Genotyped, Cryopreserved Suspensions of Human Hepatocytes as a Tool for Investigating Drug Metabolism in Polymorphic Individuals

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Introduction

An individual's responsiveness to drug therapy can be dependent upon environmental and genetic factors. For example, diet, chemical exposure (e.g. smoke) or inherent genetic polymorphisms can affect drug pharmacokinetics and pharmacodynamics. While environmental factors can be controlled to some extent, genetic factors present a challenge that lead personalized medicine. Some single nucleotide polymorphisms (SNPs) present in cytochrome P450 or phase II enzymes are known to cause decreased hepatic enzyme activity which can lead to clinically relevant consequences. For example, the anti-cancer agent Tamoxifen, a CYP2D6 substrate, is less effective in women with polymorphic CYP2D6 phenotype as compared to those with normal phenotype. Dose adjustment or alternative treatments are ways to manage polymorphic individuals. Because SNPs are so influential in drug therapy, it would be advantageous to evaluate them *in vitro* for new candidate drugs. Cryopreserved human hepatocytes which have been phenotyped and genotyped offer a viable option for studying drug disposition in polymorphic individuals and could help reduce more costly *in vivo* studies.

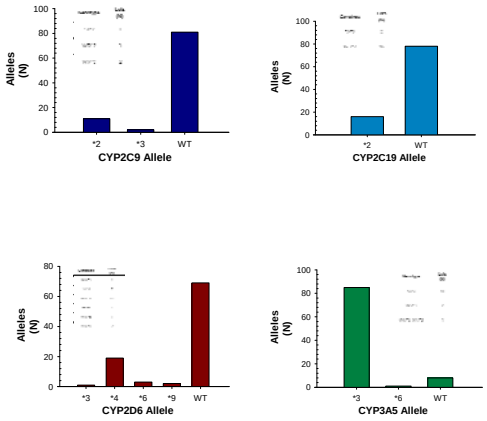
Methods

Hepatocyte Isolation and Cryopreservation. Primary human hepatocytes were isolated from resected liver tissue or whole liver tissue by a two-step collagenase perfusion method and subsequently cryopreserved. After storage at cryogenic temperatures, human hepatocytes were thawed in serum-containing Williams' E Medium (WEM), spun at appropriate conditions and re-suspended in serum-free WEM. Cell viability was assessed by Trypan blue exclusion. Acceptable post-thaw viabilities were 75% or greater.

Phenotyping. Probe substrates diclofenac, S-mephenytoin, dextromethorphan and testosterone were used to determine the enzymatic activities of CYP2C9, CYP2C19, CYP2D6 and CYP3A respectively in forty-seven lots of suspended cryopreserved human hepatocytes. Additional incubations using a low substrate concentration (1 mM) were performed to assess the intrinsic clearance of dextromethorphan. Metabolites and parent compounds were detected with LC/MS/MS analyses.

Genotyping. Genotyping for thirteen different single nucleotide polymorphisms (SNPs) shown in Table 1 was detected by DNA isolation and ABI TaqMan® primer/probe sets.

Figure 1 – CYP2C9, CYP2C19, CYP2D6 and CYP3A5 Alleles

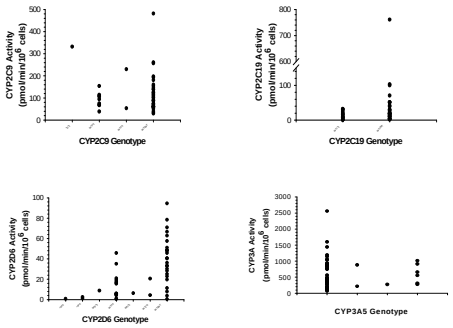


Forty-seven cryopreserved lots were screened for thirteen SNPs within the *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP3A5* genes. The number of alleles particular to each SNP are depicted in the graphs. The number of individual lots which displayed a particular genotype are depicted in the tables.

Table 1 – SNP Allele Frequencies within the Cryopreserved Lots

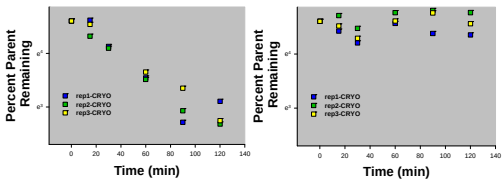
Gene	SN	Caucasians (cryo lots)	African Descent (cryo lots)	Total Population	Caucasians (literature)	African Decent (literature)	Refs
CYP2C9	*2	13.4%	0%	11.7%	8-19%	3.2%	1
	*3	1.2%	8.3%	2.1%	3.3-16.2%	1.3%	
	*6	0%	0%	0%	<1%	<1%	
CYP2C19	*2	17.1%	16.7%	17.0%	14.7%	17.3%	2,3
	*3	0%	0%	0%	<1%	<1%	
	*6	0%	0%	0%	<1%	<1%	
CYP2D6	*3	1.2%	0%	1%	<1%	<1%	4,5
	*4	22.0%	8.3%	20.2%	15-25%	2%	
	*6	3.7%	0%	3.2%	<1%	<1%	
	*9	2.4%	0%	2.1%	<1%	<1%	
CYP3A5	*3	97.6%	41.7%	91.4%	95%	27-50%	6
	*6	0%	8.3%	1.1%	<1%	13%	
	*8	0%	0%	0%	Not Reported	Not Reported	

Figure 2 – Relationship of Genotype and Phenotype



CYP2C9, CYP2C19, CYP2D6 and CYP3A specific activities were determined in forty-seven cryopreserved lots. The relationship between phenotype and genotype for all four isoforms is depicted.

Figure 3 – Parent Substrate Disappearance vs Time Profiles for Extensive and Poor CYP2D6 metabolizers



Dextromethorphan was incubated in human hepatocytes at 1 mM and monitored by LC/MS/MS. Representative parent disappearance vs time profiles are shown for (A) CYP2D6 EM phenotype and (B) CYP2D6 PM phenotype.

Table 2 – Dextromethorphan:Dextrorphan Concentration Ratios for Extensive and Poor CYP2D6 Metabolizer Lots

Lot Number (EM)	Response Ratio (Parent:Metabolite)	CYP2D6 SNP detected	Lot Number (PM)	Response Ratio (Parent:Metabolite)	CYP2D6 SNP detected
Hu4083	0.01	None	Hu4123	3	None
Hu0617	0.06	None	Hu8040	8	*4/*4
Hu0823	0.07	None	Hu0639	10	None
Hu0749	0.19	None	Hu4022	19	WT/*4
Hu4060	0.45	None	Hu0641	23	*6/*6
Hu4070	1.07	None	Hu4105	57	*4/*4

Results and Conclusions

- Assessments of polymorphic alleles in 47 lots of cryopreserved human hepatocytes revealed allelic frequencies in general agreement with population data from the literature.
- A general correlation between metabolic phenotype and wild type vs. poor metabolizer genotype was observed with CYP2C19 and CYP2D6 (CYP2C9 overall was in agreement with a single outlier *2/*2 individual appeared to show relatively high turnover that may need further evaluation)
- Intrinsic clearance studies using dextromethorphan as CYP2D6 substrate revealed median clearances of 32.0 and 2.55 mL/min/106 cells for six extensive metabolizers (EM) and six poor metabolizers (PM), respectively.
- CYP3A activity was monitored by testosterone 6ß-hydroxylase activity, which is sensitive to both CYP3A4 and CYP3A5 metabolism, therefore clear genotype/phenotype correlations with CYP3A5 PM alleles were not observed.
- Do to the small size of this growing population, homozygous CYP2C9 and CYP2C19 alleles were rarely identified in the 47-lots examined, consistent with literature.
- Dextromethorphan:Dextrorphan ratios were much lower, 0.01-1.1 in EM phenotype versus 3-57 in PM phenotype.
- Some lots displayed a polymorphic phenotype, however a SNP was not detected. Alternative PM alleles are potentially involved, and further studies to identify these alleles may elucidate these low activities.
- The use of cryopreserved human hepatocytes for drug disposition is advantageous because they are convenient, pre-characterized and representative models of liver metabolism.

References

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