

**Intestinal and hepatic metabolic activity of five cytochrome P450 enzymes –
impact on prediction of first-pass metabolism**

Aleksandra Galetin and J. Brian Houston

School of Pharmacy and Pharmaceutical Sciences, University of Manchester,
Manchester, M13 9PL, United Kingdom (A.G., J.B.H.)

a) Running title: Comparison of intestinal and hepatic P450 metabolic activity

b) **Corresponding author:** Dr Aleksandra Galetin

School of Pharmacy and Pharmaceutical Sciences,

University of Manchester,

Oxford Road,

Manchester, M13 9PL, UK

Tel: (+) 44 161 275 6886

Fax: (+) 44 161 275 8349

Email: Aleksandra.Galetin@manchester.ac.uk

c) Text: 31

Tables: 4

Figures: 5

References: 40

Abstract: 250

Introduction: 737

Discussion: 1,448

d) **Abbreviations used:** HIM, human intestinal microsomes; HLM, human liver microsomes; CL_{int} , intrinsic clearance; CL_{max} , maximum clearance, LC-MS/MS, liquid chromatography-tandem mass spectrometry

e) Absorption, Distribution, Metabolism and Excretion

ABSTRACT

The contribution of the gut is not routinely incorporated into in vitro-in vivo predictions of either clearance or drug-drug interactions and this omission may partially explain the general under-prediction trend often observed. In the current study the metabolic ability of hepatic and intestinal pooled microsomes was compared for eight CYP3A substrates (midazolam, triazolam, diazepam, alprazolam, flunitrazepam, nifedipine, testosterone and quinidine) and paclitaxel, tolbutamide, S-mephenytoin and bufuralol as CYP2C8, CYP2C9, CYP2C19 and CYP2D6 probes, respectively. A general agreement in the type of kinetics was observed between the two systems for the substrates investigated. Of the 16 pathways investigated, 75% of K_m (S_{50}) values obtained in intestinal microsomes (5.9 – 769 μM) were within 2-fold of hepatic estimates. Irrespective of the P450 investigated and normalization of V_{\max} values for the P450 abundance, clearance was 4.5 to 50-fold lower in intestinal microsomes (0.0005 – 0.51 $\mu\text{L}/\text{min}/\text{P450}$) in comparison to the hepatic estimates (0.002-5.8 $\mu\text{L}/\text{min}/\text{P450}$), whereas the rank order was consistent between the systems. Assessment of two enterocyte isolation methods (mucosal scraping or enterocyte elution) was performed at the substrate concentrations corresponding to the determined V_{\max} conditions for 11 pathways. The activity difference between the methods (3 to 29-fold) was P450-related in the following rank order - CYP2C19 > CYP3A4 > CYP2C9 ~ CYP2D6. After correction for the loss of activity between the methods, the intrinsic activities of hepatic and intestinal CYP3A4, CYP2C9, CYP2C19 and CYP2D6 were comparable for the 16 pathways. The implications of these findings on the prediction of intestinal first-pass metabolism are discussed.

Introduction

A range of P450 enzymes have been identified in human small intestine – CYP3A4, CYP3A5, CYP1A1, CYP2C9, CYP2C19, CYP2D6 and CYP2J2 (Lown et al., 1994, Madani et al., 1999, Paine et al., 1999, Zhang et al., 1999, Glaeser et al., 2002, Matsumoto et al., 2002). Analogous to liver, CYP3A4 is the predominantly expressed P450 enzyme in the small intestine, accounting for ~80% of total P450s (Lin et al., 1999, Paine et al., 2006), followed by CYP2C9 (~15%); however, the amount of CYP3A4 expressed (65.7 nmol) represents only 1% of the hepatic estimate (Paine et al., 1997, Yang et al., 2004). The expression of metabolic enzymes varies within the small intestinal villus with the highest found in mature enterocyte lining the villus tips (Kolars et al. 1994, Watkins, 1997). The zonal expression of the intestinal enzymes is more pronounced than in the liver varying along its whole length, with the highest levels of CYP3A4 found in the proximal region of the intestine that decline distally opposite to the expression of P-glycoprotein; a similar trend was observed for CYP2C9 and CYP2C19 protein expression (Thummel et al., 1997, Laple et al., 2003, Benet et al., 2004, Thorn et al., 2005).

Indirect assessment of intestinal metabolic ability (from oral and i.v. administration data) (Hall et al., 1999) indicates that the extent to which the CYP3A4 substrates are extracted by the intestine and liver varies considerably from drug to drug; in agreement with the lack of coordination in regulation of CYP3A4 expression in liver and intestine (Lown et al., 1994). In the case of alprazolam and quinidine intestinal extraction is significantly lower compared to hepatic (Damkier et al., 1999, Hirota et al., 2001), whereas for certain substrates (e.g., triazolam, atorvastatin and tacrolimus) intestinal extraction rivals or even exceeds that of the liver (Floren et al., 1997, Lennernas, 2003, Masica et al., 2004). The clinical relevance of the intestinal

metabolism depends on the relative importance of the metabolic pathway involved and possible interplay with P-glycoprotein. The latter is particularly evident for low solubility/high permeability compounds (Class 2 by Biopharmaceutics Drug Disposition Classification System) that are mutual substrates for P-glycoprotein and CYP3A4 (e.g., tacrolimus, cyclosporine), where the efflux via P-glycoprotein at the luminal membrane of the intestinal mucosa modulates intestinal CYP3A first-pass metabolism and the extent of oral bioavailability (Wu and Benet, 2005).

There have been several studies assessing the catalytic activity of intestinal P450 enzymes in comparison to the liver (Paine et al., 1997, Obach et al., 2001, von Richter et al., 2004). However, variability in the segment of the gut used (proximal or the whole length), source of the intestinal tissue (individual or pooled), different enterocyte isolation method, in addition to observed inter-individual variability in CYP3A4 expression in both liver and intestine (Kuehl et al., 2001, Lin et al., 2002), makes an unequivocal comparison difficult.

The aim of the current study was to systematically evaluate the metabolic ability of five main metabolic enzymes in both human intestinal and hepatic microsomes. Intestinal microsomes prepared by mucosal scraping of the entire length of the intestine and pooled from 10 individuals were used. The study involved eight CYP3A substrates (midazolam, triazolam, diazepam, alprazolam, flunitrazepam, nifedipine, testosterone and quinidine) and paclitaxel, tolbutamide, S-mephenytoin and bufuralol as representative probes for CYP2C8, CYP2C9, CYP2C19 and CYP2D6 activity, respectively.

Comparison of the intrinsic catalytic activity between the hepatic and intestinal enzymes was performed by normalizing the V_{\max} (and consequently the CL_{int} values) for the population relative abundance of the particular P450 enzyme. In

addition, binding affinity and consistency in kinetic behaviour were assessed for each of the 16 pathways under investigation, covering a wide range of clearance values. In order to investigate the impact of the enterocyte isolation method on the differential estimates of the P450 enzyme activity in the intestine, further studies were performed in the microsomes prepared by an enterocyte elution method. This was carried out at substrate concentrations corresponding to the previously determined V_{\max} values for 11 different pathways, including CYP3A4 and non-CYP3A4 probes.

The present study provides a comprehensive comparative analysis of the five different P450 enzymes in intestinal and hepatic microsomes using 12 different substrates and their respective pathways. A general agreement in the binding affinity and kinetic behaviour between the hepatic and intestinal enzymes observed, irrespective of the P450 enzymes investigated, provides evidence of comparable intestinal and hepatic catalytic activity (per pmol of P450 enzyme) for CYP3A4, CYP2D6, CYP2C9 and CYP2C19, conditional to enterocyte isolation method.

Materials and Methods

Chemicals. Midazolam, triazolam, alprazolam, diazepam and its metabolites, flunitrazepam, quinidine, testosterone, nifedipine, S-mephenytoin, tolbutamide, dextromethorphan, 6 β -hydroxytestosterone, NADP, isocitric dehydrogenase were purchased from Sigma Chemicals Co. (Poole, Dorset, UK). (3S)-3-hydroxyquinidine, oxidized nifedipine, 4'-hydroxymephenytoin, paclitaxel, bufuralol, 1'-hydroxybufuralol, 4-hydroxytolbutamide and midazolam metabolites were obtained from Ultrafine Chemicals (Manchester, UK). Alprazolam and triazolam metabolites were purchased from Biomol International (PA, USA) and flunitrazepam metabolites were a gift from Roche (Basel, Switzerland). All other reagents and solvents were of high analytical grade. Pooled intestinal microsomes (n=10) prepared by mucosal scraping of the entire length of the gut were purchased from InVitroTechnologies (Baltimore, MD, USA). Pooled intestinal microsomes prepared by enterocyte elution method were obtained from Xenotech (Kansas, USA). Pooled liver microsomes (n=22), 3'-hydroxy and 6 α -hydroxypaclitaxel were obtained from BD Gentest Co. (Woburn, MA, USA).

Incubation conditions in the human intestinal microsomes (HIM). The kinetic studies were performed in pooled intestinal microsomes (InVitroTechnologies) prepared by mucosal scraping of the entire length of the gut. Incubation times (10-90 mins) and protein concentrations (0.5-1.5 mg/ml) were within the linear range for each individual substrate and are shown in the Table 1. Microsomes were suspended in phosphate buffer (0.1 M, pH 7.4) with the final incubation volume of 0.25 mL. Samples were pre-incubated for 5 min in a shaking water bath at 37°C and each reaction was initiated with an NADPH regenerating system (1mM NADP⁺, 7.5 mM isocitric acid, 10 mM magnesium chloride and 0.2 units isocitric dehydrogenase). The

final concentration of the organic solvent (acetonitrile or methanol) in incubation media was less than 1 % v/v. Kinetic profiles for substrates investigated were generated from 10-12 substrate concentration points, over a 1-1000 μM range. The reaction was terminated by 0.25 mL of ice-cold acetonitrile with 1 μM of the appropriate internal standard samples were centrifuged at 13,400g for 10 min and further analyzed by LC-MS/MS.

Incubation conditions in the human liver microsomes (HLM). The kinetic studies were performed in pooled liver microsomes obtained from BD Gentest Co. (Woburn, MA, USA). Incubation times (5-60 mins) and protein concentrations (0.25-1 mg/ml) were within the linear range for each individual substrate (Table 1). The incubation procedure, NADPH regenerating system, internal standard and further analysis by LC-MS/MS were the same as described for the intestinal microsomes. The final concentration of the organic solvent (acetonitrile or methanol) in incubation media was less than 1% v/v. The substrate concentration range was the same as applied in the intestinal microsomal studies.

Comparison of the intestinal activity in microsomes prepared by enterocyte elution and mucosal scraping. In order to evaluate the impact of enterocyte isolation method on the intestinal activity additional studies were performed in pooled intestinal microsomes prepared by elution method (Xenotech, Kansas, USA). The assessment was carried out at the substrate concentrations corresponding to the previously determined V_{max} conditions for 8 different substrates (at 50, 750, 250, 250, 500, 1000, 750 and 50 μM for midazolam, alprazolam, quinidine, testosterone, diazepam, tolbutamide, S-mephenytoin and bufuralol, respectively). The 1 mg/ml protein concentration was used for all the substrates, except for midazolam where 0.5 mg/ml concentration was employed; the incubation

times were as described in Table 1. Of all the 8 substrates, only midazolam full kinetic profile was obtained at a substrate concentration range corresponding to the studies in intestinal microsomes prepared by mucosal scraping in order to compare the binding affinity and type of kinetics between 2 different methods. The incubation procedure and further analysis were as described for intestinal microsomes prepared by mucosal scraping. The extent of microsomal protein binding was determined using the microfiltration method for all substrates, as described previously (Rawden et al., 2005).

LC-MS/MS. The method used for the analysis of midazolam, triazolam, alprazolam, diazepam, flunitrazepam and quinidine were adapted from Galetin et al. (2004). The LC-MS/MS method applied for the analysis of testosterone, nifedipine, mephenytoin and tolbutamide was described in Rawden et al. (2005).

Paclitaxel. 3'-Hydroxy- and 6 α -hydroxypaclitaxel and docetaxel were quantified by LC-MS/MS. Each metabolite pair, together with docetaxel as internal standard were separated on a Luna phenyl hexyl 30x4.6 mm 5 μ m column (Phenomenex, UK) at 40 °C using a tertiary gradient maintained at 1 ml/min by a Waters Alliance 2795 HT LC system. An initial mobile phase of 78 % 0.05% formic acid/22 % acetonitrile was maintained for 1 minute before being ramped linearly to 50 % 0.05% formic acid/50 % acetonitrile at 2 minutes and then immediately to 45 % 0.05% formic acid / 55 % acetonitrile and 45% 0.001 M ammonium acetate/55 % acetonitrile before being ramped linearly from 3 to 4 minutes to 22% 0.05% formic acid/78% acetonitrile. The initial ratio was immediately re-established at 4 minutes and maintained to 5 minutes. The retention times were approximately 3.4 (3'-hydroxy paclitaxel), 3.6 (6 α -hydroxypaclitaxel) and 3.75 (docetaxel) minutes. The compounds were detected and quantified by atmospheric pressure electrospray ionisation MS/MS

using a Micromass Quattro Ultima triple quadrupole mass spectrometer. The LC column eluate was split and $\frac{1}{4}$ was delivered into the MS where the desolvation gas (nitrogen) flow rate was 600 l/hr, the cone gas (nitrogen) flow rate was 100 l/hr and the source temperature was 125 °C. Using positive ion mode, protonated molecular ions were formed using a capillary energy of 3.5 kV and cone energies of 70 V (3'-hydroxypaclitaxel), 90 V (6 α -hydroxypaclitaxel) and 55V (docetaxel). Product ions formed in argon at a pressure of 2×10^{-3} mbar and at collision energies of 10 eV (3'-hydroxypaclitaxel, m/z 870.25 \rightarrow 302.1), 20 eV (6 α -hydroxypaclitaxel, m/z 870.25 \rightarrow 286.2) and 15 eV (docetaxel, m/z 808.35 \rightarrow 226.2) were monitored as ion chromatograms which were integrated and quantified by quadratic regression of standard curves using Micromass QuanLynx 3.5 software.

Bufuralol. 1'-Hydroxybufuralol and dextromethorphan were quantified by LC-MS/MS. 1'-Hydroxybufuralol and dextromethorphan (internal standard) were separated on a Luna C18(2) 50x4.6 mm 3 μ m column (Phenomenex, UK) at 40 °C using a tertiary gradient maintained at 1 ml/min by a Waters Alliance 2795 HT LC system. An initial mobile phase of 90 % 0.001M ammonium acetate/10 % acetonitrile was maintained for 1 minute before being ramped immediately to 15.5 % 0.05% formic acid / 19.5 % acetonitrile and 42.5% 0.001 M ammonium acetate/22.5% acetonitrile. This was maintained for 2 minutes before being ramped immediately to 13.5 % 0.05% formic acid/ 1.5% acetonitrile 8.5% 0.001 M ammonium acetate/76.5% acetonitrile and maintained for a further minute. The initial ratio was immediately re-established and maintained to 5 minutes. The retention times were approximately 2.35 (1'-hydroxybufuralol) and 2.47 (dextromethorphan) minutes. The compounds were detected and quantified by atmospheric pressure electrospray ionisation MS/MS using a Micromass Quattro Ultima triple quadrupole mass spectrometer. The LC column

eluate was split and ¼ was delivered into the MS where the desolvation gas (nitrogen) flow rate was 600 l/hr, the cone gas (nitrogen) flow rate was 100 l/hr and the source temperature was 125 °C. Using positive ion mode, protonated molecular ions were formed using a capillary energy of 3.5 kV and cone energies of 70 V (1'-hydroxybufuralol) and 89 V (dextromethorphan). Product ions formed in argon at a pressure of 2×10^{-3} mbar and at collision energies of 20 eV (1'-hydroxybufuralol, m/z 278.15→186.1) and 40 eV (dextromethorphan, m/z 272.15→171.35) were monitored as ion chromatograms which were integrated and quantified by quadratic regression of standard curves using Micromass QuanLynx 3.5 software.

Data Analysis. The kinetic parameters for each substrate were obtained from untransformed data by nonlinear least squares regression using GraFit 5 (Erithacus Software, Horley, Surrey, UK) using the Michaelis-Menten equation in case of hyperbolic kinetics. When the metabolic profile was consistent with positive homotropic behaviour V_{\max} , S_{50} and Hill coefficient (n) were calculated from untransformed data using the Hill equation. For those substrates, the maximum clearance (CL_{\max}), when the enzyme is fully activated, was calculated instead of CL_{int} as described previously (Houston and Kenworthy, 2000, Galetin et al., 2004).

Normalization of V_{\max} values. To compare the intrinsic catalytic activity between the hepatic and intestinal enzymes, the V_{\max} values obtained (and consequently the CL_{int}) were normalized for the mean population relative abundance of P450 enzymes involved. The values used are listed in Table 2 and were obtained by meta-analysis of the P450 abundance in 219 (CYP3A4), 174 (CYP2C9), 126 (CYP2C19) and 98 livers (CYP2D6) (Rowland Yeo et al., 2003) and 31 intestinal samples (Paine et al., 2006).

Results

Comparison of kinetic parameters between liver and intestinal microsomes. Activity of CYP3A (3A4 and 3A5), CYP2C enzymes (2C8, 2C9 and 2C19) and CYP2D6 was investigated in pooled HLM and HIM using 12 representative probes. Paclitaxel 6 α -hydroxylation via CYP2C8 in the intestinal microsomes was negligible and was therefore not included in the further analysis.

Positive cooperativity, commonly observed with hepatic CYP3A4 (Galetin et al., 2003), was also noted in the kinetic profiles obtained in the HIM for alprazolam, triazolam, testosterone, diazepam and flunitrazepam. Alprazolam 1'- and 4-hydroxylation is shown as a representative example in Fig. 1A. The type of kinetics for the CYP3A and CYP2C19 substrates investigated was in general agreement with the previous reports (Nielsen et al., 1999, Wang et al., 2000, Hesse et al., 2001, Obach et al., 2001, Galetin et al., 2004, Rawden et al., 2005); the only significant difference was observed in the case of midazolam 4-hydroxylation where in contrast to hyperbolic kinetics in hepatic microsomes, positive cooperativity was observed in the intestine. Kinetic profiles obtained for quinidine, tolbutamide and S-mephenytoin showed standard Michaelis-Menten kinetics in both systems, whereas bufuralol followed biphasic kinetic behaviour. Initial analysis indicated significantly lower activity in HIM compared to HLM as illustrated in the comparative clearance plots for 3-hydroxyflunitrazepam in Fig. 1B (50-fold lower maximal clearance in HIM in comparison to HLM). The characteristics of positive cooperativity were observed in the clearance plots for both systems; however, the flunitrazepam concentration at which the maximum clearance (corresponding to the fully activated enzyme) was achieved differs, which may be related to inter-donor differences in the intestinal and hepatic pool.

Both K_m (or S_{50}) and V_{max} values for each individual pathway (in case of benzodiazepines) were compared between HIM and HLM. K_m (or S_{50}) values obtained in HIM were corrected for the microsomal binding (Table 3), with $f_{u(m)}$ values of 0.82, 0.64, 0.94, 0.61, 0.87, 0.67, 0.55, 0.59 and 0.31 for midazolam, diazepam, flunitrazepam, alprazolam, triazolam, quinidine, testosterone, nifedipine and bufuralol, respectively; no corrections were applied for tolbutamide and S-mephenytoin ($f_{u(m)} > 0.95$). K_m (or S_{50}) values obtained for testosterone, quinidine and bufuralol in HLM were also corrected for the microsomal binding with $f_{u(m)}$ values of 0.78, 0.8 and 0.58, respectively. A 100-fold range in K_m (S_{50}) values observed in HIM was consistent with HLM. Of the 16 pathways investigated, 75% of K_m (S_{50}) values obtained in intestinal microsomes were within 2-fold of hepatic values (Fig. 2A). The maximal disagreement was observed in the case of S-mephenytoin and 1'-hydroxymidazolam, where the intestinal K_m values were 3-fold greater than the hepatic estimates (Table 3).

In contrast, V_{max} values in HIM were two orders of magnitude lower in comparison to HLM (0.0005-0.26 vs. 0.04-6.4 nmol/min/mg protein, respectively). Nordiazepam, 1'-hydroxyalprazolam and bufuralol formation were at the lower and 1'-hydroxymidazolam and testosterone at higher activity range (Table 3, Fig. 2B). Comparison of CL_{int} ($\mu\text{L}/\text{min}/\text{mg}$ protein) or CL_{max} (for sigmoidal kinetics) obtained in both in vitro systems for all 16 pathways investigated is shown in Fig. 3A. Clearance estimates showed a wide range in both HLM (0.36-895 $\mu\text{L}/\text{min}/\text{mg}$ protein) and HIM (0.006-22 $\mu\text{L}/\text{min}/\text{mg}$ protein); in both systems alprazolam and midazolam 1'-hydroxylation resulted in the lowest and highest estimates, respectively (Table 3). Clearance obtained in HLM for CYP3A4 substrates was 16–180 fold higher (4-hydroxymidazolam and 4-hydroxyalprazolam, respectively) in comparison to HIM. In

case of CYP2D6 and CYP2C substrates, the fold difference in clearance estimates between the two in vitro systems ranged from 180 to 360 for bufuralol and tolbutamide, respectively. For most of the substrates investigated the fold difference in CL_{int} between HLM and HIM reflected the differences observed in the V_{max} values.

Pathway ratio. In order to assess the relative contribution of CYP3A5 and CYP2C19 to the overall drug clearance, differences in pathway ratios (rate of major to minor pathway) between HIM and HLM were investigated for four benzodiazepines, over a range of substrate concentrations. Midazolam (1': 4-hydroxy) and alprazolam (4: 1'-hydroxy) pathway ratios were selected as representative markers for CYP3A5 activity (Galetin et al., 2004), whereas diazepam and flunitrazepam (3-hydroxy: N-desmethyl) pathway ratios were used to assess the CYP2C19 activity in the intestine and liver. In case of flunitrazepam and diazepam the ratios showed noticeable substrate concentration-dependence; but identical trends were observed in both in vitro systems (Fig. 4). Similar trend was observed with midazolam where pathway ratio decreased with the increasing substrate concentrations, with a comparable mean ratio of 5.2 and 5.6 in liver and intestine, respectively. The most evident changes in pathway ratios were noted over the range of alprazolam concentrations. The ratio of 4- to 1'-hydroxyalprazolam decreased 5-fold in HIM resulting in 1'-hydroxyalprazolam contributing 17% to the overall alprazolam clearance in HIM, in contrast to 4% observed in HLM.

Comparison of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 hepatic and intestinal intrinsic activities. V_{max} and consequently CL values obtained in both HIM and HLM were normalized for the CYP3A4, CYP2C9, CYP2C19 and CYP2D6 relative abundance using the previously reported population values listed in Table 2 (Rowland Yeo et al., 2003, Paine et al., 2006). In contrast to expectation, the trend of

lower clearance in HIM in comparison to the estimates in HLM (4.5 to 50-fold) remained (Fig. 3B). The clearance rank order was in good agreement between the systems irrespective of the normalization for the CYP relative abundance.

Comparison of two enterocyte isolation methods (elution and mucosal scraping) showed systematically greater V_{\max} values for the CYP3A4 substrates in microsomes prepared from eluted enterocytes in comparison to mucosal scraping, ranging from 3 to 29-fold for 4-hydroxymidazolam and nordiazepam, respectively (Fig. 5A). The activity difference between the methods was CYP-related, resulting in the following rank order - CYP2C19 > CYP3A4 > CYP2C9 ~ CYP2D6. The mean activity ratio for each individual P450 is shown in Table 2. When the correction for the activity loss was applied to the clearance estimates obtained in intestinal microsomes prepared by mucosal scraping (an average 10-fold), the intrinsic catalytic activities (expressed per pmol CYP) of all four different P450 enzymes were comparable between the liver and intestine, as shown in the Fig. 3C. In case of tolbutamide, 4-hydroxyalprazolam, 3-hydroxydiazepam and 1'-hydroxytriazolam intestinal clearance represented 20-33% of hepatic estimate, whereas intestinal CL_{int} of 4-hydroxymidazolam was 2-fold greater than the hepatic counterpart (Table 4). The kinetic characteristics of 1'-hydroxymidazolam formation in both microsomes prepared by elution and mucosal scraping were comparable to the profile obtained in HLM (Fig. 5B). The enterocyte isolation method had no significant effect on midazolam 1'-hydroxylation K_m , resulting in values of 10.1 and 8.2 μM obtained in mucosal scraping and elution microsomal data, respectively.

Discussion

The current study provides a systematic overview of kinetic characteristics of 12 substrates metabolized via five different P450 enzymes in the pooled HIM. A comparison of intestinal and hepatic metabolic ability was performed for 8 CYP3A substrates; with the exception of midazolam (Paine et al., 1997) and testosterone (Obach et al., 2001), the kinetics of these substrates was previously not documented for the gut. In addition, the potential role of ‘minor’ intestinal P450s, CYP2C8, CYP2C9, CYP2C19 and CYP2D6, was assessed.

The general agreement between the atypical kinetic profiles in the HIM and HLM showed that the extent of cooperative binding to the intestinal CYP3A4 was similar to hepatic counterpart for the substrates investigated. A comparative analysis indicated no significant differences in the binding affinity between intestinal and hepatic P450s, illustrated by good agreement in the corresponding K_m values (Fig. 2A) over a 130-fold range. In contrast two orders of magnitude lower intestinal V_{max} and consequently CL values were observed (Fig. 2B and 3A, respectively). The intestinal V_{max} values obtained for the two most commonly used CYP3A4 in vitro probes, midazolam and testosterone, were comparable to the previous studies in microsomes prepared by scrapings from a variety of individual donors (5-31) (Prueksaritanont et al., 1996, Lin et al., 2002).

More than 20-fold variation in the CYP2C9 activity (assessed by either tolbutamide or diclofenac hydroxylation) has been reported in duodenal/jejunal microsomes (Prueksaritanont et al., 1996, Obach et al., 2001), consistent with the polymorphic nature of this enzyme. Although CYP2C9 represents the main CYP2C enzyme in the intestine (Läpple et al., 2003, Paine et al., 2006) its intestinal activity is indicated to be an order of magnitude lower than the hepatic complement

(Prueksaritanont et al., 1996). The tolbutamide V_{\max} obtained in our study is within the reported range; however, the tolbutamide intestinal V_{\max} and CL represented only 0.6 and 0.3% of hepatic (data not normalized for CYP2C9 relative abundance). Mephenytoin 4'-hydroxylation V_{\max} obtained in the current study was at the lower end of the reported values (0.3 – 13.1 pmol/min/mg protein) (Obach et al., 2001, Laple et al., 2003).

However, a direct comparison of the current and previously reported intestinal data is not straightforward as a number of variables may contribute to the differential estimates of the P450 activity in the intestine. Use of different segments of gut (proximal region vs. the whole length in the pooled microsomes) provides one reason for the inconsistencies seen across the studies. The enterocyte microsomal yield is a function of the gut segment used and the highest abundance and activity are observed in the proximal regions; therefore, the use of pooled intestinal microsomes from various sections of the gut might represent a limitation due to the greater intestinal heterogeneity in comparison to the liver. The effect of CYP2C polymorphism, co-regulation between CYP2C9 and CYP3A4 and impact of a number of various dietary compounds on intestinal CYP3A4 are unknown in the pooled samples used in our study; all these factors might have affected the activity of intestinal enzymes.

Most of the previous assessments of intestinal metabolic activity were performed at a single point or using a limited substrate concentration range of the representative probe (Prueksaritanont et al., 1996, Obach et al., 2001, Lin et al., 2002). In case of CYP3A4, different marker probes were used, namely, midazolam (Paine et al., 1997, Lin et al., 2002), testosterone (Prueksaritanont et al., 1996, Obach et al., 2001) and verapamil (von Richter et al., 2004), resulting in differential extent of reported inter-individual variability in the intestinal CYP3A4 activity (7 to 168-fold

difference). In addition, the most common method of assessment of intestinal activity is based on the comparison of hepatic and intestinal activity expressed per mg of microsomal protein (Paine et al., 1997, von Richter et al., 2004) rather than as catalytic activity (normalized for the relative abundance of the P450 investigated). Comparison of catalytic activity as a measure of intrinsic enzyme activity in case of verapamil (Yang et al., 2004) showed evidence of similar intestinal and hepatic CYP3A4 intrinsic activities.

The current study provides the first systematic comparison of the hepatic and intestinal intrinsic metabolic activity for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 (CYP2C8 not included due to negligible activity) for a range of substrates. Population relative abundance of the P450 enzymes (Table 2) was used for the normalization of V_{\max} and CL data; this approach was considered appropriate as pooled microsomes from different donors were used for the assessment of both intestinal and hepatic activity. Although the incorporation of the P450 relative abundance increased the overall contribution of the intestinal clearance in comparison to the liver, intestinal clearance for CYP3A4 substrates maintained 4.5 to 50-fold lower in comparison to the estimates in the liver; an 11 to 40-fold lower activity was observed for non-CYP3A4 probes, bufuralol and S-mephenytoin, respectively (Fig. 3B). The rank order in clearance was consistent between the systems over the range covered (0.002-5.8 and 0.0002-0.52 $\mu\text{L}/\text{min}/\text{pmolCYP}$ in HLM and HIM, respectively), irrespective of the P450 enzyme investigated.

The process of enterocyte isolation (mucosal scraping and enterocyte elution) was investigated as a possible reason for the discrepancy observed in hepatic and intestinal activities (Fig. 3A and 3B). Mucosal scraping (Kolars et al., 1994, Paine et al., 1997, Madani et al., 1999) is a mechanical method that generates a mixture of

cells in addition to mature enterocytes. The specific activity of P450 enzymes in microsomes prepared by this method is low as some of the enterocytes are lysed and the P450 enzymes may be denatured being exposed to the digestive enzymes (Lin et al., 1999). In contrast, elution with chemically-based protease inhibitors is a more gentle isolation technique, is less likely to damage the cells and results in higher enzyme activity (Lin et al., 1999, Zhang et al., 1999, von Richter et al., 2004).

Comparison of enterocyte isolation methods indicated on average 10-fold greater V_{\max} values in microsomes from eluted enterocytes compared to mucosal scraping. Comparison of catalytic activities (P450 relative abundance also considered) for 11 different pathways investigated (Fig. 5A), indicated that CYP2C19 activity showed the highest sensitivity on the enterocyte isolation method used (13 to 29-fold loss in the enzyme activity), followed by CYP3A4, whereas CYP2C9 and CYP2D6 were comparable (Table 2). Overall correction of the clearance estimates obtained in intestinal microsomes prepared by mucosal scraping for the activity loss resulted in comparable (within the 2-fold limit) intrinsic catalytic activities between liver and intestine for two thirds of the pathways investigated (Fig. 3C). The intestinal clearances of all standard CYP3A4 probes (midazolam, testosterone, nifedipine and quinidine) were within 61 to 136% range of hepatic estimates, whereas in the case of CYP2C9 and CYP2C19, the ratio of intestinal to hepatic clearance ranged from 33 to 73% for tolbutamide and nordiazepam, respectively. These findings represent a significant improvement in comparison to the 2 to 21 % ratio of intestinal to hepatic CL_{int} values obtained from mucosal scraping microsomal data.

Considering the liver-intestine inter-donor differences in the current study and the use of mean population values for the P450 relative abundances, we conclude that intestinal and hepatic catalytic activity (per pmol of P450 enzyme) of CYP3A4,

CYP2D6, CYP2C9 and CYP2C19 are comparable for a wide range of substrates. The current study indicates the utility of pooled intestinal microsomes in the assessment of intestinal metabolism and establishes an unequivocal relationship between the intrinsic activities of hepatic and intestinal P450 enzymes. For most of the substrates investigated here efflux transporters contribute to a minor extent in their overall disposition. Hence, it will be important to complement the current work with class 2 substrates from the Biopharmaceutics Drug Disposition Classification System (Wu and Benet, 2005) using intestinal *in vitro* systems of greater complexity (e.g., transfected cell lines and intestinal slices as in studies by Cummins et al., 2004 and van der Kerkhof et al., 2005, respectively), to adequately assess intestinal metabolism *in vitro* in presence of functional transporter proteins.

As is well documented for the liver, prediction of the extent of intestinal first-pass metabolism *in vivo* from the *in vitro* data requires an appropriate mechanistic model (e.g., well-stirred liver model) to integrate intestinal intrinsic clearance with many physiological processes. However, there are physiological complexities unique to the intestine; for example, enterocytic rather than organ blood flow needs to be incorporated in addition to the greater cellular heterogeneity and a different spatial arrangement for the P450 enzymes and P-glycoprotein in the intestine compared to the liver. Consensus has yet to be achieved on this modeling issue (Pang, 2003, Benet et al., 2004, Rostami and Tucker, 2004) and the challenges of a mechanistic approach must be met before intestinal first-pass metabolism *in vivo* can be confidently predicted from *in vitro* data on intestinal intrinsic clearance.

Acknowledgements. The Authors would like to thank Caroline Brown for her help with the intestinal microsomal work and Dr David Hallifax and Sue Murby for protein binding and analytical assays.

References:

Benet LZ, Cummins CL and Wu CY (2004) Unmasking the dynamic interplay between efflux transporters and metabolic enzymes. *Int J Pharmaceut* **277**: 3-9.

Cummins CL, Jacobsen W, Christians U and Benet LZ (2004) CYP3A4-Transfected Caco-2 cells as a tool for understanding biochemical absorption barriers: studies with sirolimus and midazolam. *J Pharmacol Exp Ther* **308**: 143-155.

Damkier P, Hansen LL and Brosen K (1999) Effect of fluvoxamine on the pharmacokinetics of quinidine. *Eur J Clin Pharmacol* **55**: 451-456.

Floren LC, Bekersky I, Benet LZ, Mekki Q, Dressler D, Lee JW, Roberts JP and Hebert MF (1997) Tacrolimus oral bioavailability doubles with coadministration of ketoconazole. *Clin Pharmacol Ther* **62**: 41-49.

Galetin A, Brown C, Hallifax D, Ito K and Houston JB (2004) Utility of recombinant enzyme kinetics in prediction of human clearance – impact of variability, CYP3A5 and CYP2C19 on CYP3A4 substrates. *Drug Metab Dispos* **32**: 1411-1420.

Galetin A, Clarke SE and Houston JB (2003) Multisite kinetic analysis of interactions between prototypical CYP3A4 subgroup substrates: midazolam, testosterone and nifedipine. *Drug Metab Dispos* **31**: 1108-1116.

Glaeser H, Drescher S, van der Kuip H, Behrens C, Geick A, Burk O, Dent J, Somogyi A, von Richter O, Griese EU, Eichelbaum M and Fromm MF (2002) Shed

human enterocytes as a tool for the study of expression and function of intestinal drug-metabolizing enzymes and transporters. *Clin Pharmacol Ther* **71**: 131-140.

Hall SD, Thummel KE, Watkins PB, Lown KS, Benet LZ, Paine MF, Mayo RR, Turgeon DK, Bailey DG, Fontana RJ and Wrighton SA (1999) Molecular and physical mechanisms of first-pass extraction. *Drug Metab Dispos* **27**: 161-166.

Hesse LM, Venkatakrisnan K, von Moltke LL, Shader RI and Greenblatt DJ (2001) CYP3A4 is the major CYP isoform mediating the in vitro hydroxylation and demethylation of flunitrazepam. *Drug Metab Dispos* **29**: 133:140.

Hirota N, Ito K, Iwatsubo T, Green CE, Tyson CA, Shimada N, Suzuki H and Sugiyama Y (2001) In vitro/in vivo scaling of alprazolam metabolism by CYP3A4 and CYP3A5 in humans. *Biopharm Drug Dispos* **22**: 53-71.

Houston JB and Kenworthy KE (2000) *In vitro-in vivo* scaling of CYP kinetic data not consistent with the classical Michaelis-Menten model. *Drug Metab Dispos* **28**: 246 – 254.

Kolars JC, Lown KS, Schmiedlin-Ren P, Ghosh M, Fang C, Wrighton SA, Merion RM and Watkins PB (1994) CYP3A gene expression in human gut epithelium. *Pharmacogenetics* **4**: 247-59.

Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA, Hall SD, Maurel P, Relling M, Brimer C, Yasuda K, Venkataramanan

R, Strom S, Thummel K, Boguski MS and Schuetz E (2001) Sequence diversity in CYP3A4 promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27**: 383-391.

Läpple F, von Richetr O, Fromm MF, Richter T, Thon KP, Wisser H, Griese E-U, Eichelbaum M and Kivistö KT (2003) Differential expression and function of CYP2C isoforms in human intestine and liver. *Pharmacogenetics* **13**: 565-575.

Lennernas H (2003) Clinical pharmacokinetics of atorvastatin. *Clin Pharmacokinet* **42**: 1141-1160.

Lin JH, Chiba M and Baillie TA. (1999) Is the role of the small intestine in first-pass metabolism overemphasized? *Pharmacol Rev* **51**:135-158

Lin YS, Dowling ALS, Quigley SD, Farin FM, Zhang J, Lamba J, Schuetz EG and Thummel KE (2002) Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal metabolism of midazolam. *Mol Pharmacol* **62**: 162-172.

Lown KS, Kolars JC, Thummel KE, Barnett JL, Kunze KL, Wrighton SA and Watkins PB. (1994) Interpatient heterogeneity in expression of CYP3A4 and CYP3A5 in small bowel. Lack of prediction of erythromycin breath test. *Drug Metab Dispos* **22**:947-955

Madani S, Paine MF, Lewis L, Thummel KE and Shen DD (1999) Comparison of CYP2D6 content and metoprolol oxidation between microsomes isolated from human livers and small intestines. *Pharm Res* **16**: 1199-1205.

Masica AL, Mayo G and Wilkinson GR (2004) In vivo comparisons of constitutive cytochrome P450 3A activity assessed by alprazolam, triazolam and midazolam. *Clin Pharmacol Ther* **76**: 341-349.

Matsumoto S, Hiramata T, Matsubara T, Nagata K, Yamazoe Y (2002) Involvement of CYP2J2 on the intestinal first-pass metabolism of antihistamine drug, astemizole. *Drug Metab Dispos* **30**: 1240-1245.

Nielsen TL, Rasmussen BB, Flinois JP, Beaune P and Brosen K (1999) In vitro metabolism of quinidine: the (3S)-3-hydroxylation of quinidine is a specific marker reaction for cytochrome P-4503A4 activity in human liver microsomes. *J Pharmacol Exp Ther* **289**: 31-37.

Obach RS, Zhang Q-Y, Dunbar D and Kaminsky LS (2001) Metabolic characterization of major human small intestinal cytochrome P450s. *Drug Metab Dispos* **29**:347-352.

Paine MF, Hart HL, Ludington SS, Haining RL, Rettie AE and Zeldin DC (2006) The human intestinal cytochrome P450 "pie". *Drug Metab Dispos*

Paine MF, Schmiedlin-ren P and Watkins PB (1999) Cytochrome P-450 1A1 expression in human small bowel: interindividual variation and inhibition by ketoconazole. *Drug Metab Dispos* **27**: 360-364.

Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, Perkins JD and Thummel KE (1997) Characterization of interintestinal and intrainestinal variations in human CYP3A-dependant metabolism. *J Pharmacol Exp Ther* **283**:1552-1562.

Pang KS Modeling of intestinal drug absorption: roles of transporters and metabolic enzymes. *Drug Metab Dispos* **31**: 1507-1519.

Prueksaritanont T, Gorham LM, Hochman JH, Tran LO and Vyas KP (1996) Comparative studies of drug-metabolizing enzymes in dog, monkey, and human small intestines, and in Caco-2cells. *Drug Metab Dispos* **24**: 634-642.

Rawden HC, Carlile DJ, Tindall A, Hallifax D, Galetin A, Ito K and Houston JB (2005) Microsomal prediction of in vivo clearance and associated inter individual variability of six benzodiazepines in humans. *Xenobiotica* **35**: 603-625.

Rostami-Hodjegan A and Tucker GT (2004) 'In silico' simulations to assess the 'in vivo' consequences of 'in vitro' metabolic drug-drug interactions. *Drug Discovery Today: Technologies* **1**: 441-448.

Rowland Yeo K, Rostami-Hodjegan A and Tucker GT (2003) Abundance of cytochrome P450 in human liver: a meta-analysis. *Br J Clin Pharmacol* **57**: 687-688.

Thörn M, Finnström N, Lundgren S, Rane A and Lööf L (2005) Cytochromes P450s and MDR1 mRNA expression along the human gastrointestinal tract. *Br J Clin Pharmacol* **60**: 54-60.

Thummel KE, Kunze KL and Shen DD (1997) Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. *Adv Drug Deliv Rev* **27**: 99-127.

van der Kirkhof EG, de Graaf IAM, de Jager MH, Meijer DKF and Groothuis GMM (2005) Characterization of rat small intestinal and colon precision-cut slices as an in vitro system for drug metabolism and induction studies. *Drug Metab Dispos* **33**: 1613-1620.

von Richter O, Burk O, Fromm MF, Thon KP, Eichelbaum M and Kivisto KT (2004) Cytochrome P450 3A4 and P-glycoprotein expression in human small intestinal enterocytes and hepatocytes: A comparative analysis in paired tissue specimens. *Clin Pharmacol Ther* **75**:172-183.

Wang RW, Newton DJ, Liu N, Atkins WM and Lu AYH (2000) Human cytochrome P-450 3A4: *in vitro* drug-drug interaction patterns are substrate-dependent. *Drug Metab Dispos* **28**: 360-366.

Watkins B (1997) The barrier function of CYP3A4 and P-glycoprotein in the small bowel. *Adv Drug Deliv Rev* **27**: 161-170.

Wu C-Y and Benet LZ (2005) Predicting drug disposition via application of BCS: Transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharm Res* **22**: 11-23.

Yang J, Tucker GT and Rostami-Hodjegan A (2004) Cytochrome P450 3A expression and activity in the human small intestine. *Clin Pharmacol Ther* **76**: 391.

Zhang Q-Y, Dunbar D, Ostrowska A, Zeisloft S, Yang J and Kaminsky LS (1999) Characterization of human small intestinal cytochromes P-450. *Drug Metab Dispos* **27**:804-809.

FOOTNOTES

Financial support for this project was provided by the following Centre for Applied Pharmacokinetic Research (CAPKR) Consortium members: Eli Lilly, GlaxoSmithKline, Novartis, Pfizer and Servier.

Address correspondence to: Dr A. Galetin, School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Oxford Rd, Manchester, M13 9PL, UK

FIGURE LEGENDS

Fig. 1. Kinetic profiles of 1'- and 4-hydroxyalprazolam in HIM (A) and comparative clearance profiles for 3-hydroxyflunitrazepam in HIM (right y-axis) and HLM (left y-axis) (B).

Fig. 2. Comparison of hepatic and intestinal K_m (A) and V_{max} values (B) for 16 individual CYP3A4 (□), CYP2C9 (▼), CYP2C19 (▲) and CYP2D6 (■) pathways investigated. In both panels the solid line represents line of unity, whereas dashed lines represent either the 2-fold (A) or 50-fold (B) difference between hepatic and intestinal values.

Fig. 3. Comparison of hepatic and intestinal CL_{int} (CL_{max}) for 16 individual CYP3A4 (□), CYP2C9 (▼), CYP2C19 (▲) and CYP2D6 (■) pathways. Clearance values are expressed either per mg of microsomal protein (A) or per pmol P450 after being normalized for the relative P450 abundance (Table 2) in the liver and intestine (B). Panel C shows the normalized CL_{int} (CL_{max}) values corrected for the activity differences between the enterocyte preparation methods. In all the panels the solid line represents line of unity, whereas dashed lines represent 50 (A), 20 (B) and 2-fold (C) difference between hepatic and intestinal values.

Fig. 4. Comparison of metabolic pathway ratios for midazolam, alprazolam, diazepam and flunitrazepam in HLM (▲) and HIM (□).

Fig. 5. Comparison of intestinal P450 activity in intestinal microsomes prepared by mucosal scraping and elution method. V_{max} corrected for the CYP population

abundance (Table 2) (A). The pathways investigated include 1'-hydroxymidazolam (1), 4-hydroxymidazolam (2), 3-hydroxydiazepam (3), nordiazepam (4), 1'-hydroxyalprazolam (5), 4-hydroxyalprazolam (6), quinidine (7), testosterone (8), mephenytoin (9), tolbutamide (10) and bufuralol (11). Comparison of 1'-hydroxymidazolam kinetic profiles in HLM (Δ), intestinal microsomes prepared by enterocyte elution (\blacksquare) and mucosal scraping (\square) (B).

TABLE 1

Incubation conditions for the 11 substrates investigated in human intestinal and liver microsomes

Substrate	HLM		HIM		Substrate concentration range (μ M)
	[Protein]	Time	[Protein]	Time	
	mg/ml	(min)	mg/ml	(min)	
Midazolam	0.25	5	0.5	10	1-200
Triazolam	0.25	15	1	30	1-1000
Alprazolam	0.25	20	1.5	30	1-1000
Diazepam	0.25	15	1.5	30	2.5-500
Flunitrazepam	0.25	20	1	30	1-1000
Quinidine	0.5	15	1	30	1-400
Testosterone	0.5	15	1	20	1-500
Nifedipine	0.25	10	1	30	1-200
S-mephenytoin	1	60	1.5	60	1-1000
Tolbutamide	0.5	60	1.5	90	1-1000
Paclitaxel	0.5	10	1	20	1-200
Bufuralol	0.5	15	1	20	1-1000

TABLE 2

Mean hepatic and intestinal population relative abundance (pmolP450/mg protein) of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 and the mean activity ratio between intestinal microsomes prepared by elution and mucosal scraping.

	Liver^a	Intestine^b	Mean enterocyte activity ratio^c
CYP3A4	155	43	8.1
CYP2C9	73	8.4	4.7
CYP2C19	14	1	21
CYP2D6	8	0.5	4.2

^aRowland Yeo et al., 2003, ^bPaine et al., 2006, ^cMean ratio of V_{max} values obtained in microsomes prepared by elution and mucosal scraping method for 11 pathways investigated

TABLE 3

Kinetic parameters for 16 pathways mediated by CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in HLM and HIM prepared by mucosal scraping

Pathway	V_{\max}		K_m or S_{50}		CL_{int} or CL_{max}		CL_{int} or CL_{max}^b	
	(nmol/min/mg protein)		(μM)		($\mu\text{L}/\text{min}/\text{mg}$ protein)		($\mu\text{L}/\text{min}/\text{pmolCYP}$)	
	HIM	HLM	HIM ^a	HLM	HIM	HLM	HIM	HLM
1'-hydroxymidazolam	0.23 ± 0.01	2.7 ± 0.2	10.1 ± 1.6	3.3 ± 0.6	22	895	0.52	5.8
4-hydroxymidazolam	0.08 ± 0.002	1.3 ± 0.04	22 ± 2	21 ± 2	3.6	59	0.08	0.38
3-hydroxydiazepam	0.041 ± 0.002	4.5 ± 0.6	213 ± 10	235 ± 54	0.095	11	0.002	0.07
Nordiazepam	0.004 ± 0.0004	0.5 ± 0.05	160 ± 29	132 ± 37	0.01	2.1	0.011	0.15
3-hydroxyflunitrazepam	0.17 ± 0.02	7.1 ± 0.3	458 ± 47	300 ± 24	0.19	12	0.004	0.08
Desmethylflunitrazepam	0.007 ± 0.001	0.4 ± 0.03	293 ± 60	174 ± 29	0.01	1.4	0.012	0.1
1'-hydroxyalprazolam	0.004 ± 0.001	0.1 ± 0.05	258 ± 62	274 ± 38	0.006	0.36	0.0002	0.002
4-hydroxyalprazolam	0.036 ± 0.008	3.9 ± 0.4	348 ± 78	309 ± 76	0.05	8.1	0.001	0.05
1'-hydroxytriazolam	0.061 ± 0.002	2.3 ± 0.5	149 ± 9	80 ± 14	0.2	29	0.005	0.19
4-hydroxytriazolam	0.12 ± 0.003	2.0 ± 0.6	242 ± 10	92 ± 34	0.25	22	0.006	0.14
Quinidine	0.01 ± 0.001	0.9 ± 0.04	69 ± 19	101 ± 12	0.19	8.7	0.005	0.06
Testosterone	0.26 ± 0.02	6.4 ± 0.3	55 ± 8	56 ± 7	2.7	68.9	0.06	0.44
Nifedipine	0.03 ± 0.003	0.89 ± 0.08	19.7 ± 4.5	10.1 ± 1.8	1.15	88.2	0.03	0.49
Mephenytoin	0.0005 ± 0.00001	0.04 ± 0.0004	106 ± 12	30 ± 1.3	0.0046	1.36	0.005	0.1
Tolbutamide	0.003 ± 0.0001	0.45 ± 0.015	769 ± 124	333 ± 29	0.0037	1.34	0.002	0.06
Bufuralol	0.0007 ± 0.00001	0.09 ± 0.01	5.9 ± 1.2	5.5 ± 1.9	0.12	25.8	0.28	3.2

^aValues corrected for microsomal binding ^bCL data ($\mu\text{L}/\text{min}/\text{mg}$) normalized for the population relative abundance for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in the liver and intestine as shown in Table 2.

TABLE 4

Relative ratio of intestinal to hepatic CL_{int} for 16 pathways investigated. Hepatic and intestinal microsomes CL_{int} values normalized for the P450 relative abundance and corrected for enterocyte isolation method activity differences

Substrate	CL_{tot} ($\mu\text{L}/\text{min}/\text{pmolCYP}$)		CL_{HIM} / CL_{HLM} (%)
	HLM	HIM	
1'-hydroxymidazolam	5.8	5.2	90
4-hydroxymidazolam	0.38	0.8	210
3-hydroxydiazepam	0.07	0.022	31
Nordiazepam	0.15	0.11	73
3-hydroxyflunitrazepam	0.08	0.04	50
Desmethylflunitrazepam	0.1	0.12	120
1'-hydroxyalprazolam	0.002	0.002	100
4-hydroxyalprazolam	0.05	0.01	20
1'-hydroxytriazolam	0.19	0.05	26
4-hydroxytriazolam	0.14	0.06	43
Quinidine	0.06	0.05	83
Testosterone	0.44	0.60	136
Nifedipine	0.49	0.30	61
Mephenytoin	0.1	0.05	50
Tolbutamide	0.06	0.02	33
Bufuralolol	3.2	2.8	88

Figure 1

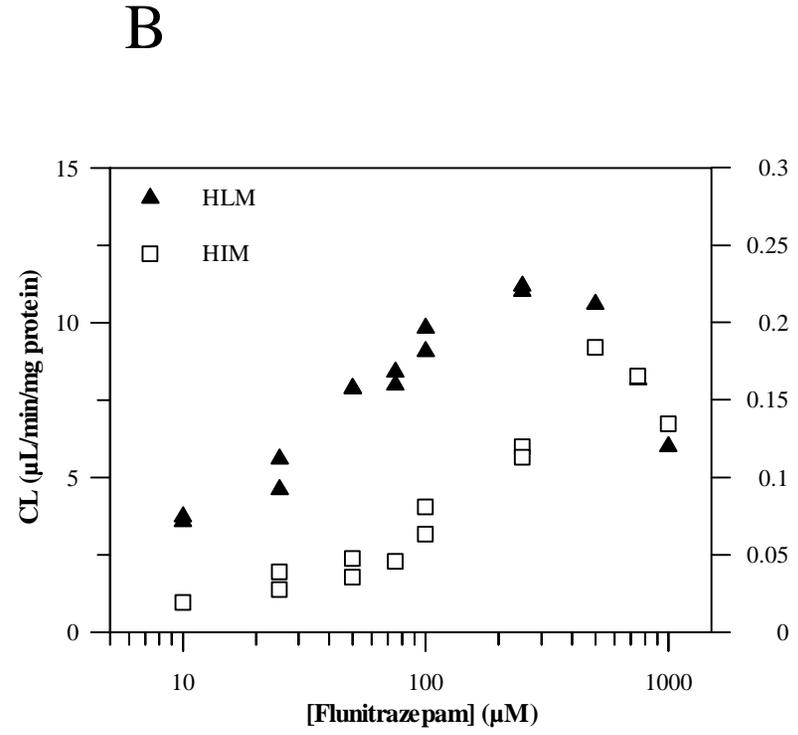
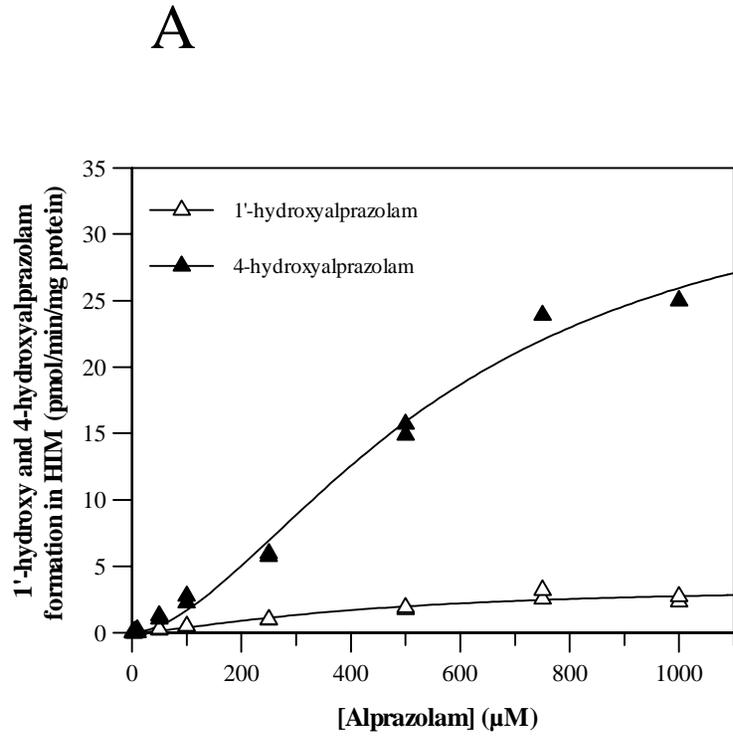


Figure 2

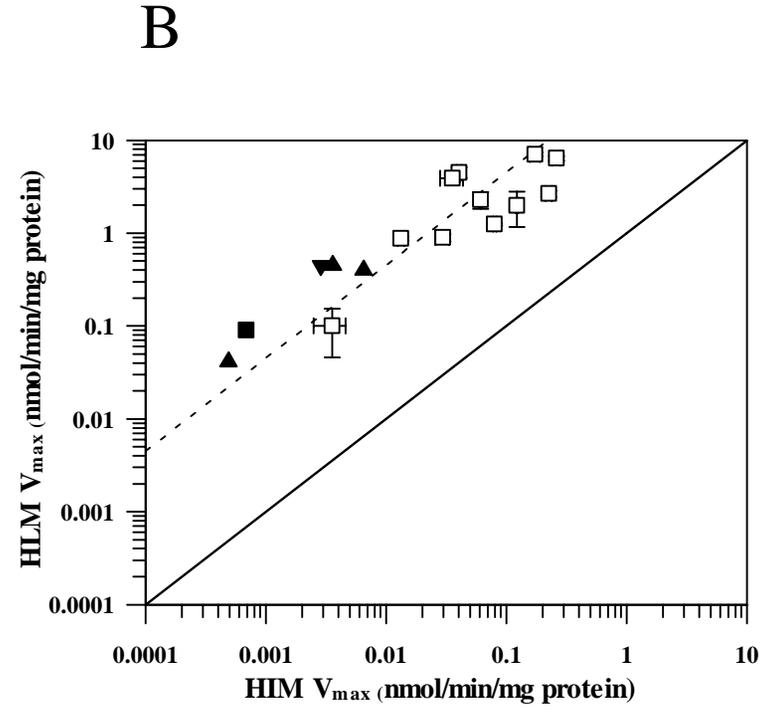
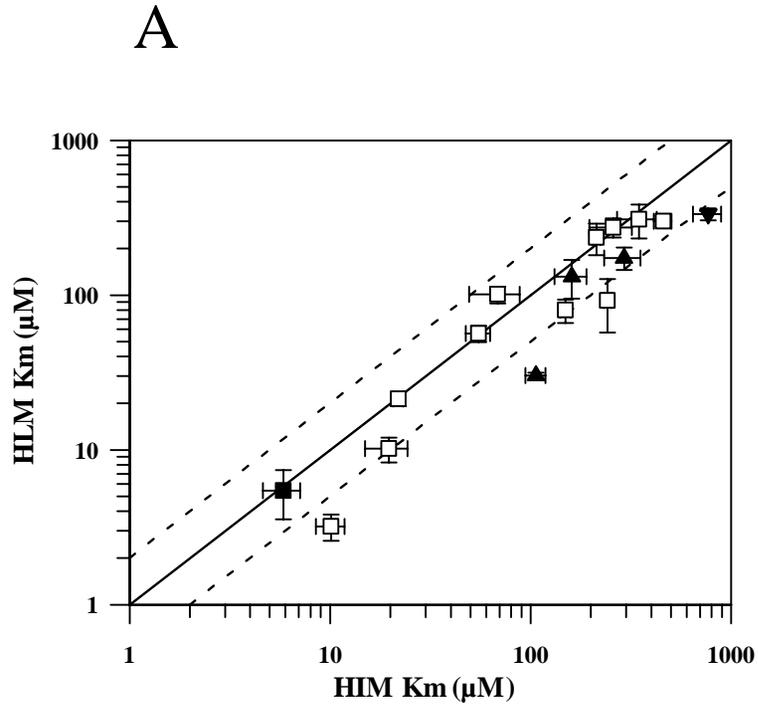


Figure 3

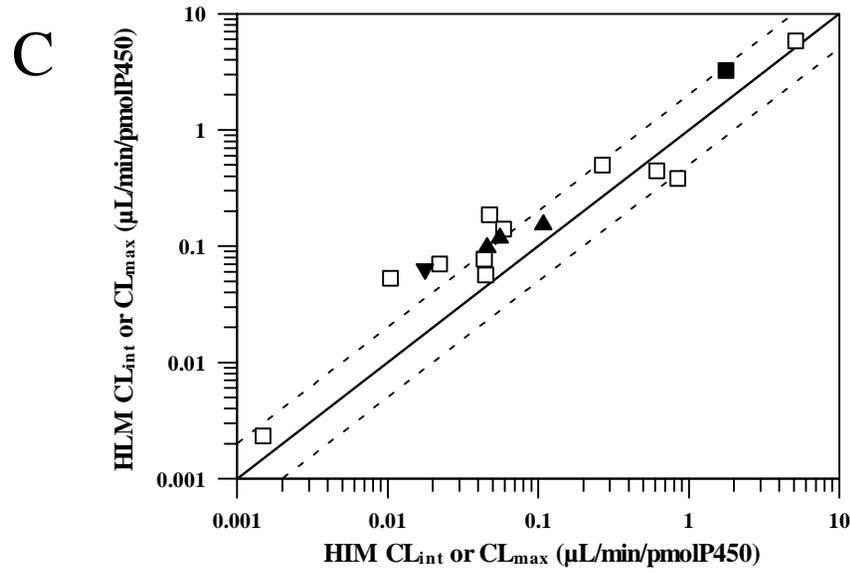
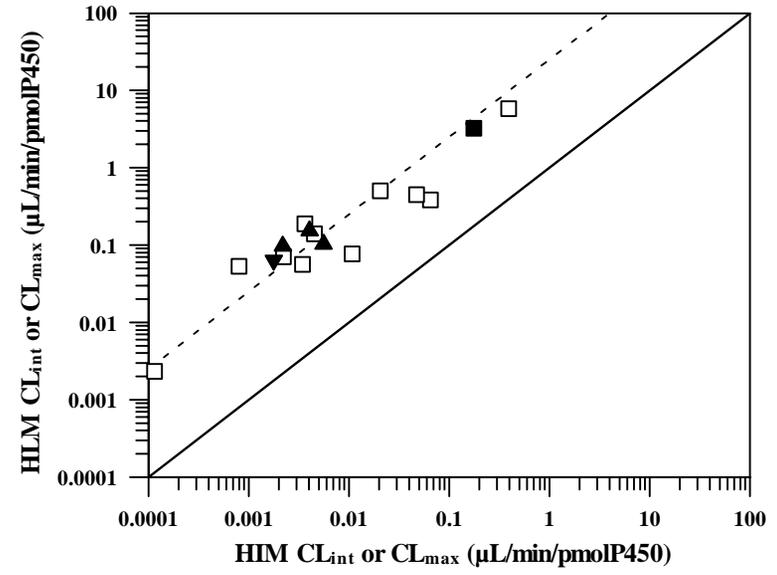
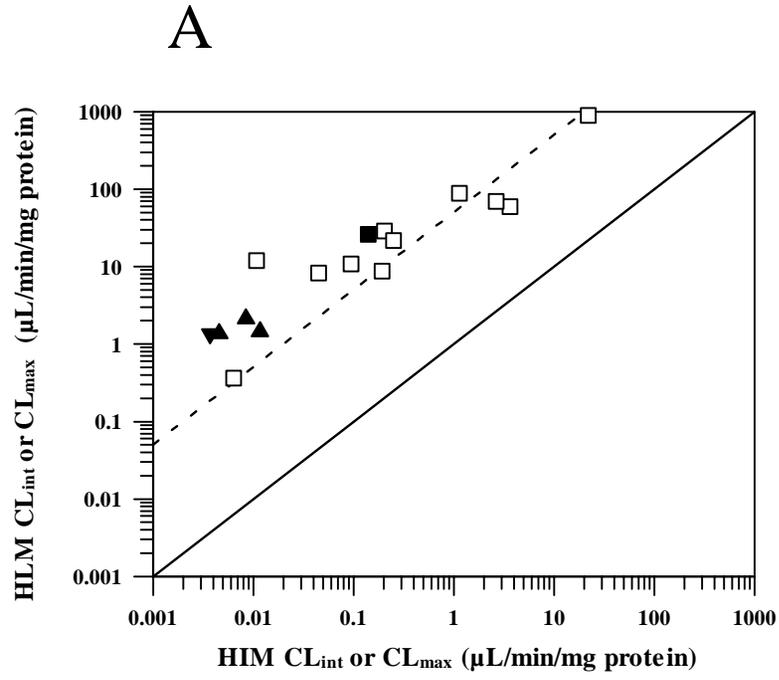
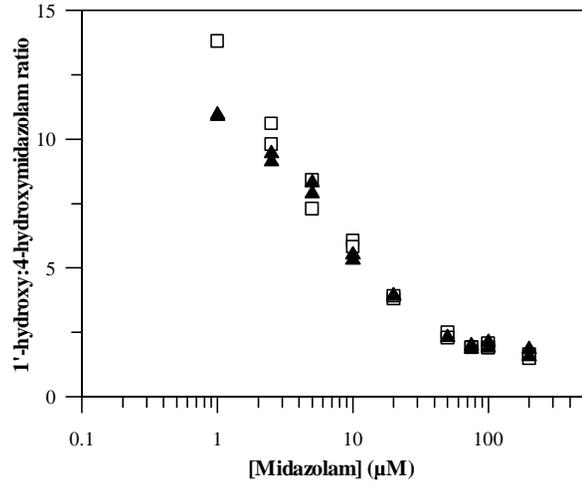
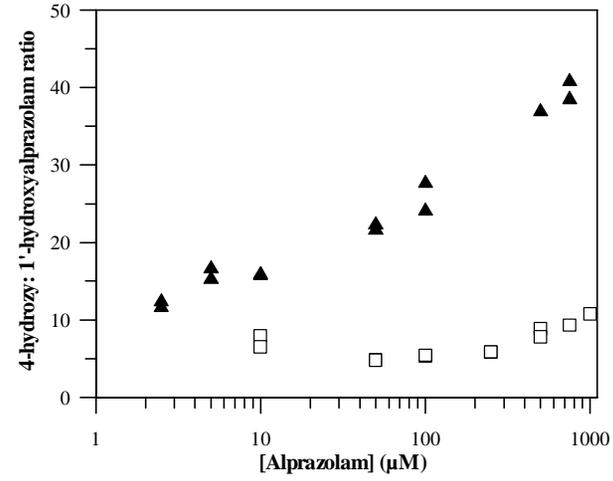


Figure 4

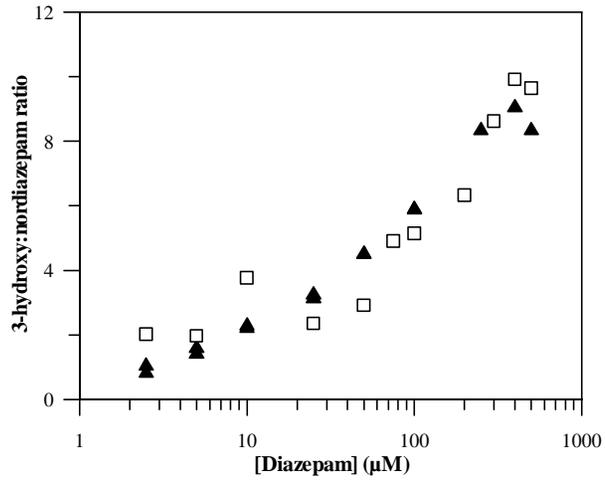
Midazolam



Alprazolam



Diazepam



Flunitrazepam

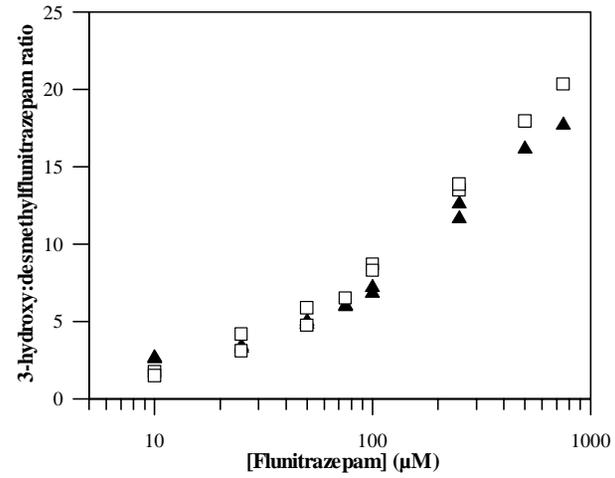


Figure 5

