Bioanalysis Form 2019, Yokohama
2019.2.13

JBF-JSSX共催セッション/ JBF-JSSX Joint Session
薬物相互作用リスク評価におけるバイオマーカーの活用とそのバイオアナリシス
Utilizing Endogenous biomarkes for drug-drug interaction evaluation and its bioanalysis

薬物トランスポーターの内在性基質を用いた薬物相互作用リスクの評価
Assessment of drug-drug interaction risks using endogenous substrates of drug transporters

東京大学大学院薬学系研究科

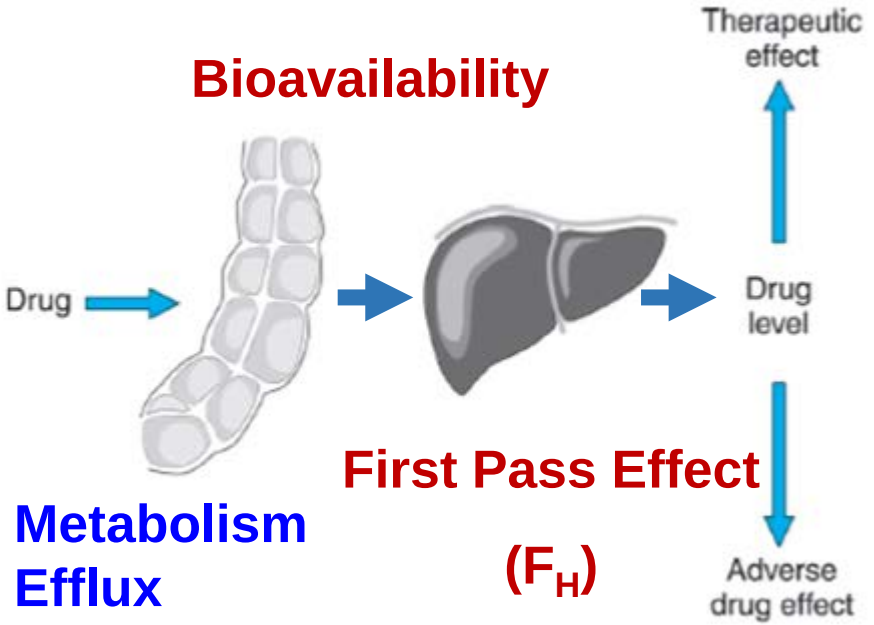
Graduate School of Pharmaceutical Sciences, the University of Tokyo

楠原　洋之/Hiroyuki Kusuhara

Factors determining pharmacokinetic properties of oral drugs

Absorption

Bioavailability



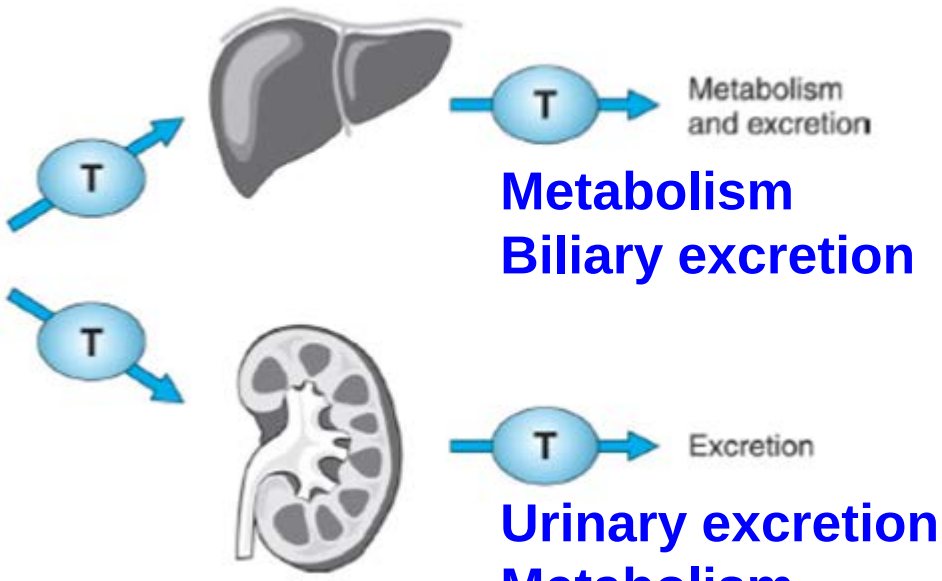
First Pass Effect

(F_H)

Metabolism
Efflux

Elimination

CL (clearance)



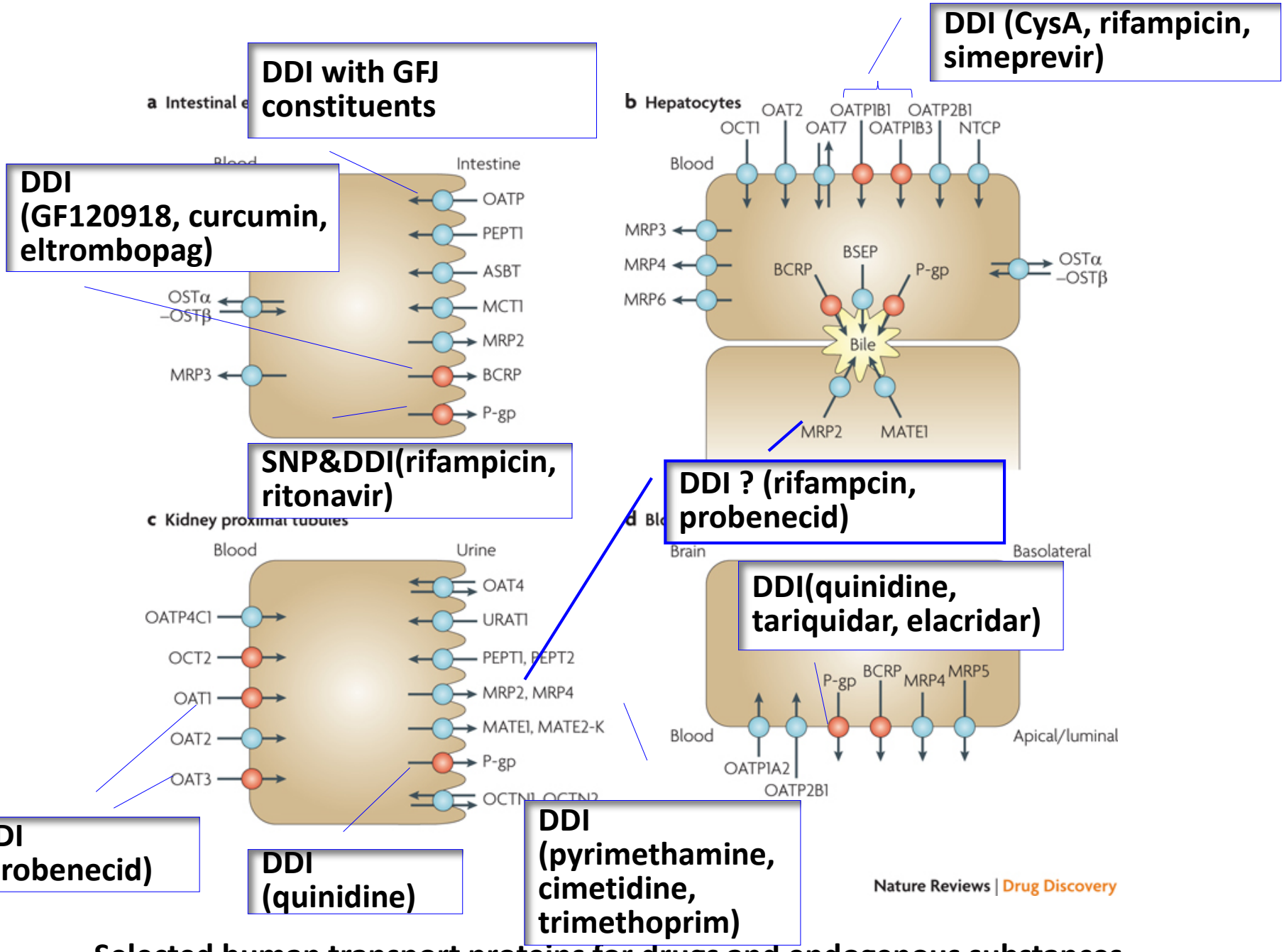
Metabolism
Biliary excretion

Urinary excretion
Metabolism

Absorption rates

Elimination rates

Activities of drug metabolizing enzymes and transporters are determinant of the fraction absorbed into the blood circulation, and clearance.

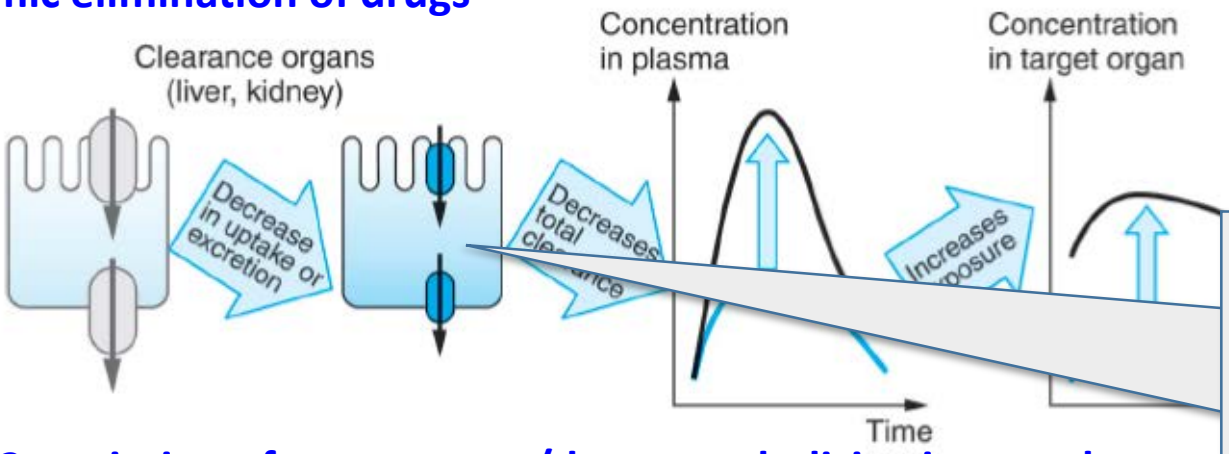


Selected human transport proteins for drugs and endogenous substances.

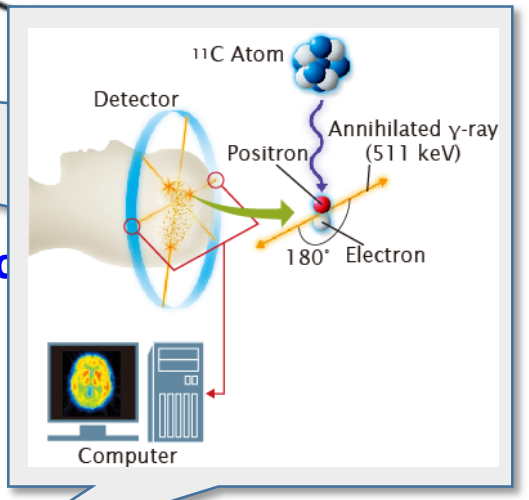
Giacomini KM. et al Nature Reviews Drug Discovery 9, 215-236 (March 2010)

Impact of drug-drug interactions involving transporters and drug metabolizing enzymes on the pharmacokinetics of drugs

Case 1. Variation of transporters/drug metabolizing enzymes involved in the absorption and systemic elimination of drugs

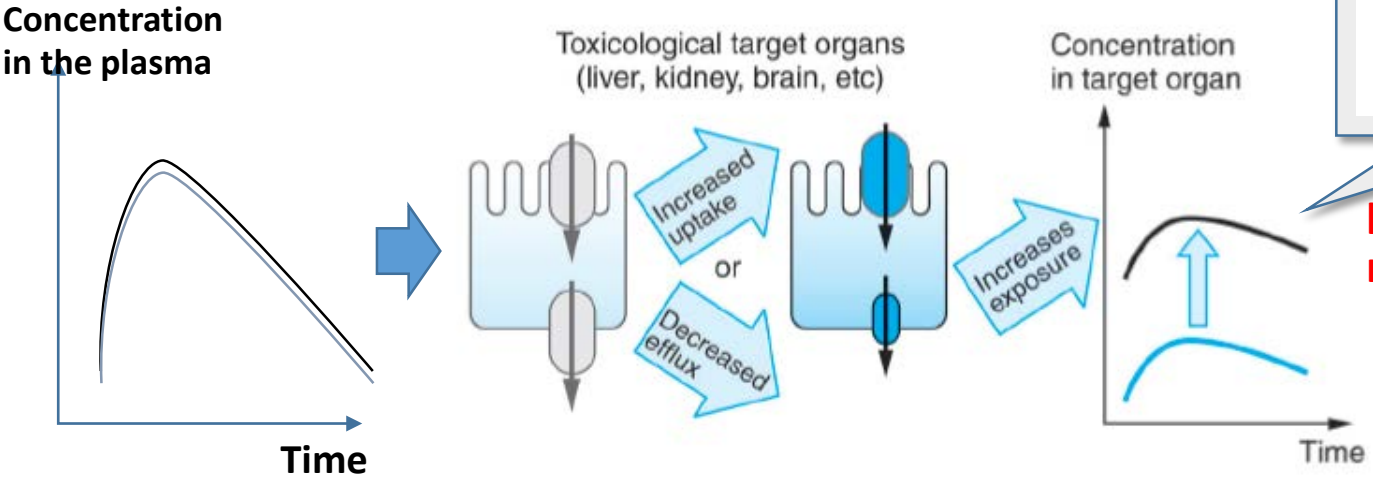


pharmacological effect↑
risk of adverse reaction↑



Case 2. variation of transporters/drug metabolizing in non-clearanc

plasma concentration is similar, however, tissue concentrations



pharmacological effect↑
risk of adverse reaction↑

Drug Development and Drug Interactions (FDA, EMA, MHLW)

OVERVIEW

Drug-drug interactions can lead to changed systemic exposure, resulting in variations in drug response of the co-administered drugs. In addition to co-administration of other drugs, concomitant ingestion of dietary supplements or citrus fruit or fruit juice could also alter systemic exposure of drugs, thus leading to adverse drug reactions or loss of efficacy. Therefore, **it is important to evaluate potential drug interactions prior to market approval as well as during the postmarketing period.**

Draft Guidelines from FDA

- **Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications**

  This guidance focuses on the conduct of clinical studies to evaluate the DDI potential of an investigational drug, including:
  (1) the timing and design of the clinical studies;
  (2) the interpretation of the study results;
  (3) the options for managing DDIs in patients.

- **In Vitro Metabolism- and Transporter- Mediated Drug-Drug Interaction Studies Guidance for Industry**

  Evaluating the DDI potential of an investigational new drug involves:
  (1) identifying the 45 principal routes of the drug's elimination;
- (2) estimating the contribution of enzymes and 46 transporters to the drug's disposition;
  (3) characterizing the effect of the drug on enzymes 47 and transporters.

Example of decision on conducting clinical DDI study using probe substrates

OATP1B1 and OATP1B3: The sponsor should conduct studies to determine the inhibition potency (i.e., IC_{50} or K_i) of the investigational drug on the uptake of a known OATP1B1 or OATP1B3 substrate in cells overexpressing the relevant transporter. Because some known OATP1B1/3 inhibitors demonstrate time-dependent inhibition, the sponsor should determine IC_{50} values following pre-incubation with the investigational drug for a minimum of 30 minutes (Amundsen, Christensen, et al. 2010; Gertz, Cartwright, et al. 2013; Izumi, Nozaki, et al. 2015). The investigational drug has the potential to inhibit OATP1B1/3 in vivo if the R value (as described in Figure 6 below) is ≥ 1.1.

Figure 6: Equation to Calculate the Predicted Ratio of the Victim Drug AUC in the Presence and Absence of the Investigational Drug to Determine the Potential to Inhibit OATP1B1/3*

$$R = 1 + ((f_{u,p} \times I_{in,max})/IC_{50}) \geq 1.1$$

R is the predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the inhibitor.
$\mathbf{f_{u,p}}$ is the unbound fraction in plasma.
$\mathbf{IC_{50}}$ is the half-maximal inhibitory concentration.
$\mathbf{I_{in,max}}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver. It is calculated as:

$$I_{in,max} = (I_{max} + (F_a F_g \times k_a \times Dose))/Q_h/R_B$$

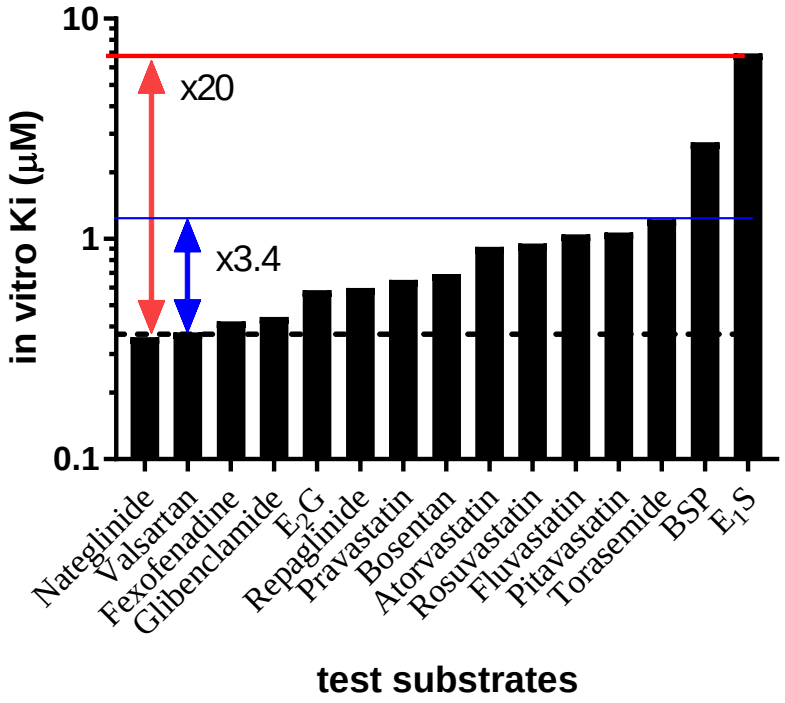
Continued

Summary of in vitro Ki values of rifampicin for OATP1B across studies

Literature information: Geometric mean 1.21 µM (0.27 - 60 µM)

cited from SJ Kim et al DMD, 2016

Ki shows substrate dependence the in vivo relevance of which remains unknown.

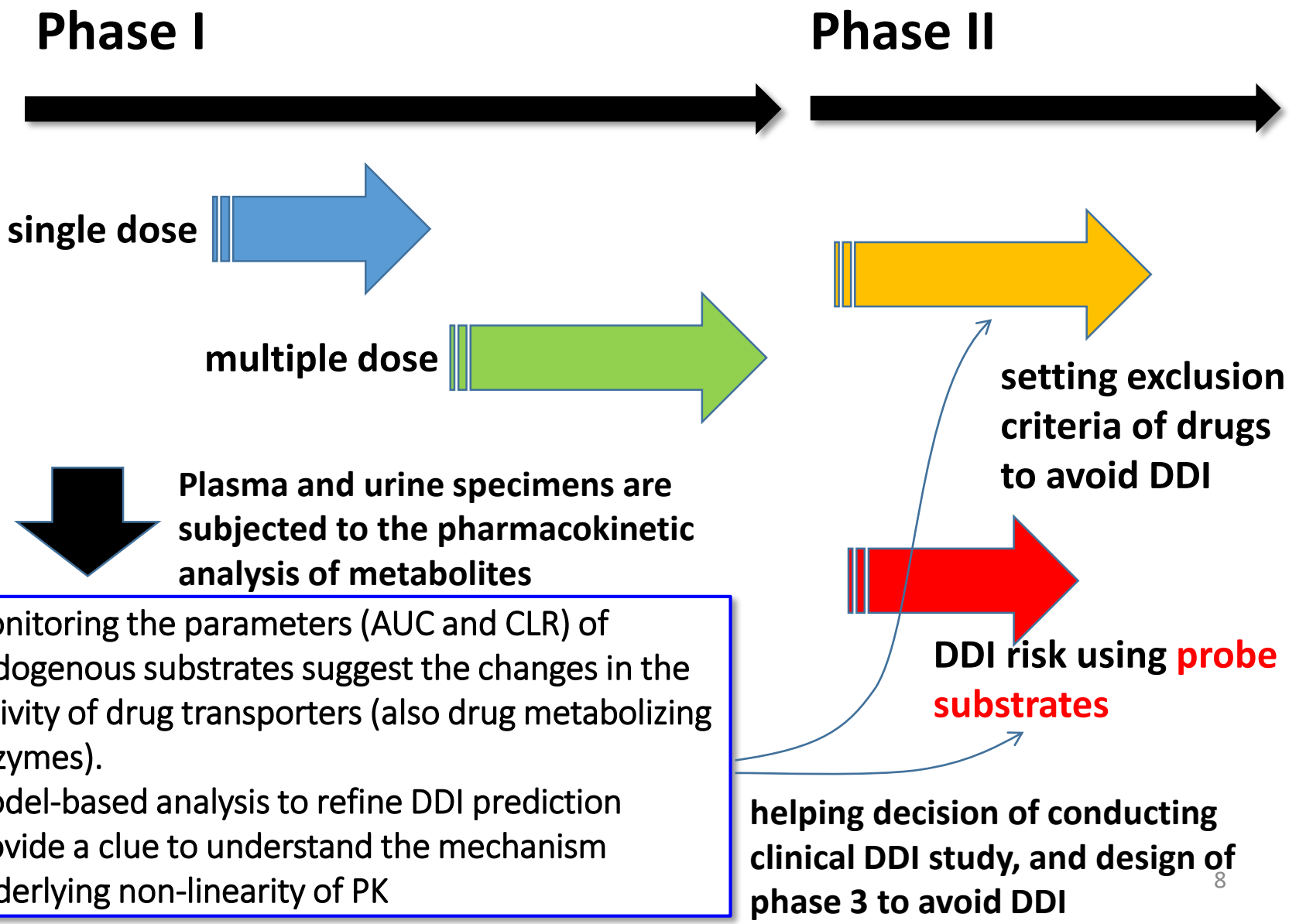


cited from Izumi et al DMD, 2015

cf Model based analysis
Ki 0.13~0.3

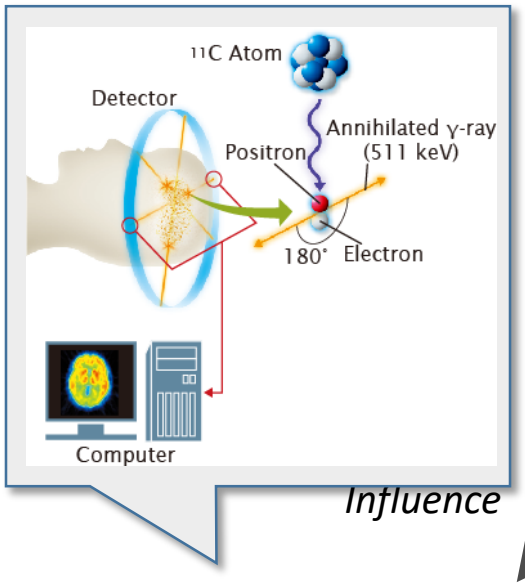
cited from
Yoshikado *et al CPT, 2016 (PTV)*
Barnett S et al., CPT, 2018 (RS and CP-I)

Application 1. Endogenous substrates will suggest DDI risk of multiple transporters in the phase I study



- Monitoring the parameters (AUC and CLR) of endogenous substrates suggest the changes in the activity of drug transporters (also drug metabolizing enzymes).
- Model-based analysis to refine DDI prediction
- Provide a clue to understand the mechanism underlying non-linearity of PK

Transporter Function and Metabolite analysis



Pharmacokinetic Drug-drug interaction

Alteration in the transporter function
(Inhibition or induction, or genetic factors)

$1+I_{u,max}/Ki$ (inhibition)

$I_{u,max}$: maximum inhibitor concentration
Ki: inhibition constant

Influence

Influence

Diagnosis?

PK profiles of drug
(exogenous substance profiles in body fluids)

Predictable?
(Pharmaco-metabonomic Hypothesis)*

Metabolites
(endogenous substance profiles in body fluids)

Inter-subject variation in drug response

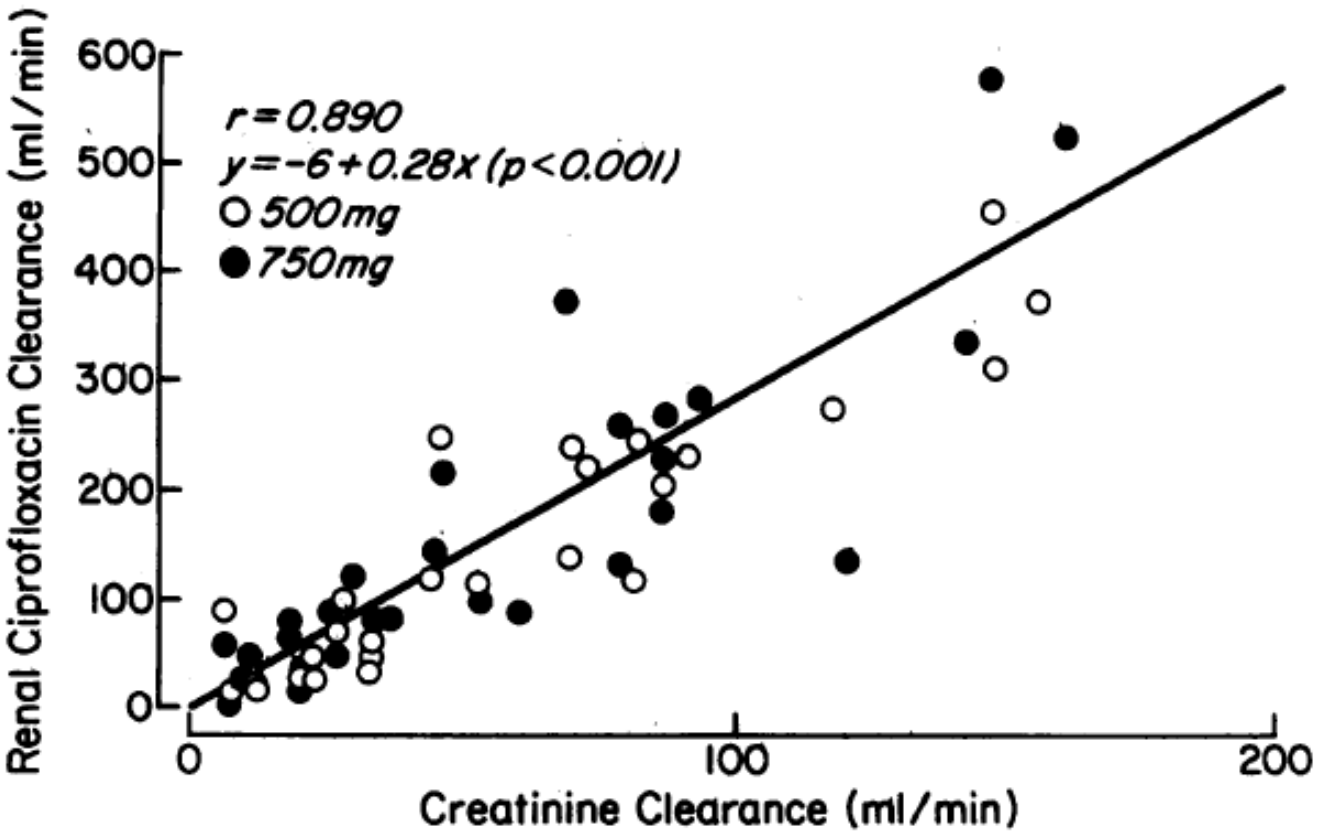
Endogenous or food-derived metabolites that could be substrates may be biomarkers of drug transporters.

Diagnosis of variation of transporter function for personalized medicine

*Clayton TA et al., *Nature* 440, 1073 (2006)

Example of PK biomarker

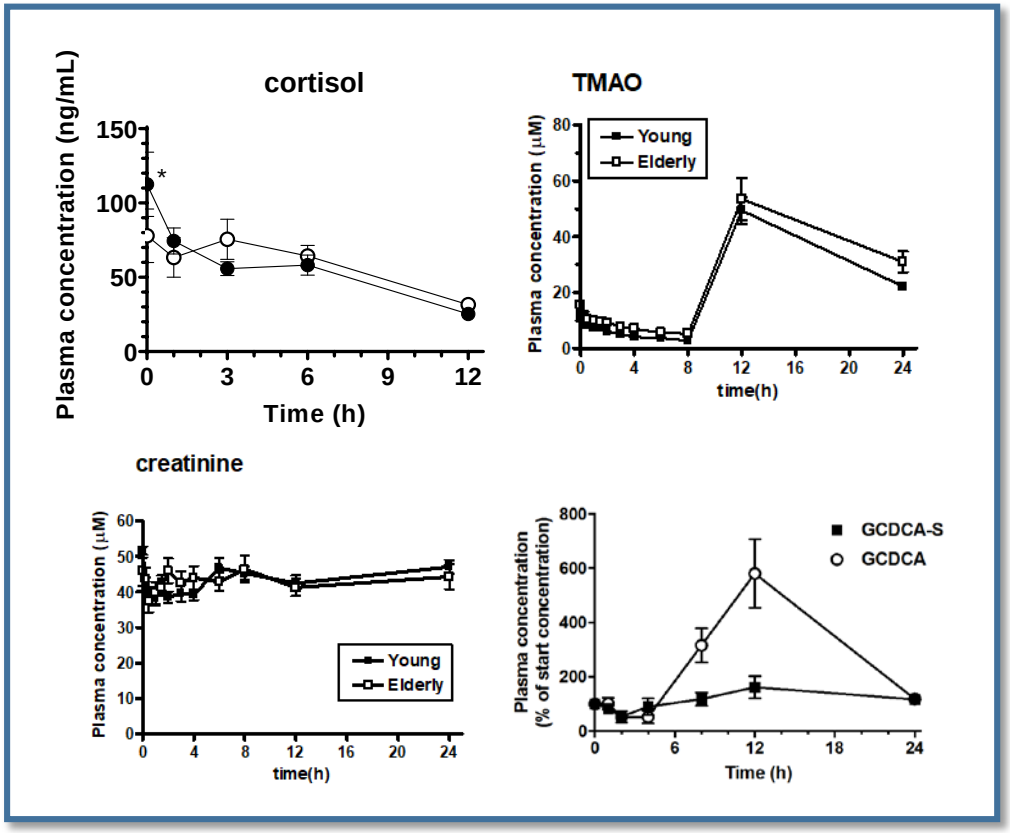
Correlation of renal clearance between ciprofloxacin and creatinine in patients with renal dysfunction



Gasser TC et al Antimicrob Agents Chemother. 1987 May;31(5):709-12.

Requirements for DDI biomarker for hepatic drug transporters

- **High specificity**
  - ✓ Predominant contribution of the drug transporter to the clearance
- **High sensitivity**
  - ✓ Major contribution of hepatic elimination to the systemic elimination
  - ✓ middle-to-low hepatic clearance (below the hepatic blood flow rate)
  - ✓ R_{dif} (contribution of active transport to the net flux)
  - ✓ Sufficiently high synthesis rate
- **Easy, accurate and reproducible for detection**
- **Small diurnal change**
  - ✓ Less inter- and intra-individual variation



Diurnal changes in the plasma concentrations of endogenous metabolites in healthy volunteers

Preclinical and clinical rationale to develop endogenous biomarkers for drug transporters

Preclinical rationale

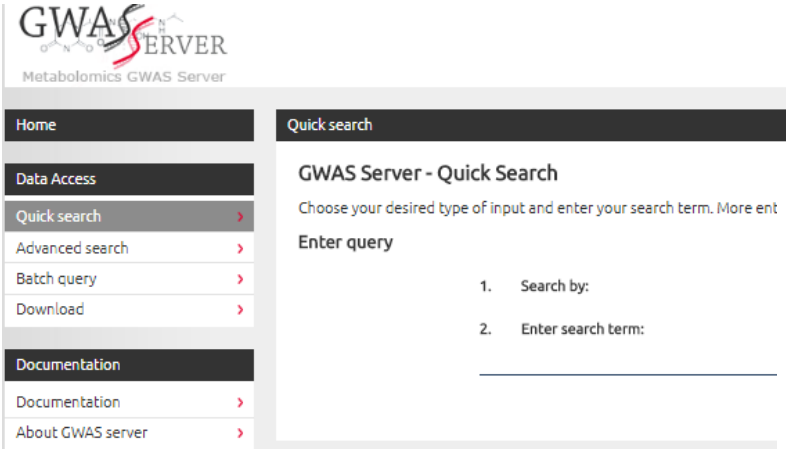
- In vitro transport experiments: overexpression system, and hepatocytes
- Animal studies: monkey, or human liver xenograft model (DDI study or knockdown)

Clinical rationale

- Pharmacogenomics; steady-state
- DDI study with the inhibitor or inducer in healthy volunteers and patients
  dose response study to find pharmacokinetic parameters sensitive to the transport activities (AUC, CL_R, etc)

Association of genetic mutations with plasma metabolite concentrations

- Clinical DDI study+Metabolomics
- GWAS+Metabolomics (database search)



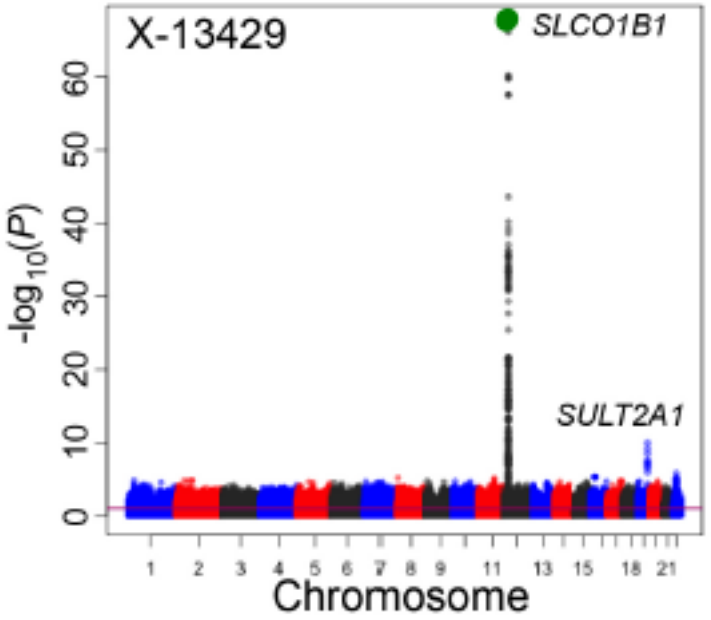
http://mips.helmholtz-muenchen.de/p

Metabolite	internal ID	Effect	Pvalue	Metabolite Links
X-11529	M32846	0.1623	1.442e-74	-
X-11538	M32855	0.0911	5.014e-72	-
X-13429	M35187	0.1025	8.834e-35	-
hexadecanedioate	M35678	0.0688	2.06e-29	hmp KEGG CAS
tetradecanedioate	M35669	0.0681	4.346e-26	hmp CAS
X-11905	M33250	0.0524	2.906e-19	-
X-12063	M33415	0.06	4.419e-19	-
X-12456	M33901	0.0515	4.108e-17	-
octadecanedioate	M36754	0.0387	9.935e-14	hmp CAS
X-11491	M32808	0.058	1.457e-13	-
X-14626	M36553	0.0282	1.971e-13	-
1-arachidonoylglycerophosphoinositol*	M34214	0.0169	9.902e-7	-
taurolithocholate 3-sulfate	M36850	0.0342	2.272e-5	hmp KEGG CAS
4-androsten-3beta,17beta-diol disulfate 2*	M37203	0.0224	8.494e-5	hmp KEGG CAS

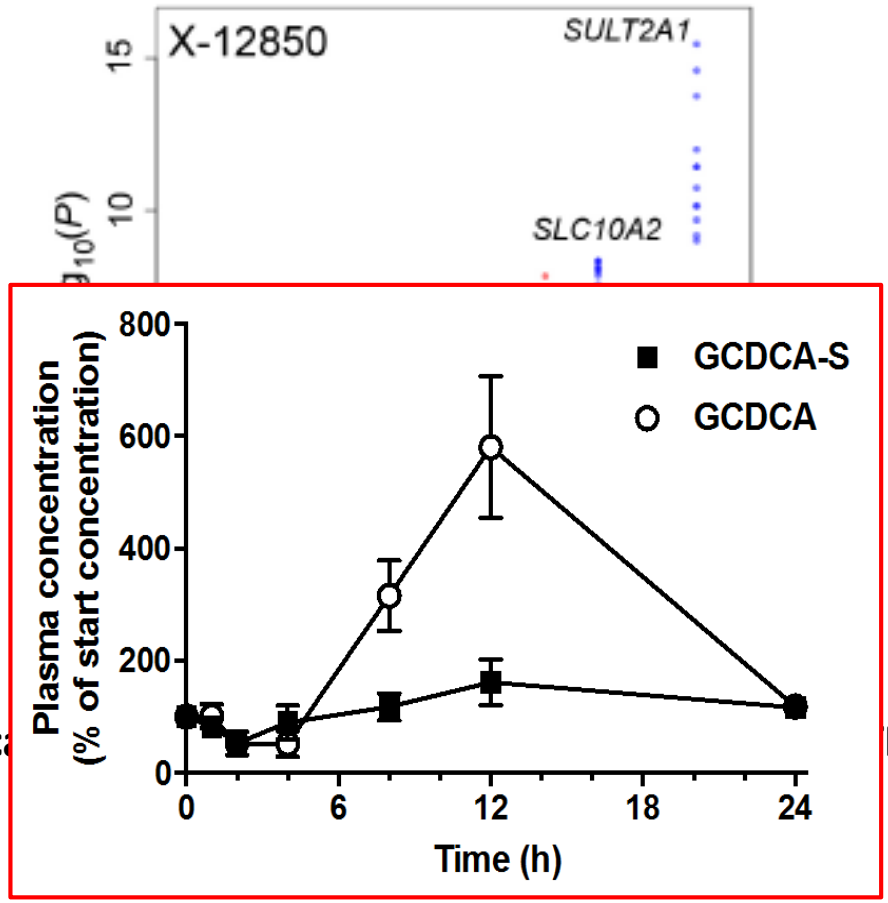
Metabolomic and Genome-wide Association Studies Reveal Potential Endogenous Biomarkers for OATP1B1

Yee et al. CPT, 2016

(ii) GDCA-S or its isomer



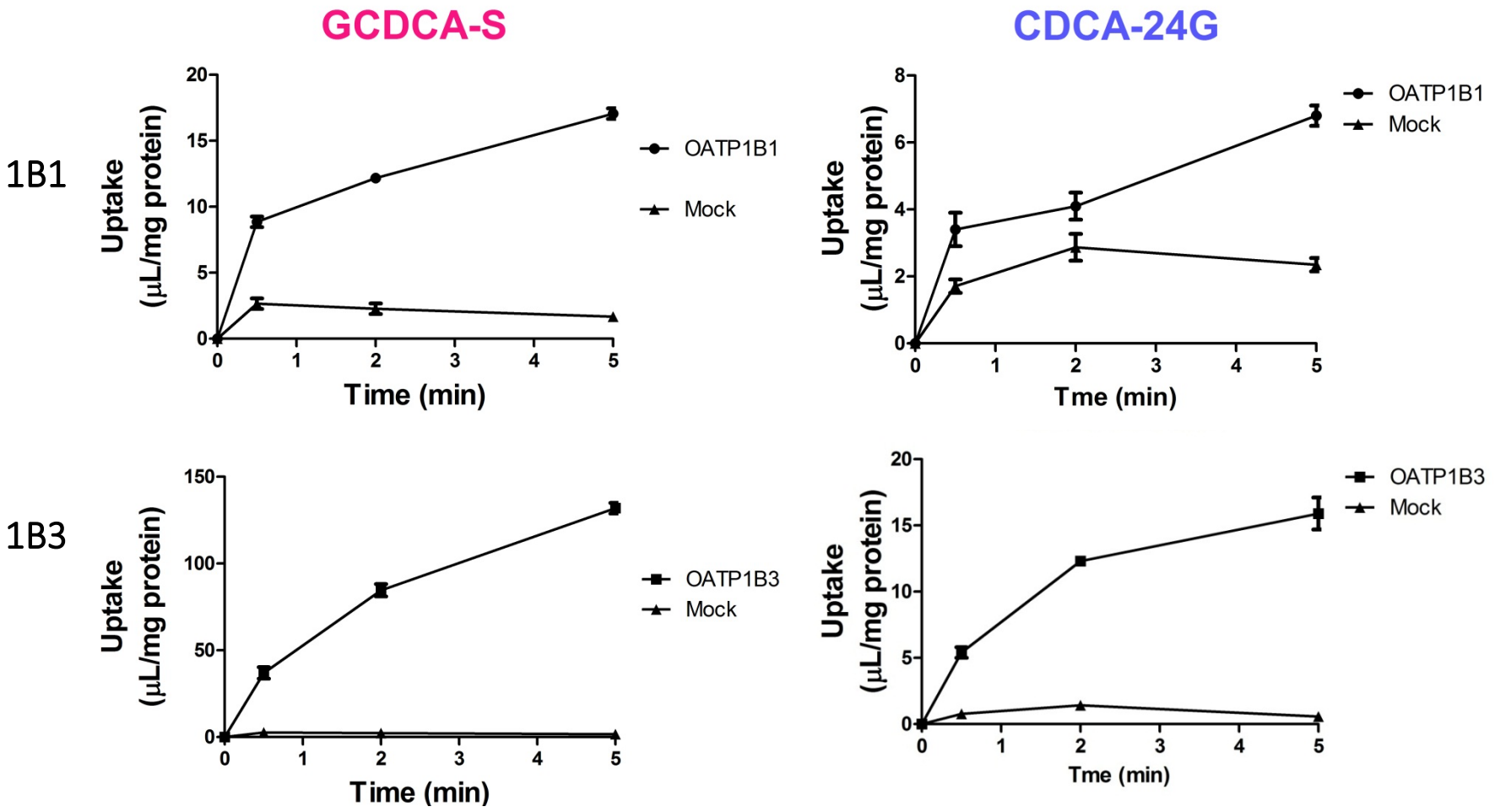
(xi) GCDCA-S or its isomer



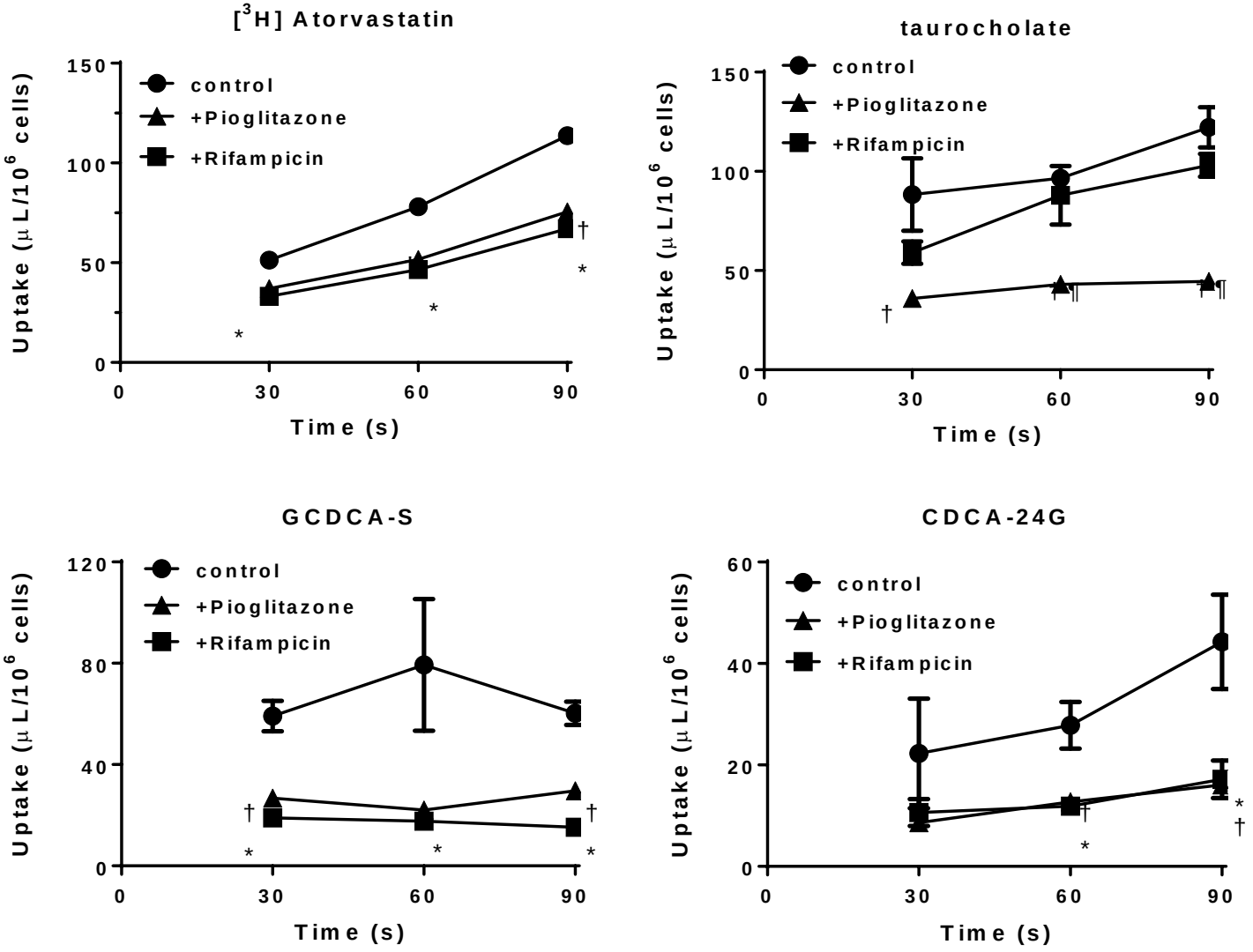
Tetradecanedioate (TDA) & Hexadeca… P1B substrates

In Vitro Transport of GCDCA-S and CDCA-24G by hOATP1Bs and Inhibition by RIF in HEK293 Cells

Time course



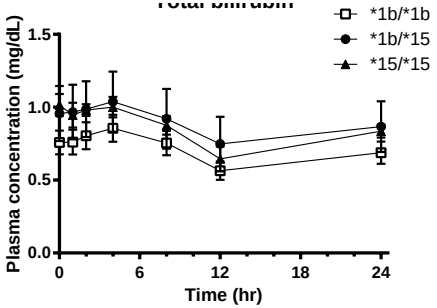
Uptake of of GCDCA-S and CDCA-24G was significantly higher in OATP-expressing cells



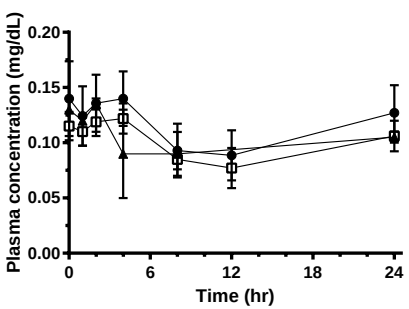
Predominant contribution of OATP1B to the uptake of GCDCA-S and CDCA-24G in cryopreserved human hepatocytes

Effect of OATP1B1 genotypes on the plasma concentrations of endogenous substrates in healthy subjects

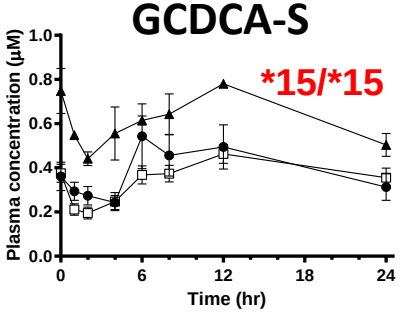
Total bilirubin



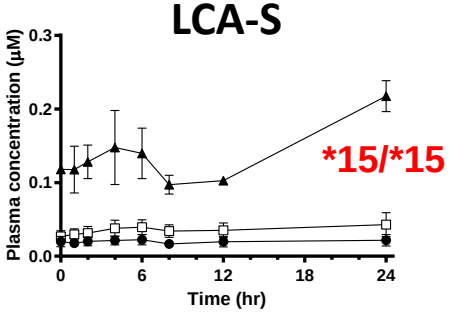
Direct bilirubin



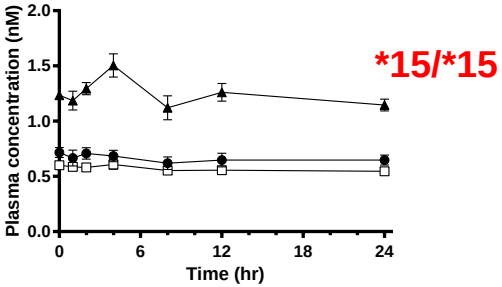
GCDCA-S



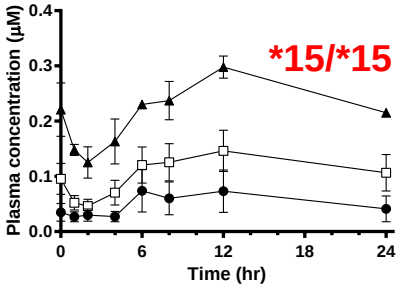
LCA-S



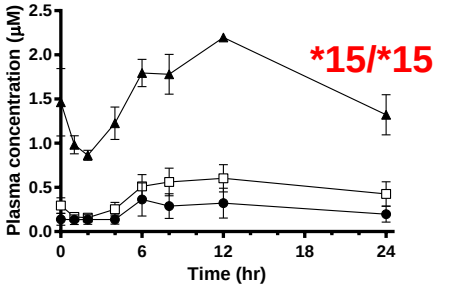
coproporphyrin I



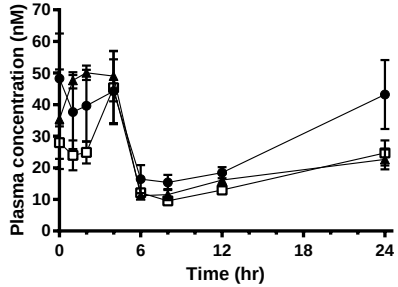
TLCA-S



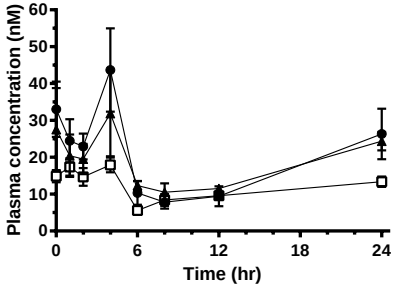
GLCA-S



Tetradecanedioic acid



Dodecanedioic acid



To investigate dose response in the effect of rifampicin on the plasma concentrations of OATP1B substrates (drugs, and endogenous substrates)

Design of clinical DDI study in healthy Japanese subjects

Term1 (Control)

Cocktails
atorvastatin
(OATP1B&CYP3A4)
pitavastatin
(OATP1B&BCRP?)
rosuvastatin
(OATP1B&MRP2, BCRP)
fluvastatin
(OATP1B&CYP2C9)

washout
(>1week)

Term2
(OATP1B inhibition)

Cocktails + **rifampicin**
(300mg p.o.)

washout
(>1week)

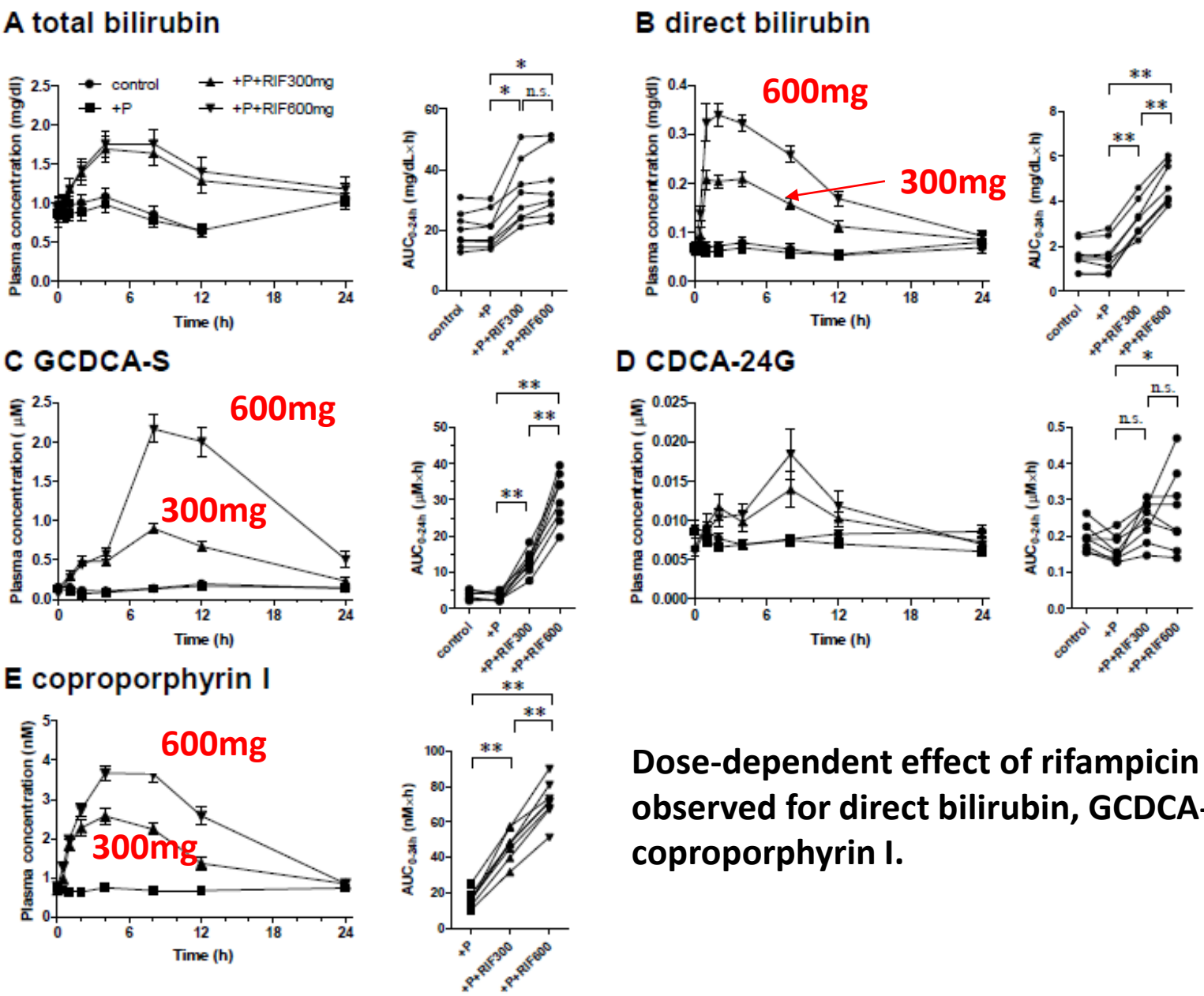
Term3
(OATP1B inhibition)

Cocktails + **rifampicin**
(600mg p.o.)

Subjects number : 8.
The clinical study was conducted in a cross over fashion.

- The protocol of this clinical study was approved by the ethics committees in the RIKEN, Faculty of Pharmaceutical Sciences, the University of Tokyo and P1-clinic.
- Plasma concentrations of drugs, bile acids, and coproporphyrin I were measured by LC-MS/MS.
- Total and direct bilirubins were measured using kit (bilirubin oxidase)

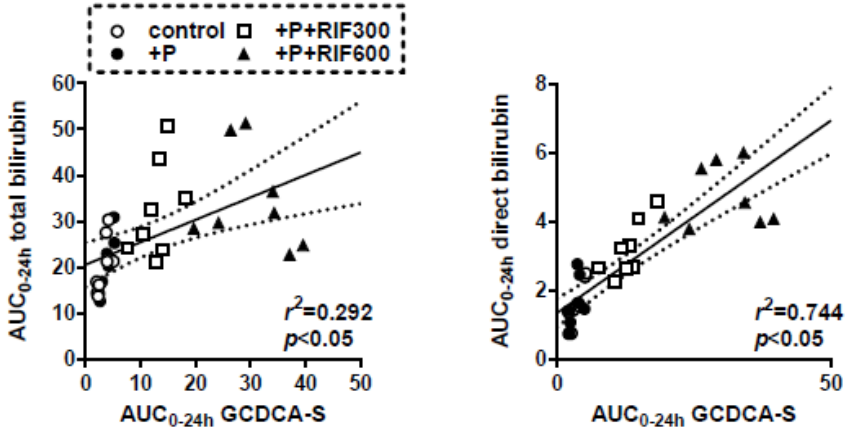
Effect of rifampicin on the endogenous OATP1B substrates in healthy subjects

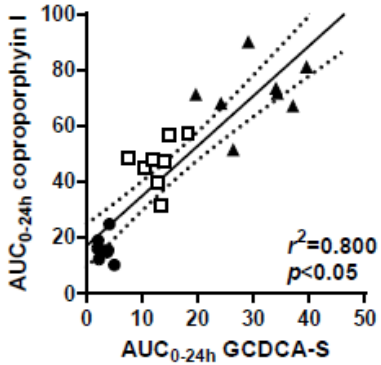


Dose-dependent effect of rifampicin was observed for direct bilirubin, GCDCA-S and coproporphyrin I.

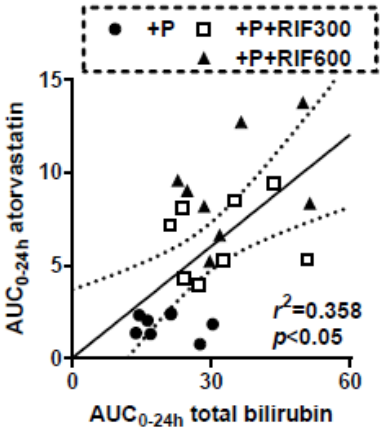
Association of AUC among OATP1B substrates

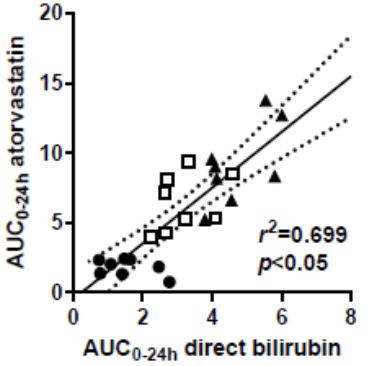
among endogenous substrates

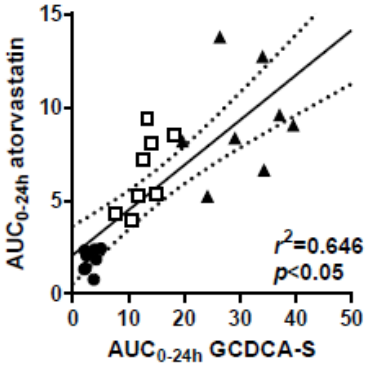


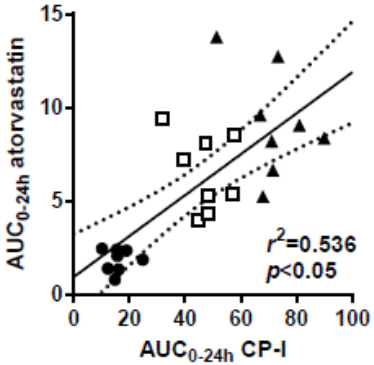


comparison with a probe drug (atorvastatin)



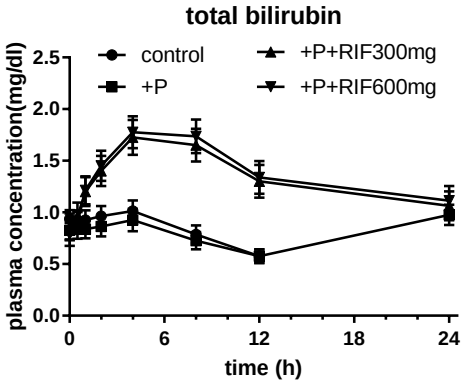


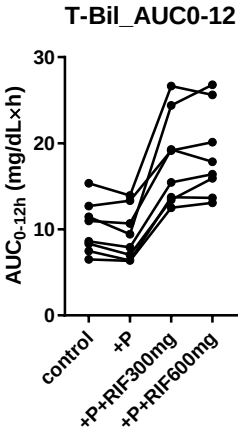




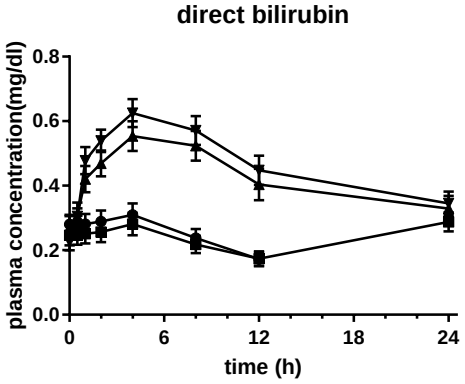
Vanadate oxidase methods

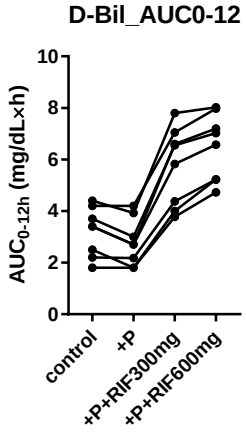
total bilirubin



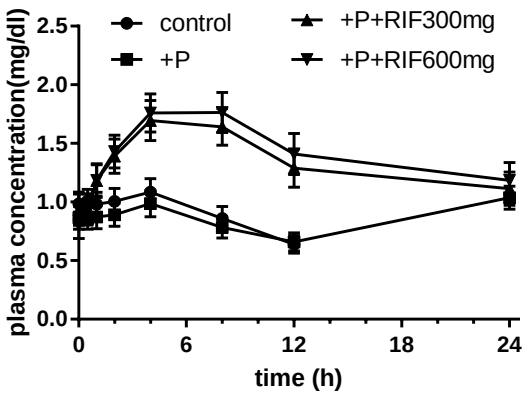


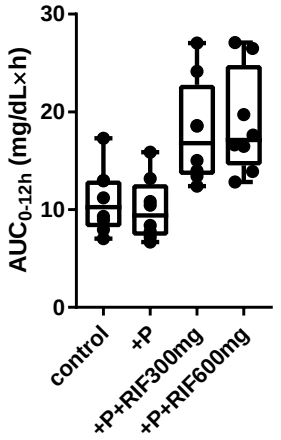
direct bilirubin

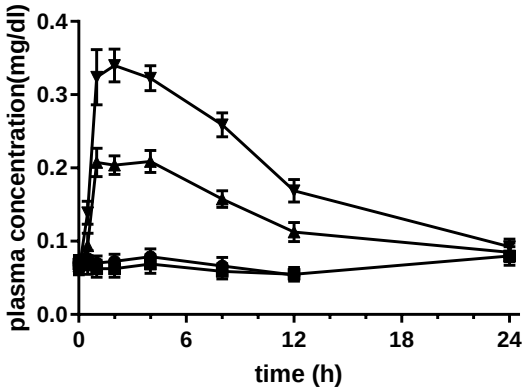


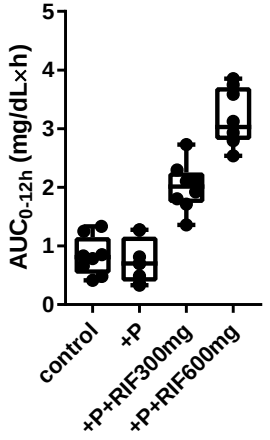


Iatro LQ T-Bil and Iatro LQ D-Bil





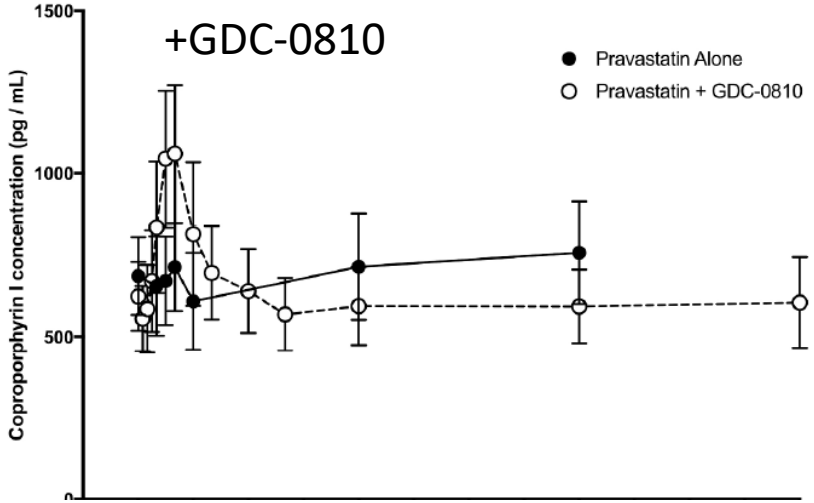




Kits with different principles (chemical reaction versus enzymatic one) provided different profiles of direct bilirubin concentration

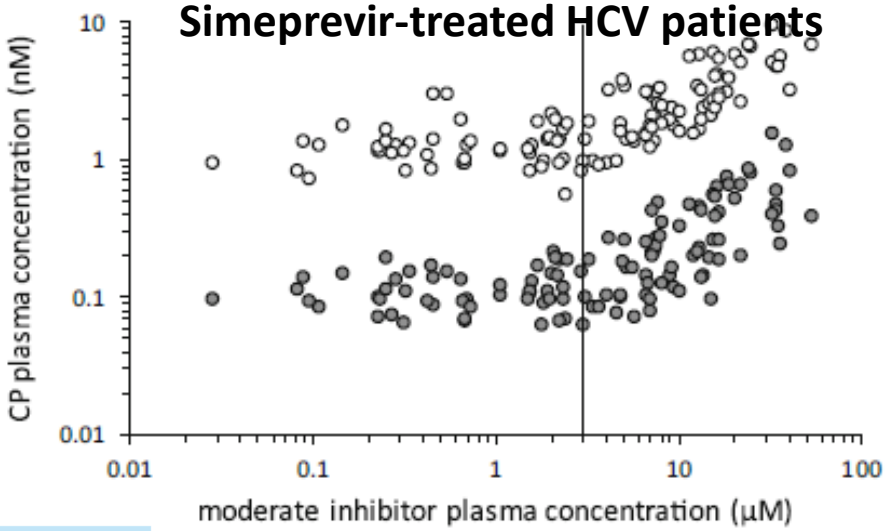
Other clinical studies using CP-I as OATP1B1 biomarker conducted by pharmaceutical industries

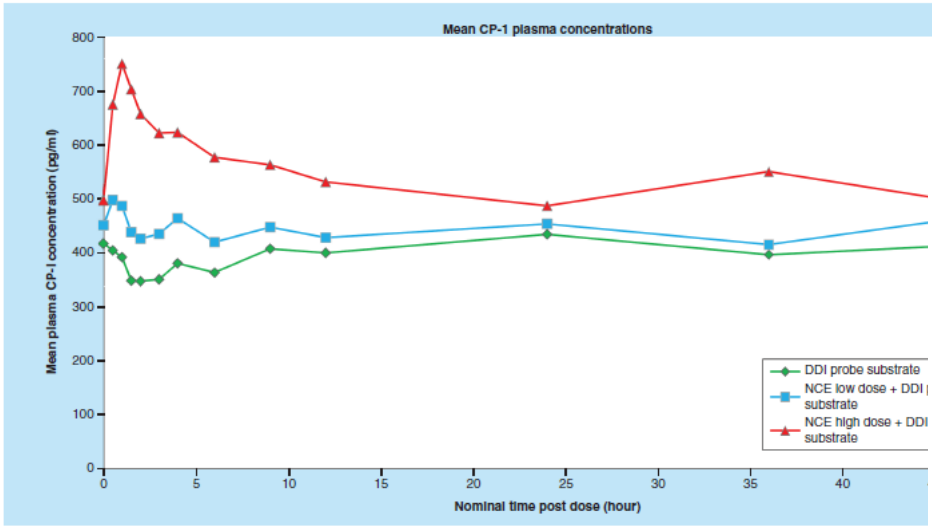
Liu et al JCP, in press



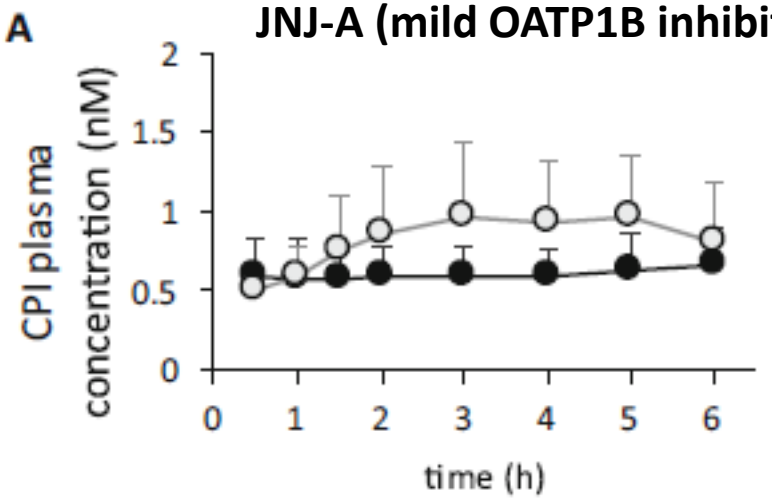
Kunze et al Clin Pharmacokinet, 2018.

Simeprevir-treated HCV patients





JNJ-A (mild OATP1B inhibitor)



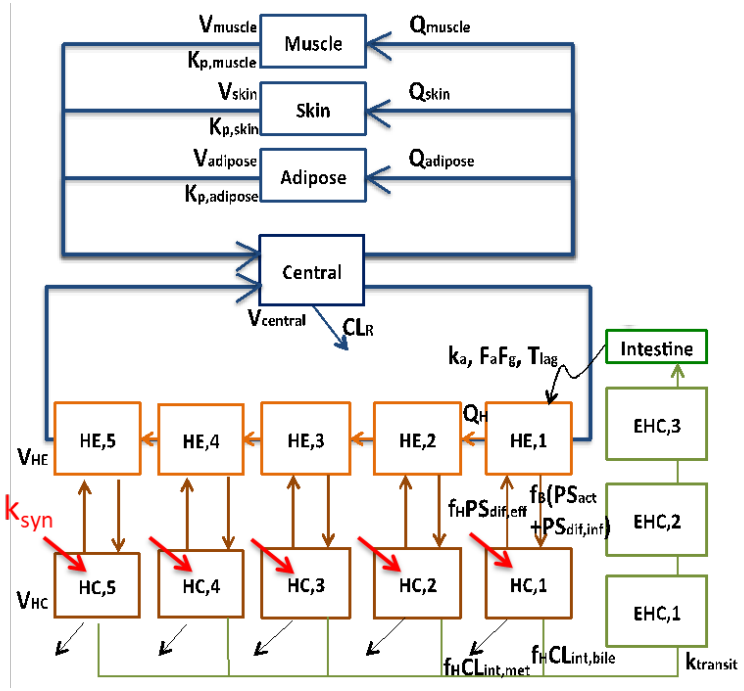
King-Ahmad et al Bioanalysis, in press

Result | PBPK modeling of the interaction between CP-I and rifampicin

We performed simulation of paclitaxel effect on CP-I plasma concentrations.

The PBPK model for CP-I and paclitaxel was constructed in Professor Sugiyama's laboratry.

CP-I



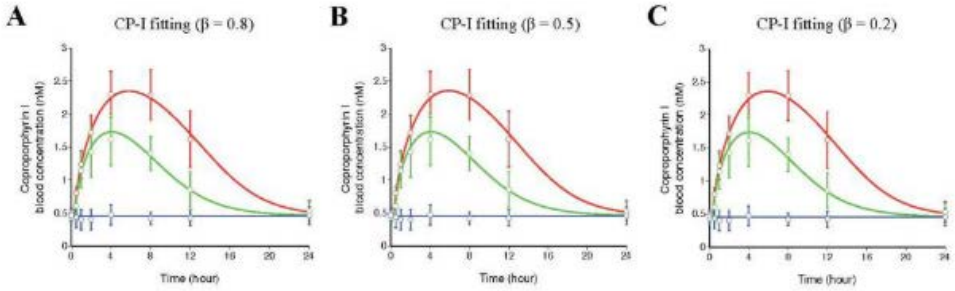


Figure 2. Simultaneously fitted blood concentration–time profiles of CP-I in the absence and presence of RIF after parameter optimization using the PBPK model incorporating the inhibition of OATP1Bs and MRP2.

(T. Yoshikado, et al., CPT PSP, 2018)

Scheme of the workflow for predicting DDI using CP-I as an endogenous biomarker

(I) Phase-1 dose escalation study for a new chemical entity (NCE) to obtain in vivo $K_{i,OATP1Bs}$ using CP-I as an endogenous probe

refine Ki value of test compound that can explain observed value

(II) Calculation of in vivo $K_{i,OATP1Bs}$ for probe substrate drugs (e.g. statins) using in vivo $K_{i,OATP1Bs}$ for CP-I obtained in (I), and in vitro $K_{i,OATP1B}$ for CP-I and probe substrate drugs

$$in\ vivo\ Ki_{OATP1Bs(Drug)} = in\ vivo\ Ki_{OATP1Bs(CPI)} \times \frac{in\ vitro\ Ki_{OATP1Bs(Drug)}}{in\ vitro\ Ki_{OATP1Bs(CPI)}}$$

(III) Prediction of changes in concentration-time profiles, AUC and C_{max} of probe substrate drugs caused by a NCE by PBPK modeling and simulation

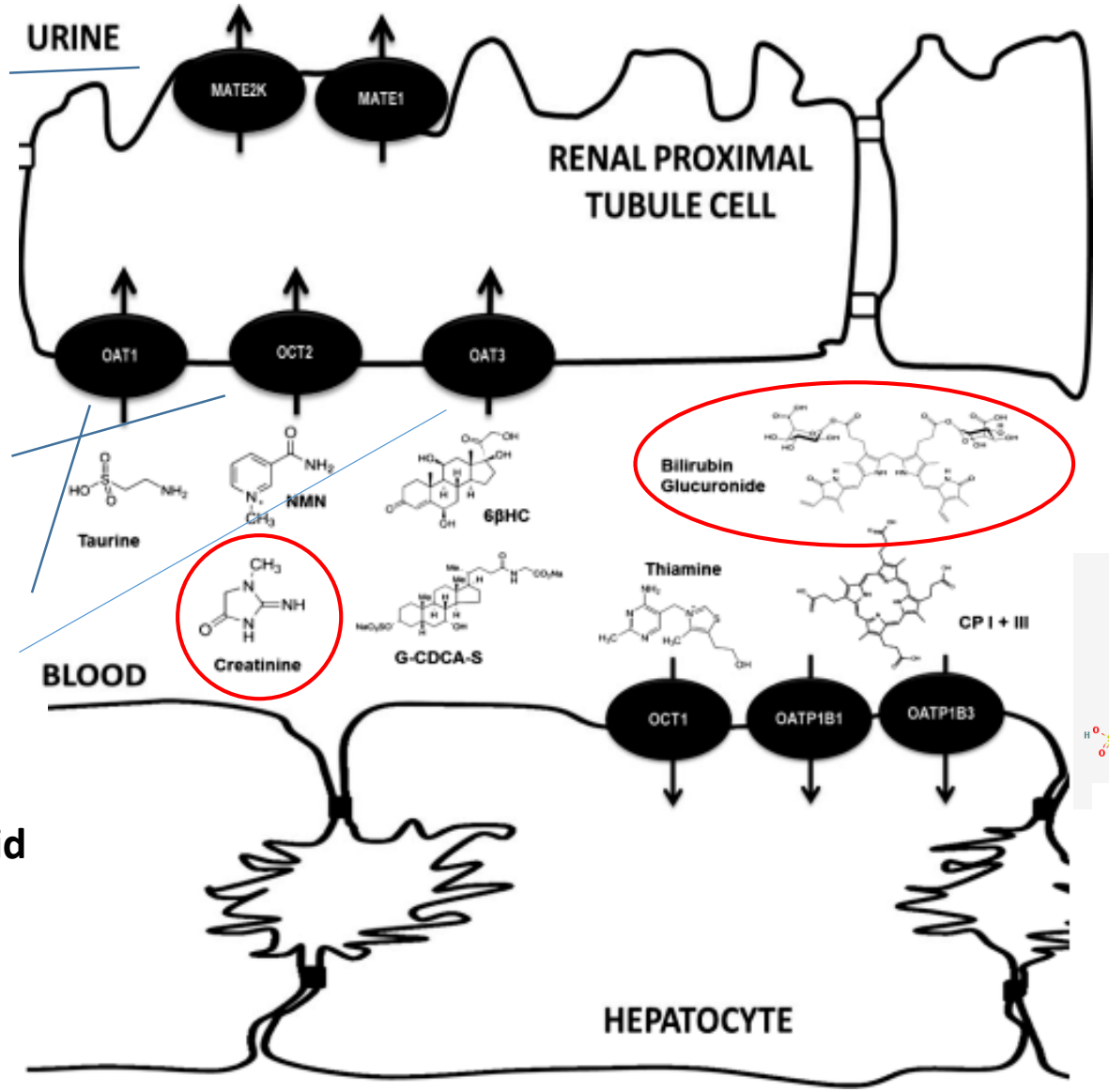
Yoshikado et al under revision

Endogenous Probes for Drug Transporters: Balancing Vision With Reality

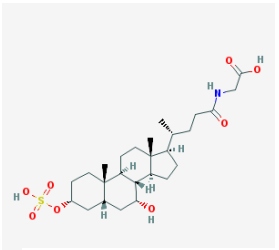
Pyrimethamine
Trimethoprim
Cobicistat
Cimetidine

Doltegravir
DX-619

probenecid



Biochemical test



GCDCA-S

In monkeys, pyridoxic acid homovalinic acid

Rodrigues AD et al CPT, 2017

Summary

- The endogenous substrates serve as surrogate DDI probes for drug transporters (multiplexed analysis of transporter-mediated DDI) in healthy volunteers and patients (with normal liver and kidney function)

  OATP1B1/1B3 : CP-I, direct bilirubin, GCDCA-S
  OCT2：creatinine, *N*-methylnicotinamide, N1-methyladenosine
  MATEs: creatinine, *N*-methylnicotinamide, thiamine, N1-methyladenosine
  OAT1:taurine
  OAT3: 6β-hydroxycortisol, glycochenodeoxycholate sulfate, (CP-III?)
  OCT1:thiamine (needs investigation in humans)
  Red colored compounds: Both plasma concentrations as well as renal clearance can be biomarkers for drug transporters.

- We can exclude the contribution of interindividual difference in oral absorption process by using endogenous substrates (for OATP1B)?
- Effect of inhibition of efflux process (for example, MRP2 inhibition for CP-I, GCDCA-S and direct blirubins) on the plasma concentration time profiles needs to be confirmed.

- **Model-based analysis** will be helpful in prediction of actual drug-drug interaction based on drug-CP-I interaction data (translation).

Acknowledgement:

Dr. Yuichi Sugiyama (Professor emeritus, University of Tokyo, & Sugiyama laboratory, RIKEN)

•Rifampicin Study

University of Tokyo: Kazuya Maeda, Hanano Terashima, Takeshi Nakayama, Daiki Mori, Tadahaya Mizuno

RIKEN: Takashi Yoshikado

Daiichi Sankyo: Issei Takehara, Nobuaki Watanabe, Osamu Ando

Pfizer: Ragu Ramanathan, Amanda J. King-Ahmad, A David Rodrigues

P-one Clinic: Ken-ichi Furihata

•Paclitaxel Study

University of Tokyo: Daiki Mori, Kazuya Maeda

Showa University: Hiroo Ishida, Ken-ichi Fujita, Sojiro Kusumoto, Yasutsuna Sasaki

Patients in Showa University Hospital

•OATP1B1 pharmacogenetic study

University of Tokyo: Daiki Mori, Kazuya Maeda

Kyushu University: Yushi Kashihara, Takeshi Hirota, Ichiro Ieiri

Fukuoka Mirai Hospital Clinical Research Center: Miyuki Kimura, Shunji Matsuki, Shin Irie

• Model based analysis:

RIKEN Sugiyama Laboratory: Takashi Yoshikado, Kota Toshimoto