0022-3565/02/3011-382–390$7.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 301, No. 1
4534/969731
Printed in U.S.A.

JPET 301:382–390, 2002

In Vitro Analysis of Human Drug Glucuronidation and Prediction of in Vivo Metabolic Clearance

M. G. SOARS, B. BURCHELL, and R. J. RILEY

Department of Molecular and Cellular Pathology, Ninewells Hospital and Medical School, Dundee, Scotland (M.G.S., B.B.); and Department of Physical and Metabolic Science, AstraZeneca Charnwood, Leics, England (R.J.R.)

Received September 7, 2001; accepted December 12, 2001 This article is available online at http://jpet.aspetjournals.org

ABSTRACT

The glucuronidation of a number of commonly used hepatic uridine diphosphate glucuronosyltransferase drug substrates has been studied in human tissue microsomes. Prediction of in vivo hepatic drug glucuronidation from liver microsomal data yielded a consistent 10-fold underprediction. Consideration of protein binding was observed to be pivotal when predicting in vivo glucuronidation for acid substrates. Studies using human intestinal microsomes demonstrated the majority of drugs to be extensively glucuronidated such that the intrinsic clearance (CL_{int}) of ethinylestradiol (CL_{int} = 1.3 μl/min/mg) was twice that obtained using human liver microsomes (CL_{int} = 0.7 μl/min/mg). The potential extrahepatic in vivo glucuronidation was calculated for a range of drug substrates from human microsomal data. These results indicate the contribution of intestinal drug glucuronidation to systemic drug clearance to be much less than either hepatic or renal glucuronidation. Therefore, data obtained with intestinal microsomes may be misleading in the assessment of the contribution of this organ to systemic glucuronidation. The use of hepatocytes to assess metabolic stability for drugs predominantly metabolized by glucuronidation was also investigated. Metabolic clearances for a range of drugs obtained using fresh preparations of human hepatocytes predicted accurately hepatic clearance reported in vivo. The use of cryopreserved hepatocytes as an in vitro tool to predict in vivo metabolism was also assessed with an excellent correlation obtained for a number of extensively glucuronidated drugs (R^2 = 0.80, $p < 0.001$).

In vitro systems, including microsomes and hepatocytes, have been routinely used in early preclinical drug metabolism studies to obtain an estimate of metabolic stability, usually expressed as intrinsic clearance (CL_{int}). Several methods have been used to predict hepatic in vivo clearance from in vitro tissue preparations. The most commonly used model has been the venous equilibrium or well stirred, which has been used to project successfully the in vivo metabolic clearance in both rat (Houston, 1994) and human (Iwatsubo et al., 1997; Obach, 1999). Interestingly, the majority of drugs used in these studies was metabolized primarily by oxidation catalyzed by members of the cytochrome P450 (P450) family. The prediction of in vivo metabolic clearance of drugs primarily undergoing phase II metabolism has been limited to several drugs studied only in the rat (Mistry and Houston, 1987). In addition, the consideration of extrahepatic metabolism may be pivotal when attempting to predict in vivo drug glucuronidation.

The kidney is the most extensively characterized extrahepatic organ to date where the glucuronidation of a range of substrates has been studied. The capacity of human kidney to glucuronidate endogenous substrates was demonstrated in a study by Matern et al. (1984) who observed the conjugation of a range of bile acids by using human kidney microsomes (HKM). In contrast, the glucuronidation of the heme catabolite bilirubin was not catalyzed by human kidney (Soars et al., 2001), confirming earlier reports of a complete absence of the major bilirubin UGT isoform UGT1A1 in human kidney (Sutherland et al., 1993).

However, the glucuronidation of a wide range of drug substrates has been observed in HKM (Soars et al., 2001). The rate of glucuronidation of the anesthetic propofol has been demonstrated to be greater in HKM than in human liver. This result was confirmed by Vietri et al. (2000) and Shipkova et al. (2001) who showed that UGT activity toward mycophenolic acid, another UGT1A9 substrate, in human kidney was twice that observed in human liver microsomes (HLM).

More recently, the role of the intestine in the first-pass metabolism of orally administered drugs has become an im-

This work was funded by AstraZeneca and The Wellcome Trust.

ABBREVIATIONS: CL_{int}, intrinsic clearance; P450, cytochrome P450; HKM, human kidney microsomes; UGT, uridine diphosphate glucuronosyltransferase; HLM, human liver microsomes; UDPGA, uridine diphosphate glucuronic acid; HIM, human intestine microsomes; HPLC, high-performance liquid chromatography; BSA, bovine serum albumin; f_u, unbound fraction in plasma; $f_{u\ inc}$, unbound fraction in in vitro microsomal incubation; HH, human hepatocyte.

portant question for the pharmaceutical industry. Zhang et al. (1999) have detected the expression of several P450s in human small intestine, including CYP2C and CYP3A4, which consolidated the earlier work of de Waziers et al. (1990). Intestinal P450s have been shown to contribute significantly to the metabolism of several drugs, including nifedipine and midazolam (Holtbecker et al., 1996; Paine et al., 1996).

The intestine may also play a crucial role in phase II drug metabolism. Strassburg et al. (2000) have studied the expression of the UGT family in the small intestine by using a quantitative duplex reverse transcription-polymerase chain reaction assay that has identified the expression of UGT1A3, UGT1A4, UGT1A10, and UGT2B15 transcripts and polymorphic regulation of UGT1A1, UGT1A6, UGT2B4, and UGT2B7 genes. Several groups have also looked at functional expression of intestinal UGTs by using a range of substrates. The intestine has been shown to catalyze the glucuronidation of a range of endogenous substrates, including bilirubin (McDonnell et al., 1996), bile acids (Parquet et al., 1985; Radominska-Pandya et al., 1998), and estrogens (Czernik et al., 2000). Further studies by Fisher et al. (2000) outlined the glucuronidation of estradiol, acetaminophen, and morphine along the length of the small intestine, perhaps suggesting the presence of UGT1A1, UGT1A6, and UGT2B7, respectively.

The aims of this article were severalfold: to attempt to predict human in vivo metabolic clearance for a range of drugs that were primarily metabolized by the phase II process of glucuronidation, comparing both microsomal and hepatocyte systems; to provide a better understanding of the potential for glucuronidation by extrahepatic tissues (kidney and intestine) for a range of drug substrates; and to assess their contribution to systemic drug glucuronidation.

Materials and Methods

Chemicals

Substrates, UDP-glucuronic acid (UDPGA), and other reagents used in the assays were purchased from Sigma Chemical (Gillingham, Dorset, UK), Aldrich Chemical (Gillingham, Dorset, UK), or BDH (Poole, Dorset, UK) and were of the highest grade available. [^{14}C]UDPGA (293.6 mCi/mmol, purity 99.7%) was purchased from PerkinElmer Life Sciences (Stevenage, Hertfordshire, UK).

Microsomal Samples

Both pooled human intestine (duodenal) microsomes (HIM) and HLM were purchased from In Vitro Technologies (Baltimore, MD). HKM were obtained as stated previously (Soars et al., 2001) Table 1 displays the characterization as provided by the commercial supplier.

Microsomal UGT Assays

UGT assays were performed as described previously (Ethell et al., 1998). Microsomes (400-μl aliquots) were optimally activated using 4×5-s bursts of sonication (Microson ultrasonic cell disruptor; Heat Technologies, Farmingdale, NY) allowing at least 1 min on ice between bursts. Tris/maleate buffer (100 mM), pH 7.4, containing 5 mM $MgCl_2$, 10 mM saccharic acid 1,4-lactone (present in all incubations), substrate (concentration dependent on substrate), 250 to 350 μg of microsomal sonicate, 2 mM UDPGA (0.1 μCi of [^{14}C]UDPGA/assay) were combined in a total volume of 100 μl. Incubations were run at 37°C for 60 min and then terminated by the addition of 100 μl of methanol that had been prechilled to −20°C. The mixture was centrifuged for 10 min at 14,000*g*. The resulting supernatant was then transferred to a high-performance liquid chromatography (HPLC) vial and 100 μl of this volume directly injected onto gradient HPLC by using solid scintillant radioactive detection as described previously (Ethell et al., 1998).

Equilibrium Dialysis

Drugs (10 μM) were mixed with HLM (at the protein concentrations similar to that used in UGT assays, approximately 2 mg/ml). The mixtures were then subjected to equilibrium dialysis against Dulbecco's phosphate-buffered saline, pH 7.4 (Sigma Chemical) at 37°C by using a DiaNorm apparatus (NBS Biologicals Limited, Cambs, UK). Diachema membranes were used (molecular weight cut-off 50 kDa, diameter 63 μm) and cells were rotated at 5 rpm for 12 h. Dialysis on each drug was performed in duplicate on at least three occasions. On completion of the dialysis period, both the microsomal and buffer fractions were removed from the cell. The microsomal fraction (100 μl) was mixed with 200 μl of ice-cold methanol and centrifuged for 15 min at 14,000*g*. An aliquot of the resultant supernatant along with the buffer fractions was then analyzed by HPLC-mass spectrometry as detailed below.

Preparation of Rat Hepatocytes

Isolation of rat hepatocytes was performed essentially using the two-step in situ collagenase perfusion method of Seglen (1976). Briefly, the hepatic portal vein of an anesthetized male Sprague-Dawley rat (weight 200–300 g) was cannulated just above the junction of the splenic and pyloric veins. Liver perfusion medium (Invitrogen, Carlsbad, CA) was perfused via the hepatic portal vein until the liver cleared to an even tan color (usually 7–8 min at a perfusion rate of 30 ml/min). Liver digestion medium (Invitrogen) was then perfused until the liver displayed evidence of extensive dissociation (usually a further 6–8 min at a perfusion rate of 30 ml/min). The liver was dissected from the rat and cells were gently teased out of the liver capsule into a beaker containing ice-cold hepatocyte suspension buffer [2.2 g $NaHCO_3$, 2.34 g Na HEPES, 2.0 g of bovine serum albumin (BSA), 1 liter of powder equivalent of Dulbecco's modified Eagle's medium (Sigma Chemical) diluted in 1

TABLE 1
Donor information for human microsomal and hepatocyte preparations

Donor	Sex	Age	Race	Cause of Death	Drug History
HIM	Pool of 10 donors				
HLM	Pool of 15 donors				
HH 1	Female	45	Caucasian	Cancer resection	None reported
HH 2	Male	47	Caucasian	Brain haemorrhage	Smoker
HH 3	Male	76	Caucasian	Unknown	None reported
HH 4	Female	60	Caucasian	Unknown	None reported
CHH 1	Female	59	Hispanic	Cerebrovascular accident	Smoker, depression, asthma
CHH 2	Male	42	Hispanic	Brain haemorrhage	Hypertension, epilepsy
CHH 3	Male	65	Caucasian	Brain haemorrhage	Smoker

CHH, cyropreserved human hepatocyte.

liter of water and adjusted to pH 7.4 with 1 M HCl]. The cell suspension was passed through a 250-μm mesh into a precooled tube and centrifuged at 50*g* for 2 min at 4°C. The supernatant was decanted, the cell pellet was resuspended in suspension buffer, and the centrifugation step was repeated. The resulting pellet of cells was resuspended in 10 ml of suspension buffer and an estimation of hepatocyte yield and viability was obtained using the trypan blue exclusion method.

Preparation of Human Hepatocytes

Human hepatocytes were prepared from an isolated lobe of human liver (obtained from local hospitals with ethical approval). Perfusion was essentially the same as described above except that an isolated lobe of liver was perfused rather than an in situ perfusion. Isolation of hepatocytes was performed as described above except that BSA was replaced with human serum albumin in the hepatocyte suspension buffer.

Determination of CL_{int} by Using Hepatocytes

Drug stocks were prepared in dimethyl sulfoxide at 100-fold incubation concentration (300 μM). Ten microliters of this 300 μM stock were added to a vial containing 490 μl of hepatocyte suspension buffer. A vial containing 250 μl of hepatocytes at a concentration of 1 million cells/ml for rat (4 million cells/ml for human) was preincubated for 5 min in a shaking water bath at 37°C along with the vial containing the drug/buffer mix. Reactions were started by adding 250 μl of drug/buffer mix to the 250 μl of hepatocytes [giving a final substrate concentration of 3 μM at 1% (v/v) dimethyl sulfoxide] and 50-μl aliquots were removed at 0, 5, 10, 20, 40, 60, and 90 min, ensuring adequate mixing. Samples were quenched in 100 μl of ice-cold methanol. Samples were subsequently frozen for 1 h at −20°C and then centrifuged for 3500 rpm for 20 min. The supernatants were removed and analyzed as described below.

Rat hepatocyte incubations were also performed without BSA as stated above, except isolated cells were recentrifuged, the supernatant was removed, and the hepatocytes were resuspended in hepatocyte suspension buffer that did not contain BSA. Hepatocyte suspension buffer without BSA was also used in drug/buffer mixes for these incubations.

Cryopreserved human hepatocytes were thawed, as stated by the commercial supplier's instructions (In Vitro Technologies) and incubated in the same manner as fresh human hepatocytes.

Analysis of Hepatocyte/Cryopreserved Hepatocyte and Protein Binding Samples

Initial mass spectrometry was conducted using a Micromass ZMD single quadrupole mass spectrometer with an HP1100 HPLC system for separation. Electrospray ionization was used for all mass spectrometry methods. Analysis of imipramine was performed in positive-ion mode monitoring at *m/z* 281.2. Negative-ion mode was used in the analysis of ethinylestradiol (*m/z* 295), hyodeoxycholic acid (*m/z* 391) propofol (*m/z* 177.1), and valproic acid (*m/z* 142.1).

Chromatographic separation was obtained using an XTerra MS C_8 column (4.6 × 50 mm, 2.5 μm) obtained from Waters (Watford, UK) by using 20 μl of each extracted sample. The mobile phase for positive-ion mode consisted of 0.25% (w/v) ammonium acetate with 0.1% (v/v) formic acid with the organic phase being methanol containing 0.1% (v/v) formic acid. The mobile phase for negative-ion mode consisted of 0.1% (v/v) triethylamine and the organic phase was methanol modified with 0.1% (v/v) triethylamine. All chromatography was performed using a generic gradient (t = 0 min % organic = 10, t = 0.5 min % organic = 10, t = 4 min % organic = 100, t = 5 min % organic = 100, t = 5.1 min % organic 10, total runtime = 5.5 min). The flow rate was set at 1.5 ml/min, which was introduced into the source at 0.4 ml/min.

Further mass spectrometry was conducted on a Micromass Quatro Ultima triple quadrapole by using an Alliance HT Waters 2790 HPLC system for separation. Analysis was by multiple reaction monitoring and conditions were optimized for each compound as follows. By using positive-ion mode, codeine was detected monitoring the transition 300.04 > 165.09 using a cone voltage of 54 V and a collision energy of 50 eV; morphine using 286.02 > 159.09, cone voltage 32 V and collision energy 50 eV, naloxone using 328.03 > 310.27, cone voltage 10 V and collision energy 22 eV; and naproxen using 231.08 > 185.11, cone voltage 21 V and collision energy 16 eV. Using negative-ion mode, furosemide was detected monitoring the transition 329.24 > 205.04 using a cone voltage of 60 V and a collision energy of 20 eV; gemfibrozil using 249.46 > 121.18, cone voltage 50 V and collision energy 20 eV; and ketoprofen using 253.38 > 209, cone voltage 40 V and collision energy 10 eV.

In these analysis chromatographic separation was achieved using a Symmetry C_8 column (2.9 × 20 mm, 5 μm) obtained from Waters by using 20 μl of each sample. The mobile phase for positive-ion mode consisted of water with 0.1% (v/v) formic acid with the organic phase being methanol containing 0.1% (v/v) formic acid. The mobile phase for negative-ion mode was as described above. All chromatography was performed using a generic gradient (t = 0 min % organic = 10, t = 0.1 min % organic = 10, t = 0.5 min % organic = 100, t = 1 min % organic = 100, t = 1.2 min % organic 10, t = 1.7 min % organic = 10, total runtime = 2 min). The flow rate was set at 1.5 ml/min, which was introduced into the source at 0.4 ml/min.

Data Analysis

Throughout this study, several approaches have been adopted to calculate CL_{int}.

Glucuronide Appearance for Intestine Microsomal Incubations. Assays were performed at substrate concentrations known to be 5-fold below the K_m of the drugs studied (data not shown). Further assays were performed at substrate concentrations at least 5-fold higher than these K_m concentrations to model V_{max} conditions. K_m and V_{max} approximations were then obtained through nonlinear regression analysis (Sharer et al., 1995).

Parent Loss for Hepatocyte Incubations. Because dose/C_0 gives a term for the volume of the incubation (expressed in ml $*$ 10^6 cells^{-1}) and the elimination rate constant $k = 0.693/t_{1/2}$, an equation expressing CL_{int} in terms of $t_{1/2}$ of parent loss can be derived:

$$CL_{int} = \frac{\text{Volume} \times 0.693}{t_{1/2}}$$

Prediction of In Vivo Clearance from in Vitro CL_{int}. Projection of human in vivo clearance was made from an adapted version of the well stirred model (Houston, 1994). Table 2 details the physiological and biochemical parameters used for the scaling of in vitro data. It should be noted that numbers for microsomal protein yield from kidney have been quoted as for liver because definitive data are currently unavailable. In addition, only the systemic contribution from intestine has been estimated because

TABLE 2
Human physiological and biochemical parameters important in scaling in vitro pharmacokinetic data
Parameters obtained from Davies and Morris (1993), Obach et al. (1997), and Lin et al. (1999).

Parameter	Value	Unit
Human liver weight	20	g/kg body of weight
Liver microsome protein yield	45	mg/g liver
Liver blood flow	20	ml/min/kg
Hepatocellularity	120	10^6 cells/g liver
Kidney weight	4.4	g/kg body of weight
Kidney microsome protein yield	45	mg/g kidney
Kidney blood flow	18	ml/min/kg
Intestine weight	30	g/kg body of weight
Intestine microsome protein yield	3	mg/g
Mucosal blood flow	4.6	ml/min/kg

models for first-pass extraction by this organ are still in their infancy (Shen et al., 1997).

$$CL_{bl} = \frac{Q \cdot CL'_{int}}{Q + CL'_{int}} \quad (1)$$

where CL_{bl} is blood clearance, Q is blood flow, and CL'_{int} is intrinsic clearance in vivo (CL_{int} was corrected for the number of cells/mg protein in the liver/kidney/intestine).

When protein binding was incorporated, the model was expanded as shown below:

$$CL_{bl} = \frac{Q \cdot \frac{f_u}{f_{u\,inc}} \cdot \frac{1}{BP} \cdot CL'_{int}}{Q + \frac{f_u}{f_{u\,inc}} \cdot \frac{1}{BP} \cdot CL'_{int}} \quad (2)$$

where f_u is unbound fraction in the plasma, $f_{u\,inc}$ is unbound fraction in in vitro microsomal incubation, B/P is blood-to-plasma ratio (assumed to be 1 for basic/neutral compounds and 1-hematocrit for acid compounds (Carlile et al., 1999).

Results

Prediction of Human Hepatic in Vivo Clearance by Using Human Liver Microsomal Studies. Previous work in this laboratory has assessed hepatic glucuronidation of drug substrates by using HLM (Soars et al., 2001). An indication of metabolic efficiency of drug glucuronidation was obtained by calculating CL_{int} and the results are shown in Table 3. The well stirred pharmacokinetic model was then used to estimate an in vivo clearance due to glucuronidation (see *Materials and Methods*, eq. 1). The in vivo total clearance and metabolic clearance due to glucuronidation for the drugs studied, obtained from an extensive literature review, are shown in Table 4. For drugs where total in vivo clearance exceeded hepatic blood flow (morphine, naloxone, and propofol) the hepatic clearance was taken as 20 ml/min/kg. In vivo drug clearance by direct glucuronidation was calculated by correcting the total in vivo clearance by the percentage of an i.v dose excreted as a direct glucuronide. The results from in vivo hepatic clearance due to glucuronidation have been compared with the estimates calculated from microsomal studies with the well stirred model (Fig. 1).

Figure 1 shows that (without the consideration of protein binding) there was a substantial underprediction of in vivo clearance for most drugs. Structural analysis suggests different trends in the data for acidic compounds compared with neural/basic compounds. Therefore, protein binding was considered as a cause of the variable prediction of in vivo hepatic drug glucuronidation.

Effect of Protein Binding on in Vivo Prediction of Drug Glucuronidation by Using Human Liver Microsomes. Equilibrium dialysis was performed with 11 drug substrates in HLM to determine the fraction of unbound drug present in each microsomal incubation ($f_{u\,inc}$) and hence the total amount of drug available for glucuronidation in in vitro incubations (Table 5). The drugs studied bound to protein to varying degrees ranging from propofol, which was highly bound ($f_{u\,inc} = 0.05$), to valproic acid, which was essentially free in the microsomal incubation ($f_{u\,inc} = 1$). The $f_{u\,plasma}$ for the drugs studied was obtained from a search of the literature (Table 5), to estimate the fraction of drug unbound in vivo. The drugs studied showed considerable variation in their binding properties, ranging from naproxen, which was highly bound to plasma proteins ($f_{u\,plasma} = 0.01$), to codeine, which was largely free in the in vivo situation ($f_{u\,plasma} = 0.93$). The drugs were classified into acidic, basic, or neutral compounds. A striking difference in protein binding for acidic drugs was revealed when comparing in vitro and in vivo situations. Acidic drugs such as naproxen and furosemide were highly bound to plasma proteins (albumin), although they were largely free in microsomal incubations, giving rise to 30- to 60-fold differences between the free fractions in vivo and in vitro. However, with the exception of ethinylestradiol, basic or neutral compounds displayed negligible differences in binding in plasma and microsomes under the conditions used. The effect of protein binding differences in the prediction of in vivo clearance is illustrated by Fig. 1. When protein binding and blood/plasma partitioning were considered in the prediction of in vivo clearance (see *Materials and Methods*, eq. 2), an excellent correlation was observed between predicted and observed hepatic clearance ($R^2 = 0.81$, $p < 0.001$; Fig. 1). However, it was interesting to note that the use of HLM consistently underpredicted the observed in vivo hepatic clearance by an order of magnitude (Fig. 1).

Glucuronidation by the Intestine. The extent of intestinal drug glucuronidation for 10 drug substrates was assessed using HIM. Table 3 shows the CL_{int} values obtained

TABLE 3

Drug glucuronidation by human tissue microsomes

CL_{int} estimates for liver are reproduced from Soars et al. (2001). Renal CL was scaled from CL_{int} estimates obtained previously (Soars et al., 2001). Values for intestine are mean ± S.D. obtained from three separate experiments. Scaled CL was determined using the well stirred model (see *Materials and Methods*).

Compound	Hepatic		Intestinal		Renal	Total
	CL_{int}	CL	CL_{int}	CL	CL	CL
	μl/min/mg	*ml/min/kg*	*μl/min/mg*	*ml/min/kg*	*ml/min/kg*	*ml/min/kg*
Codeine	0.41, 0.48	0.41	N.D.	0	—	0.41
Ethinylestradiol	0.54, 0.76	0.05	1.3 ± 0.64	0.01	N.D.	0.07
Furosemide	0.13, 0.12	0.007	0.07 ± 0.03	0.0004	—	0.007
Gemfibrozil	2.4 ± 0.3	0.20	1.83 ± 0.28	0.02	0.21	0.42
Imipramine	0.39, 0.41	0.30	—	—	0.02	0.32
Ketoprofen	0.23, 0.21	0.02	0.20 ± 0.03	0.002	0.06	0.08
Morphine	2.0, 1.9	1.3	N.D.	0	0.47	1.8
Naloxone	0.55 ± 0.1	0.37	0.21 ± 0.07	0.01	0.43	0.81
Naproxen	0.38 ± 0.04	0.01	0.31 ± 0.08	0.001	0.01	0.02
Propofol	1.4, 3.3	0.83	1.1 ± 0.18	0.04	1.3	2.2
Valproic acid	0.21, 0.19	0.03	0.06 ± 0.02	0.001	0.02	0.06

N.D., below the level of detection (8 pmol/min/mg); —, not determined.

TABLE 4
Human total in vivo clearance and clearance due to glucuronidation for 11 drugs studied

Compound	In vivo CL	% Gluc	In vivo Gluc	References
	ml/min/kg		*ml/min/kg*	
Codeine	10.7	70	7.49	Bertz and Granneman (1997), Vozeh (1988)
Ethinylestradiol	5.4	35	1.89	Chiou et al. (1998), Vozeh (1988), Maggs et al. (1983)
Furosemide	1.9	14	0.27	Vozeh (1988), Hammarlund and Benet (1989)
Gemfibrozil	1.69	40	0.68	Bertz and Granneman (1997)
Imipramine	15, 13.8	10	1.44	Chiou et al. (1998), Vozeh (1988)
Ketoprofen	1.2	90	1.08	Chiou et al. (1998), Vozeh (1988)
Morphine	24, 17.1	90	18.5	Chiou et al. (1998), Vozeh (1988)
Naloxone	25, 24.8	65	16.2	Schneider et al. (1999), Bertz and Granneman (1997), Vozeh (1988)
Naproxen	0.07	57	0.04	Vozeh (1988), Vree et al. (1993)
Propofol	27.1, 24.8	53	14.4	Simons et al. (1988), Vozeh (1988)
Valproic acid	0.12	60	0.07	Chiou et al. (1998), Vozeh (1988)

Gluc, glucuronidation.

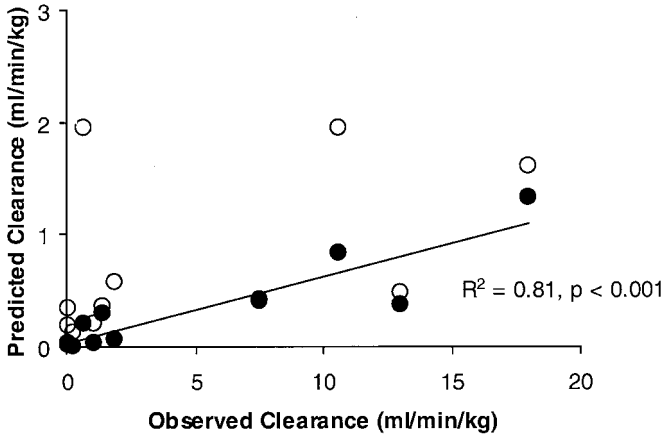

Fig. 1. Influence of nonspecific binding in the prediction of human hepatic in vivo glucuronidation from human liver microsome studies. Drug CL_{int}s determined from human liver microsome data (Table 3) were scaled using the well stirred model with (●) ($R^2 = 0.81$, $p < 0.001$) and without (○) protein binding and blood/plasma partitioning. These predicted clearances were then compared with in vivo clearances corrected for metabolic glucuronidation (Table 3).

TABLE 5
Binding parameters of 11 drugs in plasma and human liver microsomes

$f_{u\ plasma}$ values were obtained from Holfard (1998) and Cockshott et al. (1992). $f_{u\ inc}$ are mean ± S.D. determined using three separate equilibrium dialysis experiments, each performed in duplicate at 2 mg/ml protein concentration.

Compound	$f_{u\ Plasma}$	$f_{u\ inc}$	$f_{u\ plasma}/f_{u\ inc}$
Codeine[a]	0.93	0.89 ± 0.19	1.04
Ethinylestradiol[a]	0.03	0.32 ± 0.23	0.09
Furosemide[b]	0.03	0.94 ± 0.06	0.03
Gemfibrozil[a]	0.03	0.59 ± 0.22	0.05
Imipramine[b]	0.11	0.13 ± 0.04	0.85
Ketoprofen[a]	0.06	0.91 ± 0.15	0.07
Morphine[b]	0.65	0.8 ± 0.17	0.81
Naloxone[b]	0.62	0.81 ± 0.21	0.77
Naproxen[a]	0.01	0.61 ± 0.01	0.02
Propofol[b]	0.02	0.05 ± 0.04	0.4
Valproic acid[a]	0.1	1	0.1

[a] Basic/neutral drug.
[b] Acidic drug.

for the drugs in this study (using HIM) compared with CL_{int} estimates previously determined for the same drug substrates in our laboratory by using HLM.

HIM catalyzed the glucuronidation of all drugs studied except codeine and morphine where glucuronidation was below the level of detection (less than 8 pmol/min/mg). The CL_{int} values obtained using HIM varied from 0.06 μl/min/mg for valproic acid to 1.83 μl/min/mg for gemfibrozil. CL_{int} estimates obtained by HIM were of a similar magnitude to that catalyzed by HLM for the majority of drugs studied. Interestingly, the intestinal CL_{int} of the oral contraceptive ethinylestradiol was twice that in HLM.

Prediction of Human in Vivo Clearance by Using Human Tissue Microsomal Studies. To ascertain the relative importance of hepatic, renal, and intestinal drug glucuronidation, the well stirred model was used to estimate the contribution of the liver, kidney, and intestine to total systemic in vivo glucuronidation from in vitro microsomal results (Table 3). The binding of drugs in HKM and HIM was assumed to be similar to HLM (Table 5) when incorporated into the well stirred model. Figure 2 shows the proportion of predicted clearance catalyzed by human liver, kidney, and intestine. In vitro CL_{int} values suggested that intestinal drug metabolism was a significant component of systemic glucuronidation (Table 3). However, the predicted metabolic clearance estimates indicated that the role of the intestine in drug clearance by glucuronidation has been overestimated and is being overemphasized (Fig. 2; Table 3).

Prediction of Human Hepatic in Vivo Clearance by Using Human Hepatocytes. The metabolism of eight extensively glucuronidated drugs was studied in four separate preparations of human hepatocytes (Table 6). There was a large variability in the CL_{int} estimates for several drugs between the different preparations of hepatocytes (for example, the CL_{int} for naloxone varied more than 10-fold over the four preparations). In particular, human hepatocyte (HH) preparation 1 (HH1) seemed to be an extensive metabolizer for UGT2B7 substrates (codeine, morphine, and naloxone). The prediction of human hepatic in vivo clearance by using hepatocytes is shown in Fig. 3 where a significant correlation was obtained ($R^2 = 0.79$, $p < 0.005$). Hepatocytes quantitatively predicted human hepatic clearance well, in contrast to the consistent 10-fold underprediction observed using HLM (Fig. 3).

Use of Cryopreserved Hepatocytes in Prediction of in Vivo Clearance. The use of cryopreserved hepatocytes is an important alternative to the use of freshly prepared hepatocytes where availability may be restricted. Cryopreserved hepatocytes prepared from three separate donors (Table 1) were used to assess the metabolic stability of a range of drugs previously studied in freshly isolated human hepatocytes (Table 6). As for fresh human hepatocytes, an excellent correlation was obtained ($R^2 = 0.80$, $p < 0.001$) when comparing metabolic clearance determined from cryopreserved human

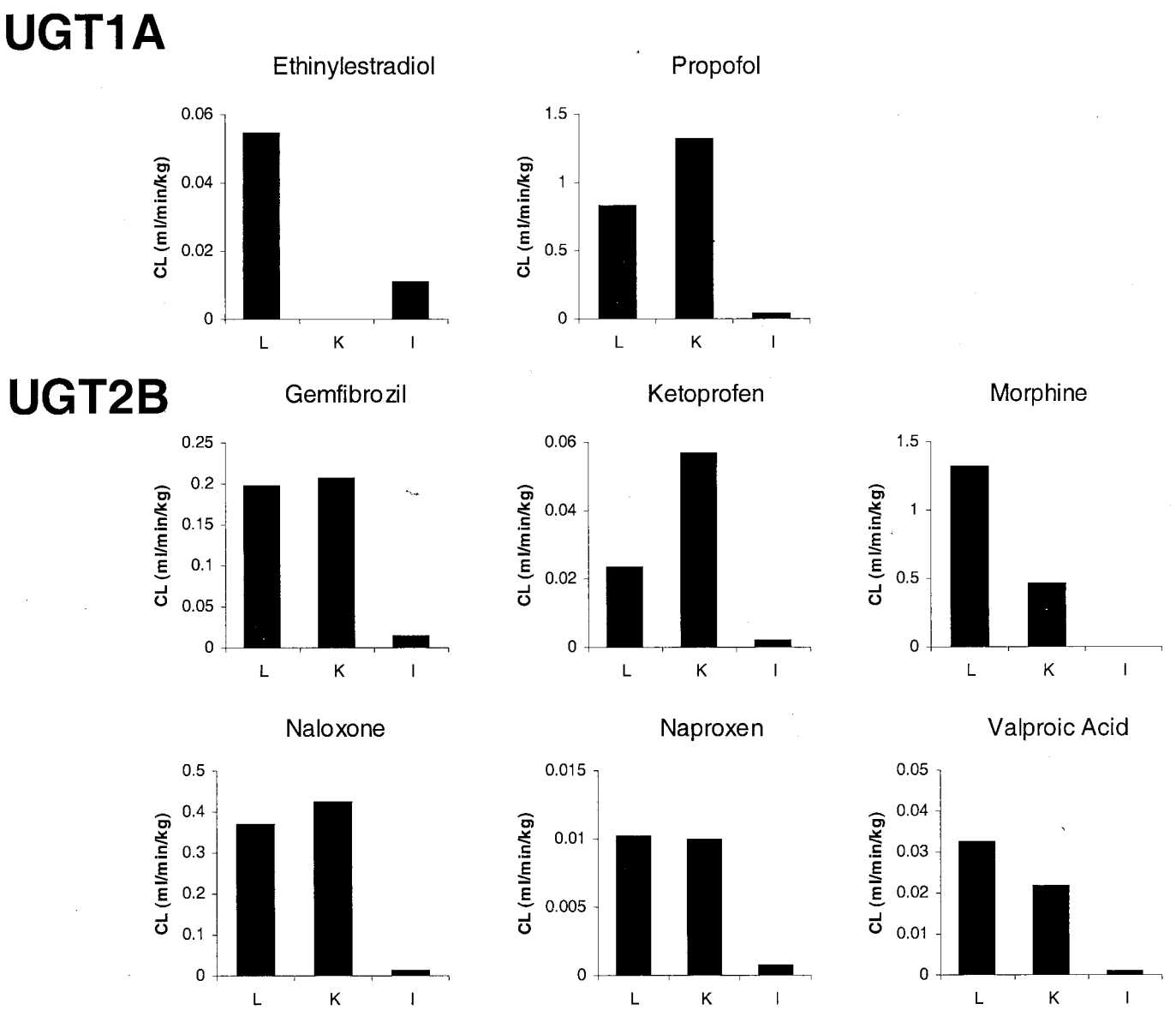

Fig. 2. Predicted contribution of liver, kidney, and intestine to human systemic in vivo glucuronidation estimated using human tissue microsomes. Drug CL_{int} data (Table 3) obtained using HLM (L), HKM (K), and HIM (I) for a range of drugs glucuronidated by both UGT1A and UGT2B isoforms were scaled using the well stirred model incorporating protein binding and blood/plasma partitioning.

hepatocytes with in vivo hepatic clearance. This suggests that cryopreserved cells may be an ideal in vitro tool from which to predict in vivo clearance, especially when fresh human tissue is in scarce supply.

Effect of Albumin on Prediction of in Vivo Clearance by Using Rat Hepatocytes. Human albumin was routinely added in human hepatocyte preparations. However, the requirement of this additional protein for drug glucuronidation was unknown. Therefore, the dependence of glucuronidation on protein binding was investigated in rat hepatocytes, due to lack of availability of human hepatocytes. Rat hepatocytes prepared/incubated with and without the presence of albumin were used to assess the effect of albumin on the CL_{int} of a range of acidic and basic/neutral drugs (Fig. 4). Figure 4 clearly shows that the clearance of basic/neutral drugs was unaffected by the presence of albumin in hepatocyte incubations. However, when the metabolism of acidic drugs was determined in the absence of albumin a 10-fold increase in clearance was observed. Interestingly, the overall CL_{int} estimates for acidic drugs were generally lower than those determined for either basic or neutral compounds (Fig. 4).

Discussion

The use of liver microsomal data to predict in vivo metabolic clearance was first demonstrated by Rane et al. (1977) and has subsequently been used by many investigators for a range of compounds (Houston, 1994; Iwatsubo et al. 1997; Obach 1999). However the majority of work in this area has focused on drugs primarily metabolized by phase I processes. Mistry and Houston (1987) attempted to predict rat in vivo clearance for three drugs highly glucuronidated by rat liver microsomes. An identical rank order of the drugs was obtained between in vitro and in vivo metabolism. However, the

TABLE 6
Intrinsic clearance estimates determined from four separate HH preparations and three human cryopreserved hepatocyte (CHH) preparations
CL_{int} values were determined using the $t_{1/2}$ method. Values set to zero were considerably less than the level of detection (0.4 μl/min/10^6 cells).

Drugs	CL_{int}						
	HH1	HH2	HH3	HH4	CHH1	CHH2	CHH3
				μl/min/10^6 cells			
Codeine	112	6	4	3	20	5	11
Furosemide	0	0	0.4	0	0	1	0
Gemfibrozil	2	0	1	0	0.4	0.4	3
Ketoprofen	0	0	0	2	0	5	0
Morphine	64	7	—	12	28	17	17
Naloxone	572	47	96	158	512	56	72
Naproxen	0	0	0	0	0	0	1
Propofol	19	47	32	48	54	45	63

—, not determined.

predicted clearance substantially underestimated the in vivo situation.

Figure 1 illustrates the potential for using microsomal incubations to predict human hepatic in vivo clearance of drugs extensively metabolized by phase II detoxification mechanisms such as glucuronidation. The consistent underprediction of human hepatic in vivo clearance produced in this work from microsomal data builds on the previous study in rat by Mistry and Houston (1987). The common theme of underprediction observed when scaling microsomal data may be due to the simplicity and assumptions made by the pharmacokinetic model used. Destruction of cellular integrity and sequential metabolic pathways may also explain this observation. Bock et al. (1976) have previously shown that addition of *N*-acetylglucosamine to rat liver microsomal incubations increased glucuronidation of naphthalene dihydrodiol to levels observed using isolated hepatocytes. It is interesting to speculate that transport of drug substrates may be more limited in microsomes than in the whole cell, which may have a part to play in the underprediction of in vivo hepatic clearance. However the consistent relationship observed between in vitro and in vivo data would aid the development of new chemical entities for which glucuronidation is known to be the major clearance mechanism.

The recent resurgence of interest in the intestine as a possible site of first-pass metabolism in humans has prompted this investigation into the significance of extrahepatic drug glucuronidation by this tissue. The in vitro CL_{int} estimates obtained for a range of drugs by using HIM (Table 3) suggest that intestinal drug glucuronidation may be significant compared with hepatic glucuronidation. The CL_{int} of ethinylestradiol was 2- to 3-fold greater with HIM than the CL_{int} determined with HLM, which agrees with studies by Fisher et al. (2000) and Czernik et al. (2000) who described higher intestinal than hepatic activity for other UGT1A1 probes such as estradiol. The intestinal activity of UGT2B7 substrates such as ketoprofen, naloxone, and valproic acid builds on the expression data of Radominska-Pandya et al. (1998) and Strassburg et al. (2000). The apparent lack of glucuronidation of morphine by HIM in this study contrasted with previous work by Fisher et al. (2000) who stated that morphine-3 glucuronidation was catalyzed by HIM albeit at low levels. Interestingly, studies in anhepatic patients have

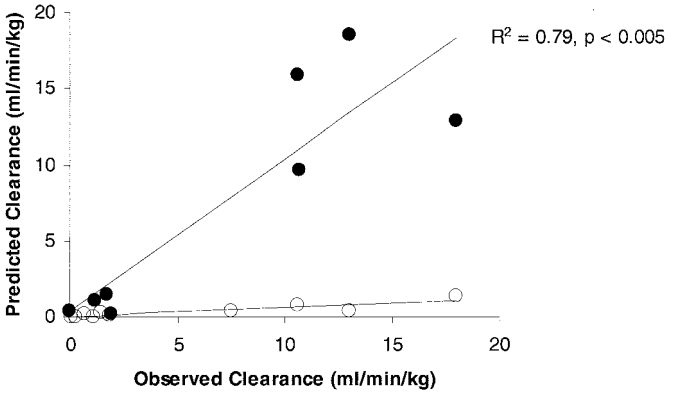

Fig. 3. Comparison of the prediction of human in vivo drug hepatic clearance by using human hepatocytes and liver microsomes. Drug CL_{int}s were determined using four separate preparations of human hepatocytes and were subsequently scaled using the well stirred model (●). These predicted clearances were correlated ($R^2 = 0.79$, $p < 0.005$) with in vivo hepatic clearance obtained from the literature (Table 4). The CL_{int} of naproxen was assumed to be 0.4 μl/min/10^6 cells. Predicted clearance from HLM studies are shown for comparison (○).

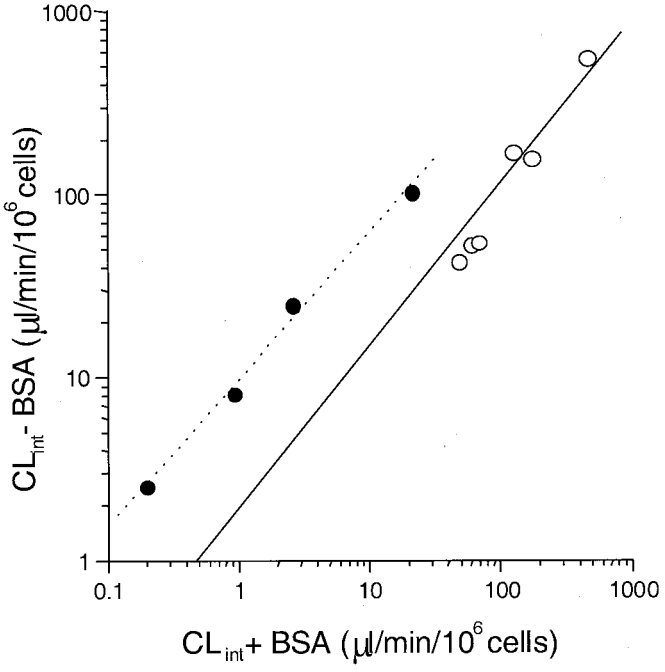

Fig. 4. Effect of albumin on intrinsic clearance estimates determined using rat hepatocytes. The effect of bovine serum albumin on CL_{int} estimates for acidic (●) and nonacidic (○) drugs was investigated using three separate preparations of rat hepatocytes with/without the presence of bovine serum albumin.

suggested minimal metabolism of morphine by the gut wall when an oral dose of morphine was administered (Mazoit et al., 1990). However, data produced by such indirect experimental techniques must be interpreted with care (Lin et al., 1999).

When in vitro intestinal drug glucuronidation was assessed using the well stirred pharmacokinetic model, the contribution of intestinal glucuronidation to systemic drug clearance appeared much less significant than hepatic glucuronidation for all drugs studied (Fig. 2; Table 3). However, renal glucuronidation was predicted to be important in the metabolism of several compounds. These data indicated an overestimation in the role of intestinal glucuronidation for systemic drug clearance might occur if drug CL_{int} estimates are taken without consideration of this in vivo contribution, particularly for drugs glucuronidated by UGT1A1. This concurs with previous work by Pacifici et al. (1988) who determined human in vitro intestinal 1-naphthol glucuronidation to be one-seventh of the hepatic glucuronidation of this substrate. However, when these microsomal activities were corrected for organ weight, hepatic glucuronidation was around 60-fold greater than glucuronidation catalyzed by the intestine.

Although in vivo data on intestinal drug glucurondiation are limited, the use of pharmacokinetic models to predict intestinal metabolism from in vitro data has been used with success previously (Mistry and Houston, 1987). Klippert et al. (1982) developed a model based on mucosal blood flow to predict the intestinal first-pass effect of phenacetin in the rat by using in vitro data. The use of such models does have certain caveats, including that metabolizing enzymes should be distributed evenly throughout the organ (Houston, 1994), in this case the mucosal layer of the intestine. However, studies by Strassburg et al. (2000) and Fisher et al. (2000) have shown UGT activity toward several substrates to vary along the length of the intestine, a pattern also observed for intestinal CYP3A4 (de Waziers et al., 1990). Interestingly, the liver, too, has well known heterogeneity in the distribution of its drug-metabolizing enzymes, and the successful prediction of a large number of drugs by using pharmacokinetic models (Iwatsubo et al., 1997; Obach, 1999) suggests that if care is taken, accurate estimates of in vivo drug clearance can be obtained.

The importance of nonspecific binding in microsomal incubations has recently received considerable attention (Obach, 1997, 1999; McLure et al., 2000). The results shown in Table 5 and Fig. 1 suggest that the consideration of differential binding of acidic drugs to plasma and microsomal proteins is imperative for an accurate assessment of in vivo hepatic clearance. In addition, the effect of nonspecific binding to serum albumin on clearance was studied in hepatocytes (Fig. 4). Only acidic drugs were affected by the presence/absence of serum albumin. The increase in clearance of acid drugs when serum albumin was removed from incubations suggests that this exclusive binding restricted their metabolism in hepatocyte incubations. The association of acidic substrates with serum albumin both in vivo (assessed by low $f_{u\ plasma}$; Table 5) and in hepatocyte incubations (Fig. 4) suggests that this in vitro system may mimic the binding of these substrates in vivo particularly well. These findings reinforce the earlier work of Shibata et al. (2000). Further benefits from the use of human hepatocytes (prepared in the presence of serum albumin) can be seen in Fig. 3 where an excellent estimate of in vivo metabolic clearance was obtained for a range of drug substrates. Interestingly, the omission of serum albumin from hepatocyte incubations may be a useful technique to differentiate between closely related acidic substrates, particularly for quantitative structure-activity relationships, and in detailed metabolite identification studies. The use of human hepatocytes allowed the consideration of both phase I and phase II metabolism to produce an accurate prediction of total human metabolic clearance.

Table 6 also shows the interindividual variation in clearance that can occur for certain drugs in human studies. For the majority of drugs studied, clearance values obtained from three of the four human hepatocyte preparations were similar; however, one outlier produced the variation in each case. There have been many factors attributed to the variation of glucuronidation rates, including genetics, age, sex, diet, and prior drug intake (Burchell et al., 2001). It is interesting to speculate that the increased clearance of certain drugs (known to be metabolized by UGT2B7; Soars et al., 2001) in HH1 may be due to the polymorphic nature of this UGT isoform. This theory is supported by the work of Patel et al., (1995) who observed a large interindividual variability in the glucuronidation of the UGT2B7 substrate oxazepam, and credited this to genetic factors. In summary, data from a significant number of individual donors (at least four) or pooled cryopreserved hepatocytes may be required to provide an accurate assessment of population variability.

This report has highlighted the relative importance of drug microsomal glucuronidation in the liver, kidney, and intestine. The well stirred model has been used to predict the in vivo implications of these tissues for drug glucuronidation. The prediction of hepatic in vivo clearance for a number of extensively glucuronidated drugs has been successfully performed in a range of in vitro systems, including microsomes, hepatocytes, and cryopreserved cells. This study suggests that care must be taken not to overemphasize the contribution of human intestinal drug glucuronidation toward systemic metabolism. However the importance of this tissue in the first-pass glucuronidation of orally administered drugs cannot be discounted, given the high concentration of unbound drug exposed to intestinal UGTs by using this route of drug delivery (Shen et al., 1997).

Acknowledgments

We thank Anthony Atkinson and Peter Littlewood for mass spectroscopy expertise.

References

Bertz RJ and Granneman GR (1997) Use of *in vitro* and *in vivo* data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin Pharmacokinet* **32:**210–258.

Bock KW, Van Ackeren G, Lorch F, and Birke FW (1976) Metabolism of naphthalene to naphthalene dihydrodiol glucuronide in isolated hepatocytes and in liver microsomes. *Biochem Pharamcol* **25:**2351–2356.

Burchell B, Ethell B, Coffey M, Findlay K, Jedlitschky G, Soars M, Smith D, and Hume R (2001) Inter-individual variation of UDP-glucuronosyltransferases and drug glucuronidation in man, in *Interindividual Variability in Drug Metabolism in Man* (Pacifici and Pelkonen eds) pp 358–394, Taylor and Francis Ltd., London, UK.

Carlile DJ, Hakooz N, Bayliss MK, and Houston JB (1999) Microsomal prediction of *in vivo* clearance of CYP2C9 substrates in humans. *Br J Clin Pharmacol* **47:**625–635.

Chiou Wl, Robbie G, Chung SM, Wu TC, and Ma C (1998) Correlation of plasma clearance of 54 extensively metabolized drugs between humans and rats: mean allometric coefficient of 0.66. *Pharmaceutical Res* **15:**1474–1479.

Holford N, editor (1998) *Clinical Pharmacokinetics: Drug Data Handbook,* 3rd ed, ADIS International Ltd., Auckland, New Zealand.

Cockshott ID, Douglas EJ, Plummer GF, and Simons PJ (1992) The pharmacokinetics of propofol in laboratory animals. *Xenobiotica* **22:**369–375.
Czernik PJ, Little JM, Barone GW, Raufman JP, and Radominska-Pandya A (2000) Glucuronidation of estrogens and retinoic acid and expression of UDP-glucuronosyltransferase 2B7 in human intestinal mucosa. *Drug Metab Dispos* **28:**1210–1216.
Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharmaceutical Res* **10:**1093–1095.
de Waziers I, Cugenec PH, Yang CS, Lerous JP, and Beaune PH (1990) Cytochrome P450 isoenzymes, epoxide hydrolase and glutathione transferase in rat and human hepatic and extrahepatic tissues. *J Pharmacol Exp Ther* **253:**387–394.
Ethell BT, Anderson G, Beaumont K, Rance DJ, and Burchell B (1998) A universal radiochemical high performance liquid chromatographic assay for the determination of UDP-glucuronosyltransferase activity. *Anal Biochem* **225:**142–147.
Fisher MB, Vandenbranden M, Findlay KAB, Burchell B, Thummel KE, Hall SD, and Wrighton SA (2000) The tissue distribution and liver bank variability of human UGT activities. *Pharmacogenetics* **10:**727–739.
Hammarlund-Udenaes M and Benet LZ (1989) Furosemide pharmacokinetics and pharmacodynamics in health and disease-An update. *J Pharmacokinet Biopharm* **17:**1–46.
Holtbecker N, Fromm MF, Kroemer HK, Ohnhms EF, and Heidermann H (1996) The nifedipine-rifampin interaction: evidence for induction of gut wall metabolism. *Drug Metab Dispos* **24:**1121–1123.
Houston JB (1994) Utility of *in vitro* drug metabolism data in predicting *in vivo* metabolic clearance. *Biochem Pharmacol* **47:**1469–1479.
Iwatsubo T, Hirota N, Ooie T, Suzuki H, Shimada N, Chiba K, Ishizaki T, Green CE, Tyson CA, and Sugiyama Y (1997) Prediction of *in vivo* metabolism in the human liver from *in vitro* metabolism data. *Pharmacol Ther* **73:**147–171.
Klippert P, Borm P, and Noordhoek J (1982) Prediction of intestinal first-pass effect of phenacetin in the rat from enzyme kinetic data-correlation with *in vivo* data using mucosal blood flow. *Biochem Pharmacol* **31:**2545–2548.
Lin JH, Chiba M, and Baillie TA (1999) Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol Rev* **51:**135–157.
Maggs JL, Grimmer SFM, Orme MLE, Breckenridge AM, and Park BK (1983) The biliary and urinary metabolites of [^{3}H]17α-ethynylestradiol in women. *Xenobiotica* **13:**421–431.
Matern S, Matern H, Farthmann EH, and Gerok W (1984) Hepatic and extrahepatic glucuronidation of bile acids in man. Characterization of bile acid uridine 5′-diphosphate-glucuronosyltransferase in hepatic, renal, and intestinal microsomes. *J Clin Invest* **74:**402–410.
Mazoit JX, Sandouk P, Scherrmann JM, and Roche A (1990) Extrahepatic metabolism of morphine occurs in humans. *Clin Pharmacol Ther* **48:**613–618.
McDonnell WM, Hitomi E, and Askari FK (1996) Identification of bilirubin UDP-GTs in the human alimentary tract in accordance with the gut as a putative metabolic organ. *Biochem Pharmacol* **51:**483–488.
McLure JA, Miners JO, and Birkett DJ (2000) Nonspecific binding of drugs to human liver microsomes. *Br J Clin Pharmacol* **49:**453–461.
Mistry M and Houston JB (1987) Glucuronidation *in vitro* and *in vivo*: comparison of intestinal and hepatic conjugation of morphine, naloxone and buprenorphine. *Drug Metab Dispos* **15:**710–717.
Obach RS (1997) Nonspecific binding to microsomes: impact on scale-up of *in vitro* intrinsic clearance as assessed through examination of warfarin, imipramine, and propranolol. *Drug Metab Dispos* **25:**1359–1369.
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.
Pacifici GM, Franchi M, Bencini C, Repetti F, Di Lascio N, and Muraro GB (1988) Tissue distribution of drug-metabolising enzymes in humans. *Xenobiotica* **18:**849–856.
Paine MF, Shen DD, Kunze KL, Perkins JD, Marsh CL, McVicar JP, Barr DM, Gillies BS, and Thummel KE (1996) First-pass metabolism of midazolam by the human intestine. *Clin Pharmacol Ther* **60:**14–24.
Parquet M, Pessah M, Sacquet E, Salvat C, Raizman A, and Infante R (1985) Glucuronidation of bile acids in human liver, intestine and kidney. An in vitro study on hyodeoxycholic acid. *FEBS Lett* **189:**183–187.
Patel M, Tang BK, Grant DM, and Kalow W (1995) Interindividual variability in the glucuronidation of (S) oxazepam contrasted with that of (R) oxazepam. *Pharmacogenetics* **5:**287–297.
Radominska-Pandya A, Little JM, Pandya JT, Tephly TR, King CD, Barone GW, and Raufman JP (1998) UDP-glucuronosyltransferases in human intestinal mucosa. *Biochim Biophys Acta* **1394:**199–208.
Rane A, Wilkinson GR, and Shand DG (1977) Prediction of hepatic extraction ratio from *in vitro* measurement of intrinsic clearance. *J Pharmacol Exp Ther* **200:**420–424.
Scheider G, Coassolo P, and Lave T (1999) Combining *in vitro* and *in vivo* pharmacokinetic data for prediction of hepatic drug clearance in humans by artificial neural networks and multivariate statistical techniques. *J Med Chem* **42:**5072–5076.
Seglen PO (1976) Preparation of isolated rat liver cells. *Methods Cell Biol* **13:**29–83.
Sharer JE, Shipley LA, Vandenbranden MR, Binkley SN, and Wrighton SA (1995) Comparisons of phase I and phase II *in vitro* hepatic enzyme activities of human, dog, rhesus monkey, and cynomolgus monkey. *Drug Metab Dispos* **23:**1231–1241.
Shen DD, Kunze KL, and Thummel KE (1997) Enzyme-catalysed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* **27:**99–127.
Shibata Y, Takahashi H, and Ishii Y (2000) A convenient *in vitro* screening method for predicting *in vivo* drug metabolic clearance using isolated hepatocytes suspended in serum. *Drug Metab Dispos* **28:**1518–1523.
Shipkova M, Strassburg CP, Braun F, Streit F, Grone HJ, Armstrong VW, Tukey RH, Oellerich M, and Wieland E (2001) Glucuronide and glucoside conjugation of mycophenolic acid by human liver, kidney and intestinal microsomes. *Br J Pharmacol* **132:**1027–1034.
Simons PJ, Cockshott ID, Douglas EJ, Gordon EA, Hopkins K, and Rowland M (1988) Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. *Xenobiotica* **18:**429–440.
Soars MG, Riley RJ, Findlay KAB, Coffey MJ, and Burchell B (2001) Evidence for significant differences in microsomal drug glucuronidation by canine and human liver and kidney. *Drug Metab Dispos* **29:**121–126.
Strassburg CP, Kneip S, Topp J, Obermayer-Straub P, Barut A, Tukey RH, and Manns MP (2000) Polymorphic gene expression and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. *J Biol Chem* **275:**36164–36171.
Sutherland L, Ebner T, and Burchell B (1993) The expression of UDP-glucuronosyltransferases of the UGT1 family in human liver and kidney in response to drugs. *Biochem Pharmacol* **45:**295–301.
Vietri M, Pietrabissa A, Mosca F, and Pacifici GM (2000) Mycophenolic acid glucuronidation and its inhibition by non-steroidal anti-inflammatory drugs in human liver and kidney. *Eur J Clin Pharmacol* **56:**659–664.
Vozeh S (1988) Pharmacokinetic drug data. *Clin Pharmacokinet* **15:**254–282.
Vree TB, Biggelaar-Martea MVD, Verwey-Van Wissen CPWGM, Vree ML, and Guelen PJM (1993) The pharmacokinetics of naproxen, its metabolite O-desmethylnaproxen, and their acyl glucuronides in humans. Effect of cimetidine. *Br J Clin Pharmacol* **35:**467–472.
Zhang QY, Dunbar D, Ostrowska A, Zeisloft S, Yang J, and Kaminsky LS (1999) Characterization of human small intestinal cytochrome P-450. *Drug Metab Dispos* **27:**804–809.

Address correspondence to: R. J. Riley, Department of Physical and Metabolic Science, AstraZeneca Charnwood, Bakewell Rd., Loughborough, Leics, LE11 5RH, England. E-mail: rob.riley@astrazeneca.com