mL/min/mL liver
(individual difference: 33%)
Liver volume: 19.5±2.2 mL/kg
Liver perfusion: 1.22±0.16 mL/min/mL liver

		observed			simulated		
		mean	SD	CV	mean	SD	CV
European American(n=20)(Tateishi,T et al., CPT 69: 333-339, 2001)							
body weight	kg	83.2	10.6		82.5	11	
CLiv	mL/min	412	124	30%	411	131	32%
	mL/min/kg	4.99	1.53	31%	4.97	1.37	28%
CLpo	mL/min	1728	1005	58%	1639	1063	65%
	mL/min/kg	20.9	12.1	58%	19.8	12.3	62%
Japanese(n=19(iv), n=22(po))							
body weight	kg	63.6	7.4		63.1	7.7	
CLiv	mL/min	311	70	23%	315	98.2	31%
	mL/min/kg	4.95	0.96	19%	4.97	1.37	28%
CLpo	mL/min	1413	730	52%	1253	808	64%
	mL/min/kg	22.4	11	49%	19.8	12.3	62%

Studies on Magnitude of Inter-Individual Variations in Blood Levels of Oseltamivira and Its Active Metabolite

■Analysis by Monte Carlo simulation



■PK parameters used for the simulation

		CV	ref
Q_h	1700 (mL/min)	19.5%	1
Q_r	1127 (mL/min)	8.3%	2
E_h	0.735	-	3
F_h	0.265	-	3
f_{b_osel}	0.460	5.8%	4
R_{b_osel}	1.270		5
$f_{b\_活性体}$	1.53	5.8%	4
$R_{b\_活性体}$	0.64		5
GFR	125 (mL/min)	30%	6
$CL_{R,\ plasma\_活性体}$	20.0 (L/hr)	-	7
$CL_{sec\_活性体}$	330 (mL/min)		8
$CL_{int\_renal\_活性体}$	305 (mL/min)	30%	
$CL_{int\_iver\_osel}$	12000 (mL/min)	30%	

■Capable of treating the drug exposure deviation generated by the combination of multiple variational parameters as a distribution map in tens of thousands scale of people.

■Drug exposures in patients, which were missed in clinical studies due to the low frequencies despite of the large functional changes, are valuable.

1. Wynne et al. Hepatology (1989) 9: 297-301
2. Muhammad et al. Journal of nuclear medicine (1969) 672- 5)
3. From the simulation results (assumed to be FaFg=1)
4. Application Materials. CV value: Kato et al. submitted
5. Application Materials. Use hematocrit level of 0.45.
6. Brian Davis et al. Pharmaceutical research (1993) 10; 7 1093-5
7. Hill et al. DMD (2002) 30: 13-9
8.Calculated by neglecting reuptake.

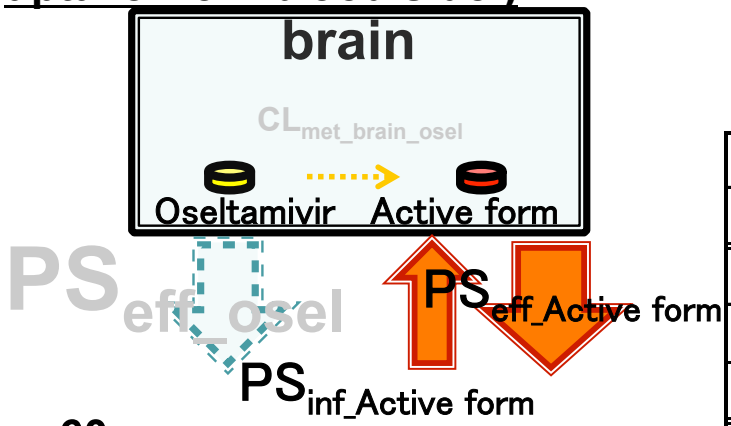
Studies on Magnitude of Inter-Individual Variation of AUC in Active Metabolite Concentration in Brain

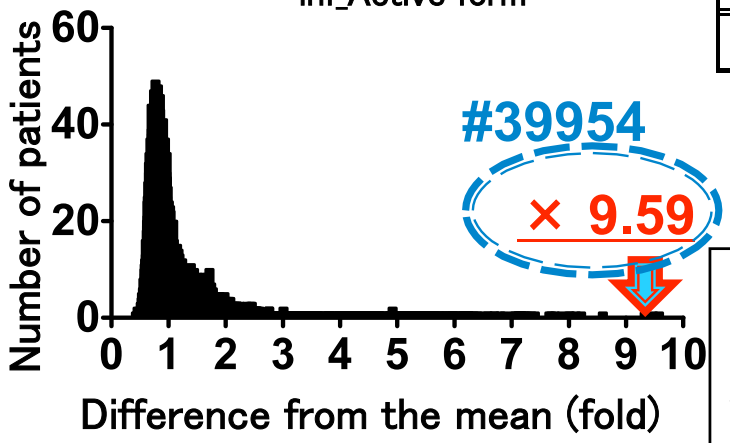
① ; Metabolism in brain　　② ; Uptake from blood

$$AUC_{brain\_active\ form} = \frac{\left(CL_{met\_brain\_osel} \times AUC_{blood\_osel} \times f_{u\_brain\_osel}\right) + \left(PS_{eff\_Active\ form} \times f_{u\_活性体} \times AUC_{blood\_活性体}\right)}{PS_{inf\_Active\ form} \times f_{u\_活性体}}$$

■Assumption 2. ①<<②

(Assuming that AUC of the active form concentration in brain is defined mainly by its uptake from blood side.)





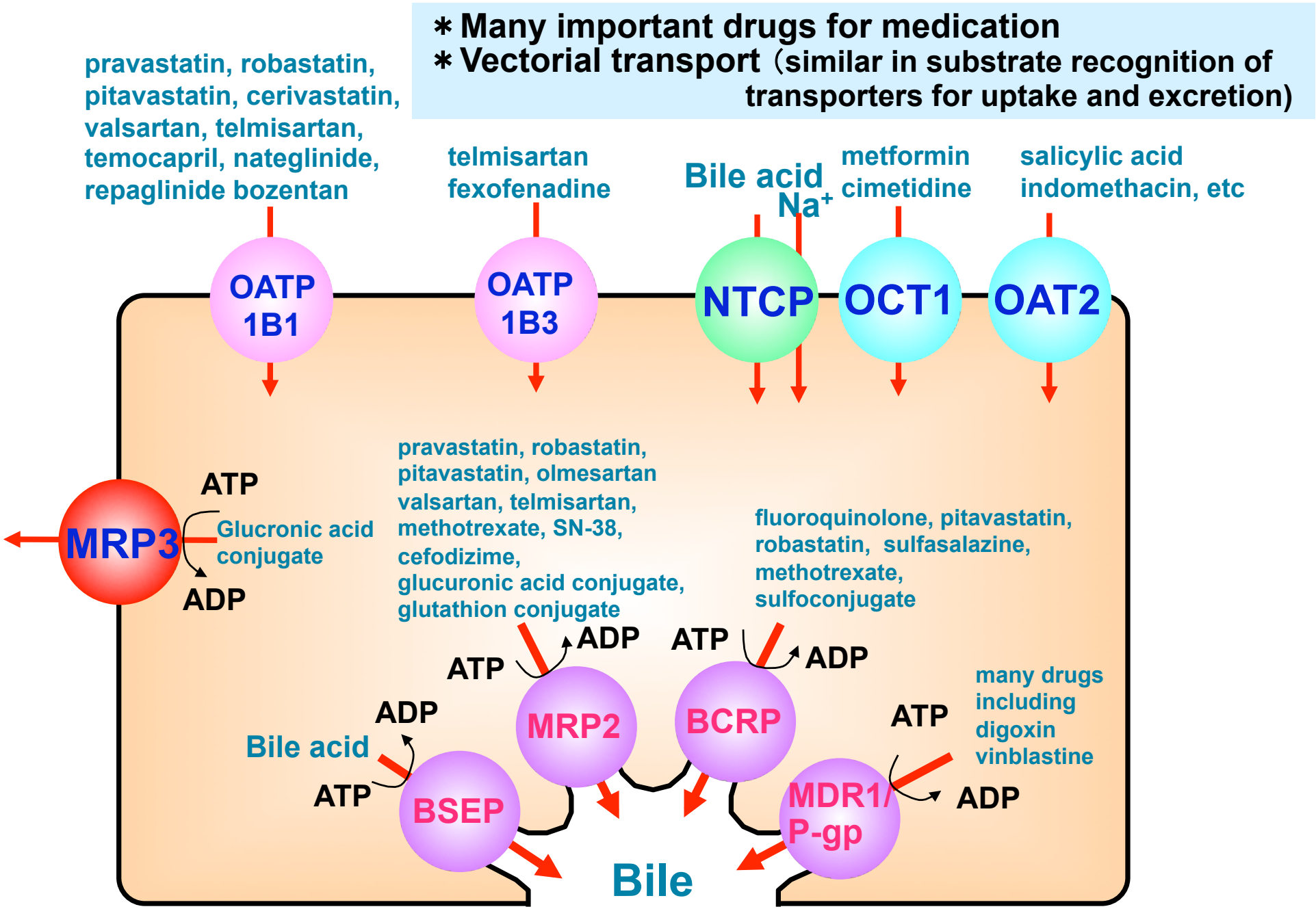
Functional change of OAT3, MRP4, MDR1, and CES1A in the subject with the highest brain exposure of active form in each assumption.

			OAT3	MRP4	MDR1	CES1A
		$AUC_{brain\_oseltamivir}$				
#38227	1 >> 2	排泄 >> 代謝	0.14	0.56	0.56	0.96
#47772	1 >> 2	排泄 = 代謝	0.12	0.79	0.09	0.53
#13386	1 >> 2	排泄 << 代謝	0.07	0.63	0.53	0.53
#39954	1 << 2	-	0.05	0.77	0.13	1.08

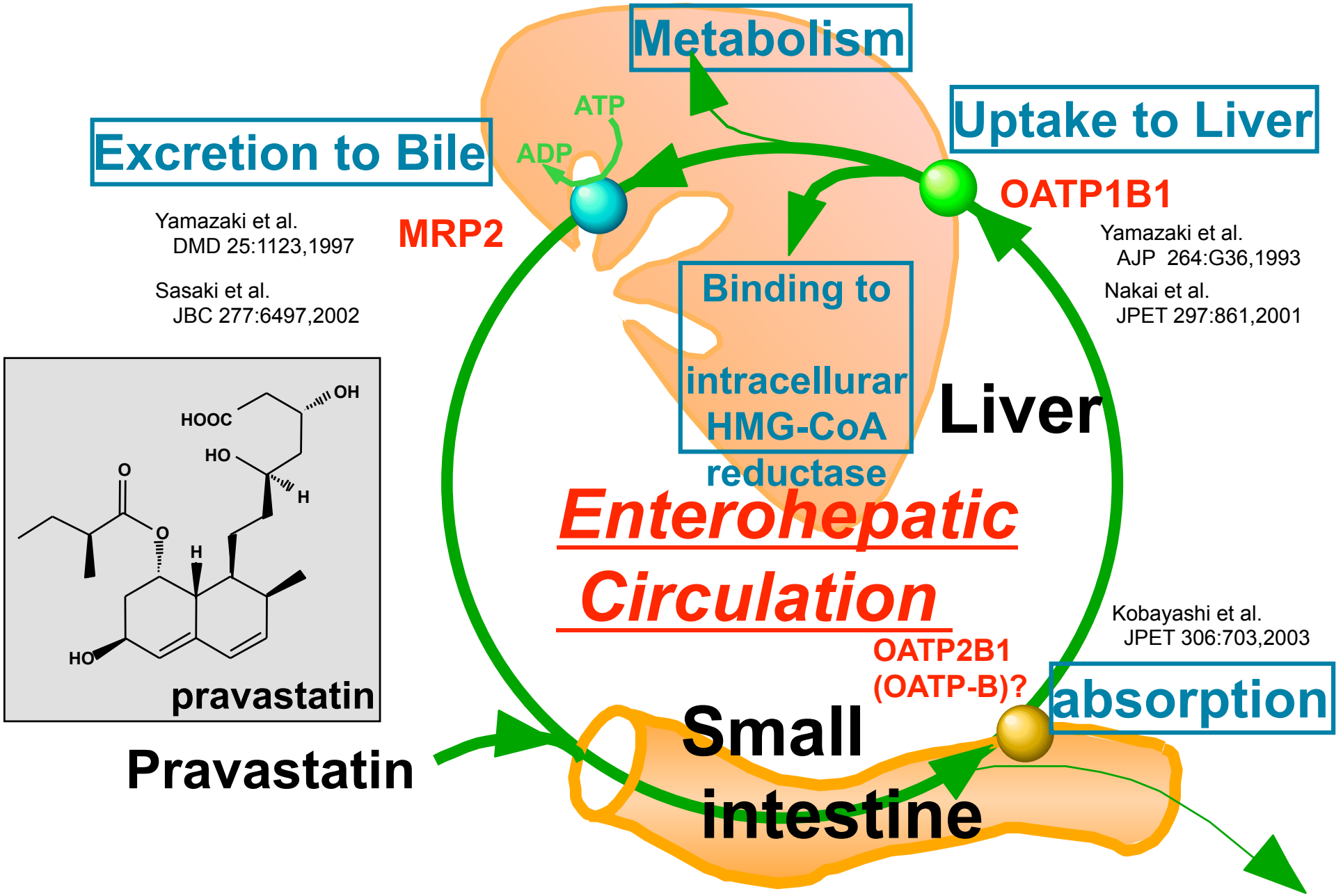
(Activity level in wild type = 1)

■AUC of oseltamivir concentration in brain increased maximum almost 10 times mean value.

■The number of subjects whose AUC increased more than 7 times were 30 out of 50000.

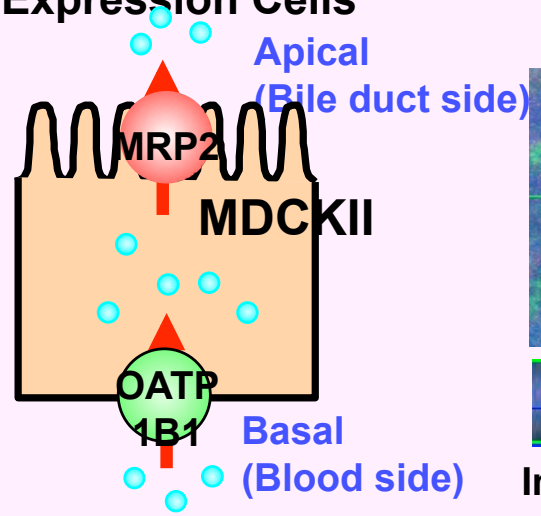


Hepatobiliary drug transporters

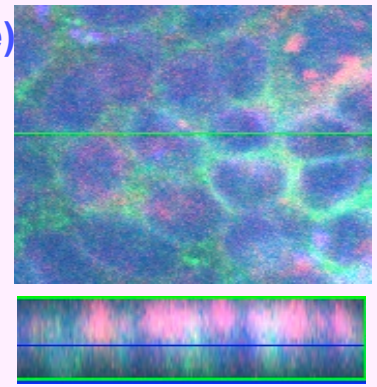


Transporter Group Involved in Enterohepatic Circulation of Pravastatin
～Efficient Transport to Liver～

Establishment of Double-Expression Cells



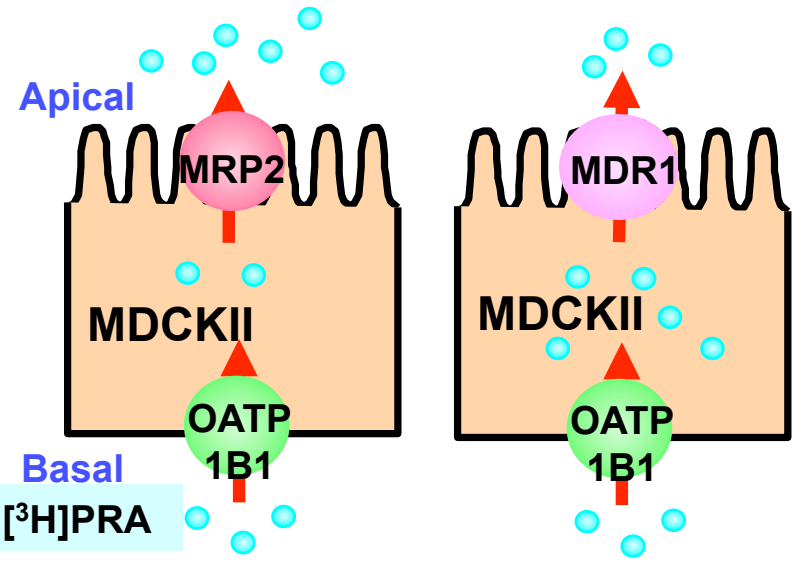
MRP2: red
OATP1B1: green
Nuclei: blue

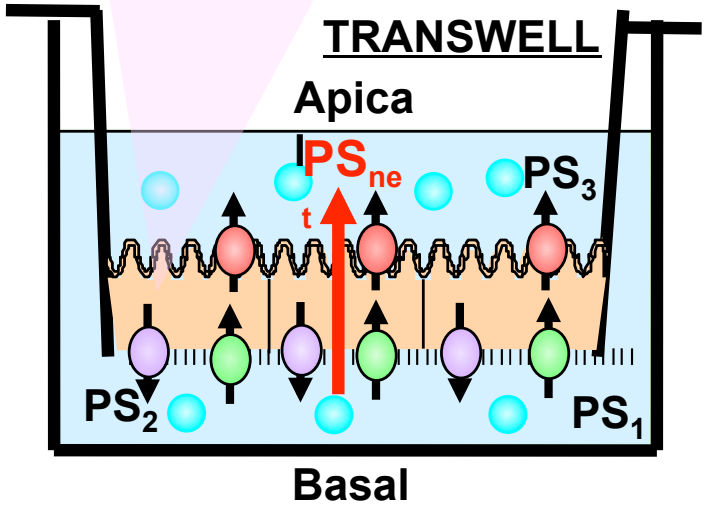


Immunostain image

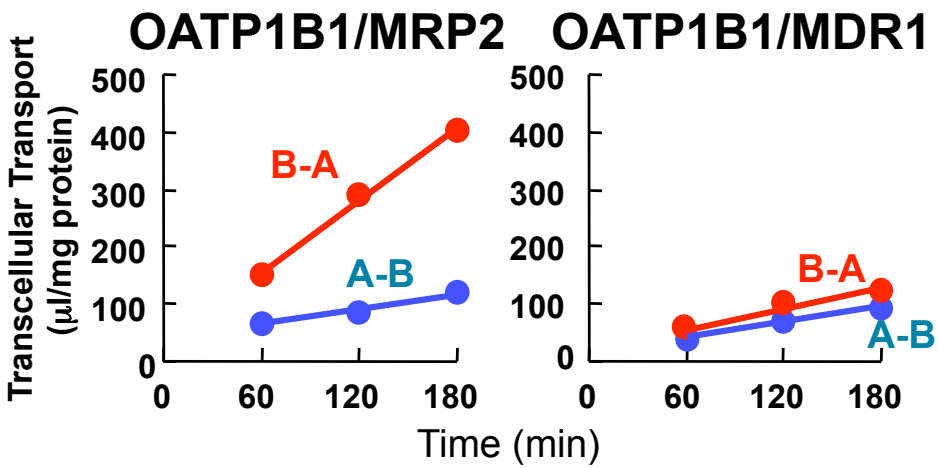
[Sasaki M et al., J Biol Chem, 277, 6497-503 (2002)]

Trans-cell Transport in Pravastatin Double-Transfectant





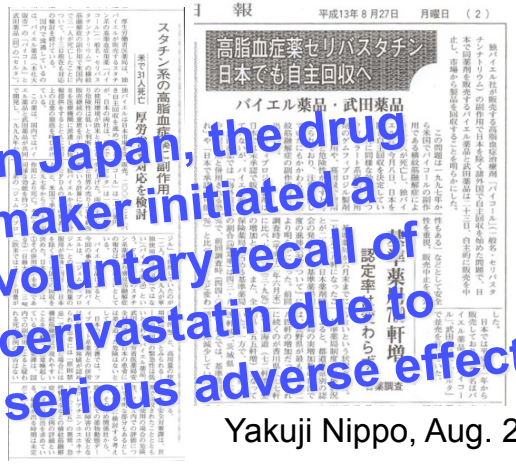
$$PS_{net} = PS_1 \times \frac{PS_3}{PS_2 + PS_3}$$



‡ Matsushima S et al., J Pharmacol Exp Ther, 314, 1059-67 (2005))

Utilization of Co-expression System of Uptake-Excretion-Transporters

Adverse Effects of Cerivastatin - Drug-Drug Interaction with Gemfibrozil -

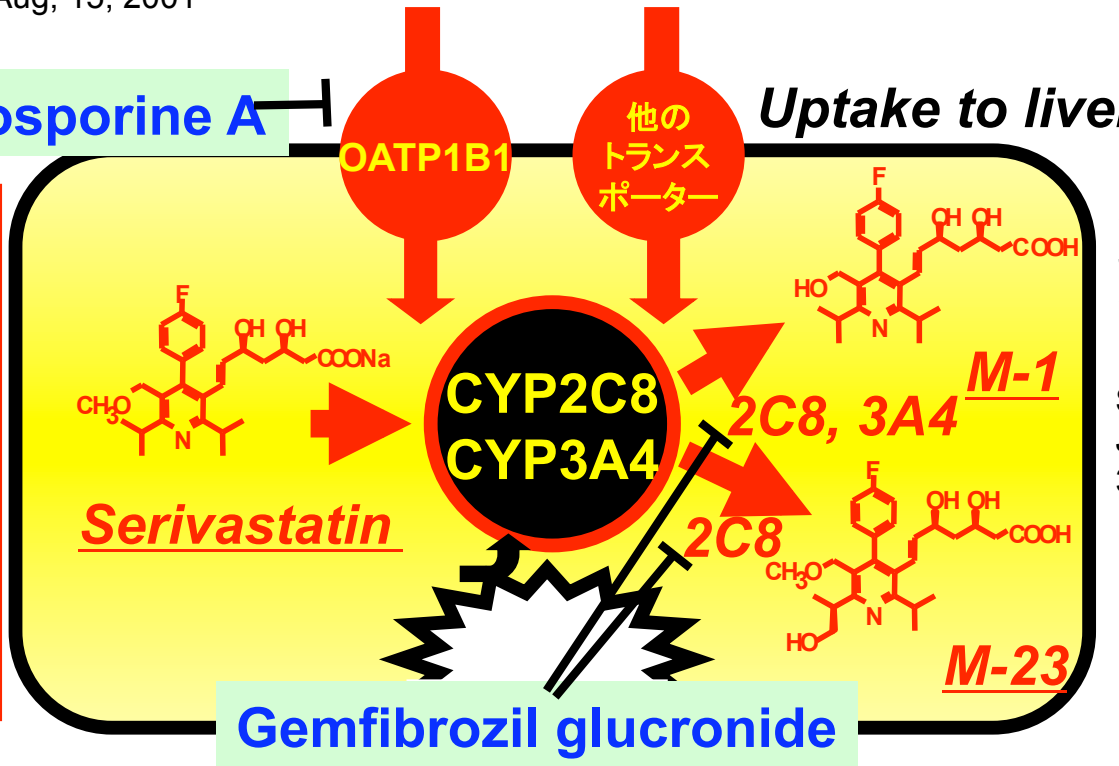


In Japan, the drug maker initiated a voluntary recall of cerivastatin due to serious adverse effects.

Yakuji Nippo, Aug. 27, 2001

Yakuji Nippo, Aug, 15, 2001

There have been 52 deaths (31 in the US) from the adverse effect rhabdomyolysis in patients taking serivastatin.
Twelve of the 31 patients in the US were confirmed to take concomitant fibrate group anti-hyperlipidemic agents.
[from British Medical Journal 323, 359 (2001) & British Medical Journal 323, 415 (2001)]



Low risk of interaction has been considered in serivastatin due to its multiple metabolic pathways .

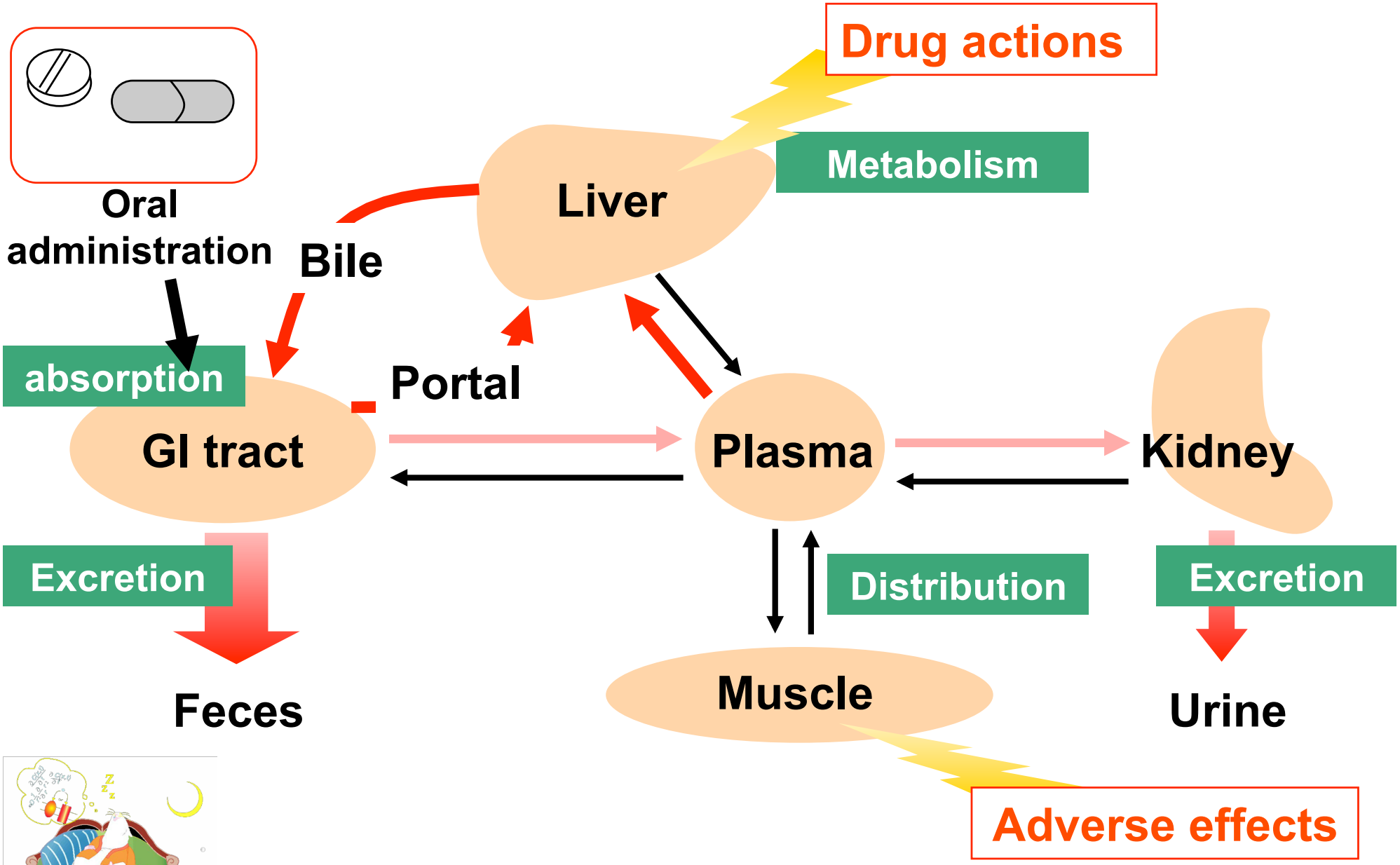
Shitara, Y. et al.
J Pharmacol Exp Ther,
304(2): 610-6 (2003)

Shitara, Y. et al.
J Pharmacol Exp Ther,
311(1): 228-36 (2004)

Shitara, Y. and Sugiyama, Y. Pharmacol Ther,
112(1): 71-105 (2006)

Elucidation of Primary Action Point in the Interacting Drug and Detoxification of Cerivastatin in Liver

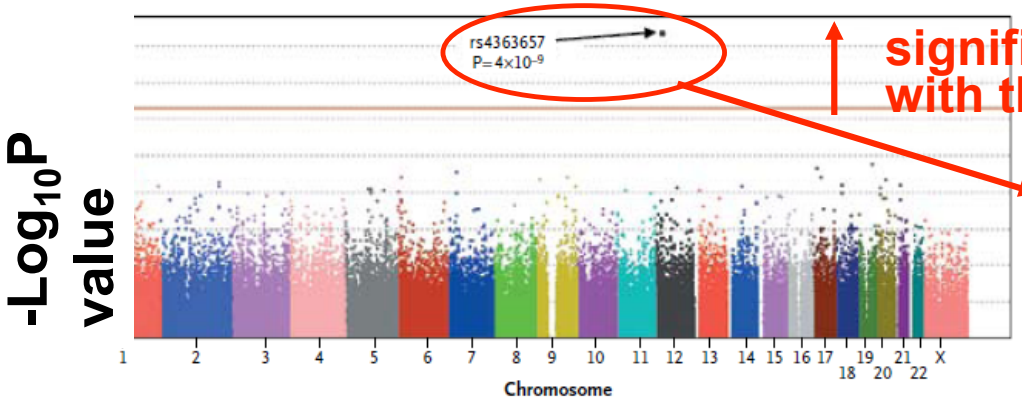
Mobilization of Pravastatin (Anti-hyperlipidemia)



‡(Modified figure from J. Azuma: 「「クスリに弱いヒト」と「困ったクスリ」たち」
(Individual Difference of Reactivity to Drug),Jiho, Inc., Tokyo, 2001

Relation between simvastatin-induced myopathy and genetic polymorphism of OATP1B1

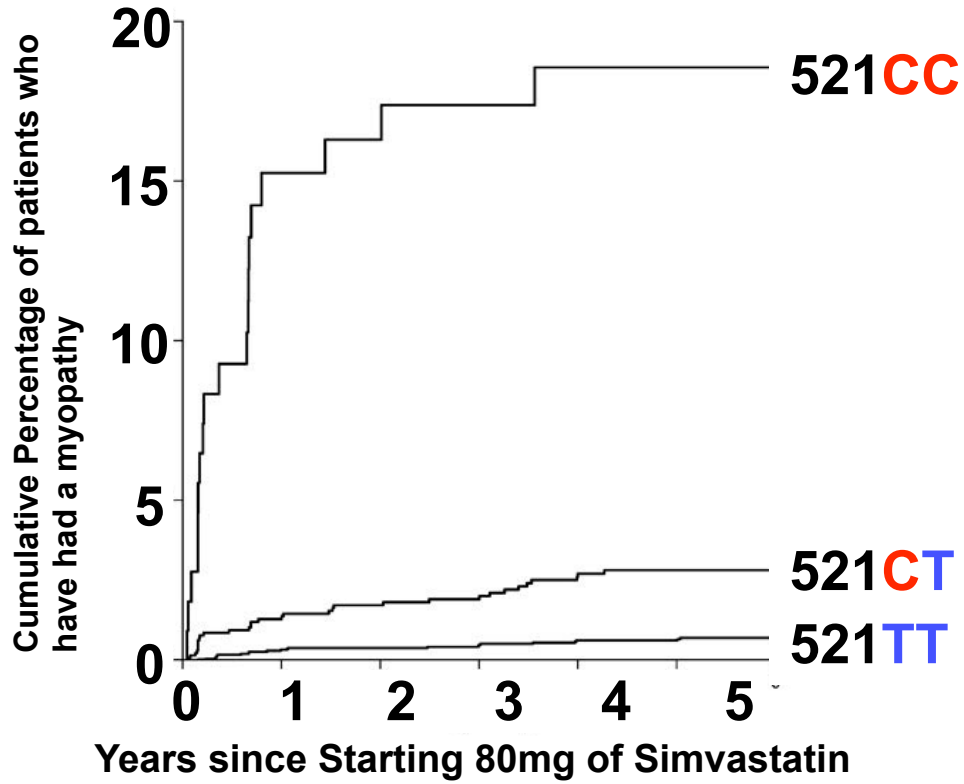
Case=85 vs control 90：ca 300,000marker SNPs



significant correlation with the event

OATP1B1 polymorphism seriously affects the adverse reactions.

Significant link r2>0.95) between Marker SNPs and SLCO1B1 T521C (V174A) !



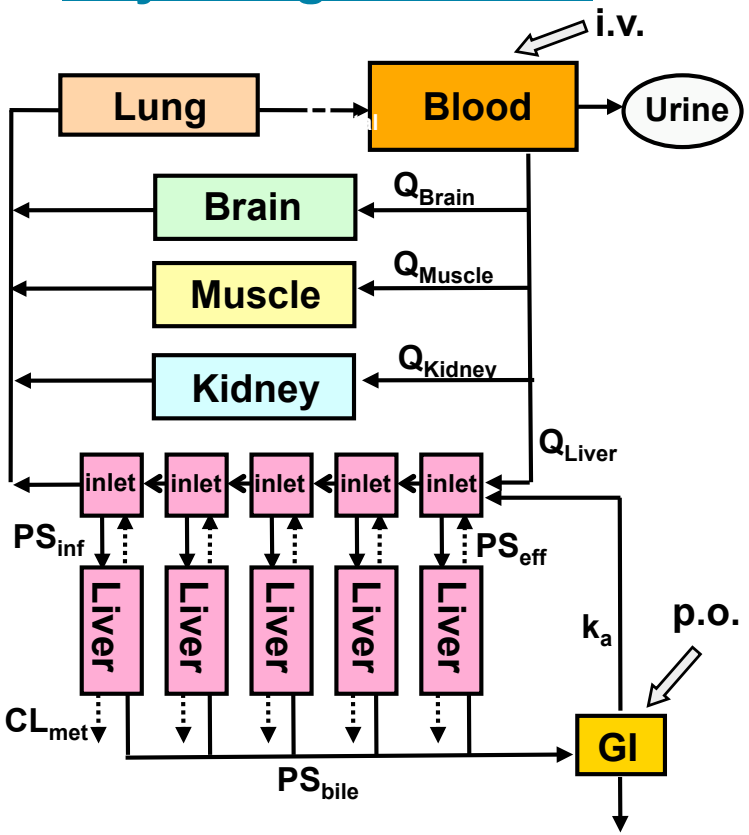
Odds ratio of this SNPs for simvastatin-induced myopathy

521CT vs TT → 4.5 fold

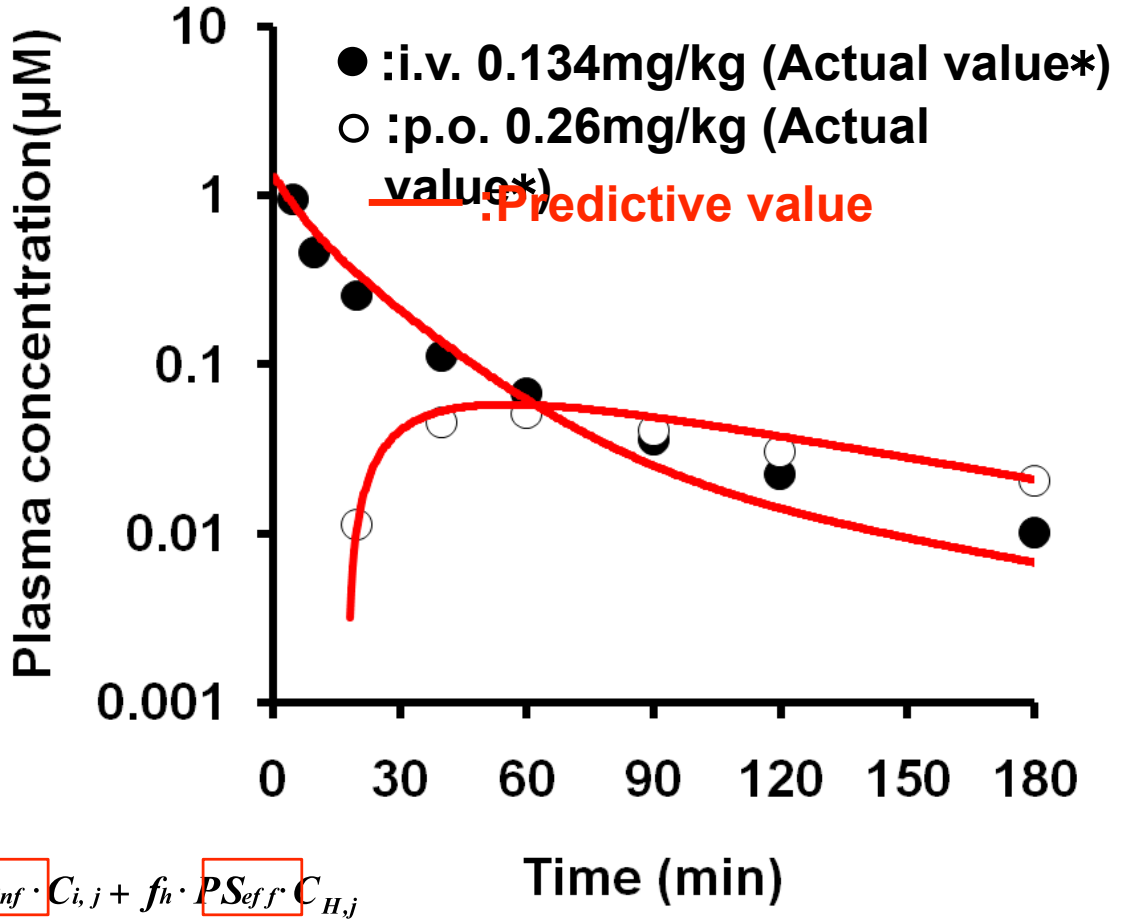
521CC vs TT →16.9 fold

‡(SEARCH Collaborative Group et al., New Engl J Med, 359, 789-99 (2008))

Physiological Model



Time Course of Plasma Concentration in Human



Blood

$$V_b \cdot \frac{dC_b}{dt} = Q_h(C_i - C_b) - CL_r \cdot C_b$$

Intra-capilary

$$V_{i,j} \cdot \frac{dC_{i,j}}{dt} = Q_h(C_{b,j} - C_{i,j}) - f_b \cdot PS_{inf} \cdot C_{i,j} + f_h \cdot PS_{eff} \cdot C_{H,j}$$

Hepatic parenchymal cells

$$V_{H,j} \cdot \frac{dC_{H,j}}{dt} = f_b \cdot PS_{inf} \cdot C_{i,j} - f_h \cdot (PS_{eff} + CL_{met} + PS_{bile}) \cdot C_{H,j}$$

Extrapolated parameters in vivo from in vitro data

Physiological Model and Prediction of Time Course in Plasma Level

Mathematical Model for Prediction of Drug Concentration

Blood

$$V_B \frac{dC_B}{dt} = Q_{LU}\left(\frac{C_{LU}}{K_{p,LU}} - C_B\right) - CL_R C_B$$

Lung

$$V_{LU} \frac{dC_{LU}}{dt} = Q_{BR} \frac{C_{BR}}{K_{p,BR}} + Q_{MU} \frac{C_{MU}}{K_{p,MU}} + Q_R \frac{C_R}{K_{p,R}} + Q_H C_{HE5} - Q_{LU} \frac{C_{LU}}{K_{pLU}}$$

Brain, Muscle, Kidney

$$V_i \frac{dC_i}{dt} = Q_i\left(C_B - \frac{C_i}{K_{p,i}}\right)$$

Liver→Efficacy Target

$$\frac{V_{Hi}}{5}\frac{dC_{Hi}}{dt} = \left(\frac{PS_{\inf}}{5}\right) f_B C_{HEi} + \left(\frac{PS_{dif}}{5}\right) f_B C_{HEi} - \left(\frac{PS_{dif}}{5}\right) f_T C_{Hi} - \left(\frac{PS_{bile}}{5}\right) f_T C_{Hi} - \left(\frac{PS_{met}}{5}\right) f_T C_{Hi}$$

Extra cellular space of liver ①

$$\frac{V_{HE1}}{5}\frac{dC_{HE1}}{dt} = Q_H\left(C_B - C_{HEi}\right) - \left(\frac{PS_{\inf}}{5}\right) f_B C_{HEi} - \left(\frac{PS_{dif}}{5}\right) f_B C_{HEi} + \left(\frac{PS_{dif}}{5}\right) f_T C_{Hi} + k_a F_a X_{GI}$$

Extracellular space of liver ②

$$\frac{V_{HEi}}{5}\frac{dC_{HEi}}{dt} = Q_H\left(C_{HE(i-1)} - C_{HEi}\right) - \left(\frac{PS_{\inf}}{5}\right) f_B C_{HEi} - \left(\frac{PS_{dif}}{5}\right) f_B C_{HEi} + \left(\frac{PS_{dif}}{5}\right) f_T C_{Hi}$$

Intra-gastrointestinal duct

$$\frac{dX_{GI}}{dt} = \sum\left(\frac{PS_{bile}}{5}\right) f_T C_{Hi} - k_a F_a X_{GI}$$

Mathematical Model for Prediction of Drug Concentration

Blood

$$V_B \frac{dC_B}{dt} = Q_{LU}\left(\frac{C_{LU}}{K_{p,LU}} - C_B\right) - CL_R C_B$$

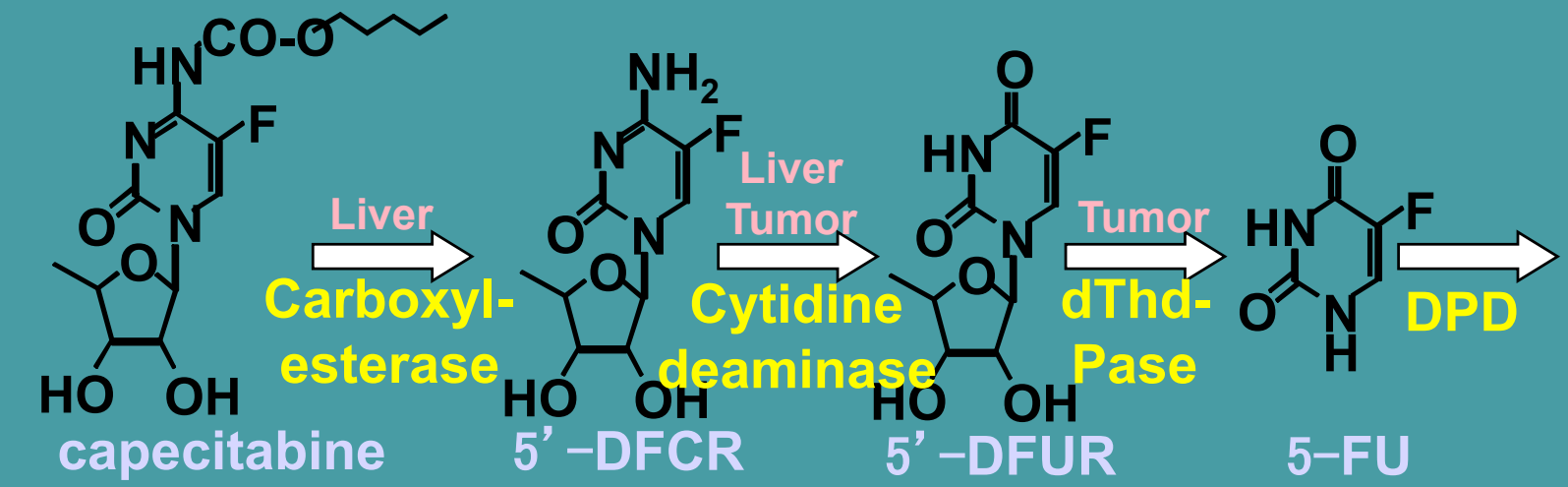
Muscle→ Adverse Effect Target

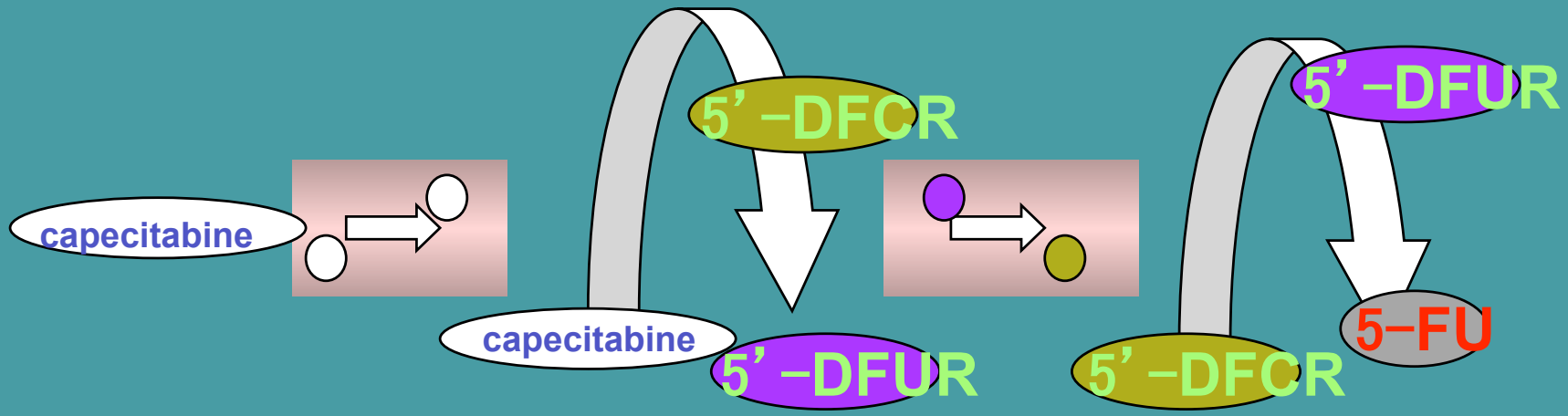
$$V_i \frac{dC_i}{dt} = Q_i\left(C_B - \frac{C_i}{K_{p,i}}\right)$$

Liver→Efficacy Target

$$\frac{V_{Hi}}{5}\frac{dC_{Hi}}{dt} = \left(\frac{PS_{\inf}}{5}\right)f_B C_{HEi} + \left(\frac{PS_{dif}}{5}\right)f_B C_{HEi} - \left(\frac{PS_{dif}}{5}\right)f_T C_{Hi} - \left(\frac{PS_{bile}}{5}\right)f_T C_{Hi} - \left(\frac{PS_{met}}{5}\right)f_T C_{Hi}$$

Phamacokinetics of Cabecitabine

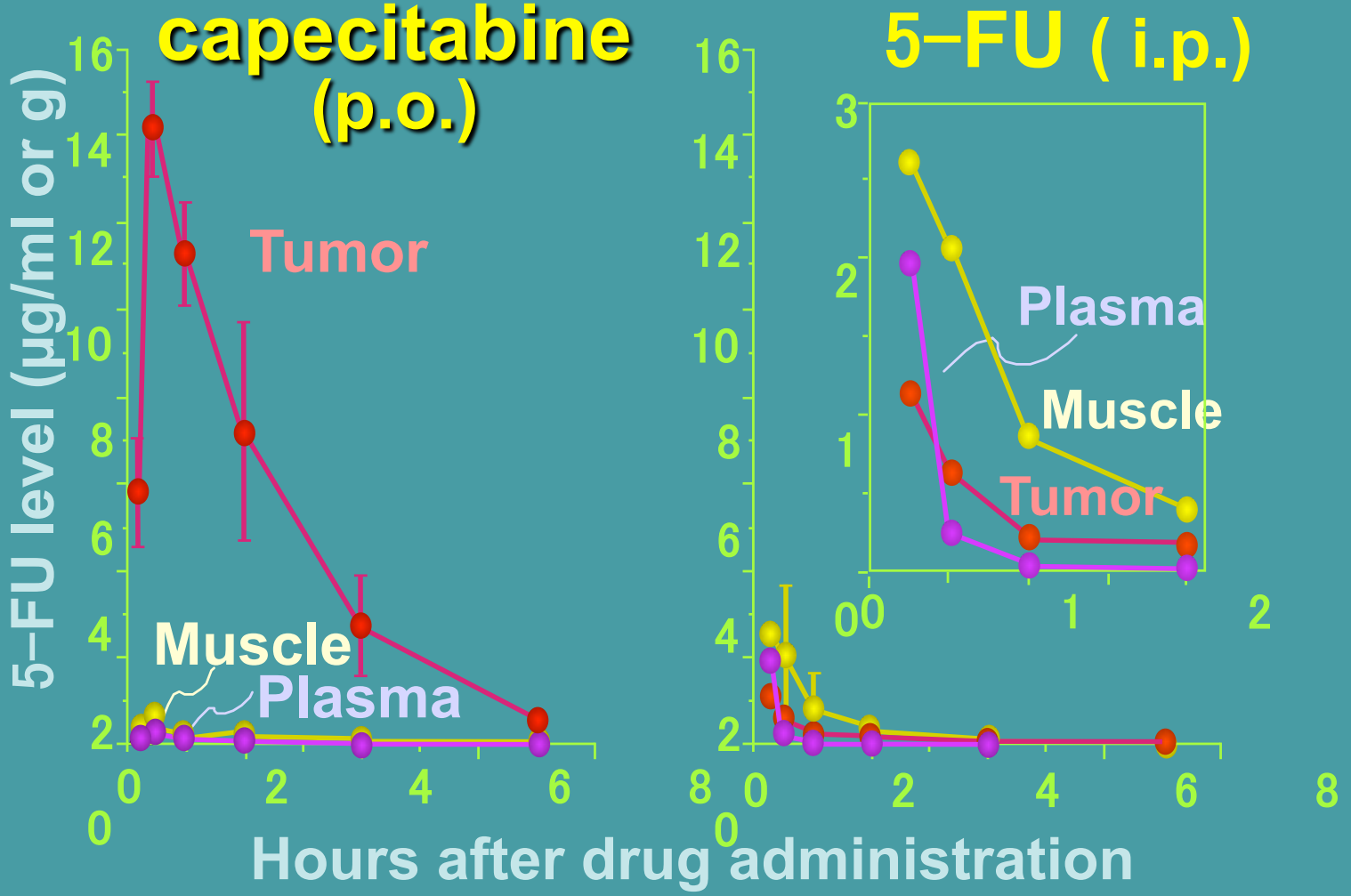




Small Intestine Liver Tumor

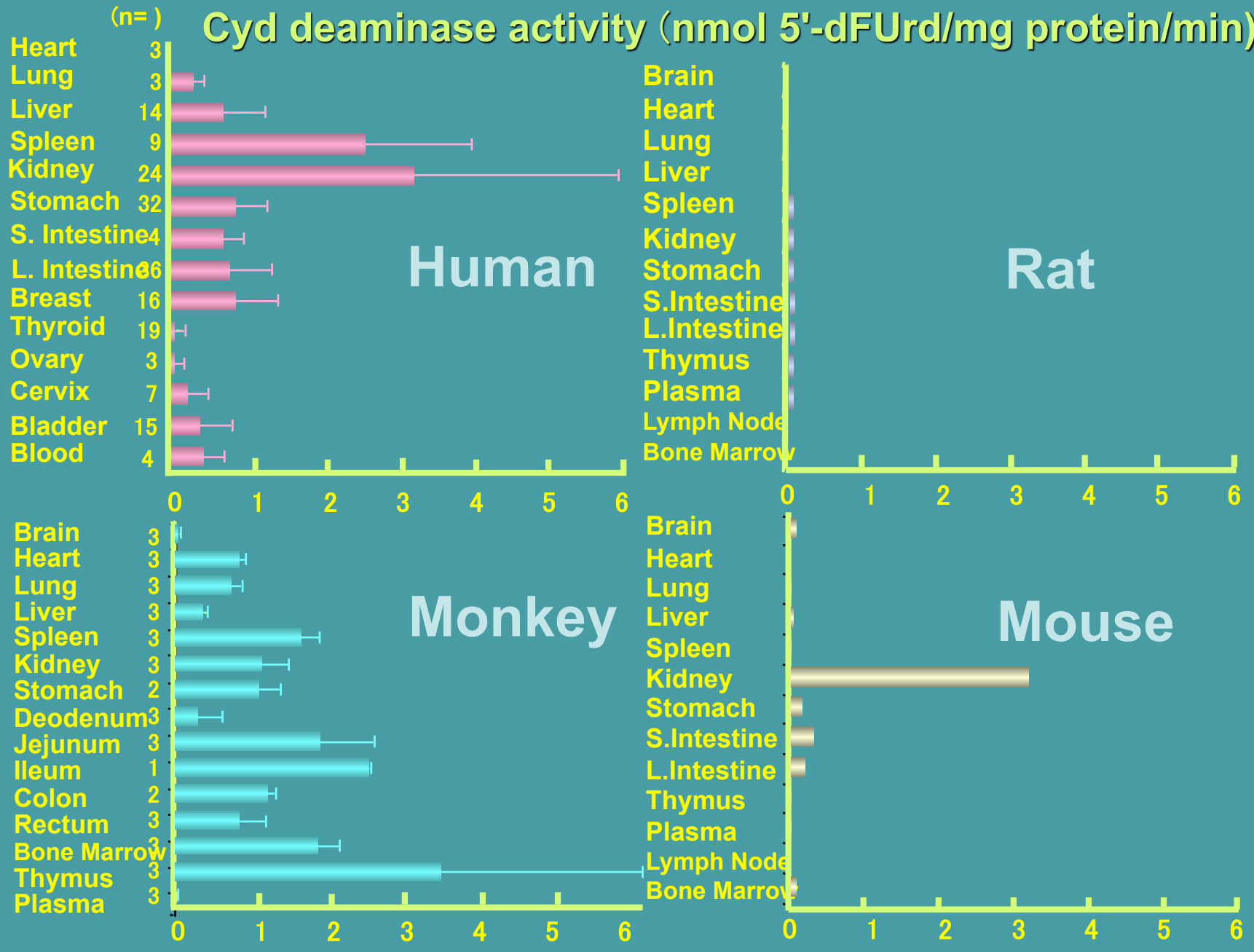
‡ H. Ishitsuka, 2000, Investigational New Drugs 18, 343-354

5-FU Levels after Drug Administration
(HCT116 human colon cancer xenograft model)



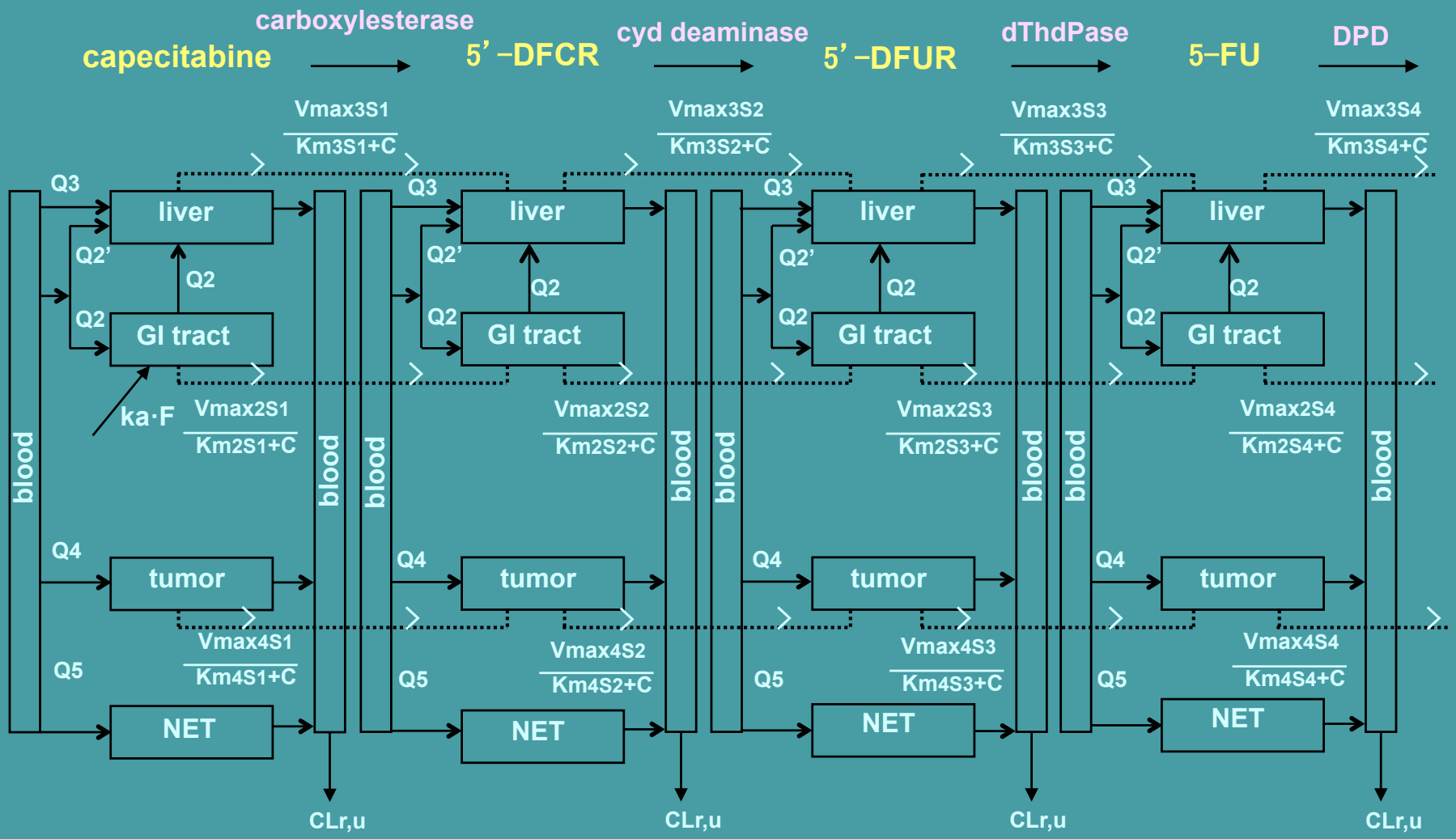
Ishikawa T. et al. Biochem J., 55, 1091-1097 (1998)

Species Difference in Tissue Distribution of Metabolic Enzyme



Onodera H. et al., 2000

PBPK model integrating metabolic enzyme activity for capecitabine



‡ Tsukamoto et al., Pharmaceutical Research. 2001. Vol. 18, Iss. 8; pg. 1190

Functional Components of DDS

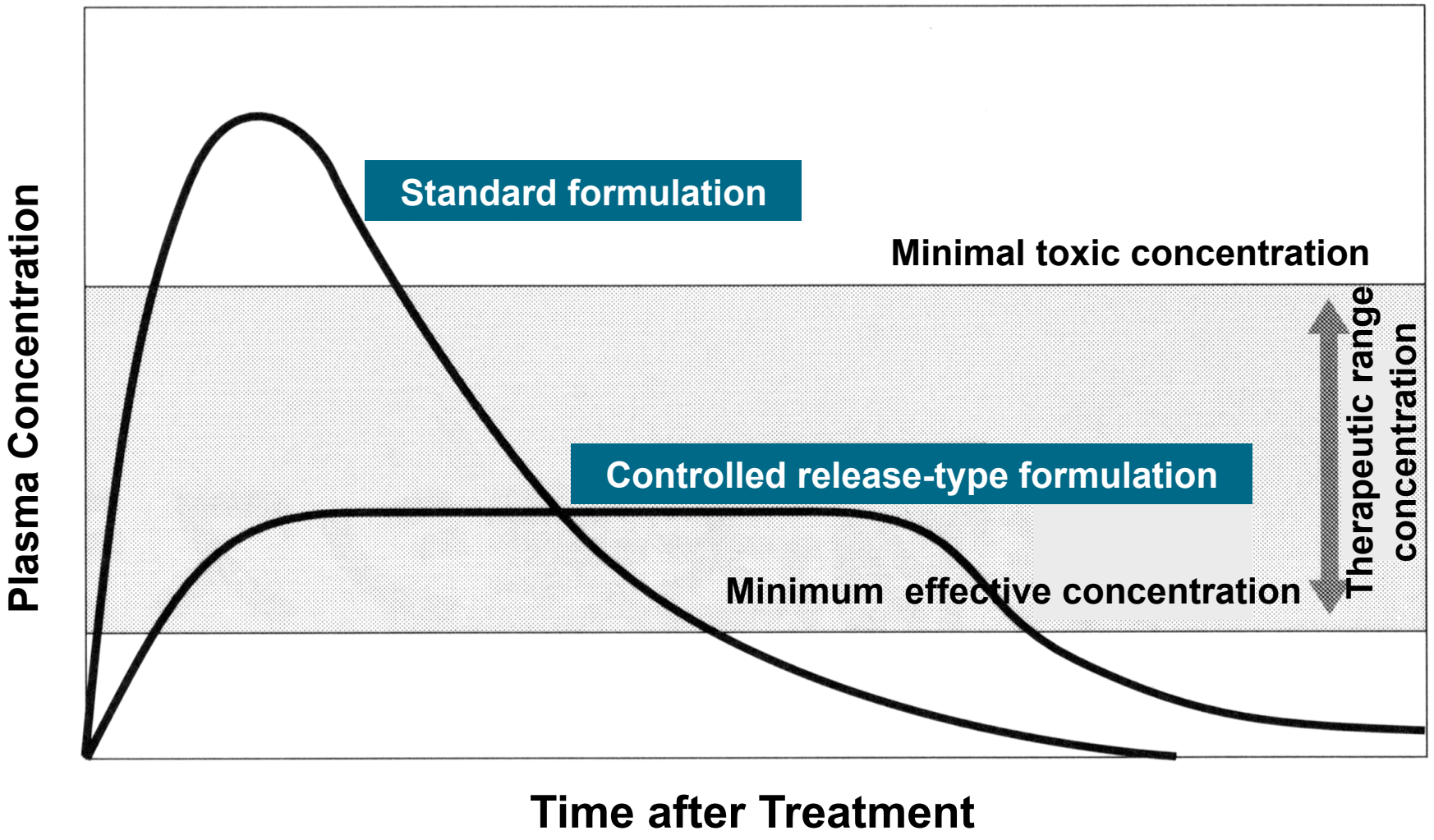
Drug Delivery System

DDS is a transport system for delivering drugs to the in vivo targeting site properly, at the right time, and in appropriate amount for requirements.

'Courier for Drugs'

Controlled Release
Improved absorption
Targeting

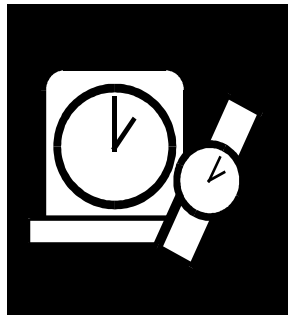
‡ Cited from Dr. M. Morishita, Department of Pharmaceutics, Hoshi University



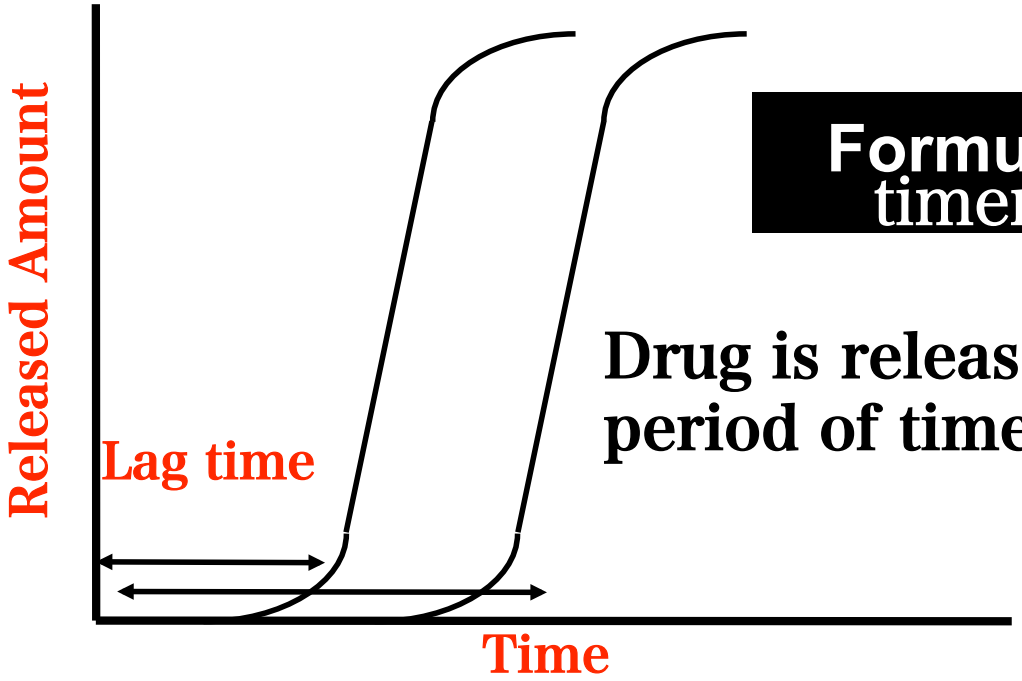
Time Course of Plasma Concentration after Treatment of Controlled Release-Type Formulation

‡ Cited from Dr. M. Morishita, Department of Pharmaceutics, Hoshi University

Time Controlled Release Formulation



Formulation with timer function

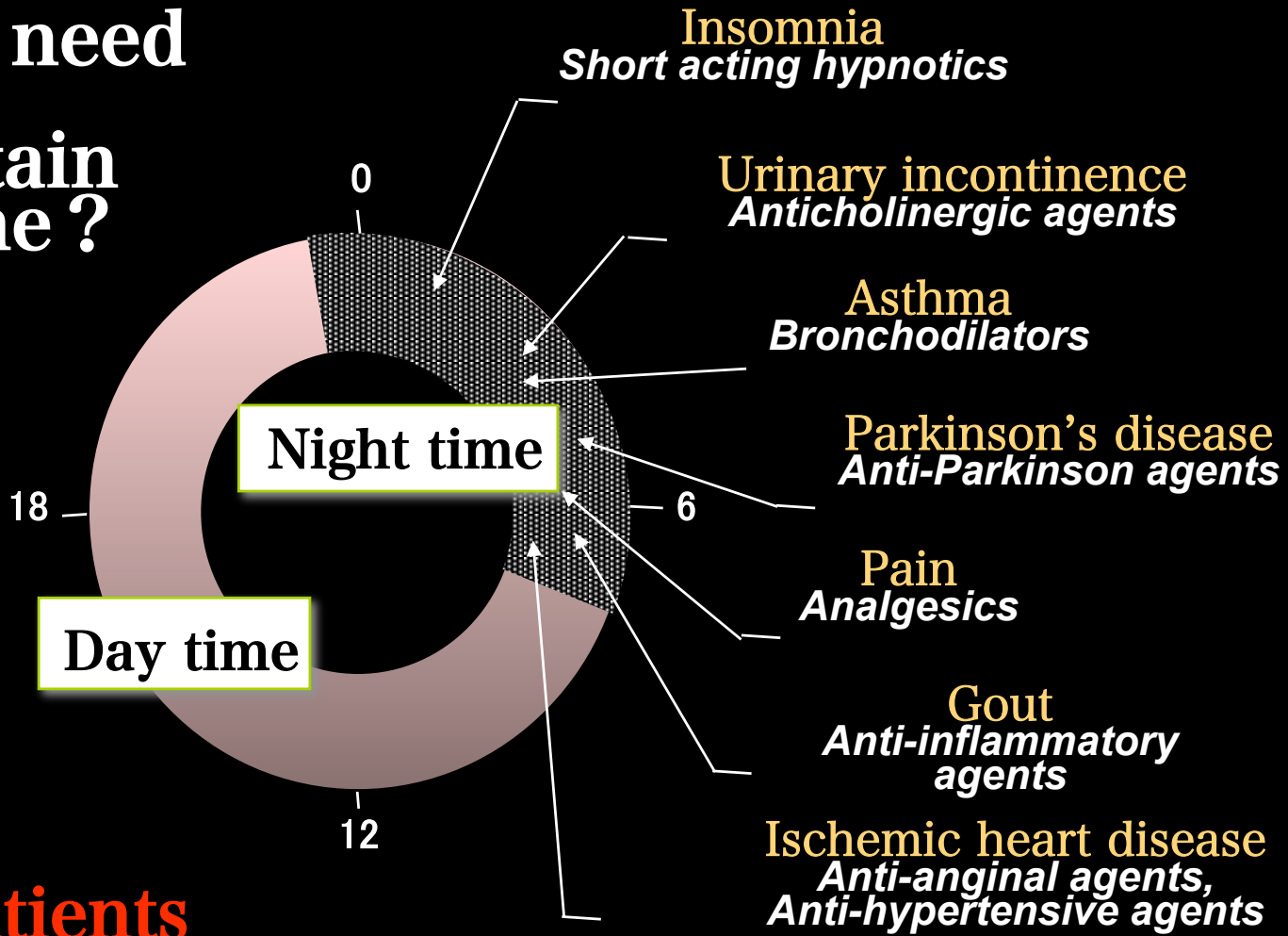


Drug is released at a certain period of time after treatment.

- New time controlled medication , in accordance with the concept of “chrono-pharacotherapy “ has been established.
- Midnight medication is avoidable.

‡ Cited from Dr. M. Morishita, Department of Pharmaceutics, Hoshi University

Do patients occasionally need medication during a certain period of time ?



Benefit for patients

- Symptoms at night or dawn are suppressible.
- With no disturbance of sleep
- No need to go up to take medicine in the middle of night.

‡ Cited from Dr. M. Morishita, Department of Pharmaceutics, Hoshi University

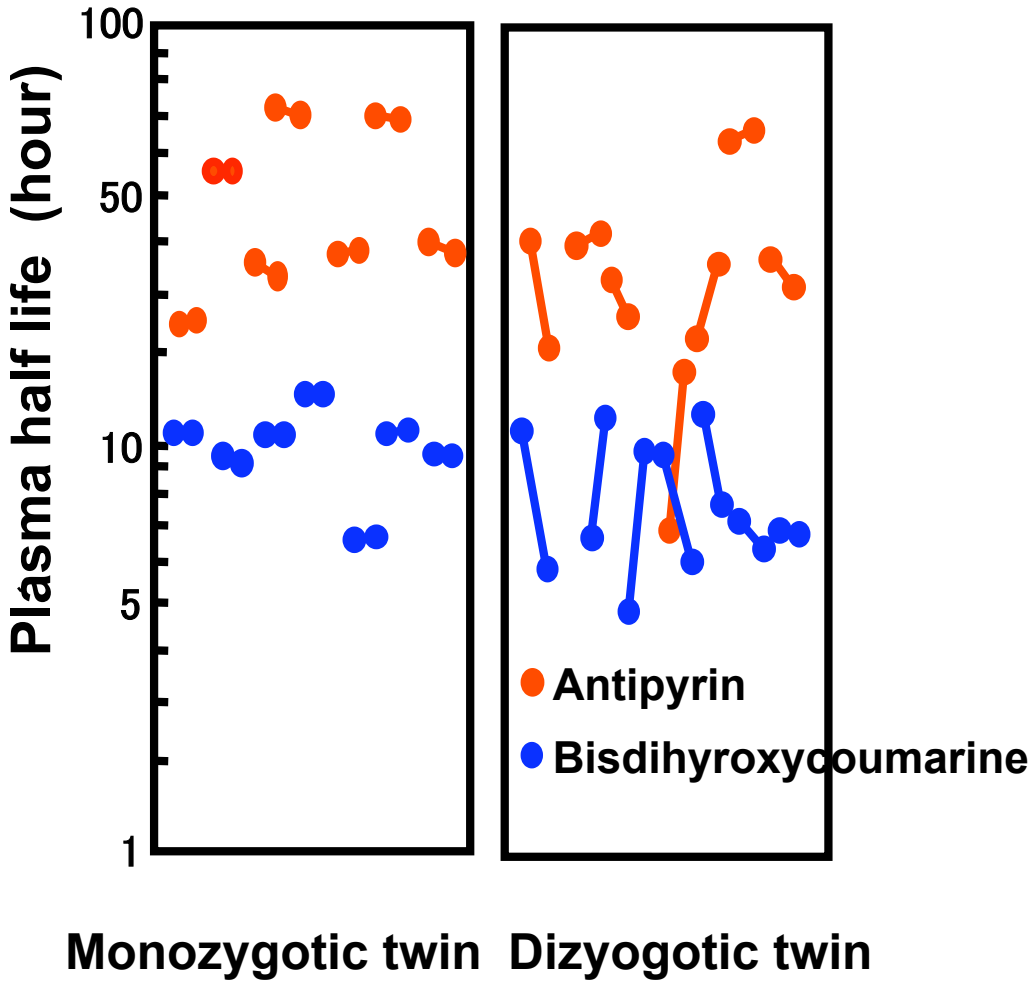
Metabolism and Efficacy of Drugs

Substances acting to body	Alcohol	Drug
Action	Intoxication	Efficacy

Isn't medication easy to work in the fast metabolizer?

e.g. Antiulcer Omeprazole (Gastric Secretion inhibitor)

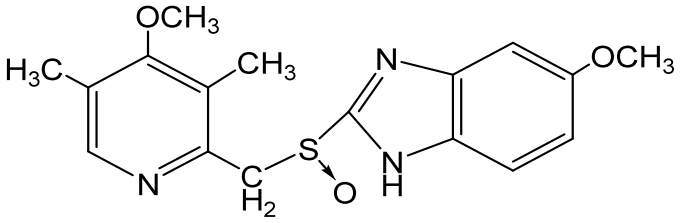
Difference between Mono- and Di-Zygotic Twin in Plasma Half Life of Antipyrin and Bisdihydroxycoumarine



‡ Cited from Professor T. Kamataki (Hokkaido University)

(Vesell, 1975)

Metabolism of Antiulser Omeprazole



Omeprazole

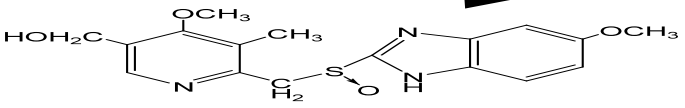
Drug metabolizing enzyme

CYP2C19

Main metabolic pathway

Drug metabolizing enzyme

CYP3A4



5-hydroxyomeprazole
(inactive)

Omeprazole sulfone
(inactive)

CYP2C19 Genotype

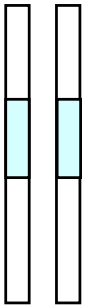
CYP2C19 gene

Rapid metabolizer：RM

Individuals with no mutation in both CYP2C19 alleles

Intermediate metabolizer：IM

Individuals with mutation in one CYP2C19 allele



Poor metabolizer: PM）

Individuals with mutation in both CYP2C19 alleles

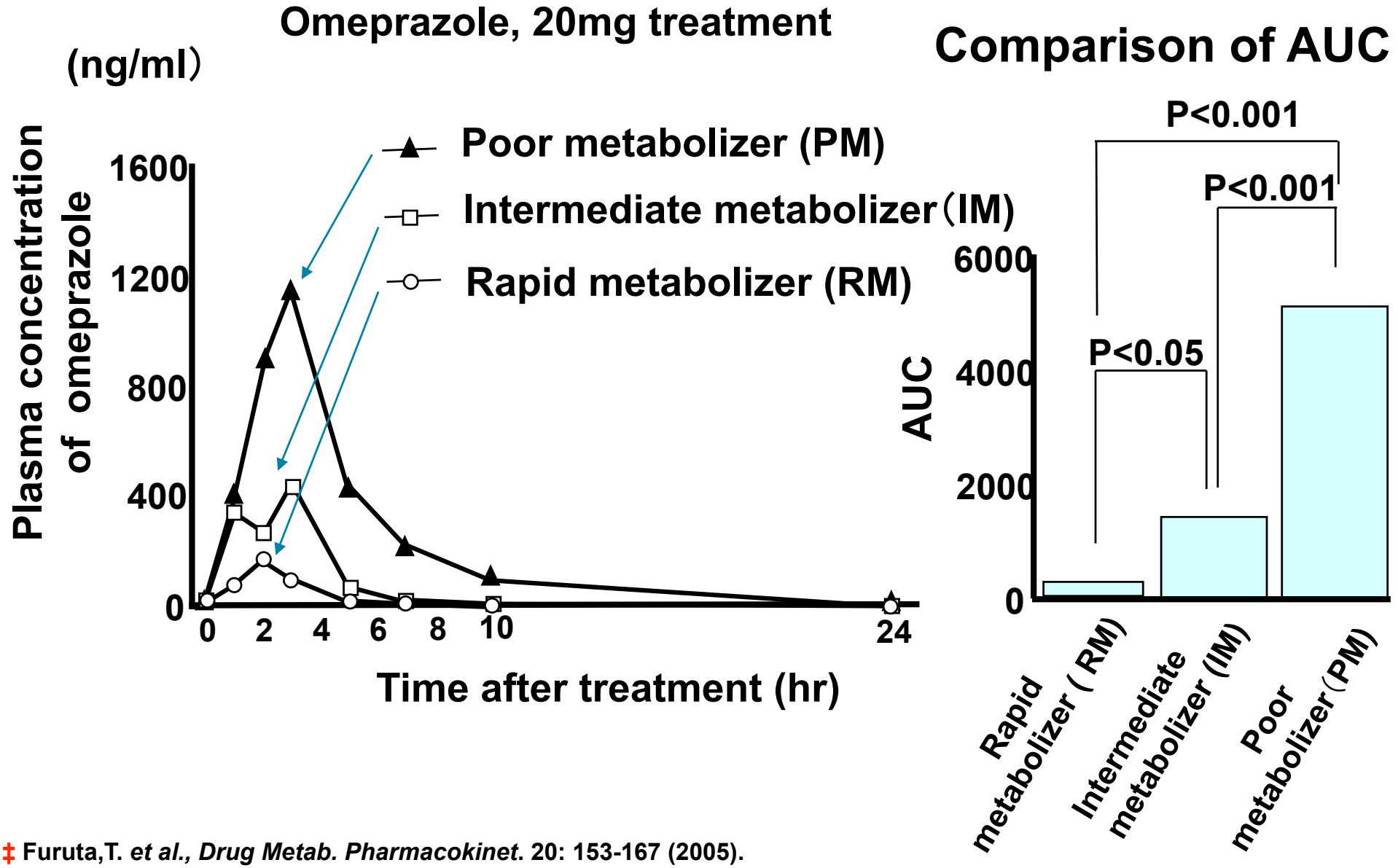
Ethnic Difference of CYP2C19 Genotype

Ethnic difference in 'Poor Metabolizer (PM)' percentage

White (US)	2.5%
White (EU)	3.0%
Black (US)	2.0%
Zimbabwean	4.8%
Chinese (Han people)	19.5%
Korean	12.6%
Japanese	18.0-22.5%

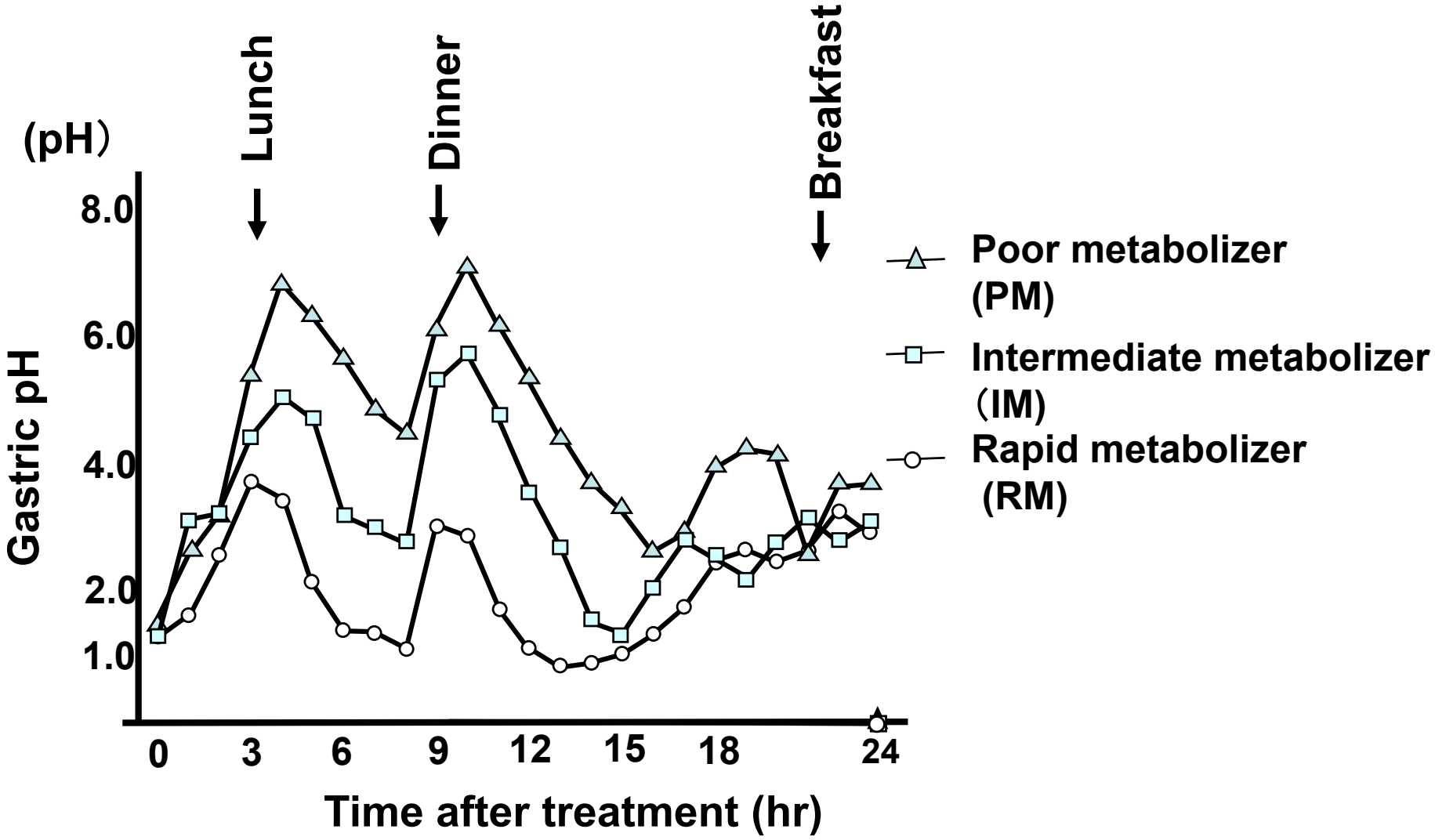
‡ Furuta,T. *et al., Drug Metab. Pharmacokinet*. 20: 153-167 (2005).

CYP2C19 Genotype and Plasma Omeprazole Concentration



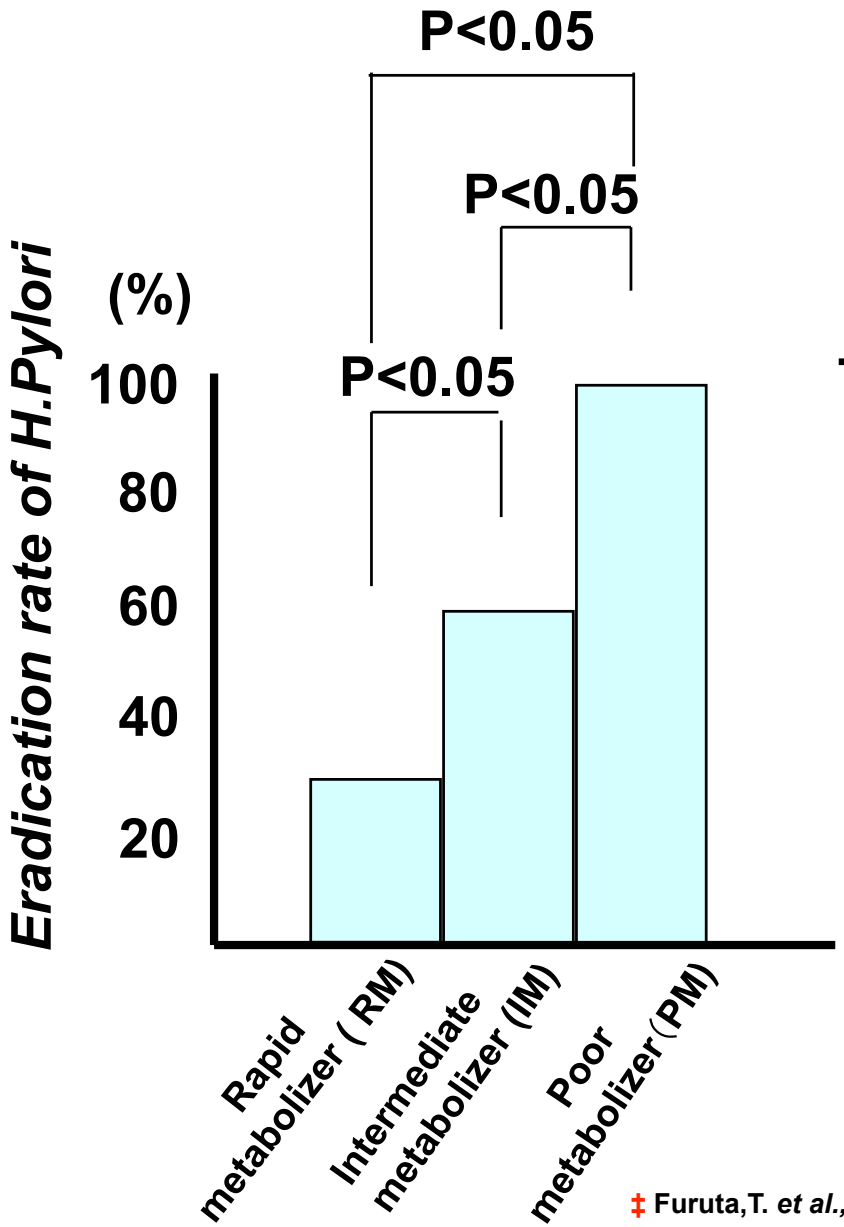
‡ Furuta,T. *et al., Drug Metab. Pharmacokinet.* 20: 153-167 (2005).

CYP2C19 Genotype and Pharmacological Effects of Omeprazole



Furuta,T. *et al., Drug Metab. Pharmacokinet.* 20: 153-167 (2005).

CYP2C19 Genotype and Decolonization of H. pylori by Omeprazole.



Omeprazole, 20mg & Amoxicilin, 2000mg Treatment for 2 weeks

‡ Furuta,T. *et al., Drug Metab. Pharmacokinet.* 20: 153-167 (2005).