The occurrence of inflammation has been shown to alter the activity of enzymes involved in drug metabolism. This altered activity demands special consideration upon co-administration of drugs due to potential pharmacological and toxicological consequences. An in vitro model that mimics liver inflammation may provide better predictive power in preclinical testing. We have developed a microperoxidase co-culture of primary hepatocytes and embryonic fibroblasts (HepatoPac™) that retains high levels of phenotypic functions such as drug metabolism enzymes for 4 weeks in vitro. Here, we supplement the HepatoPac co-cultures with primary Kupffer macrophages in order to mimic one component of inflammation. Species-matched Kupffer cells were added to human and rat HepatoPac at multiple ratios (to mimic both the normal and inflamed state of the liver) after stabilization to generate a co-culture with primary hepatocytes and embryonic fibroblasts (HepatoPac™ – Kupffer cell co-culture). Recent evidence suggests that interaction between inflammatory stress and drug metabolism may precipitate toxic responses. Here, we assess whether stimulation of HepatoPac – Kupffer cell co-cultures with LPS stimulates the cells to transcribe (TVX) toxicity. Rat or human HepatoPac cultures were treated with 50 ng/mL Lipopolysaccharide (LPS) for 20 hours and cell supernatants were analyzed for changes in cytokine secretion. Cytokine secretion were observed with Trovafloxacin (TVX) and trovafloxacin (SFLX) in serum-free media at multiple times (one, four, 9.74, and 95 hours), respectively, for a total of 72 hours in order to investigate compound toxicity. Trovafloxacin-exposed HepatoPac cultures were retreated with 50 ng/mL LPS for 24 hours after the initiation of compound dosing.

**RESULTS**

- **HepatoPac™ and HepatoPac™ - Kupffer cell co-culture.** (A) HepatoPac cultures that included rat or human Kupffer cell co-cultures for utility in inflammation drug interactions. Previously, we have shown that addition of Kupffer cells do not compromise hepatic functionality in our model (Figure 1), and remained functional (as assessed via phagocytosis, phagolysosome staining, CYP3A inhibition, and cytokine release) for up to 10 days post addition to HepatoPac. It has been proposed that inflammatory stress may precipitate an idiosyncratic adverse drug reaction (iADRs) in the liver as that observed during the administration of the fluoroquinolone antibiotic trovafloxacin (TVX) [1]. Previous work has been done in an in vivo mouse model demonstrated that LPS-induced inflammatory stress rendered TVX non-toxic in CYP3A4 activity and susceptibility to aminopyrine (in either species) data for ADRs not shown). Kupffer cells do not impair HepatoPac functionality and are responsible for the rapid destruction of TVX in vivo. The HepatoPac model system may provide a more useful tool for the evaluation of the hepatocellular toxicity of TVX. Trovafloxacin toxicity was potentiated in LPS-treated HepatoPac cell cultures in both rat and human cultures. TVX-induced depletion of CYP3A4 activity in the rat HepatoPac cell cultures was reversed by treatment with the compounds and Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immunemediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immunemediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immune-mediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immune-mediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immune-mediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immune-mediated liver toxicities.

**CONCLUSIONS & FUTURE DIRECTIONS**

- **HepatoPac™ is a multi-well (9- well plates) platform that consists of primary hepatocytes organized in periportal-pyronin-stained ducts and subsequently surrounded by JESS -murine embryonic fibroblasts. Here, both human and rat HepatoPac were supplemented with primary human or rat Kupffer cells in utility in assessment of inflammatory stress. HepatoPac human Kupffer cell co-cultures did not affect hepatocellular functionality as assessed via CYP3A activity and drug susceptibility of aminopyrine (in either species) data for ADRs not shown). Kupffer cells do not impair HepatoPac functionality and are responsible for the rapid destruction of TVX in vivo. The HepatoPac model system may provide a more useful tool for the evaluation of the hepatocellular toxicity of TVX. Trovafloxacin toxicity was potentiated in LPS-treated HepatoPac cell cultures in both rat and human cultures. TVX-induced depletion of CYP3A4 activity in the rat HepatoPac cell cultures was reversed by treatment with the compounds and Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immunemediated liver toxicities. The HepatoPac rat and human primary Kupffer cell co-culture platform could evaluate the toxicology profiles of compounds known to cause immune-mediated liver toxicities.

**REFERENCES**