Influence of Gender and Ethnicity on Drug Metabolizing Enzymes in Primary Human Hepatocytes

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Introduction

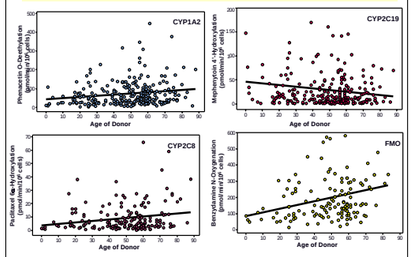
Marked differences are observed in pharmacokinetics of drugs between individuals, resulting in decreased efficacy or adverse drug reactions. Defective alleles encoding biotransformation enzymes such as CYP450s and UGTs can result in decreased drug clearance, thereby increasing the incidence of toxicity with many drugs. In some cases, polymorphic drug metabolizing enzymes may enhance efficacy e.g. omeprazole levels are maintained more efficiently in patients carrying certain alleles for CYP2C19[1]. Conversely, variant alleles of UGT1A1 are less capable of conjugating and eliminating SN-38, the active form of irinotecan, potentially causing toxicities[2]. Understanding the drug metabolizing enzymes responsible for metabolism and more importantly, elucidating the genetic basis for this variation would make drug therapy safer and more efficacious.

Polymorphism are reported in CYP2D6, 2C19, and 2C9, 1A1, 2A6, 2A13, 2C8, 3A4, and 3A5. Approximately 5-14% of Caucasians, 0-5% Africans, and 0-1% of Asians are poor CYP2D6 metabolizers. CYP1A1/2 play a role in procarcinogen bioactivation and polymorphisms can contribute to the variable susceptibility to carcinogenesis. People of African descent require higher tacrolimus doses than Caucasians to reach similar trough concentrations of Tacrolimus[3].

Sex-specific differences have also been observed where women are reported to experience more adverse drug effects than men e.g. women are more likely to experience drug-induced QT-prolongation and torsade-de-pointes arrhythmia[4]. Saquinavir and indinavir treatment results in clinically significant sex differences in plasma drug concentrations and clinical outcomes, with women having higher drug exposure[4]. Pharmacokinetics of antidepressant drugs can be substantially different, with women responding better to MAOI antidepressants and men responding better to TCAs[5]. Clinical studies indicate that CYP2E1 and CYP1A2 activity is higher in males, while CYP3A appears to have greater activity in females[6]. However, this is difficult to confirm in vivo, as multiple factors affect drug metabolism.

The molecular basis for many of these sex- and ethnicity- based differences have yet to be elucidated. In a move towards understanding these, we have characterized 254 preparations of primary human hepatocytes for the major wild type and polymorphic drug metabolizing enzymes and evaluated the influence of ethnicity, gender, age and BMI on activities.

Figure 1 – The Effect of Age on Drug Metabolizing Enzyme Activity

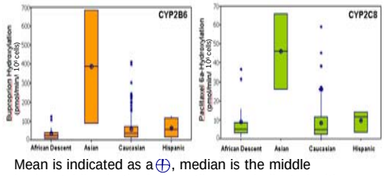


CYP1A2: Increase in activity with increasing donor age. p = 0.004; r = 0.18; n = 249
CYP2C19: Decrease in activity with increasing donor age. p = 0.075; r = -0.11; n = 245
CYP2C8: Increase in activity with increasing donor age. p = 0.008; r = 0.201; n = 175
FMO: Increase in activity with increasing age. p = 0.001; r = 0.281; n = 133
Phase I enzymes: CYP2D6, CYP2B6, CYP2C9, CYP2E1, and CYP3A did not show any statistically significant changes with age of donor.
Phase II enzymes: SULT and UGT did not show statistically significant changes with age of donor.

Statistics determined by linear regression (Pearson correlation).

The correlation between drug metabolizing enzyme activity and age is plausibly non-zero.

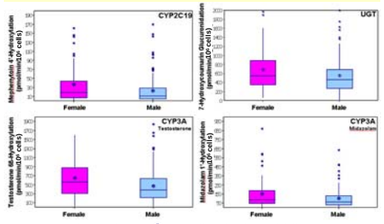
Figure 2 – The Effect of Ethnicity on Drug Metabolizing Enzyme Activity



Mean is indicated as a ⊕, median is the middle horizontal line in the box, outliers are indicated with an asterisk. Statistics determined by ANOVA.
CYP2B6: Higher activity in Asian vs. all other ethnicities studied, followed by Hispanic > Caucasian > African descent. p = 0.00; n = 15 African descent, 2 Asian, 141 Caucasian, 4 Hispanic
CYP2C8: Higher activity in Asian > Hispanic > African descent > Caucasian. p = 0.00; n = 15 African descent, 2 Asian, 155 Caucasian, 3 Hispanic
UGT activity different between Caucasian and African descent (p = 0.03)
CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A, SULT, FMO: No marked differences in activities between these different ethnicities.

Ethnicity has a non-zero effect on certain drug metabolizing enzyme activities in primary human hepatocytes.

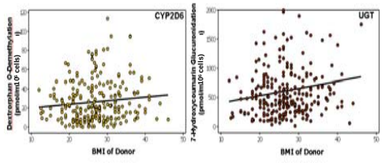
Figure 3 – The Effect of Gender on Drug Metabolizing Enzyme Activity



Mean is indicated as a ⊕, median is the middle horizontal line in the box, outliers are indicated with an asterisk. Statistics determined by ANOVA.
CYP2C19: Higher activity in female vs. male primary hepatocytes. p = 0.06; n = 118 female and 127 male
UGT: Higher activity in female vs. male primary hepatocytes p =0.03; n = 116 female and 138 male
CYP3A4 (Testosterone): Higher activity in female vs. male primary human hepatocytes. p = 0.002; n = 119 female and 130 male
CYP3A4 (Midazolam): Higher activity in female vs. male primary human hepatocytes. p = 0.05; n = 89 female and 91 male
CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, SULT, FMO: No major changes in Phase I or Phase II drug metabolizing activity by gender

The correlation between drug metabolizing enzyme activity and gender is plausibly non-zero.

Figure 4 – The Effect of Body Mass Index (BMI) on Drug Metabolizing Enzyme Activity

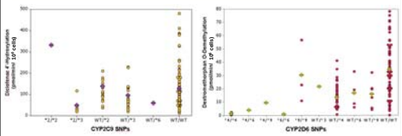


CYP2D6: Trend towards increase in activity with increasing BMI. p = 0.07; r = 0.114; n = 248
UGT: Increase in activity with increasing age. p = 0.014; r = 0.154; n=254
CYP1A2, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2E1, CYP3A, SULT, FMO: No marked changes in Phase I or Phase II drug metabolizing activity at a range of BMI.

Statistics determined by linear regression (Pearson correlation).

BMI does not appear to affect activity of the major drug metabolizing enzymes.

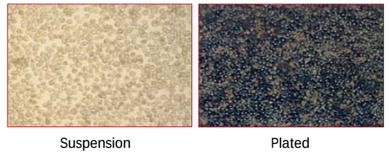
Figure 5 – The Effect of SNPs on Drug Metabolizing Enzyme Activity



Mean is indicated with a diamond. Statistics determined by ANOVA.
There are statistical differences in CYP2C9 activity between hepatocytes with certain CYP2C9 SNPs.
In CYP2C9, *2/*3, WT/*3, and WT/*6 have lower activity than the wild type. *2/*2 and WT/*2 have higher activity than the wild type (p = 0.06).
CYP2C9: n = 1 *2/*2, 19 WT/*2, 5 *2/*3, 17 WT/*2, 1 WT/*6, 93 WT/WT
Details of Donor with CYP2C9 *2/*2 polymorphism below.
Statistically lower CYP2D6 activity was observed in hepatocytes that had SNPs for CYP2D6. p = 0.00
Presence of any CYP2D6 polymorphism decreases CYP2D6 activity.
CYP2D6: n = 7 *4/*4, 2 *6/*6, 3 *9/*9, 1 WT/*3, 42 WT/*4, 1 *4/*6, 1 *4/*9, 4 WT/*6, 6 WT/*9, 94 WT/WT

The presence of SNPs has a non-zero effect on drug metabolizing enzyme activity.

Figure 6 – Cryopreserved Hepatocytes



Suspension Plated

Table 1 – Case Study of a Single Donor

26 year old Caucasian Female
SNPs: CYP2C9*2/*2; CYP3A5 *3/*3
CYP2C9*2/*2 activity: 332 pmoles/min/mg protein (mean of 128 in wt/wt)
CYP3A5 *3/*3 : 1700 pmoles/min/mg protein (mean of 236 in wt/wt, mean of 95.9 in all other donors with *3/*3)
ECOD: 31.9 pmoles/min/mg/protein (mean of 34.9 in population)
BMI: 26
Alcohol: social
Smoker: 1 packet / day for 10 years
Drug use: Yes
Prescriptions for 5 years: Effexor, Lortab, Oxycontin, Clonidine, Methadone, Bupab

Materials and Methods

Hepatocyte Isolation and Cryopreservation. Human hepatic tissues were derived from transplant reject livers and liver resections from healthy tissue (sourced through the Hepatic Research Registry), mainly from patients with hepatic cancer. Primary human hepatocytes were isolated from liver tissue by a two-step collagenase perfusion method[7] and subsequently cryopreserved. Cryopreserved hepatocytes were thawed in serum-containing medium, re-suspended in serum-free incubation medium, and assessed for cell viability by Trypan blue exclusion.

Metabolic Activity. Enzyme activities were determined in suspension hepatocytes at 0.5 x10^6 cells/mL in WEM with the probe substrates: phenactin (CYP1A2), bupropion (CYP2B6), paclitaxel (CYP2C8), diclofenac (CYP2C9), S-mephenytoin (CYP2C19), dextromethorphan (CYP2D6), chlorzoxazone (CYP2E1), testosterone & midazolam (CYP3A), benzydamine (FMO), 7-ethoxycoumarin (ECOD) and 7-hydroxycoumarin (UGT & SULT). Metabolites were identified by LC/MS/MS analysis.

Genotyping. Genotyping for thirteen different single nucleotide polymorphisms (SNPs) within four different genes, *CYP2C9, CYP2C19, CYP2D6, CYP3A5*, were detected by DNA isolation and ABI TaqMan® primer/probe sets.

Data Analysis. ANOVA and Pearson correlations (linear regression), were used to determine relationships between variables. The level of significance was 0.05 (α = 0.05). The variables were age, gender, ethnicity, BMI, presence of polymorphisms, Phase I drug metabolizing enzymatic activity (listed above), and Phase II drug metabolizing enzymatic activity (listed above).

Conclusions

•272 donor livers were evaluated for SNPs and primary hepatocyte activities

•Plausibly non-zero relationships between age and metabolic activities of CYP1A2 (increase), CYP2C8 (increase), FMO (increase); moderate decrease of CYP2C19, and no effect on CYP2D6, CYP2B6, CYP2C9, CYP2E1, CYP3A, SULT and UGT as determined by Pearson correlation.

•Minimal effect of BMI on drug metabolizing enzyme activity – moderate increase in CYP2D6 and a marked increase in UGT activities as determined by Pearson correlation.

•Gender affects activity of some of the major drug metabolizing enzymes, with higher activities observed in females for UGT and CYP3A4, moderately higher activities in CYP2C19 and no differences in CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2D6, CYP2E1, SULT & FMO as determined by ANOVA.

•Ethnicity of donor liver has an effect on enzymes in hepatocyte preparations as determined by ANOVA:

- •CYP2B6 activity: Asian >> Hispanic > Caucasian > African descent
- •CYP2C8 activity: Asian >> Hispanic > African descent > Caucasian
- •UGT activity: Caucasian >> African descent (p = 0.03).

•Statistically different enzyme activity observed in hepatocytes from livers with certain SNPs as determined by ANOVA. Drug use could change this profile.

References

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