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) shown in Table 1 was detected by DNA genotyping. For thirteen different single nucleotide 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) shown in Table 1 was detected by DNA genotyping.

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-mephentoin, 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 concentration (1 mM) were performed to assess the intrinsic clearance of dextromethorphan. Metabolites and parent concentration (1 mM) were performed to assess the intrinsic clearance of dextromethorphan.

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 6- 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:Cortisporin 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.

Table 1 – SNP Allele Frequencies within the Cryopreserved Lots

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

References