Drug-induced liver injury (DILI) is a major health problem in the United States and accounts for the majority of clinical holdups and post-marketing use restrictions by the FDA. The majority of adverse liver reactions are idiosyncratic and their underlying mechanisms are still not well understood. Better predictive models for DILI would enable the precise classification of drug candidates with hepatotoxic liabilities. We have previously developed a model in which primary hepatocytes (rat, human, dog, or monkey) are seeded onto ECM-coated domains of optimized dimensions and spatially co-cultivated with murine stellate fibroblasts (HepatoPac). HepatoPac cells in HepatoPac retain their in vivo-like morphology, express a complete complement of liver-specific genes, metabolize commercial substrates using Phase I and II drug metabolism enzymes, secrete diverse liver-specific products, and display functional bile canaliculi for several weeks in vitro. Here, we supplement the HepatoPac co-cultures with primary murine Kupffer cells for use in investigating inflammatory co-cultures. Kupffer cells were added to human HepatoPac cultures at a precise hepaticale: Kupffer cell ratio of 10:1 to mimic an infected liver state. Initial investigations were performed to address the functions of the HepatoPac platform by evaluating: (i) the phagocytosis of Staphylococcus aureus labeled bioparticles by the Kupffer macrophages, (ii) the basal CYP450 activity of the hepatocytes in the presence and absence of the Kupffer cells, (iii) secretion of cytokines by LPS-stimulated Kupffer cells, and (iv) suppression of CYP450 activity and gene expression by cytokine-stimulated HepatoPac cells. HepatoPac cultures were treated with 50 nM LPS. After 24 hours, cell supernatants were analyzed for cytokine secretion. Cytokines were measured by TofTaq (ThermoFisher) and Luminex (in the presence of fridge-free medium at multiple of their Cmax). 15.7 µM, respectively, for a total of 72 hours to investigate compound toxicity. In a follow-up study, the cultures were dosed with increasing concentrations of antiapoptotic, cytoprotective, and proinflammatory cytokines in the presence or absence of 50 nM LPS. The serum-free medium at multiple of their Cmax, up to a 100 Cmax. The Cmax values for zoclozapine and chlorpromazine were 10 ng/ml and 10 ng/ml, respectively. Furthermore, the substrates and metabolites of both zoclozapine and chlorpromazine have been reported to inhibit human-mediated hepatotoxicity.

**RESULTS**

**CONCLUSIONS**

- Previously, we showed that rat and human HepatoPac co-cultures may be used to model inflammation-driven drug interactions using Trovafloxacin as a model compound with immune-mediated toxicity. We found that Kupffer cells maintain their function in the HepatoPac co-cultures for up to 10 days in culture, exhibiting phagocytic activity and secreting cytokines when stimulated by LPS. Here, we extend the studies to show that human HepatoPac co-cultures are able to model zoclozapine- and chlorpromazine-immune-mediated toxicities.
- Consistent with literature reports, treatment of the cultures with cytokines down-regulated CYP3A4 activity and gene expression in both HepatoPac and HepatoMune cultures. The presence of the Kupffer cells intensified this effect. This shows that the Kupffer cells are needed to model complex interactions that mediate immune-mediated toxicities, which may not be modeled by hepatocytes alone.
- Trovafloxacin activity was identified in LPS-treated human HepatoPac co-cultures replicating in vivo studies in rats and mice. Furthermore, our studies in rat HepatoMune cultures showed the abrogation of TXV-mediated toxicity in the presence of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and the glucocorticoid receptor, both of which are known to be mediators of TXV toxicity.
- We showed that the HepatoPac platform is highly specific, distinguishing between Trovafloxacin and its non-toxic analog, Levofloxacin.
- Conclusion: zoclozapine and chlorpromazine toxicities were potentiated in LPS treated human HepatoPac co-cultures after repeated administration of LPS and the compounds. This finding emphasizes the need to use a long-lasting platform such as HepatoPac that enables chronic dosing of compounds to model inflammation-driven drug interactions.
- Future studies on the HepatoPac co-culture platform will seek to evaluate the toxicity profiles of more compounds known to cause immune-mediated liver toxicities.
- The HepatoPac co-culture platform could find utility in assessing of clinically relevant interactions between therapeutic biomolecules and small molecule drugs as well as evaluation of inflammation-mediated toxicities.