Assessing Chronic Toxicity of Fialuridine in A Micropatterned Hepatocyte Co-culture Model

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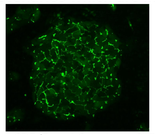
Introduction

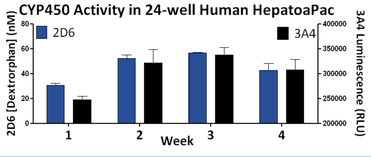
Fialuridine (FIAU), a nucleoside drug for the treatment of hepatitis B, failed in clinical trials due to hepatotoxicity leading to five patient deaths. FIAU toxicity is difficult to replicate in unstable sandwich cultures of primary hepatocytes. Liver toxicity is a major cause for attrition of pharmaceutical compounds, emphasizing the need for a more stable *in vitro* model for toxicity studies. We have utilized microfabrication tools to develop a human liver model with precise microscale cyto-architecture and optimal stromal interactions (micropatterned co-cultures) that displays phenotypic stability for several weeks *in vitro*, allowing more accurate prediction of clinically relevant toxicity[1]. Micropatterned co-cultures from two human and two rat donors were dosed twice over four days with FIAU, uridine, and four other related analogues up to 100 µM. Human hepatocytes in this model (short term dosing) experienced significant dose- and time- dependent toxicity with FIAU incubation as assessed by mitochondrial activity, morphology, albumin and urea secretion, and CYP3A4 activity. FIAU was most toxic to human hepatocytes when all endpoints were considered. There were donor dependent differences in the magnitude of toxicity as well as rank ordering of compounds. On the other hand, in short term dosing of rat hepatocytes, the same extent of FIAU toxicity was not observed as seen with human hepatocytes in micropatterned co- cultures, consistent with preclinical animal toxicity data[2]. For long-term (chronic) studies, micropatterned co-cultures with the same two cryopreserved human donors and one new rat donor were dosed with fresh compounds in culture medium every two days for three weeks (doses up to 10 µM). Results indicated that CYP3A4 activity was the most sensitive functional marker for distinguishing the effects of FIAU over other drugs at doses as low as 1 µM. Species-specific differences were observed in human and rat hepatocytes during short- vs. long-term treatment with FIAU. In the future, micropatterned co-cultures may serve as a robust model system to evaluate the chronic effects of compounds on the liver.

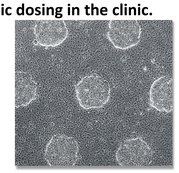
HepatoPac™

HepatoPac is a unique platform that optimizes function and life span of plated primary hepatocytes. The stability of CYP450s, and formation of liver specific structures (bile canaliculi) allows the streamlined use of one *in vitro* model for many pharmaceutical applications, compared to suspension and sandwich cultures. For metabolite identification, it has been shown that HepatoPac outperforms suspension hepatocytes, S9 fractions, and microsomes, offering a superior *in vitro* approach for generating major human metabolites[3]. Furthermore, HepatoPac has also shown utility for toxicity, high content imaging, safety, clearance, uptake and efflux studies. HepatoPac consists of primary hepatocytes attached to domains of matrix surrounded by supporting stromal cells, in industry standard plates, maintaining high throughput capabilities. This platform facilitates the long term function of hepatocytes *in vitro,* allowing clinically relevant dosing scenarios which are important for understanding toxicity that manifests over chronic dosing in the clinic.



CDF (5-and-6-carboxy-2',7'-dichlorofluorescein diacetate)





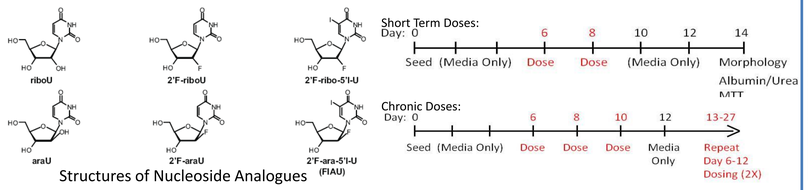
Methods

Donor	Age	Sex	Race	COD	Smoker/Alcohol/Drugs	Medications
1	57	F	C	Anoxia	Y/N/N	Clonipin, Synthroid, Paxil
2	56	M	AA	Cerebrovascular Accident	N/N/N	Insulin

•Freshly isolated primary hepatocytes from male Sprague-Dawley rat and cryopreserved primary human hepatocytes were seeded in HepatoPac 24 & 96 well plates. After attaching to micropatterned matrix domains, cultures were washed and incubated overnight. Stromal cells were seeded the following day. Media was replaced with serum supplemented proprietary medium every other day, until day 6.

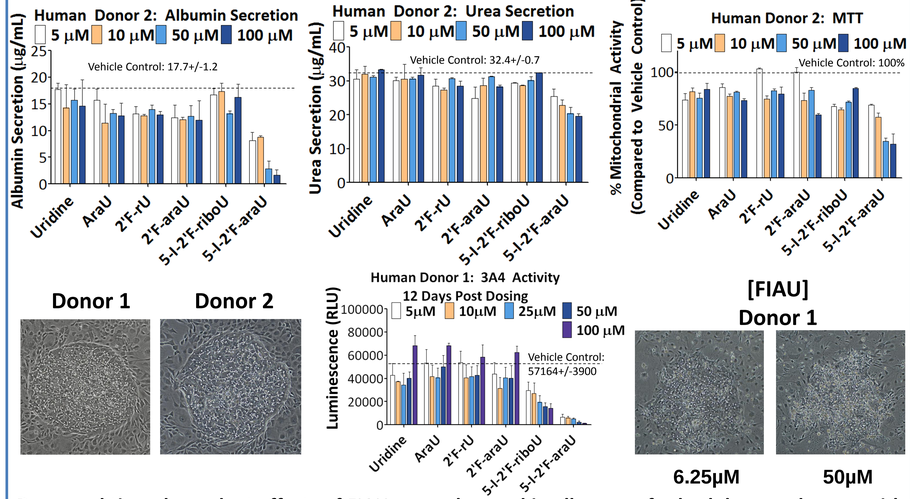
•Short term toxicity: cultures washed to remove serum and treated with FIAU and other analogues. Cultures received doses (5-100µM) in serum-free medium on days 6 & 8 from start of culturing and were switched back to medium with serum on days 10 & 12. Medium was collected to determine albumin and urea secretion[1]. On day 14, mitochondrial activity was assessed with the MTT assay.

•Chronic toxicity: HepatoPac with the same two cryopreserved human donors and one new rat donor were dosed every other day with the same compounds (1-10µM), in serum supplemented media, for up to three weeks. Albumin and urea secretion and 3A4 activity were monitored throughout the time course. CYP3A4 activity was quantified with Promega's CYP3A4-Glo assay.



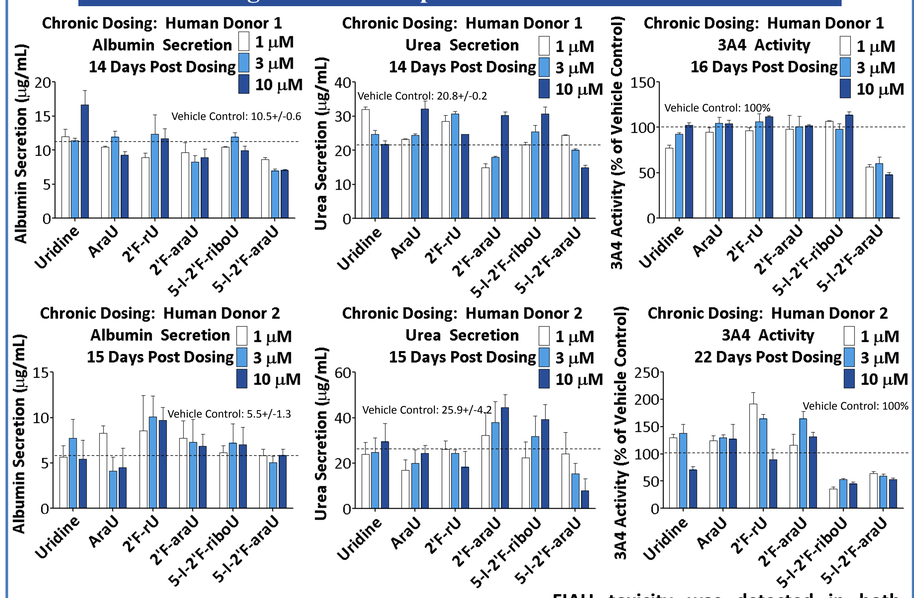
Structures of Nucleoside Analogues

Short Term Dosing in Human HepatoPac™ (8 days post dosing)



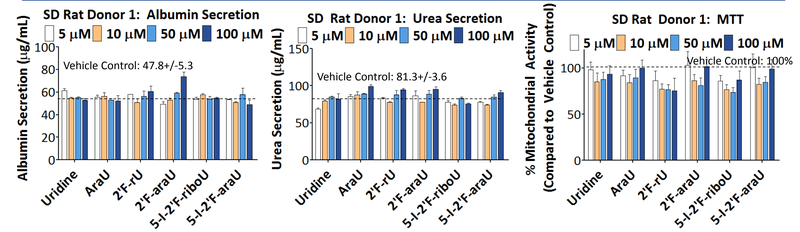
Dose- and time-dependent effects of FIAU were observed in all assays, for both human donors, with 3A4 activity being the most sensitive functional marker. Earlier time points (days 2 and 4) did not show significant toxicity (data not shown).

Chronic Dosing in Human HepatoPac™



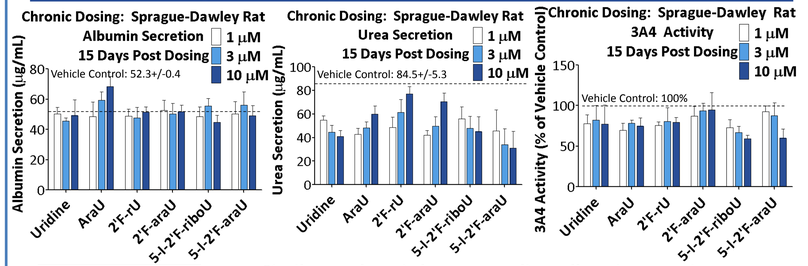
FIAU toxicity was detected in both donors. Albumin secretion was not as sensitive of a marker as it was in short term dosing. Effects of FIAU were marked by decrease in urea secretion and CYP3A4 activity. CYP3A4 activity was the most sensitive assay for determining adverse effects of FIAU over other analogues.

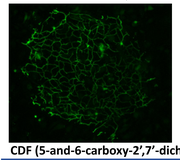
Short Term Dosing in Rat HepatoPac™ (8 days post dosing)



No apparent toxicity was seen in either of two rat donors, consistent with preclinical data (Data for second rat donor not shown).

Chronic Dosing in Rat HepatoPac™





CDF (5-and-6-carboxy-2',7'-dichlorofluorescein diacetate)

Similar to chronic human studies, albumin secretion was not a sensitive marker for detecting FIAU toxicity. However, endpoint assays for urea secretion and CYP3A4 activity did show some adverse effects when cultures were incubated with FIAU.

Conclusions

•Short term dosing: Human hepatocytes in this model experienced significant dose- and time-dependent toxicity with FIAU incubation as assessed by mitochondrial activity, morphology, albumin secretion, urea synthesis and CYP3A4 activity. FIAU was the most toxic to human hepatocytes when all endpoints were considered. There were donor dependent differences in the magnitude of toxicity as well as rank ordering of compounds. Rat hepatocytes did not exhibit the same extent of toxicity upon FIAU incubation as did human hepatocytes in micropatterned co-cultures, consistent with preclinical animal toxicity data.

•Chronic dosing: Results indicated that CYP3A4 activity was the most sensitive functional marker, compared to albumin secretion and urea synthesis to distinguish the effects of FIAU over the other analog drugs at doses as low as 1 µM.

•A functional assessment of primary rat hepatocyte health on day 14 (albumin secretion, urea synthesis, MTT) did not show toxic effects on liver specific functions during the short term dosing study. However, adverse effects were observed (CYP3A4 activity and urea synthesis) when rat cultures were subjected to long term chronic dosing. This is consistent with a 1994 *in vivo* rat study that was extended first to 4 weeks, then to 10 weeks as toxicity was not observed in the originally planned 2 weeks[2].

•Albumin was not as sensitive at detecting FIAU toxicity in chronic dosing as it in short term dosing.

•Toxicity of FIAU was seen at more clinically relevant doses (1-10µM) when cultures were able to be dosed repeatedly for 3-4 weeks.

•Species specific, dose- and time-dependent effects were observed. Mechanistic investigations are ongoing to distinguish species-specific effects of FIAU in primary hepatocytes cultured in micropatterned co-cultures.

References

1. Khetani and Bhatia. *Nature Biotech*. 26(1), 120-126 (2008)
2. Manning and Swartz. *Institute of Medicine of the National Academies*. (1995)
3. Wang *et al*. *DMD*. 38(10) 1900-1905 (2010)