TOXICITY OF SELECTED BIOACTIVATED COMPOUNDS IN PRIMARY RAT 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 [i.e. micropatterned cocultures (MPCC)]. This model retains key biochemical functions of *in vivo* liver with long term stability. Here, we assess the bioactivation and cytotoxicity of acetaminophen (APAP) and other compounds in the 96-well rat MPCC. APAP is a well-known hepatotoxin and exerts its toxic effects through bioactivation associated, in part, with cytochrome P450 3A (CYP3A). Rat MPCCs were exposed to increasing concentrations of APAP (over 5 days) and assessed for changes in hepatic ATP content, glutathione (GSH) levels 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 rat MPCCs 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 MPCCs 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. The toxicity profiles of selected bioactivated compounds are also reported here.

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 hepatocytes retain long- term functionality for several weeks *in vitro* .

The co-cultures were first allowed to stabilize functionally in serum-supplemented media for an 8-10-day period. Subsequently, one-, two-, three-, or four- week old 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 , or in the presence or absence of 10µM dexamethasone (DEX), an inducer of rat CYP3A1/2. 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.

These initial investigations were expanded to 5 compounds (cyclophosphamide, aflatoxin B1, tienilic acid, ritonavir, and isoniazid) using one week old rat HepatoPac co-cultures.

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 groups, cultures were incubated with 10µM DEX for 48 hours prior to co-administration of each respective compound and DEX to the cultures.

RESULTS

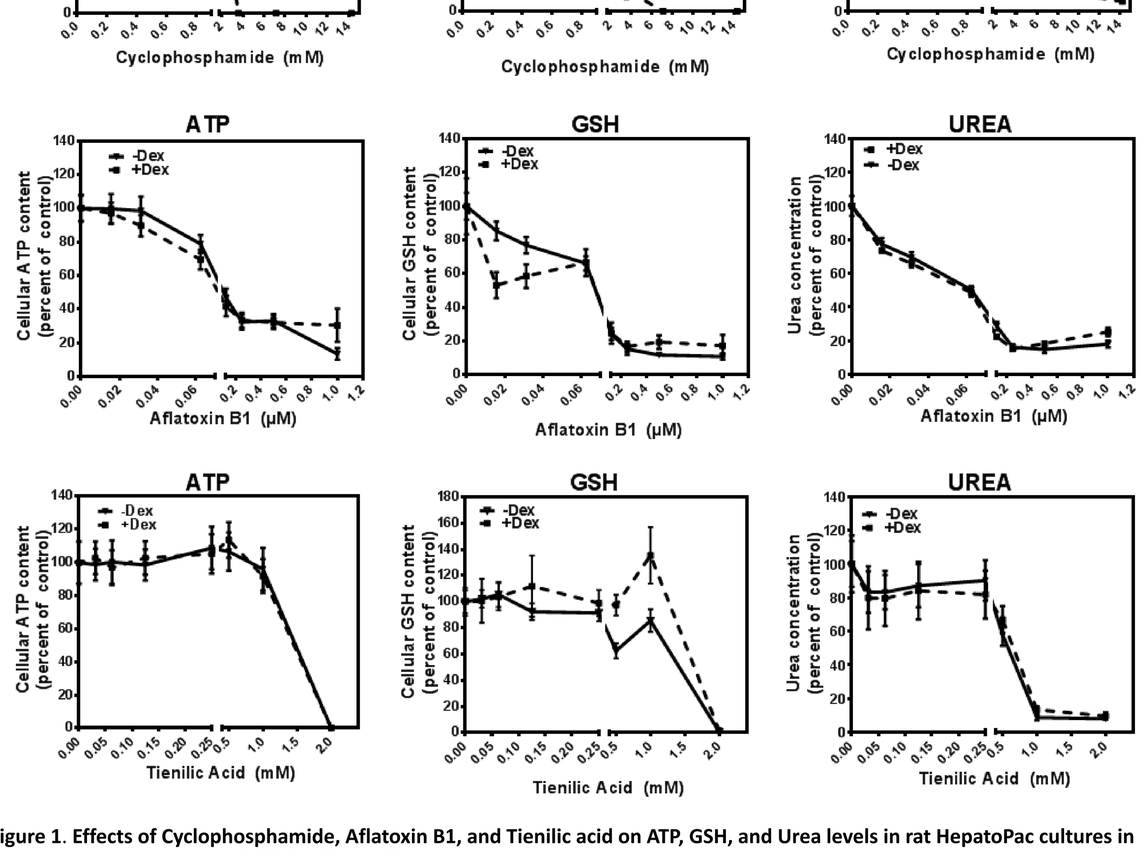

Figure 1. Effects of Cyclophosphamide, Aflatoxin B1, and Tienilic acid on ATP, GSH, and Urea levels in rat HepatoPac cultures in the presence or absence of DEX. Rat HepatoPac cultures were treated with varying concentrations of the respective compounds for five days, and ATP, GSH, and Urea concentrations were assessed afterwards. Values are the mean of triplicate wells ± S.D. of a representative culture.

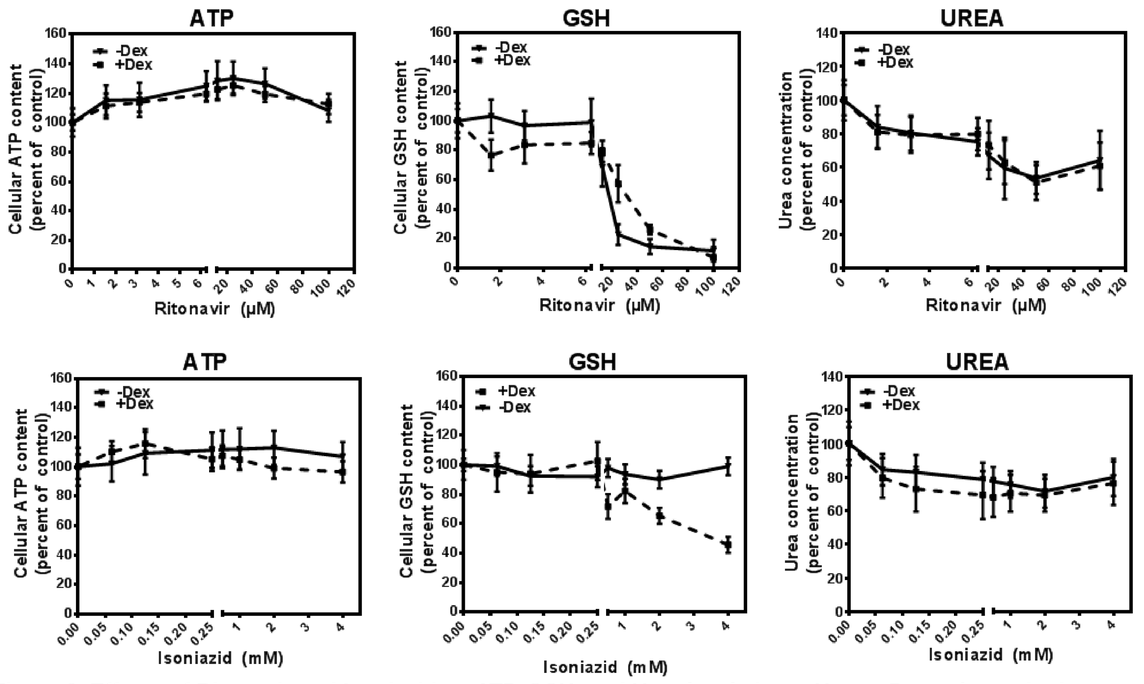

Figure 2. Effects of Ritonavir and Isoniazid on ATP, GSH, and Urea levels in rat HepatoPac cultures in the presence or absence of DEX. Rat HepatoPac cultures were treated with varying concentrations of the respective compounds for five days, and ATP, GSH, and Urea concentrations were assessed afterwards. Values are the mean of triplicate wells ± S.D. of a representative culture.

RESULTS

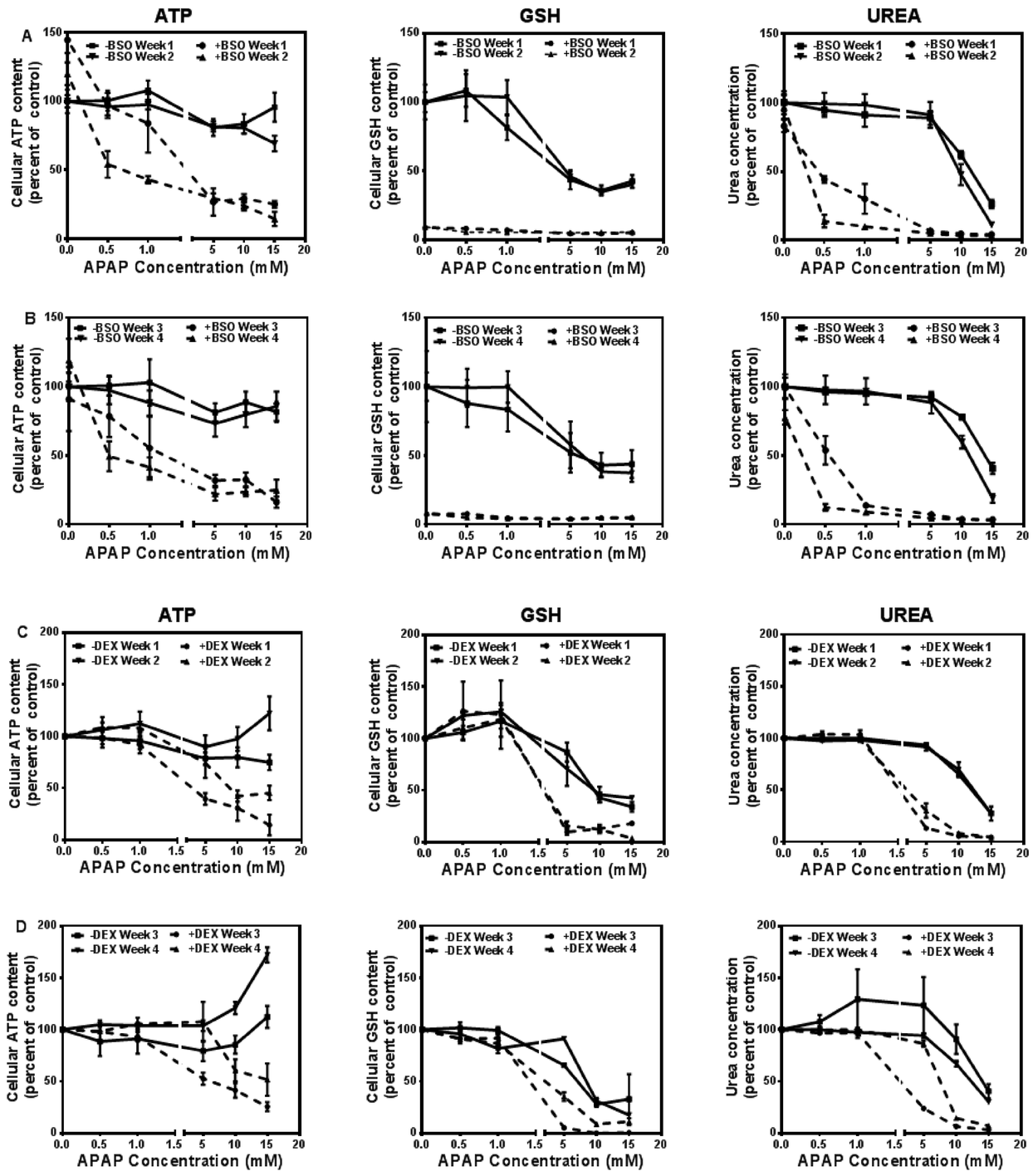

Figure 3. Effect of APAP on ATP, GSH, and Urea levels in rat HepatoPac cultures in the presence or absence of BSO or DEX determined at weekly intervals. Rat HepatoPac cultures were incubated with increasing concentrations of APAP (0 – 15 mM) in the absence or presence of 200µM BSO (A and B) or 10µM DEX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

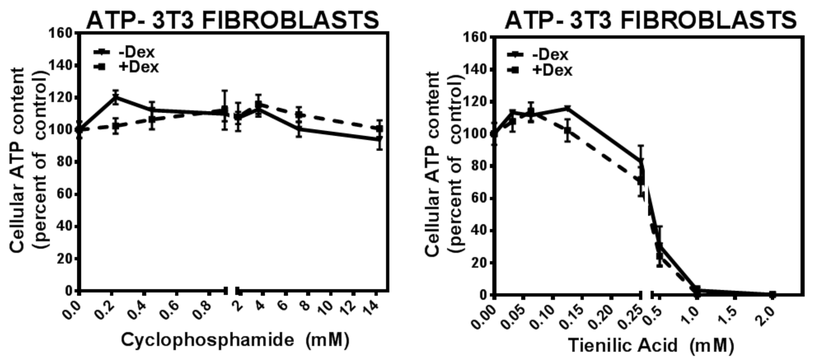

Figure 4. Effects of Cyclophosphamide and Tienilic acid on cell viability in murine 3T3 J2 Fibroblast cultures in the presence or absence of DEX. 3T3 Fibroblast cultures were treated with varying concentrations of the respective compounds for five days, and ATP content was assessed ATP-Glo assays (Promega). Values are the mean of triplicate wells ± S.D. of a representative culture. *All compounds studied, except Tienilic acid, had no adverse effects on fibroblast cell health. Tienilic acid caused concentration- dependent decreases in cellular ATP levels as well as GSH levels and urea production (data not shown) in murine 3T3 Fibroblasts.*

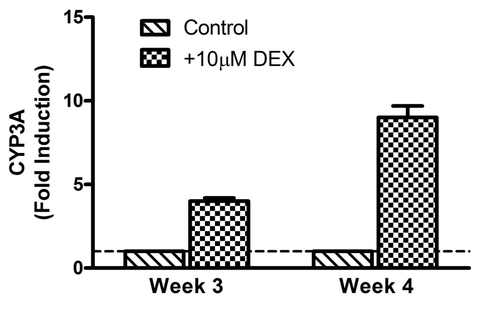

Figure 5. Dexamethasone (DEX)-mediated induction of rat CYP3A activity. Three and four week old rat HepatoPac cultures were treated for 48 hours with either vehicle alone (0.1% DMSO control) or 10µM DEX. Induction of CYP3A was subsequently assessed using the CYP3A-Glo assay (Promega). Data is normalized to control levels to show fold induction. Values are the mean of triplicate wells ± S.D. of a representative culture.

CONCLUSIONS

- 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). We also expanded the study to evaluate the toxicity profiles of selected bioactivated compounds in rat HepatoPac co-cultures.
- Rat HepatoPac cultures 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 cultures also exhibited cyclosphosphamide-, aflatoxin-, and tienilic acid- induced toxicities which may suggest that the cultures are able to generate the reactive metabolites responsible for the adverse effects of these compounds. Addition of DEX to the cultures exacerbated cyclophosphamide- induced (but not aflatoxin- or tienilic acid- induced) hepatotoxicity in the cultures.
- Ritonavir caused concentration- dependent depletion of cellular GSH content without loss of cell viability. Isoniazid on the other hand, had little or no effects on ATP, GSH, and urea levels. Co-incubation of cells with DEX and isoniazid caused a slight decrease in cellular GSH levels.
- All compounds studied, except tienilic acid, had no adverse effects on fibroblast cell health. Tienilic acid caused concentration- dependent depletion of cellular ATP levels as well as GSH levels and urea production (data not shown). The toxicity of tienilic acid may involve other mechanisms other than the generation of a reactive metabolite.
- Rat HepatoPac co-cultures 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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