BIOACTIVATION AND TOXICITY OF ACETAMINOPHEN IN RAT PRIMARY HEPATOCYTES CULTURED IN MICROPATTERNED CO-CULTURES

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ABSTRACT

Drug- induced liver injury is often caused by cytochrome P450- dependent activation of drugs into reactive metabolites. *In vitro* models, which can mimic *in vivo* responses and allow the evaluation of initial and adaptive responses to bioactivated compounds over prolonged periods, offer potentially valuable tools for toxicological assessment. We have previously developed a model in which primary hepatocytes (rat, human) are seeded onto ECM-coated domains of optimized dimensions and subsequently co-cultivated with murine embryonic fibroblasts (HepatoPac™). This model retains key biochemical functions of *in vivo* liver with long term stability. Here, we assess the bioactivation and cytotoxicity of acetaminophen (APAP) as a function of culture age in the 96-well HepatoPac format. APAP is a well-known hepatotoxin and exerts its toxic effects through bioactivation associated, in part, with cytochrome P450 3A (CYP3A). Primary rat hepatocytes cultured in the HepatoPac platform were exposed to increasing concentrations of APAP (over 5 days) and assessed for changes in hepatic ATP content, glutathione (GSH) levels, albumin secretion and urea synthesis. Similar concentration-dependent cytotoxicity profiles (AC50=8.4 ± 2.4mM for GSH depletion and 14.17 ± 3.5mM for urea synthesis inhibition) were obtained over the course of the 4-week study. Addition of 200µM L-buthionine (S, R)-sulfoximine (BSO), an inhibitor of GSH synthesis, or 10µM dexamethasone (DEX), an inducer of rat CYP3A1/2, to HepatoPac rat hepatocyte cultures potentiated APAP- induced hepatotoxicity in these cultures irrespective of culture age (over 4 weeks). These findings are consistent with the known *in vivo* mechanisms of APAP toxicity in rats. In conclusion, rat HepatoPac in a 96-well format provided reproducible APAP-induced cell cytotoxicity profiles over a 4 week period and can be used to assess the effects of chronic exposure to bioactivated compounds.

METHODS

Rat micropatterned co-cultures (HepatoPac) were created using patented microfabrication tools and consists of primary hepatocytes arranged in optimized domains and surrounded by 3T3-J2 murine embryonic fibroblasts . In this configuration, rat HepatoPac cultures retain long- term functionality for several weeks *in vitro* (Fig 1).

The co-cultures were first allowed to stabilize functionally in serum-supplemented media for a 10-day period. Subsequently, cultures were exposed for a 5-day period to different concentrations of APAP (0.5, 1, 5, 10 or 15mM) in serum-free media in the presence or absence of 200µM L-buthionine (S, R)-sulfoximine (BSO), an inhibitor of GSH synthesis (19), or in the presence or absence of 10µM dexamethasone (DEX), an inducer of rat CYP3A1/2. During the 5-day treatment period, the initial APAP treatment was administered beginning on day 10 followed by an additional treatment 2 days afterward (day 12). An identical treatment regimen as described for the first week of dosing (week 1) was initiated with a naive set of rat HepatoPac co-cultures each week for 4 consecutive weeks (i.e. dosing was initiated at 17 days [week 2], at 24 days [week 3] or at 31 days [week 4]). At the end of each 5-day treatment period, morphological and functional endpoints were analyzed to determine the stability of the HepatoPac cultures and consistency of the concentration-dependent effects of APAP treatment on hepatocellular responses.

For the BSO-treated group, cultures were pre-incubated with 200µM BSO for four hours prior to co-administration of acetaminophen and BSO to the cultures. For the DEX-treated group, cultures were incubated with 10µM DEX for 48 hours prior to co-administration of APAP and DEX to the cultures.

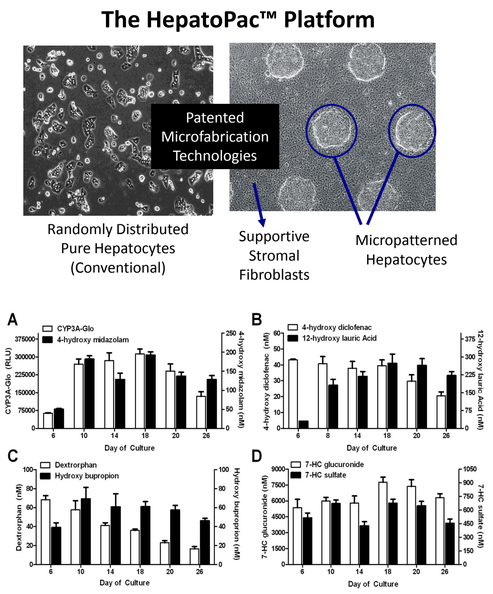

Figure 1. **The HepatoPac Platform.** HepatoPac is created using patented microfabrication tools and consists of primary hepatocytes arranged in optimized domains and surrounded by murine fibroblasts *(upper panel).* Rat HepatoPac cultures retain long- term functionality for several weeks *in vitro* [4] *(lower panel).* Select CYP450 activities are shown here. Phase II metabolism was assessed via glucuronidation/ sulfation of 7- hydroxycoumarin.

RESULTS

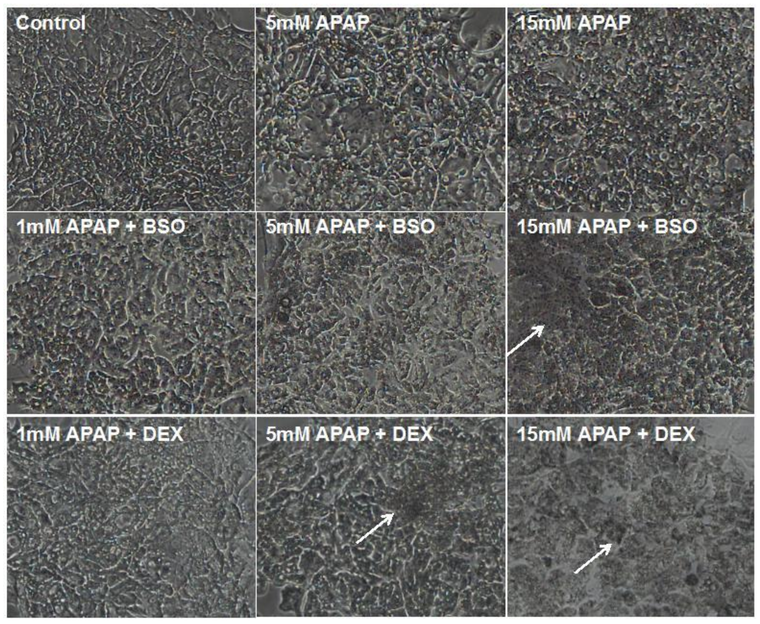

Figure 2. **Representative morphology of primary rat HepatoPac cultures treated with APAP in the absence or presence of BSO or DEX at the end of week 1.** In the presence of BSO (200µM) or DEX (10µM), concentrations of APAP of 5mM and higher caused considerable distortions in rat primary cell morphology, potentially cell necrosis (see white arrows).

	WEEK 1	WEEK 2	WEEK 3	WEEK 4
ATP (RLU/hr/hepatocyte)	518 ± 31	642 ± 20	553 ± 37	477 ± 35
GSH (RLU/hr/hepatocyte)	188 ± 23	186 ± 13	168 ± 39	178 ± 18
UREA (ng/hr/million hepatocytes)	19722 ± 833	20417 ± 972	19583 ± 278	20556 ± 1389
ALBUMIN (ng/hr/million hepatocytes)	5694 ± 694	5417 ± 1250	8333 ± 1944	5972 ± 833

Table 1. Relative ATP/GSH levels and urea/albumin production rates over time in HepatoPac rat hepatocyte cultures under control conditions. RLU= Relative Luminescence Units

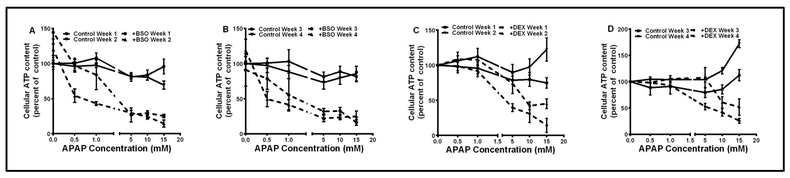

Figure 3. **Effect of APAP on ATP content in rat HepatoPac cultures in the presence or absence of BSO or DEX determined at weekly intervals.** Rat HepatoPac cultures were incubated with increasing concentrations of APAP (0 – 15 mM) in the absence or presence of 200µM BSO (A and B) or 10µM DEX (C and D) at weekly intervals for a 4-week period. After two administrations over 5 days, cell viability was determined by ATP-Glo assay. Values are the mean of triplicate wells ± S.D. of a representative culture

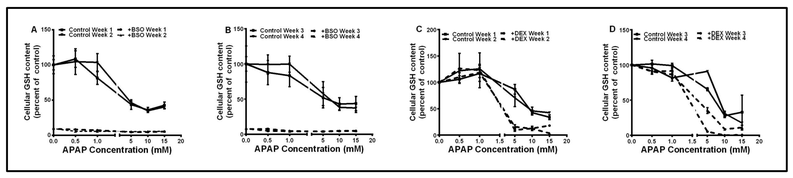

Figure 4. **Effect of APAP on GSH content in rat HepatoPac cultures in the presence or absence of BSO or DEX determined at weekly intervals.** Rat HepatoPac cultures of different ages were treated as described in Figure 3, and GSH content was assessed using the GSH-Glo assay. Values are the mean of triplicate wells ± S.D. of a representative culture.

RESULTS

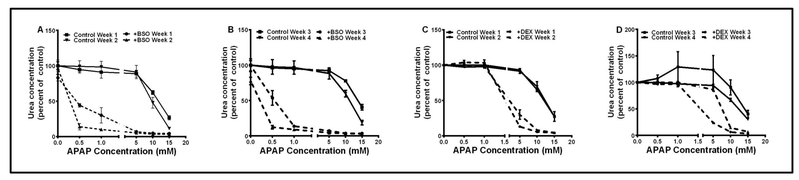

Figure 5. **Effect of APAP on urea secretion in rat HepatoPac cultures in the presence or absence of BSO or DEX determined at weekly intervals.** Rat HepatoPac cultures of different ages were treated as described in Figure 3, and urea levels were measured in supernatants. Values are the mean of triplicate wells ± S.D. of a representative culture.

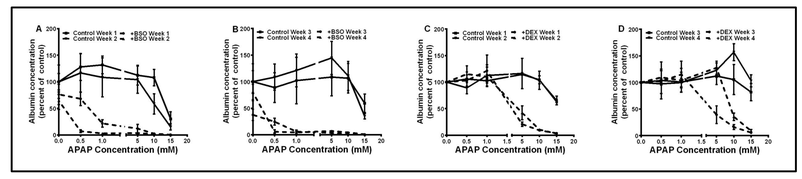

Figure 6. Effect of APAP on albumin secretion in rat HepatoPac cultures in the presence or absence of BSO or DEX determined at weekly intervals. Rat HepatoPac cultures of different ages were treated as described in Figure 3, and albumin levels were measured in supernatants using an enzyme-linked immunosorbent assay (ELISA). Values are the mean of triplicate wells ± S.D. of a representative culture.

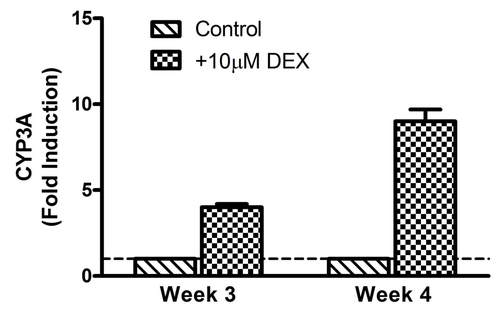

Figure 7. Dexamethasone (DEX)-mediated induction of rat CYP3A. Three and four week old rat HepatoPac cultures were treated for 48 hours with either vehicle alone (0.1% DMSO control) or 10µM DEX. Induction of CYP3A was subsequently assessed using the CYP3A-Glo assay (Promega). Data is normalized to control levels to show fold induction. Values are the mean of triplicate wells ± S.D. of a representative culture.

CONCLUSIONS

- Rat HepatoPac™ is a multi-well platform that consists of primary hepatocytes organized in empirically optimized clusters and subsequently surrounded by 3T3-J2 murine embryonic fibroblasts.
- Here, we assessed the concentration-dependent toxicity of APAP in rat HepatoPac as a function of culture age and in the presence or absence of a prototype CYP3A inducer, dexamethasone, and GSH depleting agent, L-buthionine (S, R)-sulfoximine (BSO).
- Rat HepatoPac can reproduce the key steps in the hepatocellular toxicity of the bioactivated compound APAP, which is exacerbated by GSH depletion and CYP induction. Cultures exhibited reproducible APAP-induced toxicity over a 4-week period
- Rat HepatoPac may prove useful for assessing acute and chronic effects of other bioactivated compounds and comparing species differences in bioactivation pathways for human risk assessment.

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