

in humans by scaling-up the human in vitro parameters using the PBPK model and the SFs determined in rats. Sensitivity analyses showed that variations in the hepatic uptake ability markedly altered the plasma concentration of pravastatin, but had only a small effect on the liver concentration, which is in good agreement with clinical observations. Furthermore, variations in the canalicular efflux ability markedly altered the liver concentration of pravastatin, but had only a minimal effect on the plasma concentration. Variations in MRP2 activity may have large and small impacts on the therapeutic efficacy and adverse effects of pravastatin, respectively.

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**Prediction of the overall renal tubular secretion and hepatic clearance of anionic drugs and a renal drug-drug interaction involving OAT3 in humans by in vitro uptake experiments**

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**Running Title Page**

**Running title: Uptake-limited renal and hepatic elimination of anionic drugs**

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**Abbreviations:**

OATP, organic anion transporting polypeptide; OAT(Oat), organic anion transporter; statin, HMG-CoA reductase inhibitor; sartan, angiotensin receptor blocker; CL, clearance;  $CL_{int,all}$ , overall intrinsic clearance; DDI, drug-drug interaction; PAH, p-aminohippurate; LC, liquid chromatography; MS, mass spectrometry

**Abstract**

The present study investigated the prediction of the overall renal tubular secretion and hepatic clearances of anionic drugs based on in vitro transport studies. The saturable uptake of eight drugs, most of which were OAT3 substrates (rosuvastatin, pravastatin, pitavastatin, valsartan, olmesartan, trichlormethiazide, *p*-aminohippurate, and benzylpenicillin) by freshly prepared human kidney slices underestimated the overall intrinsic clearance of the tubular secretion; therefore, a scaling factor of 10 was required for in vitro–in vivo extrapolation. We examined the effect of gemfibrozil, and its metabolites, gemfibrozil glucuronide and the carboxylic metabolite, gemfibrozil-M3, on the pravastatin uptake by human kidney slices. The inhibition study using human kidney slices suggests that OAT3 plays a predominant role in the renal uptake of pravastatin. Comparison of unbound concentrations and  $K_i$  values (1.5, 9.1 and 4.0  $\mu\text{M}$ , for gemfibrozil, gemfibrozil glucuronide and gemfibrozil-M3, respectively) suggests that the mechanism of the interaction is ascribed mainly to an inhibition by gemfibrozil and gemfibrozil glucuronide. Furthermore, extrapolation of saturable uptake by cryopreserved human hepatocytes predicts the clearance comparable with the observed hepatic clearance although fluvastatin and rosuvastatin required a scaling factor of 11 and 6.9, respectively. This study suggests that in vitro uptake assays using human kidney slices and hepatocytes provide a good prediction of the overall tubular secretion and hepatic clearances of anionic drugs, and renal drug-drug interactions. It is also recommended to perform in vitro–in vivo extrapolation in animals to obtain more reliable prediction.

## Introduction

Prediction of the pharmacokinetic properties of drugs in humans in the preclinical stages of drug development is very important in order to avoid failure in the subsequent clinical stages because of poor pharmacokinetic properties. The liver and kidney are the major systemic clearance organs for drugs in the body. Drug-metabolizing enzymes and transporters play significant roles in the renal and hepatic elimination of drugs from the systemic circulation and, therefore, these activities are a critical factor determining systemic drug exposure. It is well accepted that, because of large species differences in drug metabolism, the metabolic clearance determined in animal studies cannot always be directly extrapolated to humans. *In vitro* systems, such as liver microsomes and hepatocytes, have been developed to replace animal studies and provide reliable predictions of the hepatic metabolic clearance of drugs (Obach, 1999; Kilford et al., 2008; Stringer et al., 2008; Chiba et al., 2009).

Animal scale-up has been widely used to predict the renal clearance of drugs in humans (Adolph, 1949; Boxenbaum, 1982). Renal elimination occurs in the glomeruli and proximal tubules where filtration and secretion occur, respectively. Animal scale-up is undoubtedly useful for drugs that are eliminated in the urine by glomerular filtration because glomerular filtration rate (GFR) depends on the molecular size and conforms to allometric scaling across species. However, Mahmool (1998) reported outliers of this prediction. This may be because of species difference in the tubular secretion of drugs mediated by renal transporters. We reported a species difference in the transport activity of the basolateral renal organic anion transporter OAT3 (Tahara et al., 2005b), and in the contribution of organic anion and cation transporters to the renal uptake of H<sub>2</sub> blockers (Tahara et al., 2005a). Therefore, an approach based on *in vitro* studies with a human-derived *in vitro* system will likely provide more reliable predictions of the renal clearance of drugs in humans.

Tubular secretion of organic anions involves uptake and subsequent efflux into the urine. Recently, we demonstrated that the renal uptake clearance of anionic drugs is close to the



tubular secretion clearance in rats (Watanabe et al., 2009b). Hence, the uptake is the rate-determining process as observed in the hepatic elimination of statins (Watanabe et al., 2010a), indicating that in vitro-in vivo extrapolation of tissue uptake clearance could provide a reasonable estimate of renal clearance. Kidney slices have been used as an in vitro system to investigate the contribution of OAT1 and OAT3 to the net renal uptake of drugs in rats and humans (Hasegawa et al., 2003; Nozaki et al., 2007a). Although it was clear that the uptake clearance in rat kidney slices underestimates the in vivo renal clearance of drugs (Hasegawa et al., 2003), the introduction of a scaling factor improved the predictability (Watanabe et al., 2009b).

The present study evaluated the predictability of renal clearance of anionic drugs, statins and sartans in freshly prepared human kidney slices as well as their hepatic clearance in cryopreserved hepatocytes. Human kidney slices have been used as an in vitro system to investigate the mechanisms of drug-drug interactions (DDI) involving renal clearance (Nozaki et al., 2007b). The DDI between gemfibrozil and pravastatin, where concomitant use of gemfibrozil reduced the renal clearance of pravastatin by approximately 40% (Kyrklund et al., 2003), is a unique transporter-based DDI; studies with human OAT-expressing systems suggest that the interaction involves inhibition of OAT3-mediated uptake of pravastatin not only by gemfibrozil but also by its metabolites (Nakagomi-Hagihara et al., 2007). In the present study, we investigated the mechanism of the DDI between pravastatin and gemfibrozil using human kidney slices.

## Methods

### Materials

[<sup>3</sup>H]-Pravastatin (44.5 Ci/mmol) and [<sup>3</sup>H]-olmesartan (79 Ci/mmol), and unlabeled pravastatin and olmesartan were kindly donated by Daiichi-Sankyo Co. (Tokyo, Japan). [<sup>3</sup>H]-Valsartan (81.0 Ci/mmol) and unlabeled valsartan were kindly donated by Novartis Pharma (Basel, Switzerland). [<sup>3</sup>H]-Pitavastatin (16 Ci/mmol) was kindly donated by Kowa Co. (Tokyo, Japan), and [<sup>3</sup>H]-rosuvastatin (79 Ci/mmol) was kindly donated by AstraZeneca (London, UK). Unlabeled pitavastatin was synthesized by Nissan Chemical Industries (Chiba, Japan). Unlabeled rosuvastatin and candesartan were purchased from Toronto Research Chemicals (North York, Canada). [<sup>3</sup>H]-*p*-Aminohippurate (PAH) (4.1 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). [<sup>14</sup>C]-Benzylpenicillin (59 mCi/mmol) was purchased from GE Healthcare UK (Buckinghamshire, England). Unlabeled PAH, gemfibrozil and trichlormethiazide were purchased from Sigma-Aldrich (St. Louis, MO). Unlabeled benzylpenicillin was purchased from Wako Pure Chemicals (Osaka, Japan). A carboxylic metabolite of gemfibrozil, M3 (purity: 99.6%), was chemically synthesized in KNC Laboratories, Co. Ltd. (Kobe, Japan) (Shitara et al., 2004). Gemfibrozil 1-O-β-glucuronide was biosynthesized with dog liver microsomes by XenoTech, LLC (Lenexa, KS), as described previously (Hirouchi et al., 2009). All other chemicals were of analytical grade and commercially available.

### Preparation of Human Kidney Slices and Uptake of Organic Anions by Human Kidney Slices

This study protocol was approved by the Ethics Review Boards at Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan and Tokyo Women's Medical University, Tokyo, Japan. All participants provided written informed consent. Intact renal cortical tissues were obtained from surgically nephrectomized patients with renal cell carcinoma

at Tokyo Women's Medical University. Samples of human kidney from subjects were stored in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) on ice immediately after kidney removal. After 30-min transportation, kidney slices were prepared as described below.

Uptake studies by human kidney slices were carried out as described in previous reports (Watanabe et al., 2009b). Kidney slices (300  $\mu\text{m}$  thick) from intact human cortical tissue were kept in ice-cold buffer before use. The buffer consisted of 120 mM NaCl, 16.2 mM KCl, 1 mM  $\text{CaCl}_2$ , 1.2 mM  $\text{MgSO}_4$ , and 10 mM  $\text{NaH}_2\text{PO}_4$  /  $\text{Na}_2\text{HPO}_4$  adjusted to pH 7.5. One slice, weighing 2 to 10 mg, was incubated at 37°C on a 12-well plate with 1 ml oxygenated buffer containing drugs in each well after a 5 min preincubation at 37°C. After incubation for designated periods, the uptake was terminated by transferring the slice to ice-cold drug-free buffer followed by washing twice with ice-cold buffer. The wet weight of the slice was measured before solubilization. Concentrations of the test drugs in the uptake studies were as follows: 0.1  $\mu\text{M}$  and 100  $\mu\text{M}$  for rosuvastatin and pitavastatin; 0.1  $\mu\text{M}$  and 500  $\mu\text{M}$  for pravastatin; 0.01  $\mu\text{M}$  and 100  $\mu\text{M}$  for valsartan and olmesartan; 0.1  $\mu\text{M}$  and 1000  $\mu\text{M}$  for PAH; 1  $\mu\text{M}$  and 1000  $\mu\text{M}$  for benzylpenicillin; 10  $\mu\text{M}$  and 1000  $\mu\text{M}$  for trichlormethiazide. The trace concentrations of all the drugs in the uptake assays were much lower than the  $K_m$  (IC50) values for the basolateral uptake transporters, and the excess concentrations were sufficiently high enough to saturate the transporter-mediated uptake process.  $K_m$  values of olmesartan, PAH, and benzylpenicillin for the uptake by human kidney slices are 0.12, 31.1–47.8, and 13.9–89.9  $\mu\text{M}$ , respectively (Nozaki et al., 2007a; Yamada et al., 2007).  $K_m$  values of rosuvastatin, pravastatin, and pitavastatin for human OAT3 are 7.4, 27.7, and 3.3  $\mu\text{M}$ , respectively (Fujino et al., 2005; Nakagomi-Hagihara et al., 2007; Windass et al., 2007). The IC50 value of valsartan for OAT3 is 0.2  $\mu\text{M}$  and IC50 values of trichlormethiazide for OAT1 and OAT3 are 19.2 and 71.2  $\mu\text{M}$ , respectively (Hasannejad et al., 2004; Sato et al., 2008).

The concentrations of pravastatin, pitavastatin, rosuvastatin, valsartan, olmesartan, benzylpenicillin and PAH were determined by measuring their radioactivity. The slice was

dissolved in 1 mL Soluene-350 (Perkin Elmer Life Science). The radioactivity in the scintillation cocktail (Hionic-Fluor; Perkin Elmer Life Sciences) was determined by liquid scintillation counting (LS6000SE; Beckman Coulter). The concentrations of candesartan and trichlormethiazide were determined by LC/MS, as described below. PBS (100  $\mu$ L) was added to the slices followed by sonication to break them down, and then these samples were used for the measurement of drug concentrations by LC/MS.

#### **Uptake Study Using Human Cryopreserved Hepatocytes**

Cryopreserved human hepatocytes were purchased from XenoTech LLC (Lenexa, KS), the Research Institute for Liver Disease (Shanghai, China) and In Vitro Technologies (Baltimore, MD). The uptake study was performed using a rapid separation method as described previously (Watanabe et al., 2010a). Briefly, the uptake reaction was initiated by adding an equal volume of buffer containing drugs (final concentration: 0.1  $\mu$ M and 100  $\mu$ M for rosuvastatin; 0.1  $\mu$ M and 500  $\mu$ M for valsartan; 5  $\mu$ M and 100  $\mu$ M for candesartan) to the hepatocyte suspension after a 3-min preincubation at 37 °C. After a designated time, the reaction was terminated by separating the cells from the medium using a centrifugal filtration technique. For this purpose, a 100- $\mu$ L aliquot of incubation mixture was placed in a 0.4 mL centrifuge tube (Sarstedt, Numbrecht, Germany) containing 50  $\mu$ L of 2N sodium hydroxide for radiolabeled compounds or 100  $\mu$ L of 5M ammonium acetate for unlabeled compounds under a 100  $\mu$ L layer of an oil mixture (density, 1.05; mixture of silicone oil and mineral oil, Sigma-Aldrich). Samples were then centrifuged for 10 s in a Microfuge (Beckman Coulter, Fullerton, CA). During this process, the hepatocytes pass through the oil layer into the aqueous solution (2N sodium hydroxide or 5M ammonium acetate). In the case of unlabeled compounds, tubes were frozen in liquid nitrogen immediately after centrifugation and stored at  $-20^{\circ}\text{C}$  until drug measurement.

The concentrations of rosuvastatin, valsartan and olmesartan were determined by measuring their radioactivity. After overnight incubation at room temperature to dissolve the

cells in alkali, the centrifuge tube was cut and each compartment was transferred to a scintillation vial. The compartment containing dissolved cells was neutralized with 50  $\mu$ L of 2N hydrochloric acid, mixed with scintillation cocktail (Clearsol II; Nakalai Tesque, Kyoto, Japan), and the radioactivity was determined in a liquid scintillation counter (LS6000SE; Beckman Coulter). The concentration of candesartan was determined by LC/MS. An aliquot was taken from the upper portion of the medium and quenched in methanol, and the cells were taken from the centrifuge tube and sonicated in a new tube, containing methanol, to disintegrate them. The samples were vortexed and centrifuged, and supernatant fractions from both the medium and cell portions were analyzed by LC/MS as described below.

#### LC/MS Analysis

Protein was precipitated with three volumes of methanol and removed by centrifugation at 15,000 g at 4°C for 10 min. The supernatant fractions were subjected to LC/MS. The appropriate standard curves were prepared in the equivalent blank matrix and used for each analysis. The analysis of candesartan was performed with an LCMS-2010 EV equipped with a Prominence LC system (Shimadzu, Kyoto, Japan), whereas the analysis of trichlormethiazide was performed with an Alliance HT 2795 separation module with an autosampler (Waters, Milford, MA), and a Micromass ZQ mass spectrometer (Waters). Chromatographic separation was performed on a CAPCELL PAK C<sub>18</sub> MGII column (3  $\mu$ m, 3.0 x 50 mm; Shiseido, Tokyo, Japan) in gradient mode. The column temperature and flow rate were 40°C and 0.4 ml/min, respectively. For the analysis of candesartan, 0.05% (v/v) formic acid and acetonitrile were used as the mobile phase. The acetonitrile concentration was 25% at 0 min, 50% at 3 min, and 25% from 3.01 to 5 min. Candesartan was detected at *m/z* of 441 in electrospray positive ionization mode. The interface voltage was 3.5 kV, and the nebulizer gas (N<sub>2</sub>) flow was 1.5 L/min. The heat block and curved desolvation line temperatures were 200°C and 150°C, respectively. For the analysis of trichlormethiazide, 0.05% (v/v) formic acid and acetonitrile

were used as the mobile phase. The acetonitrile concentration was 5% at 0 min, 90% at 4 min, and 5% from 4.01 to 7 min. Trichlormethiazide was detected at  $m/z$  of 378 in electrospray negative ionization mode. The desolvation temperature, capillary voltage, and cone voltage were 350°C, 3200 V, and 20 V, respectively.

### Determination of Kinetic Parameters

The *in vitro* intrinsic uptake clearance was calculated by dividing the initial uptake velocity by the drug concentration in the incubation buffer. The initial uptake velocity of the drugs was calculated as the slope of the uptake volume at 0.5 and 1 min or 0.5 and 2 min in hepatocytes, and at 15 min in kidney slices. Because of the limitation in the supply of the human kidney, the uptake was determined only at one point (15 min). The linearity of the uptake of five drugs, rosuvastatin, pravastatin, pitavastatin, valsartan, and olmesartan, up to 15 min was examined using one batch of kidney slices for each drug. Intrinsic uptake clearances were scaled up to the *in vivo* value per body weight using the following physiological scaling factors,  $1.2 \times 10^8$  cells/g liver, 24.1 g liver/kg body weight and 4.43 g kidney/kg body weight (Davies and Morris, 1993).

*In vivo* hepatic and renal secretion overall intrinsic clearance ( $CL_{h,int,all}$  and  $CL_{sec,int,all}$ ), which represents the elimination of drugs from circulating blood, were calculated from Eqs 1 – 4 using a dispersion model, because a drug showing blood flow-limited clearance was included:

$$CL_H \text{ (or } CL_{sec}) = Q \cdot (1 - F) \quad (1)$$

$$F = \frac{4a}{(1+a)^2 \cdot \exp\{(a-1)/2/D_N\} - (1-a)^2 \cdot \exp\{-(a+1)/2/D_N\}} \quad (2)$$

$$a = (1 + 4R_N \cdot D_N)^{1/2} \quad (3)$$

$$R_N = f_B \cdot \frac{CL_{h,int,all} \text{ (or } CL_{sec,int,all})}{Q} \quad (4)$$

where  $CL_H$ ,  $CL_{sec}$ ,  $Q$ ,  $D_N$  and  $f_B$  represent the hepatic clearance, renal secretion clearance, organ blood flow rate, dispersion number and blood unbound fraction, respectively. The blood flow rate in the liver was set at 20.7 ml/min/kg body weight and in the kidney at 15.7 ml/min/kg body weight (Davies and Morris, 1993), and  $D_N$  was set at 0.17 (Roberts and Rowland, 1986; Iwatsubo et al., 1996).  $CL_{sec}$  was estimated by subtracting  $f_B \times GFR$  from the observed in vivo renal clearance with regard to the blood concentration assuming that only unbound drug was cleared from plasma ( $CL_R$ ) ( Eq. 5 )

$$CL_{sec} = CL_R - f_B \times GFR. \quad (5)$$

Inhibition constants ( $K_i$ ) for gemfibrozil-related compounds were calculated from the following equation (Eq. 6), which is applicable to both competitive and non-competitive inhibition provided the substrate concentration is well below its  $K_m$  value (Ito et al., 1998):

$$\Delta CL_{uptake(+inhibitor)} = \frac{\Delta CL_{uptake(control)}}{1 + I / K_i} \quad (6)$$

where  $\Delta CL_{uptake}$  represents the  $CL_{uptake}$  for transporter-mediated uptake which is the  $CL_{uptake}$  of radiolabeled pravastatin (0.1  $\mu M$ ) minus that measured in the presence of an excess of non-radiolabeled pravastatin (500  $\mu M$ ),  $\Delta CL_{uptake(+inhibitor)}$  and  $\Delta CL_{uptake(control)}$  are the  $\Delta CL_{uptake}$  values estimated in the presence and absence of inhibitors, respectively, and  $I$  represents the inhibitor concentration. Fitting of the data to Eq.6 was carried out by an iterative nonlinear least squares method with use of the program "MULTI" (Yamaoka et al., 1981) to obtain the  $K_i$  value based on the nominal concentration which was assumed to represent the free concentration. The input data were weighted as the reciprocal of the observed values, and algorithm for the fitting used the Damping Gauss-Newton Method.

The degree of inhibition of the renal tubular secretion clearance of pravastatin in humans was estimated by calculating the following R value, which represents the ratio of the renal tubular secretion clearance in the absence of inhibitor to that in its presence (Eq. 7),

$$R = 1 + \frac{f_u \cdot C_{\max}}{K_i} \quad (7)$$

where  $f_u$  represents the protein unbound fraction of the inhibitor in plasma and  $C_{\max}$  represents the maximal plasma concentration of the inhibitor.



## Results

### Uptake Clearance of anionic drugs by Human Kidney Slices

Time profiles of the uptake of five drugs, rosuvastatin, pravastatin, pitavastatin, valsartan, and olmesartan in human kidney slices are shown in Fig. 1. Uptake was linear for all the drugs up to 15 min at trace concentration. The uptake was saturated in the presence of excess concentration of drugs, whereas the fraction of saturable component of pitavastatin uptake at 100  $\mu$ M was quite small. Table 1 shows the uptake clearance of trichlormethiazide, PAH, and benzylpenicillin as well as the five drugs in human kidney slices.

### Comparison between in vitro Uptake Clearance by Human Kidney Slices and in vivo Renal Secretion Overall Intrinsic Clearance

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 since OAT3 is predominantly involved in the renal uptake of benzylpenicillin (Nozaki et al., 2007a). The saturable uptake clearance of anionic drugs in human kidney slices was corrected by the ratio of the saturable uptake of benzylpenicillin in the same batch of human kidney to the average value to take account of the uptake by different batches of kidney slices (Table 1). This correction was based on our previous finding that the interbatch difference was very great, however, the uptake varied along with the mRNA expression of OAT3 (Nozaki et al., 2007a). The uptake of benzylpenicillin by the kidney slices ranged from 1.56 to 4.26 ml/g kidney/15min in this study.

The corrected saturable uptake clearance was extrapolated to the in vivo value per body weight ( $CL_{\text{uptake,slice}}$ ) using the physiological scaling factor, 4.43 g kidney/kg body weight (Davies and Morris, 1993) (Table 1) and was compared with the in vivo renal secretion overall intrinsic clearance ( $CL_{\text{sec,int,all}}$ ) (Fig.2A). The uptake of methotrexate and fexofenadine by human kidney slices was taken from previous reports (Nozaki et al., 2007b; Matsushima et al., 2009).  $CL_{\text{uptake,slice}}$  underestimated  $CL_{\text{sec,int,all}}$ , namely,  $CL_{\text{sec,int,all}}$  was 10-fold greater than

$CL_{\text{uptake,slice}}$  on average (Fig.2A and Table 2). Because the renal clearance of PAH is close to the blood flow rate, no reliable intrinsic clearance can be estimated. Therefore, PAH was not included in Fig. 2A. The renal secretion clearance of the drugs was predicted from  $CL_{\text{uptake,slice}}$  multiplied by the scaling factor of 10 using the dispersion model, and was plotted against the observed renal secretion clearance ( $CL_{\text{sec}}$ ) (Fig. 2B). The in vivo  $CL_{\text{sec}}$  of the test compounds including PAH, which has a blood-flow-limited renal clearance, correlated with the uptake clearance by kidney slices. Because plasma protein binding of PAH is very low,  $CL_{\text{sec,predicted}}$  of PAH was the highest of all the drugs even though it has moderate uptake clearance by kidney slices.

#### **Effects of PAH, benzylpenicillin and gemfibrozil and its metabolites on the Uptake of Pravastatin by Human Kidney Slices**

The inhibitory effects of PAH and benzylpenicillin (selective inhibitors of hOAT1 and hOAT3, respectively) on the uptake of pravastatin by fresh human kidney slices were examined. PAH and benzylpenicillin inhibit both OAT1 and OAT3; however, for OAT1 substrates, PAH shows significant inhibition at lower concentration than benzylpenicillin, and vice versa for OAT3 substrates (Nozaki et al. 2007a). Both compounds inhibited the uptake of pravastatin by human kidney slices in a concentration-dependent manner, and almost completely inhibited the saturable component of pravastatin uptake at 1 mM (Fig. 3). The  $IC_{50}$  value of benzylpenicillin was between 10  $\mu\text{M}$  and 100  $\mu\text{M}$ , which was lower than that of PAH (100  $\mu\text{M}$  – 1 mM).

The effect of gemfibrozil, gemfibrozil-M3 and gemfibrozil-glucuronide on the uptake of pravastatin (0.1  $\mu\text{M}$ ) by human kidney slices was examined (Fig. 4). Gemfibrozil and its two metabolites inhibited pravastatin uptake in a concentration-dependent manner. Since the concentration of pravastatin used in this inhibition study was 0.1  $\mu\text{M}$ , and much lower than the  $K_m$  value for OAT3 (28  $\mu\text{M}$ ) (Nakagomi-Hagihara et al., 2007), the  $IC_{50}$  values of the inhibitors approximate their  $K_i$  values. The  $K_i$  values of gemfibrozil and its metabolites are summarized

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 ( $CL_{\text{uptake,hep}}$ ,  $\mu\text{L}/\text{min}/10^6\text{cells}$ ) and found to be as follows: rosuvastatin,  $5.21 \pm 0.929$  and  $0.932 \pm 1.32$ ; valsartan,  $6.53 \pm 1.14$  and  $2.42 \pm 0.642$ ; olmesartan,  $4.20 \pm 0.120$  and  $2.07 \pm 0.0333$ ; candesartan,  $15.5 \pm 0.743$  and  $1.98 \pm 0.863$  at trace and excess concentrations, respectively. The  $CL_{\text{uptake,hep}}$  of each drug was considerably lower in the presence of an excess of the drug.  $CL_{\text{uptake,hep}}$  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:  $1.2 \times 10^8$  cells/g liver and 24.1 g liver/kg body weight (Table 2). The  $CL_{\text{uptake,hep}}$  values of the nine drugs were similar to or somewhat lower than in vivo hepatic overall intrinsic clearance ( $CL_{\text{h,int,all}}$ ) (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 ( $CL_{\text{uptake,slice}}$ ) 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).