NUCLEAR RECEPTOR-MEDIATED CHANGES IN GENE EXPRESSION IN A HUMAN HEPATIC MICROPATTERNED CO-CULTURE MODEL FOLLOWING TREATMENT WITH HEPATOTOXIC COMPOUNDS

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INTRODUCTION

- In recent years there have been multiple research efforts exploring use of *in vitro* data for identification of chemical hazards to predict *in vivo* toxicity and prioritize compounds for conventional toxicity testing.
- The mechanism of action leading to many adverse health effects due to toxicity of chemical entities is a result of direct binding of the chemicals to nuclear receptors and they are often targets for high throughput screening.
- Many high throughput screening approaches, such as the current suite of *in vitro* ToxCast assays, also may not represent complex biochemical and multi-cellular responses observed *in vivo* with adequate fidelity.
- An organotypic model that retains physiological functions in a higher throughput platform and importantly for a longer time than standard sandwich hepatocyte cultures is needed to facilitate *in vitro* hepatocyte research.
- We recently demonstrated that a novel hepatic culture model in which cryopreserved primary human hepatocytes are seeded onto micropatterned 96-well plates and co-cultured with murine embryonic fibroblasts (MPCC; HepatoPac™) retains key biochemical functions of the liver *in vivo*, including metabolic capacity.
- The purpose of this study is to evaluate the retention of functional nuclear receptor pathways and to evaluate activity of the nuclear receptors in the human micropatterned co-cultured model.

METHODS

- The compounds used in this study were selected from the U.S. Environmental Protection Agency's ToxCast Phase I and European Joint Research Centre datasets, along with positive controls for nuclear receptors.
- Two human hepatocyte donors were utilized for the study.
- Human micropatterned co-cultures (MPCC) were created using patented microfabrication tools and consist of primary hepatocytes arranged in optimized domains and surrounded by 3T3-J2 murine embryonic fibroblasts (Hepregen, Medford, MA).
- The co-cultures were first allowed to stabilize functionally in serum-supplemented maintenance media for a 10-day period as described in the manufacturer's protocol. On Day 13, maintenance medium was replaced with toxicity application medium (lacking serum) for 4 hours prior to treatment. Cells were treated once with compounds diluted in fresh toxicity application medium and were incubated for 24 or 72 hours.
- RNA was extracted using RNeasy 96 well Kit (Qiagen, Valencia, CA), reverse transcription was carried out using High Capacity RNA-to-cDNA Kit (Applied Biosystems/Life Technologies, Grand Island, NY), and qPCR was carried out in 96-well format utilizing manufacturer's recommended "best coverage" Taqman® Gene Expression Assays for genes of interest along with Taqman® Universal PCR Master Mix (Applied Biosystems).

RESULTS

Compound	Gene	Nuclear Receptor	Hu30	Hu4021
Rifampin	CYP3A4	PXR	0-100 µM diluted 3 fold	0-100 µM diluted 10 fold
PB	CYP3A4	PXR	0-2 mM diluted 3 fold	0-100 µM diluted 10 fold
PB	CYP2B6	CAR	0-2 mM diluted 3 fold	0-100 µM diluted 10 fold
CITCO	CYP2B6	CAR	0-50 µM diluted 3 fold	0-50 µM diluted 10 fold
3-MC	CYP1A2	AhR	0-10 µM diluted 3 fold	0-10 µM diluted 10 fold
OME	CYP1A2	AhR	0-100 µM diluted 3 fold	0-100 µM diluted 10 fold
GW-7647	HMGCS2	PPARα	0-10 µM diluted 5 fold	0-100 µM diluted 10 fold
PFOA	HMGCS2	PPARα	0-100 µM diluted 3 fold	0-100 µM diluted 10 fold
WY-14,643	All	PPARα	0-100 µM diluted 5 fold	0-100 µM diluted 10 fold
Unknowns	All	Unknown	0-100 µM diluted 3 fold	0-100 µM diluted 10 fold

Table 1: Treatment design for dose-response study of each compound for both human donors.

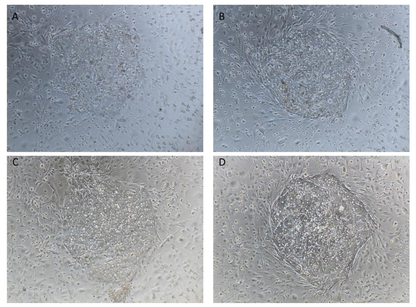

Figure 1: Photomicrographs exhibiting morphology of hepatocytes from 2 different human donors seeded onto HepatoPac micropatterned co-culture plates (MPCC). A: Hu30 vehicle treated after 24 hours of exposure (day 14 of culture). B: Hu30 vehicle treated after 72 hours of exposure (day 16 of culture). C: Hu4021 vehicle treated after 24 hours of exposure day 14 of culture). D: Hu4021 vehicle treated after 72 hours day of exposure (day 16 of culture). All images shown at 100X magnification.

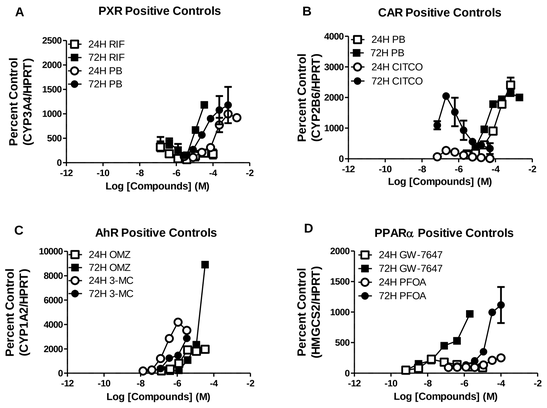

Figure 2: Effect of known nuclear receptor activators on gene expression using hepatocytes from donor Hu30 seeded into MPCC. MPCCs were exposed to activators of PXR, AhR, CAR, PPARα for 24 and 72 hours as described in Methods. All gene expression data was normalized to HPRT and cultures were exposed to 24 hours (white symbols) or 72 hours (black symbols). A: PXR positive controls for CYP3A4 were rifampin (RIF) and phenobarbital (PB). B: CAR positive controls for CYP2B6 used were PB and CITCO. C: AhR positive controls for CYP1A2 were omeprazole (OMZ) and 3-methylcholantrene (3-MC). D: PPARα positive controls for HMGCS2 were GW-7647 and perfluorooctanoic acid (PFOA).

Compound	Gene	Nuclear Receptor	Hu30 24H	Hu30 72H	Hu4021 24H	Hu4021 72H
Rifampin	CYP3A4	PXR	ND	ND	0.03541	0.1856
PB	CYP3A4	PXR	130.7	31.67	ND	ND
PB	CYP2B6	CAR	29.04	151.2	ND	13.78
CITCO	CYP2B6	CAR	ND	1.743	0.08813	ND
3-MC	CYP1A2	AhR	0.5388	ND	0.2087	ND
OMZ	CYP1A2	AhR	1.374	ND	ND	97.42
GW-7647	HMGCS2	PPARα	ND	ND	0.002608	0.2795
PFOA	HMGCS2	PPARα	20.28	17.26	24.24	ND

Table 2: Calculated AC_{50} values from Figure 2 and 3 graphs. Table represents calculated AC_{50} values in µM for effects of each positive control compound on gene expression of genes of interest and concurrent nuclear receptor. ND indicates not determined.

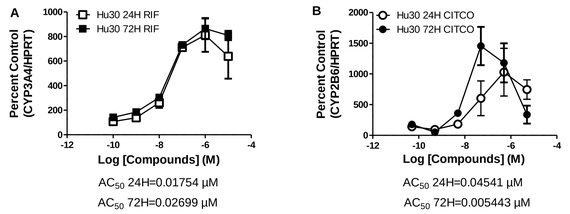

Figure 3: Effects of increased exposure dilutions on curve shape and AC_{50}. Values for Hu30 micropatterned co-cultures treated with A: Rifampin for 24 hours (white squares) or 72 hours (black squares), or B: CITCO for 24 hours (white circles) or 72 hours (black circles). Calculated AC_{50} values are under each graph.

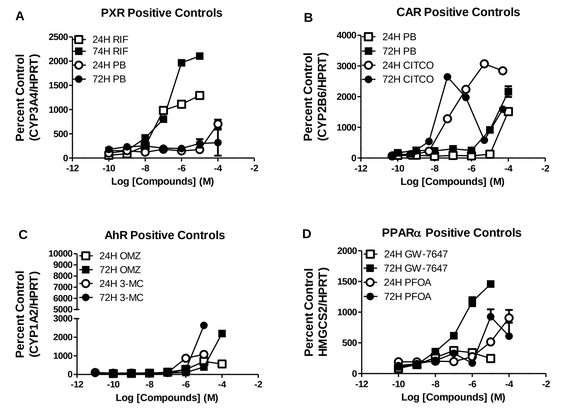

Figure 4: Effect of known nuclear receptor activators on gene expression using hepatocytes from donor Hu4021 seeded into MPCCs. See legend to Figure 2 for details.

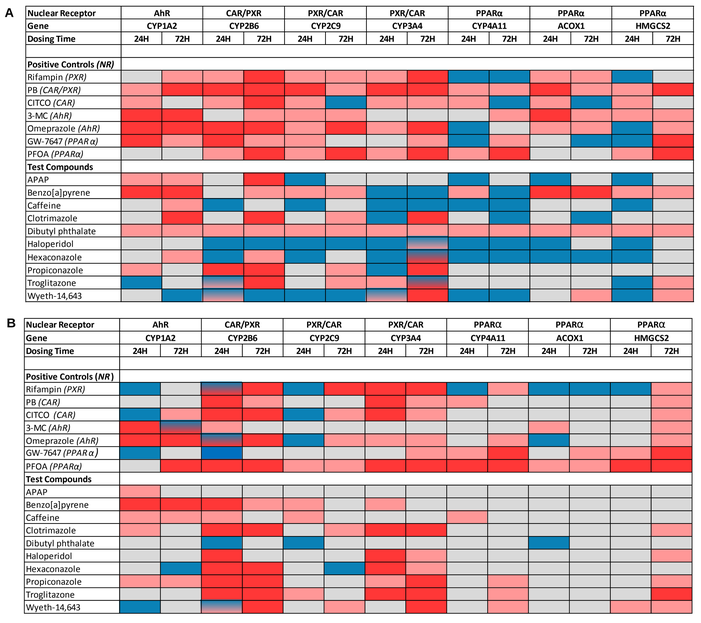

Figure 5: Heat map showing effects of all compounds tested on gene expression A. Hu30 and B. Hu4021 micropatterned co-culture for 24 and 72 hours after exposure. Data shown is normalized gene expression for genes of interest, RQ as percent of control. The point of departure (POD) is defined as an increase of 2-5 fold over vehicle treated (pink cell), 5-10 fold over vehicle treated (red cell), or 50% reduction of vehicle treated (blue cell). All data is expressed as µM at the point which a change was first observed. Mixed color cells showed both a reduction (blue) and increase of 2-5 fold (pink) or 5-10 fold (red) at different concentrations of compound. Grey cells indicate no change within the defined points of departure.

SUMMARY

- Previous work has demonstrated that this model exhibits consistent responses to nuclear receptor agonists in cryopreserved hepatocytes from rat and this study is one of the first to employ cryopreserved human hepatocytes.
- Nuclear receptor signaling pathways in this model are retained and responded to positive controls as expected, with greater sensitivity of response for CAR and PXR than is evident in traditional sandwich-cultured hepatocytes.
- AC_{50} values were calculable for positive controls and responses to most of the test compounds were evident for many of the genes tested.
- Sandwiched cryopreserved hepatocytes historically show an AC_{50} for CYP3A4 gene expression of 0.2-0.4 µM for 72 hours RIF dosing of 0.01-10 µM. By comparison, calculated AC_{50} values from Figure 3A (Table 2) for Hu4021 and Figure 4A for Hu30 exhibited AC_{50} values in the MPCC model of 0.2 µM for Hu4021 and 0.03 µM for Hu30 when treated for 72 hours with lower concentrations of rifampin, thus displaying an equal or greater sensitivity as compared to the sandwich-cultured model.

CONCLUSIONS

- The ease of use and the robust nature of the micropatterned co-culture system offer many benefits, especially for routine assessment for nuclear receptor agonists.
- The co-culture is stable for several weeks and responds in an equivalent or more sensitive manner to compound exposures as we have demonstrated in this study.
- The higher throughput capacity of this model combined with the retained physiological capabilities of hepatocytes and longevity in culture offers possibilities for exploring the effects of multiple chemical entities on nuclear receptor activation and adaptive responses.

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