

Development of *in vitro* Toxicogenetic Models for Hepatotoxicity

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Abstract

Numerous studies support the fact that a genetically diverse mouse population may be useful as an animal model to understand and predict toxicity in humans. We hypothesized that cultures of hepatocytes obtained from a large panel of inbred mouse strains can produce data indicative of inter-individual differences in *in vivo* responses to hepato-toxicants. In order to test this hypothesis and establish whether high-throughput *in vitro* studies using cultured hepatocytes from genetically distinct mouse strains are feasible we aimed to: standardize cell isolation and culture conditions, determine whether the near-physiological maintenance of the cells isolated from different mouse inbred strains can be achieved and assess whether the reproducibility of functionality can be attained within a given strain over subsequent isolations. Hepatocytes were isolated from 15 strains of mice and cultured for up to 7 days in traditional 2D culture. The cells have been assessed for viability and functionality on a daily basis by measuring production of lactate, pyruvate, and urea, as well as leakage of lactate dehydrogenase. We also employed calcein and ethidium fluorescence staining to assess cell viability at 1, 3, 5 and 7 days of culture. Our data shows that high yield (48 to 87 million hepatocytes/mouse) and viability (86 to 98%) can be achieved across a panel of strains. Total RNA was isolated from the cells harvested on day 1 and 3 of culture and RT-PCR analysis was carried out to evaluate mRNA levels representative of liver specific genes. Furthermore, we conclude that cell function of hepatocytes isolated from different strains and cultured under standardized conditions is comparable and cells remain viable and metabolically active as indexed by lactate, pyruvate and urea production. These experiments open new opportunities for high-throughput and low-cost *in vitro* assays that may be used for studies of toxicity in a genetically diverse population. (Supported, in part, by grants from NIEHS R01-ES015241 and US EPA RD-833825)

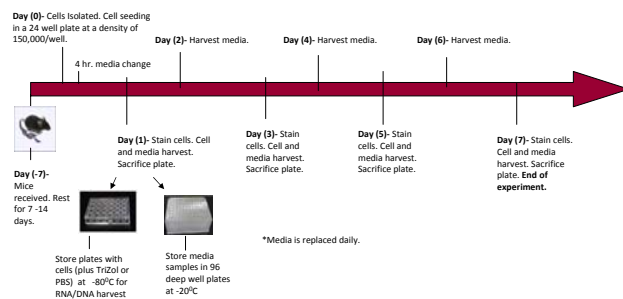


Figure 1. Schematic of work flow. Primary hepatocytes were isolated from mice and cultured for up to 7 days. At time points culture day 1, 3, 5 and 7 cell viability was assessed using calcein and ethidium fluorescence staining. Cells were harvested on days 1, 3, 5 and 7 and media was harvested daily.

Strain	1/1HCHB	B6HMD	C57BL/6J	DBA/2J	B6/DBA	B6/129	B6/129	B6/129	B6/129	A/J	PH/DOH	NR1/HR	NR1/HR
Final Yield	75.5	65.5	79	54	40	51	57.5	38.5	52	57	64	75.5	97.5
Final Viability	94%	93%	95%	95%	98%	92%	93%	92%	93%	97%	93%	94%	96%

Table 1. Table summarizing liver parenchymal cell yields (in millions) and viability after isolation.

Introduction

Drug-induced liver injury (DILI) is a primary determinant of hepatic dysfunction and a major concern for the development and approval of new pharmaceuticals. Inter-individual susceptibility, governed by genetic and environmental factors, may play a key role in determining outcome. Mice are widely used in toxicology research as a model organism. Mouse models are considered to be helpful in addressing the potential drug-induced toxicities and exploring mechanisms of injury. In addition mouse models are useful for identifying unique genetic determinants which might infer inter-individual differences in susceptibility. We hypothesized that a panel of inbred mouse strains is representative of a genetically diverse population and can be used as a model for human diversity. Recent studies performed in our laboratories validated an *in vivo* strategy using a mouse diversity panel. Data generated from these studies support the fact that a genetically diverse mouse population may be useful as an animal model to understand and predict rare adverse drug events in humans. The focus of this study is to identify whether a culture hepatocyte model can be developed from a mouse diversity panel.

Materials and Methods

Primary mouse hepatocytes were isolated from 4-6 week old male mice from Jackson Labs and maintained by CellzDirect. Isolation and maintenance protocols were performed according to CellzDirect's standard operating procedures for mouse hepatocytes. Cultured hepatocytes were plated on collagen matrix in monolayers and were fluorescently labeled through incubation (20 min. at 37°C) with calcein-AM (Invitrogen) and ethidium homodimer-1 (Invitrogen). Harvested media was stored at -20°C. Urea concentration was assayed using a colorimetric endpoint assay using a chromogenic reagent (QuantiChrom™ Urea Assay, BioAssay Systems). Lactate dehydrogenase (LDH) was assayed using a spectrophotometric kinetic assay. Pyruvate and lactate levels were measured using a spectrophotometric endpoint assay. The total RNA from cultured cells was prepared using TRIzol reagent (Invitrogen) and a Qiagen Mini Kit (Qiagen). Synthesis of first-strand cDNA from total RNA was performed with 2ug of total RNA and recommended volumes of reagents from High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Transcripts encoding ALB, CPS-1, CYP1A2, CYP3A11, CYP4A10, GSTA2, HNF4a, UGT1A1 and SLC01B2 were quantified by RT-PCR analysis (TaqMan Gene Expression Assays, Applied Biosystems).

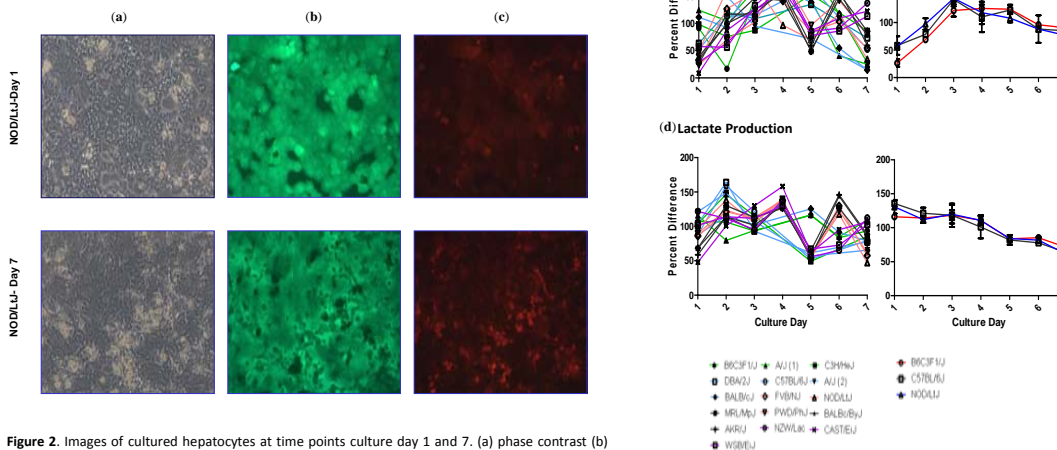


Figure 2. Images of cultured hepatocytes at time points culture day 1 and 7. (a) phase contrast (b) calcein-AM stain (c) ethidium homodimer-1 stain.

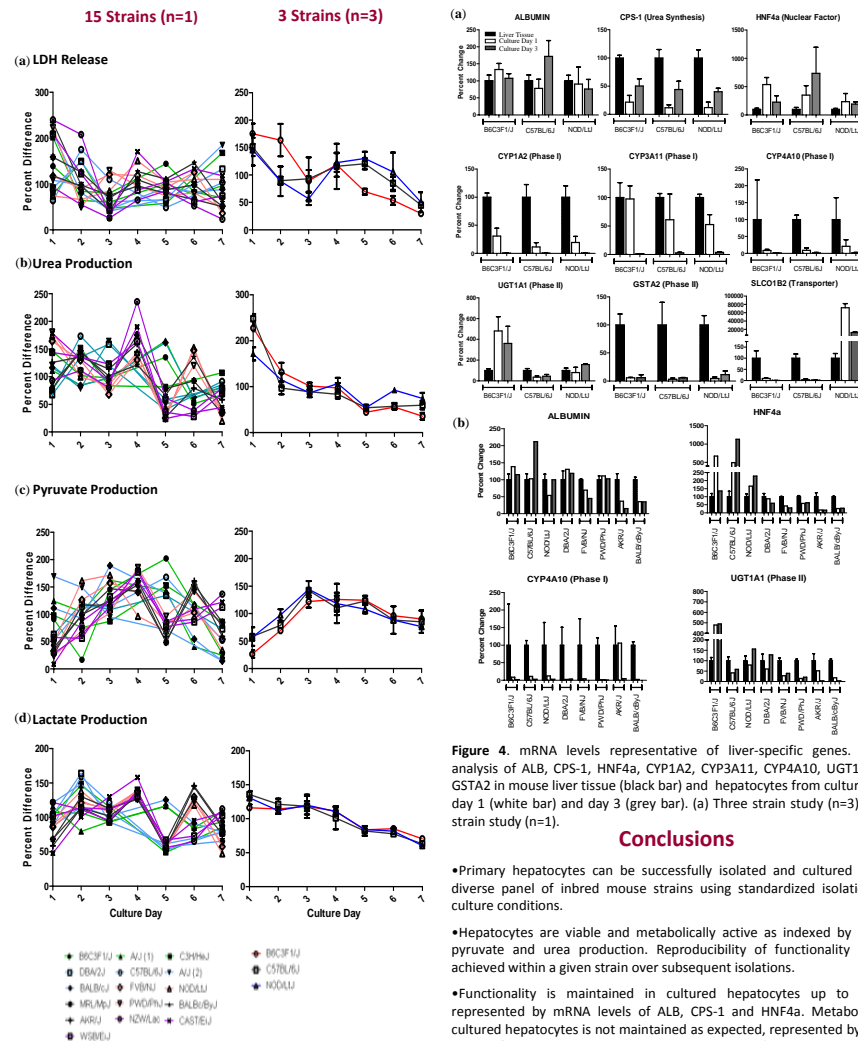


Figure 3. Functional characterization of cultured mouse hepatocytes. (a) Rates of LDH release. (b) Rates of urea production (c) Rates of pyruvate production (d) Rates of lactate production. Data was normalized to the mean of daily release/synthesis of each biochemical parameter.

Figure 4. mRNA levels representative of liver-specific genes. RT-PCR analysis of ALB, CPS-1, HNF4a, CYP1A2, CYP3A11, CYP4A10, UGT1A1 and GSTA2 in mouse liver tissue (black bar) and hepatocytes from cultured cells day 1 (white bar) and day 3 (grey bar). (a) Three strain study (n=3); (b) 15 strain study (n=1).

Conclusions

- Primary hepatocytes can be successfully isolated and cultured from a diverse panel of inbred mouse strains using standardized isolation and culture conditions.
- Hepatocytes are viable and metabolically active as indexed by lactate, pyruvate and urea production. Reproducibility of functionality can be achieved within a given strain over subsequent isolations.
- Functionality is maintained in cultured hepatocytes up to day 3, represented by mRNA levels of ALB, CPS-1 and HNF4a. Metabolism of cultured hepatocytes is not maintained as expected, represented by mRNA levels of CYP1A2, CYP3A11, CYP4A10 and GSTA2 (with the exception of UGT1A1).

Acknowledgements

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