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 plates of optimized dimensions and subsequently co-cultivated with murine embryonic fibroblasts (i.e., micropatterned cocultures [MPCCs]). 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 micropatterned cocultures (MPCCs). APAP is a well-known hepatotoxicant and exerts its toxic effects through bioactivation associated, in part, with cytochrome P450 2E1 (CYP2E1). Rat MPCCs were exposed to increasing concentrations of APAP for 5 days and assessed for changes in hepatic ATP content, glutathione (GSH) levels and urea synthesis. Similar concentration-dependent cytotoxicity profiles (AP50-4.4 ± 2.4 µM for GSH depletion and 19.3 ± 5.6 mM for urea synthesis) 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 CYP3A12, to rat MPCCs potentiated APAP-induced hepatotoxicity in these cultures irrespective of culture age (up to 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 optimal domain 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- to 10-day period. Subsequently, one-, two-, three-, or four-week old cultures were exposed for 4 to 5 days to different concentrations of APAP (0.5, 1.5, 10 or 15µM) 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 CYP3A12. 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 (BSO) to the cultures for five days, and ATP, GSH, and urea synthesis were assessed afterwards. Values are the mean of triplicate wells ± S.D. of a representative culture.

RESULTS

APAP concentrations (µM) 0 1 2 3 4 5 6 ATP, GSH, and Urea levels in rat HepatoPac cultures in the presence or absence of BSO or DEX were determined at weekly intervals. Rat HepatoPac cultures were incubated with increasing concentrations of APAP (0–15 µM) in the presence or absence 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 were the mean of triplicate wells ± S.D. of a representative culture.

CONCLUSIONS

Here, we assessed the concentration-dependency 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 hepatotoxicity 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 cytochrome P450-dependent毒xicities which may suggest that the cells are able to generate the reactive metabolites responsible for the adverse effects of these compounds. Addition of DEX to the cultures exacerbated cytochrome P450-dependent toxicity (but not aflatoxin- or tienilic acid-induced hepatotoxicity in the cultures).

Rat HepatoPac cultures showed concentration-dependent decreases in cellular ATP levels as well as GSH levels and urea production (data not shown) in murine 3T3 fibroblasts.

All compounds studied, except tienilic acid, had no adverse effects on fibroblast cell health. Isoniazid caused concentration-dependent decreases in 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.

multi-drug resistance proteins.

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.

REFERENCES


