A Comparison of Suspension, Plated Monoculture, and Hµrel® Co-Culture Hepatocyte Models for Estimating Intrinsic Clearance of Low-Turnover Drugs

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Purpose
To compare in vitro hepatocyte models (suspension, plated and Hµrel® co-culture) using the same lot of cryopreserved hepatocytes for calculating intrinsic clearance of low-turnover drugs.

Methods
Drugs with diverse metabolic pathways and a range of intrinsic clearances (±warfarin, timolol, theophylline, alprazolam, prednisolone, meloxicam, diazepam, tolbutamide, metoprolol, verapamil, glimepiride, efavirenz, and diclofenac) were selected for incubation in three hepatocyte models. Incubations were performed with the same lot of pooled cryoplateable hepatocytes (5 donors) in a 96-well format. Cell densities of 50,000 (suspension), 45,000 (plated monoculture), and 30,000 cells/well (Hµrel® co-culture) were utilized based on optimization studies or literature. For plated studies, hepatocytes were plated, and dosing occurred 4 hours later. Hµrel® co-culture plates were prepared at Hµrel Corporation, and shipped for immediate use 6 days later. Maximum incubation times for each hepatocyte system were 4 hours (suspension), 24 hours (plated), and 72 hours (co-culture) with six time points collected for clearance rate determinations. Incubations were quenched at each time point with ice-cold ACN containing internal standard, and samples were centrifuged and analyzed by LC/MS/MS. Depletion rates were calculated and converted to an in vitro intrinsic clearance for comparison between hepatocyte systems and to the back-calculated in vivo intrinsic clearance values for correlation analysis.

Results
Substrate depletion greater than 20% by the terminal time point was the criteria set for calculation of intrinsic clearance. Thus, intrinsic clearance lower limits were 11.9 mL/min/kg for suspended hepatocytes, 1.1 mL/min/kg for plated hepatocytes, and 0.56 mL/min/kg for the Hµrel® co-culture model. Using these limits, intrinsic clearance for 8 of the 13 compounds tested in suspended hepatocytes was able to be calculated. Compounds with no observable clearance included low-turnover drugs ±warfarin, timolol, theophylline, alprazolam and prednisolone. With the plated hepatocyte monoculture model, only 4 of 9 compounds incubated had enough depletion to enable intrinsic clearance calculations. Using the Hµrel® co-culture system, intrinsic clearance for 11/13 compounds was able to be calculated, the exceptions being theophylline and alprazolam, which may require longer incubation time.

Conclusion
To the best of our knowledge, no lab has compared various hepatocyte models using the same lot of hepatocytes for direct comparison. For incubations utilizing suspended hepatocytes, intrinsic clearance for moderate/high-turnover compounds were predicted within 2- to 3-fold of the in vivo intrinsic clearance. Thus, suspended hepatocytes provided the most accurate estimation of clearance. However, for low-turnover compounds, a model providing longer incubation times is required. The 24 hour plated hepatocyte incubations did not provide added benefit for low-turnover compounds with markedly less activity compared to suspension incubations. Using the Hµrel® co-culture system, intrinsic clearance for most of the low-turnover compounds was able to be measured, and with activity 2- to 7-fold higher than simply plating hepatocytes in monoculture, although under-prediction of in vivo intrinsic clearance was observed for higher turnover compounds. However, the Hµrel® co-culture system, with the ability to incubate at least 72 hours, would be a preferable model for estimating intrinsic clearance of low-turnover compounds such as ±warfarin, tolbutamide and prednisolone.