2-P-45

COMPARISON OF TRANSPORTER–MEDIATED UPTAKE, EFFLUX AND METABOLIC FUNCTION IN FRESH AND CRYOPRESERVED HEPATOCYTES, IN SUSPENSION AND PRIMARY CULTURE

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Purpose: Hepatocytes in suspension or primary culture are good models for transporter, metabolism and safety. To assess the affect of cryopreservation on human hepatocytes, uptake and hepatic clearance ($CL_{H}$) were assessed in individual donors before and after cryopreservation. Experiments were conducted in suspension and attached cells.

Methods: Hepatocytes prepared by our standard methods were kept in suspension or in culture or cryopreserved. Intrinsic clearance of midazolam, phenacetin, dextromethorphan, benzydamine, tolbutamide and diazepam was compared in fresh and cryopreserved hepatocytes. For the suspension uptake experiments, fresh and cryopreserved hepatocytes were incubated with prototypical probe substrates (taurocholate, estrone sulfate, digoxin and MPP) and harvested by the oil filtration method. For biliary efflux measurements, hepatocytes were plated to form healthy confluent monolayers with intact bile canaliculi and assessed using standard B-CLEAR® methodologies.

Results and Discussion: For the intrinsic clearance studies, predicted clearances from hepatocyte suspensions of moderate- to high-turnover compounds were similar between fresh and cryopreserved hepatocytes isolated from four donors (e.g. midazolam $CL$: 12.3 vs. 14.4 mL/min/kg; diazepam: 3.50 and 1.73 mL/min/kg). Predicted clearance from hepatocyte cultures were also similar (mean midazolam $CL$: 10.8 (fresh) vs 6.09 (cryo) mL/min/kg). Short-term suspension hepatocytes did not give reliable $CL_{H}$ data for low-turnover compounds. There was up to a 10-fold variability in uptake and biliary clearance of prototypical substrates between different preparations of human hepatocytes and no significant difference between the individually frozen and cryopreserved lots.

Conclusions: Attached hepatocytes may provide better metabolic clearance predictions than suspensions. Cryopreserved human hepatocytes possess transporter and metabolic capacities comparable to fresh isolated cells.

2-P-46

EVALUATION OF FOOD-DRUG INTERACTIONS BY IN VITRO INHIBITION ASSAY OF P450-DEPENDENT DRUG METABOLISM

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[Purpose] In order to evaluate the food-drug interactions, we have developed in vitro inhibition assay system of cytochrome P450(CYP)-dependent drug metabolism. In this study, we investigated the inhibition of CYP-dependent drug metabolism (four major CYP isoforms: CYP1A2, 2C9, 2D6 and 3A4) by several commercially available drinks containing various plant-derived compounds, and evaluated the food-drug interactions.

[Methods] Ten commercially available drinks containing grapefruit juice as a positive control were used in inhibition assay. Each sample was evaporated and dissolved in reaction solution at final concentration of 0.5 to 5% (v/v). The inhibition effects of the drinks on the CYP1A2 (Phenacetin), 2C9 (Diclofenac), 2D6 (Bufuralol) and 3A4 (Testosterone) activity were examined with pooled human liver microsomes and recombinant CYP enzymes (Baculosomes).

[Results and Discussion] Grapefruit juice as a positive control showed significant inhibition for all of CYP-dependent drug metabolism in human liver microsomes. CYP3A4-dependent testosterone hydroxylation was inhibited by ten commercially available drinks (IC50: 0.5%-5%), and significantly inhibited by several drinks containing catechin as green and oolong tea extracts. The samples containing herbal extract and tomato lycopene showed the significant inhibition effect on 1A2-dependent phenacetin $O$-deethylation and 2C9-dependent diclofenac hydroxylation, respectively. This in vitro inhibition assay system of CYP-dependent drug metabolism using a convenient HPLC method allows us to evaluate the food-drug interactions.