Development of a Novel Micropatterned Co-Culture Model with Primary Dog Hepatocytes to aid in Accurate Prediction of DILI Risk

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ABSTRACT

Drug Induced Liver Injury (DILI) is a major concern in drug development for pharmaceutical companies and is one of the foremost reasons for post-marketing withdrawal of drugs. Accurate prediction and early detection of DILI remains a challenge in preclinical safety studies. Although preclinical species may not adequately represent the biochemical and molecular mechanisms present in humans, they remain a critical tool for assessing DILI risk, and ensuring the safety of patients undergoing drug therapy. In addition, *in vitro* models using primary hepatocytes have been developed as a necessary complement to *in vivo* investigations. We have developed a novel model in which primary dog hepatocytes are seeded onto ECM-coated domains of optimized dimensions and subsequently co-cultivated with fibroblasts (i.e. micropatterned co-cultures (MPCCs)), thus retaining key biochemical functions of *in vivo* liver. Here, we assess the ability of the dog MPCC platform to accurately predict DILI potential by treating the co-cultures with representative compounds of DILI and non-DILI concerns. Dog MPCCs were exposed to increasing concentrations of Aspirin (0-300µM), Amiodarone (AMD, 0-80µM) and Aflatoxin B1 (AFB, 0-1µM) over a 5-day period and evaluated for changes in cellular ATP levels, albumin synthesis, and ALT leakage from the treated cultures. Aspirin, a non-toxicant, did not have any adverse effects on the cultures. In contrast, AFB, a known hepatotoxicant to human and rat hepatocytes, caused decreases in cellular ATP levels (to ~40% of control levels) and albumin production (less than 10% of control levels). Interestingly, AMD, a human hepatotoxicant, was mildly toxic to the dog cultures resulting in significant decreases in only albumin production (to ~30% of control levels) and minimal impact on ATP levels. Also, ALT leakage in the treated cultures was significantly increased (greater than 2-fold) in AFB-treated cultures but not in Aspirin- or AMD-treated cultures. These results validate the use of the dog MPCC model for assessing clinical DILI.

METHODS

Dog micropatterned co-cultures (HepatoPac®) were created using patented microfabrication tools and consist of primary hepatocytes arranged in optimized domains and surrounded by human BJ fibroblasts. In this configuration, the HepatoPac co-cultures retain *in vivo* functionality *in vitro* (Fig 1). The co-cultures were first allowed to stabilize functionally in serum-supplemented media for an 8-10 day period prior to dosing. Subsequently, cultures were exposed, for a 5-day period, to different concentrations of Aspirin (0, 4.7, 9.4, 18.75, 37.5, 75, 150 or 300µM), Amiodarone (0, 1.25, 2.5, 5, 10, 20, 40 or 80µM) or Aflatoxin B1 (0, 0.0156, 0.031, 0.062, 0.125, 0.25, 0.5 or 1µM) in serum-free medium. At the end of the 5-day treatment period, morphological and functional endpoints were analyzed to ensure that the HepatoPac co-cultures were phenotypically stable and to determine the concentration-dependent, drug-induced effects on hepatocellular health/function. Functional endpoints evaluated included cellular ATP content, albumin secretion and ALT leakage. ATP levels in cell lysates were measured using the CellTitre-Glo® luminescent kit (Promega Corp.) and albumin secretion was assayed in the culture medium using Dog Albumin ELISA kits (ICL). ALT leakage in the spent medium was measured using Sigma-Aldrich's ALT Activity Assay Kit.

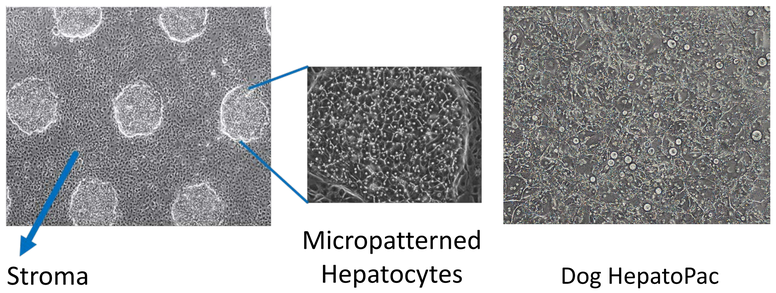

Figure 1. HepatoPac Platform. HepatoPac® micropatterned co-culture platform is created using patented microfabrication tools and consists of primary hepatocytes arranged in optimized domains and surrounded by stromal fibroblasts, which support the highest level of performance observed in the HepatoPac. HepatoPac cultures retain long- term functionality for several weeks *in vitro.* Human HepatoPac shown, left and center. Dog HepatoPac shown, right.

RESULTS

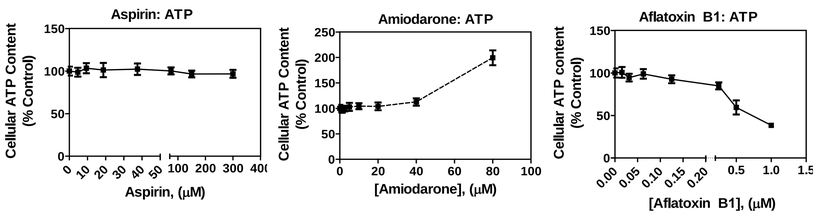

Figure 2: Effects of Aspirin, Amiodarone and Aflatoxin B1 on cellular ATP levels. Dog HepatoPac co-cultures were treated with varying concentrations of compounds - Aspirin, Amiodarone or Aflatoxin B1 – for five days. ATP contents were assessed 5 days post-drug treatment. Responses are represented as percentage of vehicle controls. Values are the mean of triplicate wells ± S.D. of a representative culture. TC50 values for ATP depletion are >300µM, >80µM and 0.7µM for Aspirin, Amiodarone and Aflatoxin B1, respectively.

RESULTS

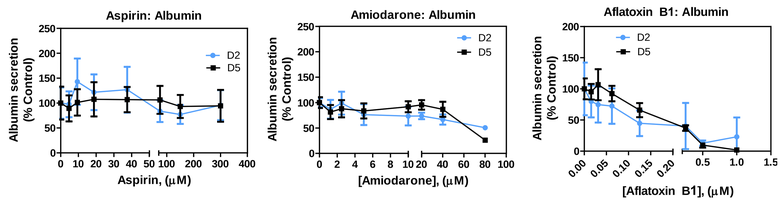

Figure 3: Effects of Aspirin, Amiodarone and Aflatoxin B1 on albumin secretion in dog HepatoPac® co-cultures. Dog HepatoPac co-cultures were treated with varying concentrations of the compounds - Aspirin, Amiodarone or Aflatoxin B1 – for five days. Albumin concentration in the spent culture medium were assessed 2 (D2) and 5 (D5) days post-drug treatment. Responses are represented as percentage of vehicle controls. Values are the mean of triplicate wells ± S.D. of a representative culture. TC50 values for suppression of albumin secretion were i) >300µM on D2 and D5 for Aspirin, ii) >100µM and 62.3µM on D2 and D5 for Amiodarone, respectively, and iii) 0.12µM and 0.18µM on D2 and D5 for Aflatoxin B1, respectively.

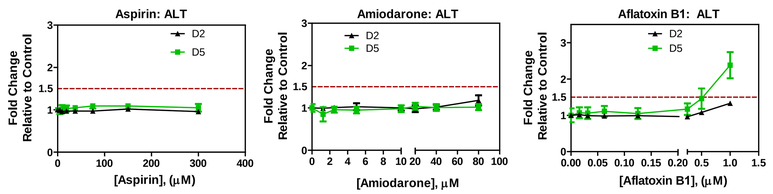

Figure 4: Effects of Aspirin, Amiodarone and Aflatoxin B1 on ALT leakage from dog hepatocytes. Dog HepatoPac co-cultures were treated with varying concentrations of the compounds - Aspirin, Amiodarone or Aflatoxin B1 – for five days. ALT concentration in the medium were assessed 2 (D2) and 5 (D5) days post-drug treatment. Responses are represented as fold change relative to vehicle controls. Values are the mean of triplicate wells ± S.D. of a representative culture. ALT leakage from the hepatocytes was considered significant if it was ≥ 1.5 fold of controls. There was significant ALT leakage from Aflatoxin B1-treated cultures alone.

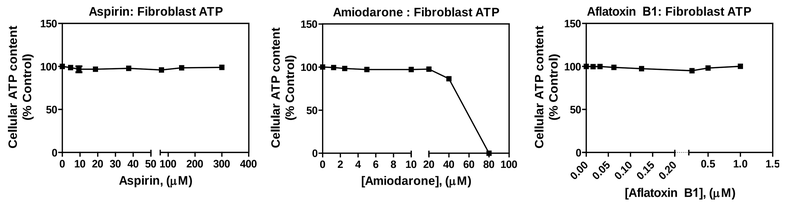

Figure 5: Effects of Aspirin, Amiodarone and Aflatoxin B1 on cellular ATP levels in fibroblast cultures. Fibroblast-only cultures were treated with varying concentrations of compounds - Aspirin, Amiodarone or Aflatoxin B1 – for five days. ATP contents were assessed 5 (D5) days post-drug treatment. Responses are represented as percentage of vehicle controls. Values are the mean of triplicate wells ± S.D. of a representative culture. Treatment with Aspirin and Aflatoxin B1 had negligible effect on the fibroblast cultures while treatment with Amiodarone resulted in significant ATP depletion at the maximum test concentration.

COMPARISON OF TOXICITY PROFILES BETWEEN SPECIES – HISTORICAL HEPATOPAC® DATA

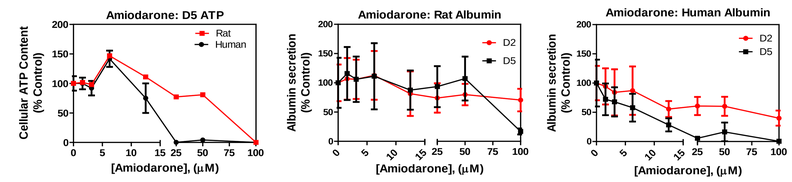

Figure 6: Effect of Amiodarone treatment on ATP levels and albumin secretion in Rat and Human HepatoPac co-cultures. HepatoPac co-cultures were treated with varying concentrations of Amiodarone up to a maximum test concentration of 100µM. Responses are represented as percentage of vehicle controls. Values are the mean of triplicate wells ± S.D. of a representative culture.

COMPARISON OF TOXICITY PROFILES BETWEEN SPECIES – HISTORICAL HEPATOPAC® DATA

AMIODARONE		
	TC50 (µM)	
	Human	Rat
ATP (D5)	13.2	59.4
Albumin (D2; D5)	44; 5.7	>100; >100

Table 1: TC50 values for ATP depletion and suppression of albumin secretion in rat and human HepatoPac co-cultures treated with Amiodarone. Rat and human HepatoPac co-cultures were treated with varying concentrations of Amiodarone for five days. There was clear species-related differences in toxicity profiles – Amiodarone was more toxic to human hepatocytes (with lower TC50 values) than to the rat hepatocytes.

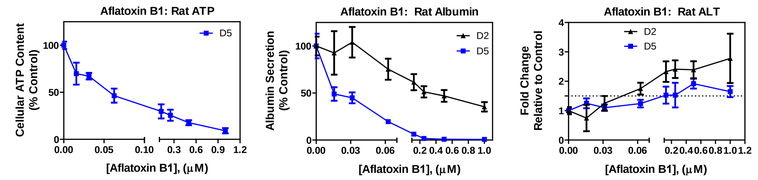

Figure 7: Effect of Aflatoxin B1 treatment on ATP levels, albumin secretion and ALT leakage in Rat HepatoPac co-cultures. Rat HepatoPac cultures were treated with varying concentration of Aflatoxin B1 (up to 1µM) for 5 days. Values are the mean of triplicate wells ± S.D. of a representative culture. TC50 values were determined to be i) 0.06µM for ATP depletion; ii) 0.33µM and 0.02µM for suppression of albumin secretion on days 2 (D2) and 5 (D5) respectively; and iii) ALT leakage from the hepatocytes was considered significant if it was ≥ 1.5 fold of controls. There was significant ALT leakage from Aflatoxin B1-treated cultures alone.

CONCLUSIONS

- Dog MPCCs treated with reference compounds, produced hepatocellular responses that were expected
- Aspirin, a non-hepatotoxic compound, had no impact on cell health and viability while the two hepatotoxicants, Amiodarone and Aflatoxin, did have effects on markers of hepatic health/function and cellular viability –albumin secretion, ALT leakage and cellular ATP levels.
- Hepatocyte-specific responses to the test compounds were accurately delineated from the effects seen in the dog HepatoPac co-cultures and the effects in fibroblast-only cultures.
- Results from these experiments, when compared to historical data showed that there were species specific responses to toxicants:
  - Amiodarone was more toxic to human hepatocyte than to rat or dog hepatocytes.
  - Aflatoxin B1 was more toxic to rat hepatocytes than it was to dog hepatocytes.
- These data highlight the superiority of a long-term, functional tissue-engineered liver model of the dog MPCC platform, over traditional models in predicting clinically relevant toxicities.

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