Cytochromes P450, UDP-Glucuronosyltransferases and Carboxylesterases In Human Small Intestine Microsomes: Characterization and Inter-Individual Variability Among Donors

Sweta N. Parikh, Lucien F. Tarabelsi, Catherine M. Chandler, Kristen J. Lohnes, Peter V. Gagne, Charles L. Crespi*, and Christopher J. Patten*
BD Biosciences Discovery Labware, Woburn, MA 01801, USA

BD
BD Biosciences

Abstract

Epithelial cells of the small intestine, enterocytes, are enriched in both P450 and UGT activity, and are often the first site for metabolism of orally administered drugs. The duodenum and first half of the jejunum are reported to have the highest drug metabolism activity of the small intestine, while the ileum and second half of the jejunum have been shown to possess significantly lower activity. In this communication we report the isolation of intestinal microsomes from the duodenum and first half of the jejunum using the EDTA elution method. Intestinal microsomes were prepared from five donors. Each duodenum and jejunum section was tested for the following activities: testosterone 6β-hydroxylation (CYP3A4), diclofenac 4'-hydroxylation (CYP2C9), astemizole *O*-demethylation (CYP2J2/4F12/3A4), estradiol 3-glucuronidation (UGT1A1/8/10) and p-nitrophenolacetate hydrolysis (hCE1/CE2). Significant differences in activity were observed between donors (e.g. testosterone hydroxylase varied as much as 8-fold between donors). Kinetic analysis demonstrated similar Km's between intestine and liver microsomes. Data is presented which shows multiple P450s are involved in the *O*-demethylation of astemizole in HIM (CYP2J2, 4F12 and 3A4). The results of this study further demonstrate the utility of using microsomes from small intestine (duodenum and first half of the jejunum) to study the first pass effect of drug metabolism.

Introduction

The small intestine is enriched in P450 and other drug metabolism enzymes (e.g. UGT, SULT, CE), and can contribute significantly to first pass metabolism of drugs. For example, terfenadine, ebastine, and astemizole are H-antihistamine drugs that are extensively metabolized by P450s in the small intestine.[1,2] The small intestine is about seven meters and consists of: proximal duodenum (1 foot), jejunum section (8 feet), ileum (12 feet). P450/UGT activity is highest in duodenum and initial foot sections of jejunum. P450 and UGT profiles are somewhat different between human intestinal and liver microsomes, as is the case with other extra hepatic tissues (e.g. CYP1A1 is present in intestine but not in liver). In the current study we show the characterization (P450, UGT, CE) of single donor HIM and a pooled sample. The activities in HIM were compared to HLM. Pooled HIM is shown to be a useful tool to study intestinal first pass metabolism.

Materials and Methods

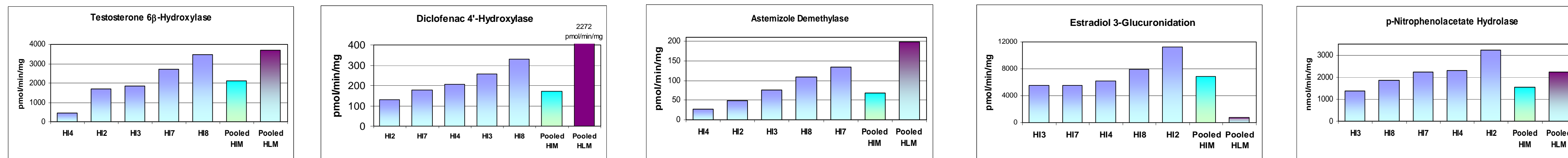
Enzyme Preparations

BD Supersomes™ Enzymes from insect cells expressing human CYP2J2, CYP3A4 and CYP4F12 membrane protein were obtained from BD Biosciences Discovery Labware (Woburn, MA). Pooled human liver microsomes (HLM) were prepared from 25 donor HLM samples (Cat. No. 452161). Enterocytes were obtained from the duodenum and jejunum sections of small intestine from five donors using a modification of the EDTA-elution method.[3] Pooled intestinal microsomes were prepared by equal mixing of microsomes from each of the five donors. The inhibitory CYP3A4 monoclonal antibody (MAB) was from BD Biosciences Discovery Labware.

Enzyme Assays

P450 activities were carried out with an NADPH generating system in either phosphate (testosterone, astemizole) or Tris (diclofenac) buffer. Incubations for estradiol glucuronidation were conducted in Tris buffer and contained alamethicin to overcome latency. Metabolites were analyzed by HPLC using previously established methods. p-Nitrophenolacetate (p-NPA) hydrolysis was carried out by previously described methods.[4] For routine assays the substrate concentrations were as follows: testosterone, 200 µM; diclofenac, 100 µM; astemizole, 40 µM; estradiol, 100 µM; p-NPA, 1000 µM. For the CYP3A4 MAB inhibition experiments the astemizole concentrations were 4 µM and 40 µM. All assays were conducted under linear conditions for both time and protein concentration. Kinetic parameters (Km and Vmax) were determined using Sigma Plot-Enzyme Kinetics software.

1 P450, UGT and Carboxylesterase Activity in a Panel of Human Intestinal Microsomes, Pooled Human Intestinal Microsomes, and Pooled Human Liver Microsomes



2 Kinetic Analysis (Km and Vmax) of HIM and HLM Catalytic Activities

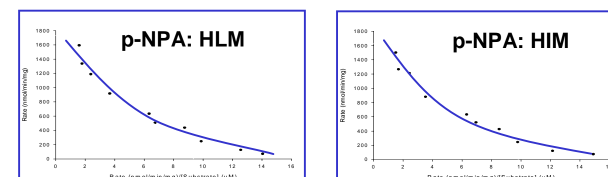
Substrate/Activity	Enzyme	Intestinal Microsomes			Liver Microsomes		
		Kinetic Model	Km (µM)	Vmax (pmol/mg/min)	Kinetic Model	Km (µM)	Vmax (pmol/mg/min)
Testosterone 6beta-Hydroxylase	CYP3A4	MM	70	2709	MM	62	5680
Diclofenac 4'-Hydroxylase	CYP2C9	MM	6.9	187	MM	2.5	3707
Astemizole Demethylase	CYP2J/4F/3A4	MM	3.7	118	MM	3.5	491
Estradiol 3-Glucuronidation	UGT1A1/8/10	MM	6.2	7054	Hill	26 (^{1}n = 2)	1352
Estradiol 17-Glucuronidation	UGT's	ND	ND	ND	MM	11	185
[2,3] p-NPA Hydrolase (High Affinity)	Carb. Esterase	MM	42	653	MM	44	680
[2,3] p-NPA Hydrolase (Low Affinity)	Carb.Esterase	MM	172	988	MM	136	844

[1] Hill Coefficient
[2] Vmax units for hydrolase activity are nmol/min/mg
[3] p-NPA demonstrated multiple Km values on Eadie-Hofstee Plot
MM: Michaelis-Menten Kinetics
ND: Metabolite peak was not detected in HIM

3 Eadie-Hofstee Plots of p-NPA Hydrolysis and Estradiol 3-Glucuronidation

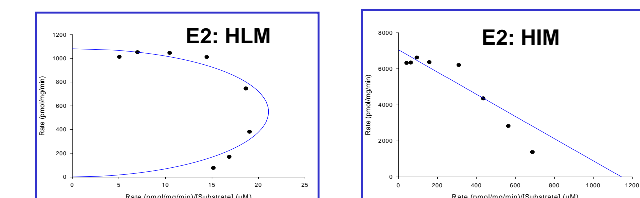
I) p-NPA Hydrolysis

- Curvature of line suggests multiple Carboxylesterase enzymes
- Similar Km values in HLM and HIM suggests same CE isoforms



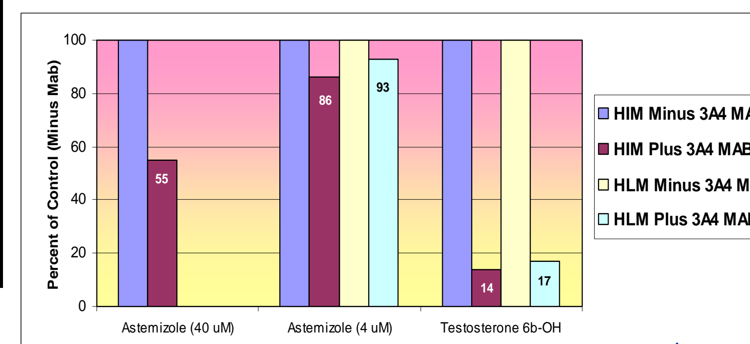
II) Estradiol 3-Glucuronidation

- HLM graph suggests allosteric kinetics (Hill coefficient = 2)
- HIM graph is consistent with simple Michaelis-Menten kinetics



4 P450 Isoforms Responsible for Astemizole *O*-Demethylation in HIM and HLM: CYP2J2, 4F12 and 3A4

I) Inhibition of Astemizole *O*-Demethylase in HIM and HLM by CYP3A4 Inhibitory MAB (monoclonal antibody)



II) Astemizole *O*-Demethylation by P450 Supersomes: CYP2J2, 3A4 and 4F12

P450 Supersome	Kinetic Model	Km (uM)	Vmax (pmol/min/pmol)	Vmax/Km
CYP2J2	MM	0.8	4.3	5.4
CYP3A4	MM	10	11	1.1
CYP4F12	Sub. Inhibition	2	49	25

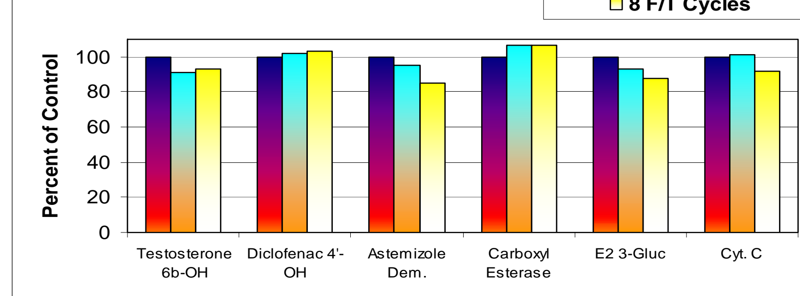
I) CYP3A4 MAB Inhibition Experiment:

- CYP3A4 MAB did not inhibit astemizole *O*-demethylation at low (4 µM) concentrations in either HIM or HLM.
- The 3A4 MAB produced ~45% inhibition at high astemizole concentrations (40 µM).

II) Kinetic Analysis:

- CYP2J2 and 4F12 have low Km values and high intrinsic clearance values.
- CYP3A4 is a higher Km, lower clearance isoform.

5



References

1. Hashizume, T., et al. *JEPT*, **300**:298 (2002).
2. Matsumoto, S., et al. *Drug Metab. Disp.*, **30**:1240 (2002).
3. Zhang, Q., et al. *Drug Metab. Disp.*, **27**:804 (1999).
4. Morgan, E., et al. *Arch. Biochem. Biophys.*, **315**:495 (1994).

Conclusions:

All donor HIM were active for testosterone 6beta-OH (3A4), diclofenac 4'-OH (2C9), astemizole *O*-demethylation (2J/4F/3A), estradiol 3-glucuronidation and p-nitrophenolacetate hydrolysis.

Testosoterone 6beta-OH (200 µM) and astemizole *O*-demethylase (40 µM) showed a significant correlation (r = 0.88), suggesting CYP3A4 involvement in astemizole *O*-demethylation at high, 40 µM astemizole concentrations. All other activities in HIM varied independently from one another.

Kinetic analysis demonstrated that pooled HIM has similar Km values as HLM for the activities tested. Only the Km for estradiol 3-glucuronidation varied significantly between HIM and HLM.

Both HIM and HLM demonstrated multiple Km values for p-NPA hydrolysis. The Vmax for esterase activity was similar between HIM and HLM.

Astemizole is *O*-demethylated by multiple P450s: CYP2J2, 3A4 and 4F12. However, the results in Section 4 suggest that CYP2J2 and CYP4F12 (and not CYP3A4) are the most important P450s at low astemizole concentrations (~4 µM) in both HIM and HLM. This conclusion is consistent with others.[2]

All HIM catalytic activities were stable after repeated freeze/thaw cycles.

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Not for resale.
BD, BD Logo, and all other trademarks are the property of Becton, Dickinson and Company. ©2004BD