in Table 3. Considering the unbound concentration, gemfibrozil had the most potent effect on the renal uptake of pravastatin, while the metabolites produced a slight inhibition at the clinical dose of gemfibrozil (Table 3).

Comparison between in vitro Uptake Clearance by Human Hepatocytes and in vivo Hepatic Overall Intrinsic Clearance

The uptake clearance of rosuvastatin, valsartan, olmesartan and candesartan by cryopreserved human hepatocytes was determined ($CL_{uptake,hep}$, μL/min/10^6cells) and found to be as follows: rosuvastatin, 5.21 ± 0.929 and 0.932 ± 1.32; valsartan, 6.53 ± 1.14 and 2.42 ± 0.642; olmesartan, 4.20 ± 0.120 and 2.07 ± 0.0333; candesartan, 15.5 ± 0.743 and 1.98 ± 0.863 at trace and excess concentrations, respectively. The $CL_{uptake,hep}$ of each drug was considerably lower in the presence of an excess of the drug. $CL_{uptake,hep}$ at a trace concentration of drugs determined in this study as well as in the previous studies (total of nine drugs) was scaled up to the in vivo value per body weight with the following physiological scaling factors: 1.2×10^8 cells/g liver and 24.1 g liver/kg body weight (Table 2). The $CL_{uptake,hep}$ values of the nine drugs were similar to or somewhat lower than in vivo hepatic overall intrinsic clearance ($CL_{h,int,all}$) (Fig. 5).

Discussion

We previously reported that hepatic uptake is the rate-determining process in the hepatic elimination of statins in rats and humans, and this likely holds true for the renal elimination of anionic drugs in rats although there are exceptions (Watanabe et al., 2009a; Watanabe et al., 2010a; Watanabe et al., 2010b). These indicate the importance of measuring the uptake clearance for the prediction of hepatic and renal clearance based on in vitro experiments. The present study examined the predictability of renal and hepatic clearance using in vitro tools, such as freshly prepared human kidney slices and cryopreserved human hepatocytes.

The uptake of all drugs tested by kidney slices was saturable (Table 1). The clearance for the saturable uptake of the drugs by kidney slices ($CL_{uptake,slice}$) underestimated the in vivo intrinsic clearance for renal tubular secretion (Fig. 2A). Because the kidney slices consist of multilayered epithelial cells, limited diffusion into the slices from the incubation buffer may cause such an underestimation (Watanabe et al., 2009b). Introducing the scaling factor of 10 for in vitro-in vivo extrapolation (IVIVE), the renal tubular secretion clearance of the test compounds including PAH correlated with the uptake clearance by kidney slices (Fig. 2B). As observed in rats (Watanabe et al., 2009b), these results suggest that the uptake is also rate-determining in the tubular secretion in the human kidney, and IVIVE will help to predict the renal clearance of anionic drugs. Among the tested drugs, valsartan was an outlier of the correlation for some unknown reason (Fig 2), and it was also an outlier in rats (Watanabe et al., 2009b). We speculate that valsartan may undergo reabsorption from the urine to blood, and/or significant basolateral efflux in the tubular secretion. Since it has been reported that the apical transporter, OAT4 interacts with valsartan, OAT4 may mediate the reabsorption of valsartan in the kidney (Yamashita et al., 2006). Because of the large interbatch difference in the uptake of valsartan, further studies are necessary to elucidate the underlying mechanism. Most of the drugs used in the present study were reported to be substrates of human OAT3, but not OAT1 (Fujino et al., 2005; Nakagomi-Hagihara et al., 2007; Windass et al., 2007; Yamada et al., 2007).

PAH is the only OAT1-specific substrate in the present study, and thus, further studies are necessary to elucidate the predictability of the tubular secretion of OAT1 substrates using kidney slices and to examine whether the scaling factor of 10 can be applied to OAT1- and OCT2 substrates.

The variations in the uptake activity in the major elimination organs greatly affect the systemic exposure (Kusuhara and Sugiyama, 2009). Inhibition of uptake has been reported as the underlying mechanism of drug-drug interactions (DDI) in the renal elimination (Tahara et al., 2006a; Tahara et al., 2006b; Nozaki et al., 2007b; Matsushima et al., 2009). In the present study, we investigated the mechanism of the DDI in the kidney between pravastatin and gemfibrozil using human kidney slices. The contribution of OAT-mediated uptake was examined using PAH and benzylpenicillin as inhibitors of OAT1 and OAT3. Benzylpenicillin was a more potent inhibitor of pravastatin uptake by human kidney slices than PAH (Fig. 3), suggesting that OAT3 is the main transporter (Nozaki et al., 2007a). This is consistent with a previous report which shows that OAT3, but not OAT1, can accept pravastatin as a substrate (Nakagomi-Hagihara et al., 2007). Consistent with a previous report, not only gemfibrozil but also its metabolites, gemfibrozil-M3 and gemfibrozil-glucuronide, could inhibit the uptake of pravastatin by human kidney slices with Ki values similar to those for OAT3 (3.4 μM, 2.7 μM, and 9.9 μM) (Nakagomi-Hagihara et al., 2007) (Table 3). The unbound plasma maximum concentrations ($C_{max,u}$) of gemfibrozil and its glucuronide at clinical dosages (600 mg twice a day) (Okerholm et al., 1976; Backman et al., 2002; Nakagomi-Hagihara et al., 2007) were enough to cause significant inhibition of pravastatin uptake (R values were 0.60-0.69 and 0.8, respectively), whereas that of gemfibrozil-M3 was not high enough (the R value was 0.93). Therefore, inhibition of OAT3 by gemfibrozil and gemfibrozil glucuronide could account for the DDI. It is worth mentioning that the inhibition by gemfibrozil glucuronide might appear to be biphasic (Figure 4C) although such biphasic inhibition was not observed in OAT3-transfected cells (Nakagomi-Hagihara et al., 2007). Further studies are necessary to elucidate the mechanism

underlying this discrepancy. Today, there is a growing interest in the safety of metabolites. The FDA has issued Human Metabolites in Safety Testing (MIST), guidance to assure the safety of major drug metabolites. Caution must be paid to the incidence of DDI caused by drug metabolites.

Taken together, freshly prepared kidney slices serve to predict the renal clearance, and DDI quantitatively. Figure 5 shows the reliability of the prediction of hepatic clearance of anionic drugs using cryopreserved human hepatocytes where the extrapolated hepatic uptake clearance of most of anionic drugs was comparable with the overall hepatic intrinsic clearance. Rosuvastatin as well as fluvastatin was a outlier of the correlation (Figure 5), and this holds true in rats by unknown reason (Watanabe et al., 2009b). As reported previously for fluvastatin (Watanabe et al., 2010), rat scaling factor may be able to improve the prediction. Unfortunately, because the hepatic elimination of rosuvastatin is blood-flow limited, the scaling factor could not be precisely obtained by rat studies. Taken together, in vitro uptake studies using human hepatocytes often lead to accurate prediction, however, it is recommended to perform IVIVE in animal study to obtain highly reliable prediction. In vitro transport experiments would be able to predict the elimination pathway of anionic drugs from the systemic circulation in early stage of drug development.

In conclusion, the present study suggests that hepatic and renal clearances of anionic drugs are predictable based on in vitro uptake studies using human cryopreserved hepatocytes and kidney slices. Moreover, kidney slices can be used for predicting clinically relevant DDI caused by alterations in renal uptake activity. These in vitro experimental systems will be applicable to drug discovery and development studies for the prediction of organ clearances in the liver and kidney.

Authorship Contributions

Participated in research design: Ta Watanabe, To Watanabe, Kusuhara, Maeda and Sugiyama.

Conducted experiments: Ta Watanabe, To Watanabe, Debori, kondo, Nakayama and Horita.

Contributed new reagents or analytic tools: Ogilvie, Parkinson and Hu.

Performed data analysis: Ta Watanabe, To Watanabe and Debori.

Wrote or contributed to the writing of the manuscript: Ta Watanabe, Kusuhara, Ogilvie, Parkinson and Sugiyama.

Other: Kusuhara and Sugiyama acquired funding for the research.

References

Adolph EF (1949) Quantitative Relations in the Physiological Constitutions of Mammals. *Science* **109**:579-585.

Backman JT, Kyrklund C, Neuvonen M and Neuvonen PJ (2002) Gemfibrozil greatly increases plasma concentrations of cerivastatin. *Clin Pharmacol Ther* **72**:685-691.

Boxenbaum H (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. *J Pharmacokinet Biopharm* **10**:201-227.

Chiba M, Ishii Y and Sugiyama Y (2009) Prediction of hepatic clearance in human from in vitro data for successful drug development. *Aaps J* **11**:262-276.

Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res.* **10**:1093-1095.

Fujino H, Saito T, Ogawa S and Kojima J (2005) Transporter-mediated influx and efflux mechanisms of pitavastatin, a new inhibitor of HMG-CoA reductase. *J Pharm Pharmacol* **57**:1305-1311.

Hasannejad H, Takeda M, Taki K, Shin HJ, Babu E, Jutabha P, Khamdang S, Aleboyeh M, Onozato ML, Tojo A, Enomoto A, Anzai N, Narikawa S, Huang XL, Niwa T and Endou H (2004) Interactions of human organic anion transporters with diuretics. *J Pharmacol Exp Ther* **308**:1021-1029.

Hasegawa M, Kusuhara H, Endou H and Sugiyama Y (2003) Contribution of organic anion transporters to the renal uptake of anionic compounds and nucleoside derivatives in rat. *J Pharmacol Exp Ther* **305**:1087-1097.

Hirouchi M, Kusuhara H, Onuki R, Ogilvie BW, Parkinson A and Sugiyama Y (2009) Construction of triple-transfected cells [organic anion-transporting polypeptide (OATP) 1B1/multidrug resistance-associated protein (MRP) 2/MRP3 and OATP1B1/MRP2/MRP4] for analysis of the sinusoidal function of MRP3 and MRP4. *Drug Metab Dispos* **37**:2103-2111.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H and Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50**:387-412.

Iwatsubo T, Hirota N, Ooie T, Suzuki H and Sugiyama Y (1996) Prediction of in vivo drug disposition from in vitro data based on physiological pharmacokinetics. *Biopharm Drug Dispos* **17**:273-310.

Kilford PJ, Stringer R, Sohal B, Houston JB and Galetin A (2008) Prediction of drug clearance by glucuronidation from in vitro data: Use of combined P450 and UGT cofactors in alamethicin activated human liver microsomes. *Drug Metab Dispos* **2**:2.

Kyrklund C, Backman JT, Neuvonen M and Neuvonen PJ (2003) Gemfibrozil increases plasma pravastatin concentrations and reduces pravastatin renal clearance. *Clin Pharmacol Ther* **73**:538-544.

Matsushima S, Maeda K, Inoue K, Ohta KY, Yuasa H, Kondo T, Nakayama H, Horita S, Kusuhara H and Sugiyama Y (2009) The inhibition of human multidrug and toxin extrusion 1 is involved in the drug-drug interaction caused by cimetidine. *Drug Metab Dispos* **37**:555-559.

Nakagomi-Hagihara R, Nakai D and Tokui T (2007) Inhibition of human organic anion transporter 3 mediated pravastatin transport by gemfibrozil and the metabolites in humans. *Xenobiotica* **37**:416-426.

Nozaki Y, Kusuhara H, Kondo T, Hasegawa M, Shiroyanagi Y, Nakazawa H, Okano T and Sugiyama Y (2007a) Characterization of the uptake of organic anion transporter (OAT) 1 and OAT3 substrates by human kidney slices. *J Pharmacol Exp Ther* **321**:362-369.

Nozaki Y, Kusuhara H, Kondo T, Iwaki M, Shiroyanagi Y, Nakayama H, Horita S, Nakazawa H, Okano T and Sugiyama Y (2007b) Species difference in the inhibitory effect of nonsteroidal anti-inflammatory drugs on the uptake of methotrexate by human kidney slices. *J Pharmacol Exp Ther* **322**:1162-1170.

Obach RS (1999) Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: An examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab Dispos.* **27**:1350-1359.

Okerholm RA, Keeley FJ, Peterson FE and Glazko AJ (1976) The metabolism of gemfibrozil. *Proc R Soc Med* **69 Suppl 2**:11-14.

Roberts MS and Rowland M (1986) A dispersion model of hepatic elimination: 1. Formulation of the model and bolus considerations. *J Pharmacokinet Biopharm.* **14**:227-260.

Sato M, Iwanaga T, Mamada H, Ogihara T, Yabuuchi H, Maeda T and Tamai I (2008) Involvement of uric acid transporters in alteration of serum uric acid level by angiotensin II receptor blockers. *Pharm Res* **25**:639-646.

Shitara Y, Hirano M, Sato H and Sugiyama Y (2004) Gemfibrozil and its glucuronide inhibit the organic anion transporting polypeptide 2 (OATP2/OATP1B1:SLC21A6)-mediated hepatic uptake and CYP2C8-mediated metabolism of cerivastatin: analysis of the mechanism of the clinically relevant drug-drug interaction between cerivastatin and gemfibrozil. *J Pharmacol Exp Ther* **311**:228-236.

Shitara Y, Itoh T, Sato H, Li AP and Sugiyama Y (2003) Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J Pharmacol Exp Ther* **304**:610-616.

Stringer R, Nicklin PL and Houston JB (2008) Reliability of human cryopreserved hepatocytes and liver microsomes as in vitro systems to predict metabolic clearance. *Xenobiotica.* **38**:1313-1329.

Tahara H, Kusuhara H, Chida M, Fuse E and Sugiyama Y (2006a) Is the monkey an appropriate animal model to examine drug-drug interactions involving renal clearance? Effect of probenecid on the renal elimination of H2 receptor antagonists. *J Pharmacol Exp Ther* **316**:1187-1194.

Tahara H, Kusuhara H, Endou H, Koepsell H, Imaoka T, Fuse E and Sugiyama Y (2005a) A

species difference in the transport activities of H2 receptor antagonists by rat and human renal organic anion and cation transporters. *J Pharmacol Exp Ther* **315**:337-345.

Tahara H, Kusuhara H, Maeda K, Koepsell H, Fuse E and Sugiyama Y (2006b) Inhibition of oat3-mediated renal uptake as a mechanism for drug-drug interaction between fexofenadine and probenecid. *Drug Metab Dispos* **34**:743-747.

Tahara H, Shono M, Kusuhara H, Kinoshita H, Fuse E, Takadate A, Otagiri M and Sugiyama Y (2005b) Molecular cloning and functional analyses of OAT1 and OAT3 from cynomolgus monkey kidney. *Pharm Res* **22**:647-660.

Watanabe T, Kusuhara H, Maeda K, Shitara Y and Sugiyama Y (2009a) Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. *J Pharmacol Exp Ther.* **328**:652-662.

Watanabe T, Maeda K, Kondo T, Nakayama H, Horita S, Kusuhara H and Sugiyama Y (2009b) Prediction of the hepatic and renal clearance of transporter substrates in rats using in vitro uptake experiments. *Drug Metab Dispos* **37**:1471-1479.

Watanabe T, Kusuhara H, Maeda K, Kanamaru H, Saito Y, Hu Z and Sugiyama Y (2010a) Investigation of the rate-determining process in the hepatic elimination of HMG-CoA reductase inhibitors in rats and humans. *Drug Metab Dispos* **38**:215-222.

Watanabe T, Kusuhara H and Sugiyama Y (2010b) Application of physiologically based pharmacokinetic modeling and clearance concept to drugs showing transporter-mediated distribution and clearance in humans. *J Pharmacokinet Pharmacodyn* **37**:575-590.

Windass AS, Lowes S, Wang Y and Brown CD (2007) The contribution of organic anion transporters OAT1 and OAT3 to the renal uptake of rosuvastatin. *J Pharmacol Exp Ther* **322**:1221-1227.

Yamada A, Maeda K, Kamiyama E, Sugiyama D, Kondo T, Shiroyanagi Y, Nakazawa H, Okano T, Adachi M, Schuetz JD, Adachi Y, Hu Z, Kusuhara H and Sugiyama Y (2007)

Multiple human isoforms of drug transporters contribute to the hepatic and renal transport of olmesartan, a selective antagonist of the angiotensin II AT1-receptor. *Drug Metab Dispos* **35**:2166-2176.

Yamaoka K, Tanigawara Y, Nakagawa T and Uno T (1981) A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn.* **4**:879-885.

Yamashita F, Ohtani H, Koyabu N, Ushigome F, Satoh H, Murakami H, Uchiumi T, Nakamura T, Kuwano M, Tsujimoto M and Sawada Y (2006) Inhibitory effects of angiotensin II receptor antagonists and leukotriene receptor antagonists on the transport of human organic anion transporter 4. *J Pharm Pharmacol* **58**:1499-1505.

Legends for Figures

Fig. 1. Time profiles of the uptake of [^{3}H]-rosuvastatin, [^{3}H]-pravastatin, [^{3}H]-pitavastatin, [^{3}H]-valsartan, and [^{3}H]-olmesartan by human kidney slices. The uptake of these drugs was determined at two concentrations at 37°C. Final concentrations of the drugs were as follows: 0.1 μM and 100 uM for rosuvastatin, pitavastatin and valsartan; 0.1 μM and 500 μM for pravastatin; 0.01 μM and 100 μM for olmesartan . Each point represents the mean ± SE (n = 3 slices from one kidney).

Fig. 2. Comparison between the uptake clearance by human kidney slices ($CL_{uptake,slice}$) and the observed renal secretion intrinsic clearance ($CL_{sec,int,all}$) (Panel A) and between the predicted renal secretion clearance ($CL_{sec,\ predicted}$) and the observed one (CL_{sec}) (Panel B). $CL_{sec,int,all}$ was calculated based on Eqs. 1 to 4. $CL_{sec,predicted}$ was estimated from Eqs. 1 to 4, assuming that $CL_{uptake,slice}$ was equal to the renal overall intrinsic clearance. Plots represent the following: 1, rosuvastatin; 2, pravastatin; 3, pitavastatin ; 4, valsartan ; 5, olmesartan; 6, Trichlormethiazide; 7, PAH; 8, fexofenadine; 9, methotrexate; and 10, benzylpenicillin. The solid line and the dashed lines represent the line of unity and the lines of the 1:3 and 3:1 correlations, respectively. Circles and diamonds indicate drugs taken up into the kidney by mainly OAT3 and OAT1, respectively. Triangles show drugs, the dominant transporter of which is unclear.

Fig. 3. Inhibitory effect of PAH and benzylpenicillin on the uptake of pravastatin by human kidney slices. The uptake of [^{3}H]-pravastatin (0.1 μM) was determined in the presence and absence of unlabeled PAH (open columns) and benzylpenicillin (closed columns), and in the presence of excess unlabeled pravastatin (500 μM) for 15 min at 37°C. The values are shown as a percentage of the uptake in the absence of inhibitors and an excess of pravastatin. Each value represents the mean ± SE (n = 6 slices). The absolute uptake clearance of

pravastatin at 0.1 μM in the absence of inhibitors was 2.86 ± 0.11 ml/g kidney/15 min (mean ± SE).

Fig. 4. Inhibitory effect of gemfibrozil and its metabolites, gemfibrozil-M3 and gemfibrozil-glucuronide on the uptake of pravastatin by human kidney slices. The uptake of [^{3}H]-pravastatin (0.1 μM) was determined in the presence and absence of unlabeled gemfibrozil (A), gemfibrozil-M3 (B) and gemfibrozil-glucuronide (C) for 15 min at 37°C. The values are shown as a percentage of the uptake in the absence of inhibitors. Each value represents the mean ± SE (n = 6 slices). The absolute uptake clearance of pravastatin at 0.1 μM in the absence of inhibitors was 2.86 ± 0.11 ml/g kidney/15 min (mean ± SE). Dashed lines represent the ratio of the uptake of [^{3}H]-pravastatin determined in the presence of excess unlabeled pravastatin to that in the absence of excess pravastatin.

Fig. 5. Comparison between the uptake clearance by hepatocytes ($CL_{uptake,hep}$) and the observed hepatic overall intrinsic clearance ($CL_{h,int,all}$)

Plots represent the following: 1, rosuvastatin; 2, pravastatin; 3, valsartan; 4, olmesartan; 5, candesartan; 6, pitavastatin; 7, atorvastatin; 8, fluvastatin and 9, cerivastatin. The solid line and the dashed lines represent the line of unity and the lines of 1:3 and 3:1 correlations, respectively.

Table 1. Uptake clearance of drugs by human kidney slices

	Batch Nos.	uptake of test drug			normalized value [a]	$CL_{uptake,slice}$ [b]
		tracer (T)	excess (E)	T-E		
		ml/g kidney/15 min				ml/min/kg
rosuvastatin	1	8.02 ± 1.55	2.69 ± 0.06	5.33 ± 1.55	11.9	3.30
pravastatin	2	2.86 ± 0.11	0.84 ± 0.05	2.02 ± 0.12	1.77 ± 0.13	0.447
	3	2.62 ± 0.31	0.76 ± 0.04	1.86 ± 0.31	1.68 ± 0.38	
	4	2.15 ± 0.10	0.71 ± 0.03	1.43 ± 0.10	1.71 ± 0.27	
	5	2.05 ± 0.40	1 ± 0.12	1.05 ± 0.42	1.29 ± 0.23	
pitavastatin	6	20.6 ± 1.36	15.3 ± 1.52	5.27 ± 2.04	2.55	0.957
	8	15.3 ± 2.34	11.8 ± 1.04	3.50 ± 2.56	4.36 ± 0.24	
valsartan	1	8.28 ± 1.06	2.34 ± 0.34	5.94 ± 1.11	13.3	2.38
	7	6.38 ± 1.88	3.34 ± 0.12	3.04 ± 1.89	3.88 ± 0.24	
olmesartan	6	16.0 ± 0.72	1.67 ± 0.11	14.3 ± 0.73	6.93 ± 0.66	3.13
	7	13.9 ± 1.59	1.65 ± 0.04	12.2 ± 1.59	15.6 ± 0.76	
trichlormethiazide	3	5.18 ± 0.79	2.12 ± 0.06	3.06 ± 0.79	2.77 ± 0.63	1.01
	4	6.72 ± 0.86	2.94 ± 0.33	3.78 ± 0.92	4.50 ± 0.71	
PAH	2	2.24 ± 0.31	0.55 ± 0.01	1.69 ± 0.31	1.48 ± 0.11	0.482
	3	2.14 ± 0.22	0.60 ± 0.03	1.54 ± 0.22	1.40 ± 0.32	
	4	1.63 ± 0.17	0.63 ± 0.00	1.00 ± 0.17	1.19 ± 0.19	
	5	2.98 ± 0.36	0.74 ± 0.32	2.24 ± 0.49	2.75 ± 0.49	
	6	4.98 ± 0.21	1.12 ± 0.06	3.86 ± 0.22	1.87 ± 0.18	
fexofenadine	(1)	2.60 ± 0.29	0.91 ± 0.21	1.68 ± 0.36	1.25 ± 0.19	0.347
methotrexate	(2)	1.26	0.51	0.75	0.61	0.168
benzylpenicillin	1	1.56 *	0.74 *	0.82 *		0.508
	2	2.70 ± 0.15	0.61 ± 0.02	2.09 ± 0.15		
	3	2.71 ± 0.46	0.69 ± 0.07	2.02 ± 0.46		
	4	2.21 ± 0.24	0.68 ± 0.00	1.54 ± 0.24	1.72[c] ± 0.09	
	5	2.00 ± 0.11	0.51 ± 0.24	1.49 ± 0.26		
	6	4.26 ± 0.27	0.46 ± 0.23	3.79 ± 0.36		
	7	2.17 ± 0.07	0.74 ± 0.02	1.43 ± 0.07		
	8	2.31 *	0.84 *	1.47 *		

Uptake clearance of drugs by human kidney slices was determined at 37°C at two (trace and excess) concentrations. Final concentrations of the drugs were as follows: 0.1 μM and 100 μM for rosuvastatin, pitavastatin and valsartan; 0.1 μM and 500 μM for pravastatin; 0.01 μM and 100 μM for olmesartan; 0.1 μM and 1000 μM for PAH; 1 μM and 1000 μM for benzylpenicillin; 10 μM and 1000 μM for trichlormethiazide. The uptake clearance was calculated by dividing

the uptake volume at 15 min by the drug concentration in the incubation buffer. Each value represents the mean ± SE (n = 3 slices). Benzylpenicillin was used as the standard compound throughout the study for normalization of the uptake of the test drugs by different batch of kidney slices.

[a] Uptake of test drugs was corrected by the ratio of the saturable uptake of benzylpenicillin in the same batch of human kidney to the average value (1.83)

[b] Normalized uptake clearance were scaled up to the in vivo value per body weight using the following physiological scaling factors, 4.43 g kidney/kg body weight (Davies and Morris, 1993). Average values were used for the extrapolation for rosuvastatin, pravastatin, pitavastatin, valsartan, olmesartan, trichlormethiazide and PAH.

[c] average value used for the normalization

* average value of two slices

(1) (Matsushima et al., 2009) T-E of benzylpenicillin was 2.47 ml/g kidney/15 min

(2) (Nozaki et al., 2007b) T-E of benzylpenicillin was 2.26 ml/g kidney/15 min

Table 2. Kinetic parameters of drugs regarding hepatic and renal clearance.

	$CL_{uptake,hep}$	$CL_{uptake,slice}$ [a]	f_u [b]	R_B [b]	f_B	$CL_{h,int,all}$ [b]	$CL_{sec,int,all}$ [b]	Scaling factor [c]	CL_{sec} [b]
	ml/min/kg	*ml/min/kg*				*ml/min/kg*	*ml/min/kg*		*ml/min/kg*
Rosuvastatin	15.1	3.30	0.12	0.69	0.174	104	27.0	8.2	3.92
Pravastatin	13.8 [d]	0.447	0.55	0.56	0.989	23.3	16.6	37	9.47
Pitavastatin	446 [d]	0.957	0.0052	0.58	0.009	411	3.34	3.5	0.03
Valsartan	18.9	2.38	0.04	0.55	0.073	8.95	1.80	0.7	0.129
Olmesartan	12.1	3.13	0.01	0.55	0.018	18.6	11.1	3.5	0.204
Trichlormethiazide	-	1.01	0.29	0.55	0.527	-	10.7	11	4.54
PAH	-	0.482	0.83	0.70	1.19	-	N.A.	N.A.	13.6
Fexofenadine	-	0.347 [f]	0.31	0.55	0.564	-	1.52	4.4	0.658
Methotrexate	-	0.168 [g]	0.37	0.83	0.446	-	1.40	8.3	1.13
benzylpenicillin	-	0.508	0.56	0.66	0.843	-	5.71	11	5.26
Candesartan	44.8	-	0.0027	0.55	0.005	67.2	-	-	-
Atorvastatin	169 [d]	-	0.0511	0.61	0.084	329	-	-	-
Fluvastatin	351 [d]	-	0.0037	0.52	0.007	3760	-	-	-
Cerivastatin	746 [e]	-	0.007	0.58	0.012	485	-	-	-

$CL_{uptake,hep}$, uptake clearance by human hepatocytes; $CL_{uptake,slice}$, corrected saturable uptake clearance by human kidney slices; $CL_{h,int,all}$, observed hepatic overall intrinsic clearance; $CL_{sec,int,all}$, observed renal secretion overall intrinsic clearance; CL_{sec}, observed renal secretion clearance; N.A., not applicable

[a] saturable uptake clearance corrected by uptake of benzylpenicillin. The details of this estimation are described in the text and Table 1.

[b] references cited are listed in the Appendix (Supplemental Table 1).

[c] Scaling factors were calculated by dividing $CL_{sec,int,all}$ by $CL_{uptake,slice}$.

[d] (Watanabe et al., 2010a)

[e] (Shitara et al., 2003)

[f] (Matsushima et al., 2009)

[g] (Nozaki et al., 2007b)

Table 3. Kinetic parameters of gemfibrozil-related compounds to predict the DDI between pravastatin and gemfibrozil.

	Ki	C_{max}	fu [d]	$C_{max,u}$	R
	μM	μM			
Gemfibrozil	1.47 ± 0.48	100-150 [a, b]	0.00648	0.65-0.97	0.60-0.69
Gemfibrozil-M3	3.97 ± 1.49	24 [c]	0.0123	0.30	0.93
Gemfibrozil-glucuronide	9.05 ± 8.43	20 [b]	0.115	2.3	0.80

Inhibitory effect of gemfibrozil-related compounds on the uptake of pravastatin by human kidney slices was examined. The Ki values were determined by non-linear regression analysis and are represented as the mean ± S.D.

[a] (Backman et al., 2002)

[b] (Okerholm et al., 1976)

[c] (Nakagomi-Hagihara et al., 2007)

[d] (Shitara et al., 2004)

Fig. 1

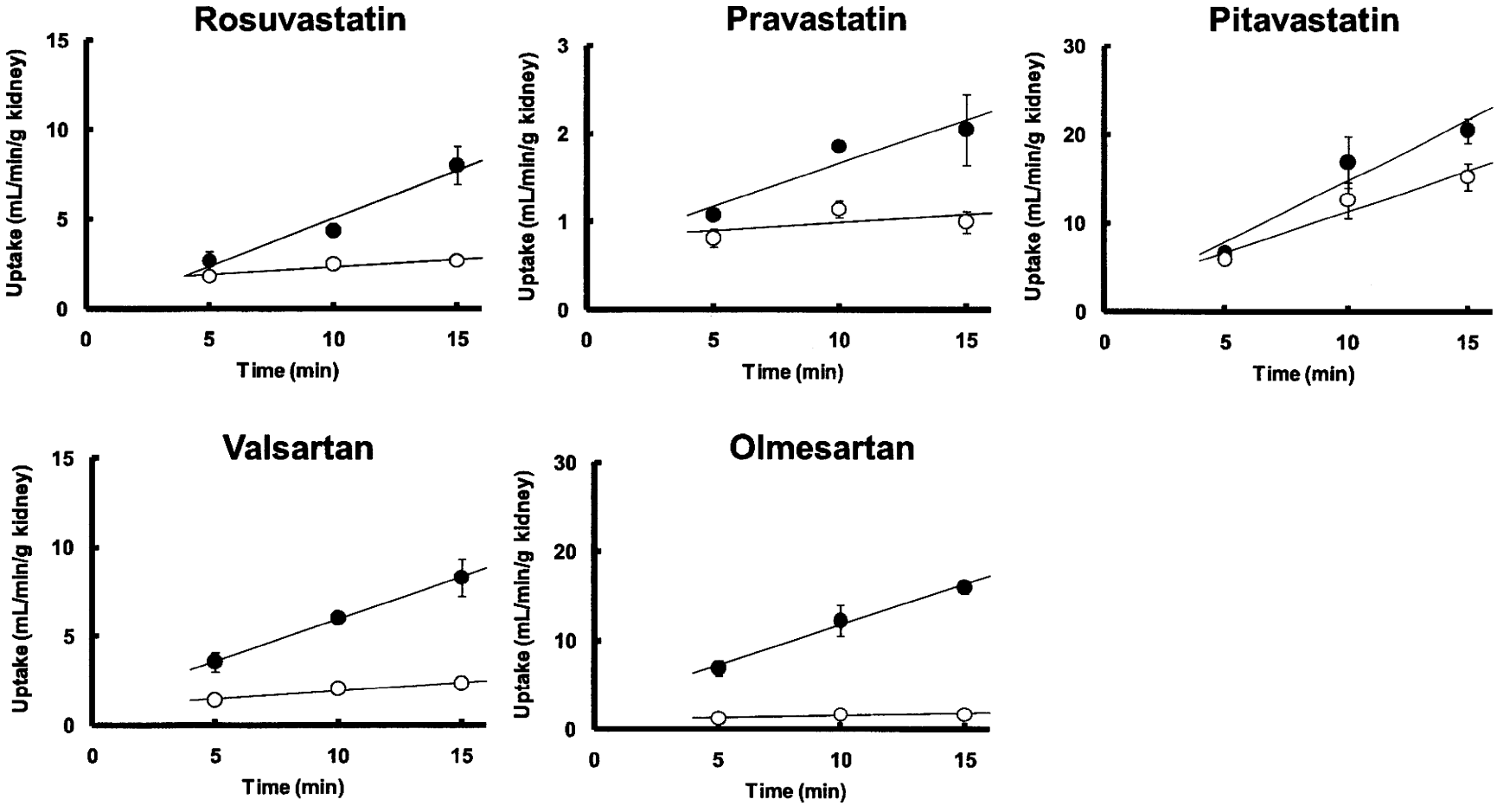

Rosuvastatin
Uptake (mL/min/g kidney)
Time (min)
Pravastatin
Uptake (mL/min/g kidney)
Time (min)
Pitavastatin
Uptake (mL/min/g kidney)
Time (min)
Valsartan
Uptake (mL/min/g kidney)
Time (min)
Olmesartan
Uptake (mL/min/g kidney)
Time (min)

Fig. 2

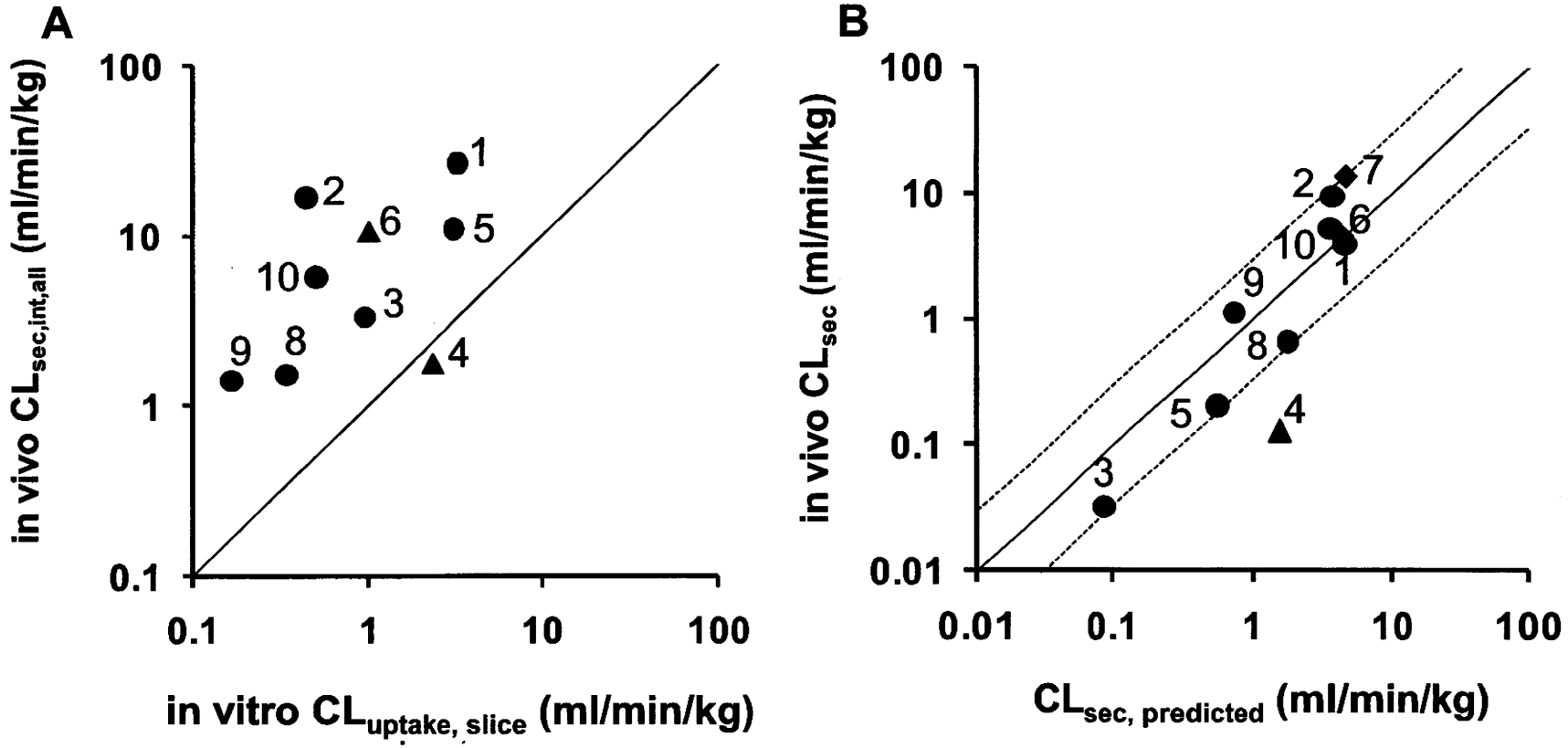
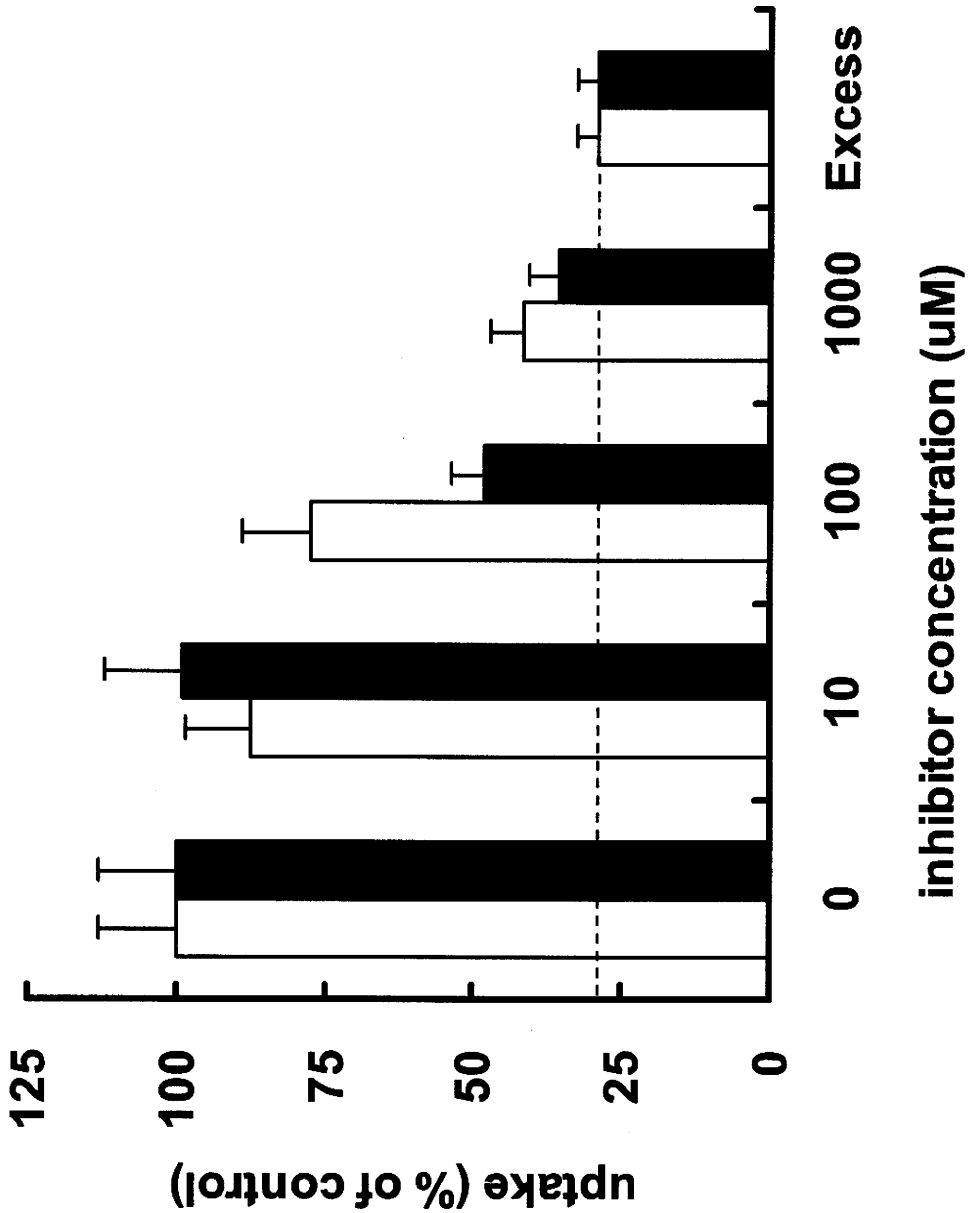

A
100
10
1
0.1
0.1
1
10
100
in vivo $CL_{sec,int,all}$ (ml/min/kg)
in vitro $CL_{uptake, slice}$ (ml/min/kg)
1
2
3
4
5
6
8
9
10
B
100
10
1
0.1
0.01
0.01
0.1
1
10
100
in vivo CL_{sec} (ml/min/kg)
$CL_{sec, predicted}$ (ml/min/kg)
1
2
3
4
5
6
7
8
9
10

Fig. 3


uptake (% of control)
125
100
75
50
25
0
0
10
100
1000
Excess
inhibitor concentration (uM)