Comparison of CYP Activities from Human Liver Microsome Pools Based on Weight, Gender and Age

LaHoma Easterwood[1], Matt Palmer[1], Kevin Lehnert[1], Jason Wright[1], Lyndee Lowrance[2], Ammy Santiago[1] and Jeanette Hill[1], (1)CellzDirect, Inc./Life Technologies, 1624 Headway Circle, Austin, TX 78754, (2)CellzDirect, Inc./Life Technologies, 4301 Emperor Blvd., Durham, NC 27703

Abstract / Introduction

Liver microsomes are subcellular fractions containing common drug metabolizing enzymes including cytochrome P450 (CYPs), flavin-monooxygenases (FMOs), carboxylesterases, epoxide hydrolase, UDP-glucuronosyltransferases (UGTs). Because liver microsomes are abundant in enzymes that are involved in the biotransformation of xenobiotics, they serve as a useful biological system for assessing the intrinsic clearance (CL_{INT}) of a particular drug, for identifying which CYPs are involved in the metabolism of a given xenobiotic, and for identifying potential drug interactions associated with inhibition of CYPs or UGTs. Large differences in CYP activity are often observed between individual donors. To determine whether levels of CYP activity correlate to specific donor phenotypes, microsomal pools were produced based on **weight** (normal weight [Body Mass Index (BMI)<25], overweight [BMI=25-30], obese [BMI=30-40] and morbidly obese,[BMI≥40]), **gender** (male or female), and **age** (18-35 years, 40-60 years, >75 years). CYP activities for each pool were characterized and kinetic parameters (V_{max} and K_m) were determined for the major drug metabolizing CYP enzymes (CYP1A2, phenacetin *O*-dealkylation; CYP2A6, coumarin 7-hydroxylation; CYP2B6, bupropion hydroxylation; CYP2C8, paclitaxel 6α-hydroxylation; CYP2C9, tolbutamide hydroxylation; CYP2C19, (S)-mephenytoin 4'-hydroxylation; CYP2D6, dextromethorphan demethylation; CYP2E1, chlorzoxazone 6-hydroxylation; CYP3A4, testosterone 6β-hydroxylation and midazolam 1'-hydroxylation). The results obtained demonstrate that differences in CYP activity correlate with donor phenotypes based on weight, gender and age. These results provide a better understanding of factors that may contribute to individual differences in drug metabolism.

Methods

Microsomes were prepared from frozen liver tissue from individual donors using standard methods (ref. 1). Microsomal pools were prepared based on donor phenotype as described in **Table 1**. Metabolic activity was determined in 0.1 M potassium phosphate buffer, pH 7.4, using conditions described in **Table 2**.

V_{max} (nmol/min/mg) values for each microsomal pool were compared to those generated from a microsomal pool representative of the Average Population and the calculated percentages are shown as % Activity of the Average Population Pool in **Figures 1, 2,** and **3**.

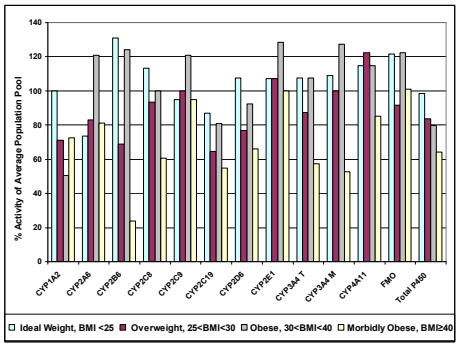
Table 1 – Microsomal Pool Description

Lot #	Pool Description	# of Donors
	Body Mass Index	
PL033	Ideal Weight, BMI <25	15
PL034	Overweight, 25<BMI<30	15
PL031	Obese; 30<BMI<40	10
PL032	Morbidly Obese, BMI≥40	5
	Age	
PL036	18 - 39 years	15
PL037	40 - 60 years	25
PL035	Geriatric, >75yrs	10
	Gender	
PL038	Female	20
PL039	Male	20
	CYP Activity	
PL040	High CYP3A4	20
PL041	High CYP2D6	20
	Average Population Pool	
PL050	50 donor	50

Table 2 –Conditions for Microsomal Activity Assessment

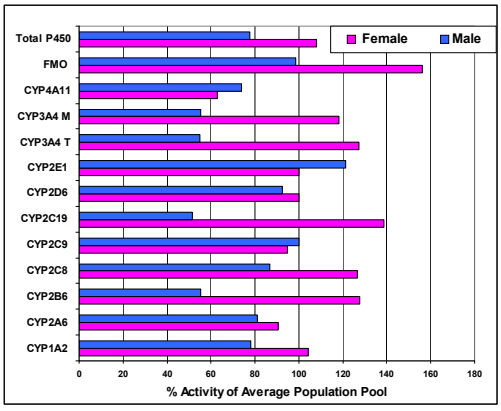
Enzyme Monitored	Marker Substrate	Incubation Time (min)	Protein Conc. (mg/mL)	Metabolite
CYP1A1/2	Phenacetin	30	0.1	Acetaminophen
CYP2A6	Coumarin	5	0.025	7-Hydroxycoumarin
CYP2B6	Bupropion	20	0.25	Hydroxybupropion
CYP2C8	Paclitaxel	10	0.075	6α-Hydroxypaclitaxel
CYP2C9	Tolbutamide	20	0.1	Hydroxytolbutamide
CYP2C19	(S) Mephenytoin	30	0.1	4'-Hydroxymephenytoin
CYP2D6	Dextromethorphan	15	0.2	Dextrorphan
CYP2E1	Chlorzoxazone	20	0.1	6-Hydroxychlorzoxazone
CYP3A4/5	Midazolam	4	0.025	1'-Hydroxymidazolam
CYP3A4/5	Testosterone	7	0.05	6ß-Hydroxytestosterone
CYP4A11	Lauric Acid	15	0.2	12-Hydroxydecanoic Acid
FMO	Methyl p-Tolylsulfide	15	0.05	Methyl p-Tolylsulfoxide
UGT	7-Hydroxycoumarin	30	0.2	7-Hydroxycoumarin glucuronide

Figure 1 – Metabolic Activities for Microsomal Pools Based on Donor Weight



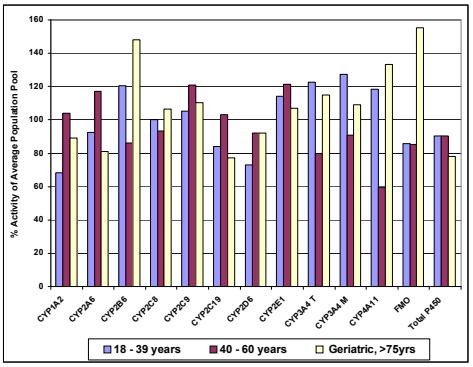
Pooled microsomes from Morbidly Obese donors (BMI ≥ 40) show decreased metabolic activity as compared to the Average Population

Figure 2 – Metabolic Activities for Microsomal Pools Based on Donor Gender



Pooled microsomes from Female donors showed higher metabolic activities than pooled microsomes from Male donors

Figure 3 – Metabolic Activities for Microsomal Pools Based on Donor Age



Microsomal pools from Geriatric donors (>75 years) show higher CYP2B6, CYP4A11, and FMO activity than other age groups

Results and Conclusions

- Pooled microsomes from **morbidly obese** (BMI ≥ 40) donors showed decreased activity for multiple metabolic enzymes as compared to an average population pool. Activities showing >20% decrease were:

  CYP1A2, 2B6, 2C8, 2C19, 2D6, 3A4 and Total P450

- **Female** pooled microsomes showed higher metabolic activity than **male** pooled microsomes for many enzymes including:

  CYP1A2, 2A6, 2B6, 2C8, 2C19, 2D6, 3A4, FMO, and Total P450

- CYP2B6, CYP4A11 and FMO activities were elevated in **Geriatric (>75 years)** pooled microsomes.

- These results suggest that metabolic activities due to donor phenotypes can vary and may be dependent upon body weight, gender and age.
- These results provide a better understanding of some factors that may contribute to individual differences in drug metabolism.

References

Hill, JR (2004) In Vitro Drug Metabolism Using Liver Microsomes, Current Protocols in Pharmacology, Ed. John Wiley and Sons, Unit 7.8, 7.8.1-7.8.12.

Ethics Statement

CellzDirect/Life Technologies acquires consented, rejected-for-transplant livers and discarded surgical remnants from Research Tissue Organizations and participating medical centers. CellzDirect/Life Technologies adheres to all patient-consent, HIPAA, and ethical rules and regulations in its procurement and handling of human tissues. CellzDirect/Life Technologies only obtains human liver tissue from non-profit organizations that follow these same rigorous regulations. The specimens are de-identified upon procurement and there is no linkage between the consent form with the donor's name or personal information and internal files. The identities of the donors are not disclosed to the staff of CellzDirect/Life Technologies.