Prolonged Culturing of Human Hepatocytes in Human Plasma for P450 Induction and *In Vitro* Hepatotoxicity Studies

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Introduction

- **In vitro toxicology studies are routinely performed with the target cells cultured in protein free medium**
- **In vivo target cells are exposed to toxicants in the presence whole plasma**
- **An ideal in vitro model therefore should consist of the target cells cultured in whole plasma**
- **We hypothesize that evaluation of drug toxicity with a physiologically relevant target cell cultured in human plasma may allow direct extrapolation of in vitro findings to human in vivo**
- **Based on this hypothesis, we initiated the evaluation of the ability of human plasma to support the culturing of human hepatocytes for in vitro hepatotoxicity studies**

Materials & Methods

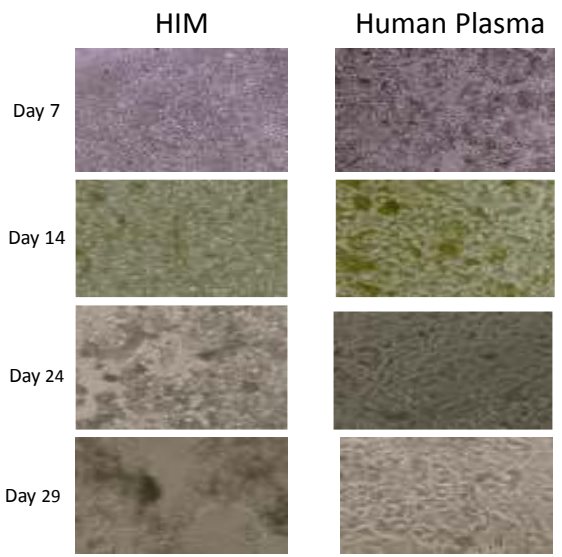
Cell Culture: Hepatocytes from donor HH1062 (IVAL, Columbia, MD) were plated in both human plasma (minimally-modified human plasma (HPZ-A), IVAL) and protein free hepatocyte media (HIM, IVAL) for a total duration of 29 days. The hepatocytes were plated in 96-well collagen-coated tissue culture plates using Universal Cryopreservation Plating Medium (UCPM, IVAL). After 4 h of attachment, the medium was changed to HPZ-A and HIM containing 0.25 mg/mL Matrigel. Medium was changed to HPZ-A without Matrigel on the next day, and every 2 – 3 days afterwards for a culture duration of 29 days.

For CYP induction comparisons, 4 lots of human hepatocytes (HH1007, HH1026, HH1051, HH1053) were used. The hepatocytes were plated in 96-well plates as described above in both HIM and HPZ-A, with treatment of prototypical inducers beginning on day 5 after plating. The treatment duration was 3 days. Gene expression by RT-PCR was used to measure induction of CYP1A2 (by omeprazole), CYP2B6 (by phenobarbital), and CYP3A4 (by rifampin).

For long-term cytotoxicity, lot HH1062 was cultured in HPZ-A as described above, and treated with seven concentrations of aflatoxin B1 (concentrations ranging from 1 uM to 90 uM) on day 2 and day 10. Viability was quantified based on cellular ATP contents (ATPLite, Perkin-Elmer). Results are expressed as Relative Viability using the following equation:

$$\text{Relative Viability (\%)} = \frac{\text{ATP (Treatment)}}{\text{ATP (Solvent Control)}} \times 100$$

Morphology of Human Hepatocytes Cultured in Human Plasma



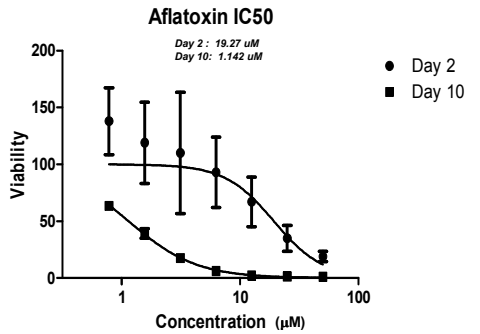
P450 Induction in Human Plasma

Fold induction in four lots of human hepatocytes cultured in protein free hepatocyte medium (HIM) and modified human plasma (HPZ-A) for CYP1A2, CYP2B6, and CYP3A4

	Fold Induction (mRNA)					
	CYP1A2		*CYP2B6*		*CYP3A4*	
Lots	HIM	HPZ-A	HIM	HPZ-A	HIM	HPZ-A
HH1007	3	6	3	2	9	20
HH1026	4	7	4	2	13	25
HH1051	4	5	7	4	10	18
HH1053	4	8	3	2	9	16

In Vitro Hepatotoxicity Evaluation in Human Plasma

Application of Human Hepatocyte/Human Plasma Culture for the Evaluation of Acute and Subchronic Hepatotoxicity:
Proof-of-Principle Study with Aflatoxin B1



Summary and Conclusions

- We have successfully developed a 100% human plasma medium, HPZ-A with which human hepatocytes could be cultured for a prolonged period of >29 days.
- Human hepatocytes cultured in HPZ-A are responsive to CYPs 1A2, 2B6, and 3A4 induction, demonstrating the retention of intact P450 induction pathways
- Aflatoxin B1, which requires metabolic activation for hepatotoxicity, was found to be cytotoxic to human hepatocytes cultured in human plasma. Aflatoxin IC_{50} value after a10-day treatment duration was 19X lower than that observed after a 2-day treatment duration. **The results suggest that human hepatocytes cultured in human plasma could activate a protoxicant and can be used for the evaluation of chronic hepatotoxicity.**

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