P40. Understanding The Performance Of Cellular Models For Intrinsic Clearance Determination: Case Studies For Assessment Of Low-Turnover Compounds Using HμREL*human*Pool™ Coculture System

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INTRODUCTION

HμREL*human*Pool™ Coculture system is a flexible less labor-intensive Hepatocyte culture system when compared to other reported methodologies, such as relay method and HepatoPac. This system, following a 7-day acclimation period, maintains its peak enzyme activity even after several weeks in culture, allowing discovery screening efforts that are otherwise severely impacted by reduced hepatocyte enzyme activity after a few hours. We had previously enabled HμREL*human*Pool™ Coculture system as standard screening platform to assess the intrinsic clearance of low-turnover drugs. Here we present a fit for purpose three phase approach to understand the performance of these cellular models for intrinsic clearance and their usage in our group, assay performance and case studies using this Coculture system.

METHODS

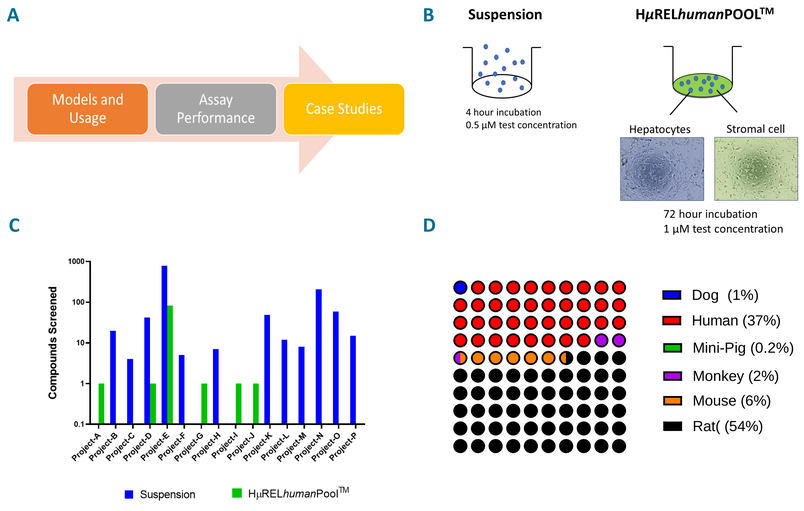

Figure-1: (A) Heuristic three phase approach to understand assay performance metrics (B) Cellular models for intrinsic clearance (C) Usage profile by projects of various in vitro cellular clearance models over a period of 2 years. (D) Usage profile by species of suspension hepatocytes over the past two years.
Key Message: Suspension hepatocytes usage at 90%. HμREL*human*Pool™ hepatocytes usage at 10%. Species usage for suspension: Rat (54%), Human (37%), Mouse (6%), others (<5%)

ASSAY PERFORMANCE

Drug	Ion Class	Metabolizing Enzyme	Cyprotex	Internal
Diazepam	Neutral	CYP2C19, CYP3A4	0.54	0.78
Tolbutamide	Acid	CYP2C9	1.04	1.37
Verapamil	Base	CYP3A4, CYP1A2, CYP2C9	14.5	10.1
Theophylline	Base	CYP1A2	LLQ	LLQ
Ketoprofen	Acid	UGT	4.36	4.8
Bupropion	Base	CYP2B6,CYP1A2, CYP2A6, CYP3A4, CYP2E1	6.42	2.7
Carvedilol	Base	CYP2B6, CYP1A2	54.12	37.3
Disopyramide	Base	CYP3A4	0.22	0.13
Prednisolone	Neutral	CYP3A4	0.35	0.39
Quinidine	Base	CYP3A4	0.36	0.56
Warfarin	Neutral	CYP2C9, CYP3A4	0.54	0.56
Imipramine	Base	CYP2C9, CYP2D6. CYP3A4, CYP1A2	1.2	1.9
Diclofenac	Acid	CYP2C9, UGT2B7	30.6	42.1

Table-1: Internal assessment of human in vitro intrinsic clearance (μL/min/10^6 cells) of 13 commercial compounds compared to that generated by Cyprotex in HμREL*human*Pool™

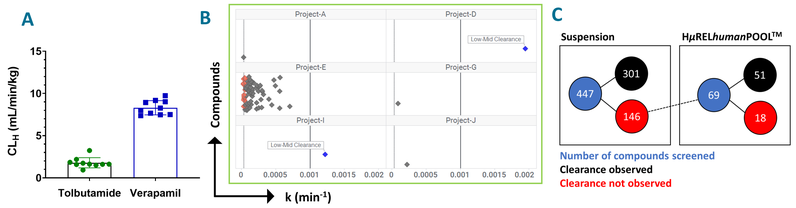

Figure-2: Assay Performance (A) Controls (B) Rate Constant of low turnover compounds using HμREL*human*Pool™ (C) Clearance under detection limits (red) as compared to above detection limit (black) in Suspension and HμREL*human*Pool™ In vitro systems. **Key Message:** Detection limit (k) for suspension hepatocyte is 0.00093 min-1. Detection limit for HμREL*human*Pool™ hepatocytes is 0.000052 min-1. HμREL*human*Pool™ hepatocytes successfully estimated clearance for 74% of compounds for which rate was under the limit of resolution in suspension assay

RESULTS

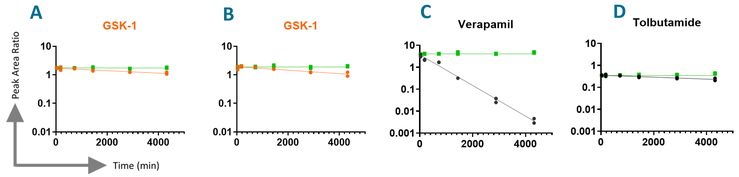

Figure-3: Clearance curves of (A) GSK-1 (B) Repeat of GSK-1 (C) Verapamil-Control (D) Tolbutamide-Control in HμREL*human*Pool™ (orange, black) stromal control cell (green) from standard 3-day study

RESULTS

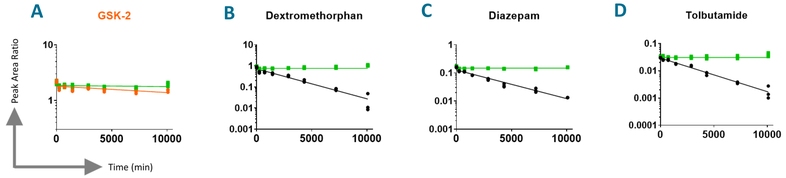

Figure-4: Clearance curves of (A) GSK-2 (B) Dextromethorphan-Control (C) Diazepam-Control (D) Tolbutamide-Control in HμREL*human*Pool™ and in stromal control cell (green) from non-standard 7-day study

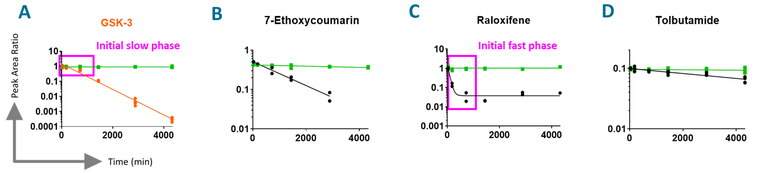

Figure-5: Clearance curves of (A) GSK-3 (B) 7-Ethoxycoumarin-Control (C) Raloxifene-Control (D) Tolbutamide-Control in HμREL*human*Pool™ and in stromal control cell (green) from standard 3-day study.
Key Message: HμREL*human*Pool™ hepatocytes studies were successfully used to estimate clearance of Project-E compound GSK-1 (Figure-3, high reproducibility), Project-A compound GSK-2 (Figure-4, improved resolution) and Project-D compound GSK-3 (Figure-5, different mechanism).

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents under an IRB/EC approved protocol. Compounds are identified as GSK-1, 2 and 3 and Projects are identified as A, B, C, D, E, F, G, H, I, J, K, L, M, N, O and P to protect confidential information.

CONCLUSIONS

The reproducibility and flexibility to improve the detection limit by extending (standard duration - 72 hours) incubation times makes HμREL*human*Pool™ Coculture system a very useful model to measure clearance of low –turnover drugs.

REFERENCES

1. Hultman, et.al., Mol. Pharmaceutics. 2016, 13 (8):2796-2807.
2. Bonn, et.al., Drug Metab. 2016,44(4):527-33.