CRYOPRESERVED HEPATOCYTES

Hepatocyte Suspensions. Fresh Human Hepatocyte Suspensions (FHHS), Individual Cryopreserved Human Hepatocytes (ICHH; 5 donors), and Pre-Pooled Cryopreserved Human Hepatocytes (PPCHH; LiverPool) were used to predict the metabolic stability of pharmaceuticals.

Preparation of Cryopreserved Human Hepatocyte Suspensions. Vials were thawed in a 37°C water bath, and cells were diluted in InViroGRO® CF medium. ICHH from 5 donors were pooled at the time of thawing. Cells were centrifuged and resuspended in supplemented Krebs-Beaufort buffer. Cell counts and viability were determined by Trypan blue exclusion. The cell suspensions were then diluted to 2 million viable cells per mL with supplemented Krebs-Beaufort buffer.

Incubations. The chemicals were prepared in water, methanol, or acetonitrile at 0.5 mM concentrations and diluted with supplemented Krebs-Beaufort buffer to dosing concentrations of 10 µM. Aliquots (10 µL) of the 10 µM chemical solutions were transferred to uncoated 96-well plates. Hepatocytes (150 µL) of the 10 µM chemical solutions were transferred to uncoated 96-well plates. Hepatocytes (150 µL) were added to the wells and the mixtures were incubated in a 37°C, 5% CO2, humidified environment. In vivo clearance (CLin vivo) is the amount of drug eliminated by the liver per unit time and can be calculated by the Well Stirred, Parallel Tube, or Parallel Plate models. The predicted hepatic clearance values were within 2-fold of the actual hepatic clearance.

Table 1. Calculation of predicted in vitro hepatic clearance (CLH, in vitro) by the trapezoidal rule.

Table 2. Calculation of predicted in vivo hepatic clearance (CLH, in vivo) using the Well Stirred model, and the actual in vivo clearance (CLin vivo).

Figure 1. Metabolic stability of model pharmaceuticals in human hepatocytes.

Figure 2. Classification of pharmaceuticals based on their in vitro intrinsic clearance.

Figure 3. Correlation of predicted in vivo hepatic clearance obtained using Well Stirred model and actual in vivo clearances.

Results

• No significant differences were observed with freshly isolated human hepatocytes, individual cryopreserved hepatocytes, and pre-pooled cryopreserved hepatocytes in the prediction of high, moderate, and low clearance compounds (unpublished data).

• Calculation of predicted in vivo hepatic clearance by the Well Stirred or the Parallel Tube models resulted in similar correlation. The predicted hepatic clearance values were within 2-fold of the actual clearance values for 65% of the compounds and within 3-fold for 61% of the compounds.

Conclusions

• Pre-pooled cryopreserved hepatocytes were comparable to freshly isolated hepatocytes in determining intrinsic clearance.

• In vitro intrinsic clearance was used in the classification of 65% of compounds into low, moderate, and high clearance groups and in the prediction of 21% of compounds accurately (data not shown).

• Separation of the compounds into 3 classes (A, B, or C) or neutral (N) compounds did not increase the correlation of predicted in actual clearance values (data not shown).

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