Modeling of Cytokine-Induced Liver Hepatotoxicity of Trovafloxacin Using Co-cultures of Hepatocytes and Kupffer Cells

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### Introduction

Drug Induced Liver Injury (DILI) is a major health problem that accounts for over 50% of acute liver failures in the United States. It is usually caused by the idiosyncratic nature of liver enzymes resulting in drug toxicity in liver parenchymal cells. The other reason for DILI, often overlooked in pharmacological testing due to the unavailability of proper in vitro models, is indirect toxicity resulting from immune cells activating liver inflammatory responses. In fact, the liver contains a large amount of both innate and adaptive immune cells with the largest population of resident macrophages, Kupffer cells, in the body, making it one of the primary organs of immunity. As such, liver immune cells play a critical role in hepatotoxicity and resulting liver injury. For this reason, we have established a co-culture system of liver parenchymal cells, hepatocytes, and Kupffer cells that can be used to model drug-induced immune reactions resulting in acute hepatotoxicity. In this study, this system was used to model the hepatotoxicity of Trovafloxacin (TVX) and resulting necrotic death of hepatocytes due to blunted IL-6 inhibition of P450s and increased production of cytotoxic TNFα.

### Materials and Methods

Hepatocytes and Kupffer cells were obtained from Life Technologies (Carlsbad, CA). Cells were analyzed for purity using the Attune Acoustic Focusing Cytometer. Kupffer cell morphological assessments with CD14, CD68 and CD163 were performed utilizing standard IHC methodologies. Cytokines were assessed by ELISA for IL-6, IL-10 and TNFα in response to 24hr, 48hr and 72hr treatment with various concentrations of E. coli (0127:B8) LPS and TVX (Sigma). Kupffer cells and hepatic co-cultures were cultured in Adv. DMEM supplemented with ITS+, and Pen/Strep (GIBCO®, Life Technologies). For plating media, an additional 10% FBS was used. Cultures: Cryopreserved cells were thawed and centrifuged. Supernatants were removed and cells were resuspended in approximately 1 mL of plating media, then diluted to approximate seeding densities and analyzed for viability via Trypan Blue exclusion. For co-cultures of Kupffer cells and hepatocytes, Kupffer cells were first seeded onto 24-well Collagen I coated plates, and cultured overnight. Hepatocytes were seeded approximately 24 hours after Kupffer cells.

### Results

Cytometric analysis of primary Kupffer cells displayed at least 90% purity of two distinctive subpopulations of CD68/CD163 cells. In addition, variable expression of CD14 was observed following culture for 24, 48, and 72hr after LPS treatment. During these time points, cytokine analysis in hepatocyte and Kupffer cell co-cultures for IL-6, IL-10, and TNFα was used to analyze the effect of LPS on hepatic metabolism and showed markedly down-regulated activity of various P450s (for example, CYP3A4 showed nearly 50 and 80% inhibition at 48 and 72hr after LPS treatment, respectively). Inhibition of metabolism correlated with IL-6 and IL-10 up-regulation. This response was blunted in co-cultures with LPS and TVX showing no changes to CYP3A4 activity and significantly lower production of IL-6; however, TNFα production was unaffected in co-cultures and markedly up-regulated in Kupffer cell monocultures. Additional ATP and LDH data correlated with observed necrotic cytotoxicity in hepatocytes after TVX treatment.

### Conclusion

Co-culture of hepatocytes and Kupffer cells can be used to predict DILI resulting from indirect adaptive immune reaction due to drug hepatotoxicity. As shown in this work, co-culture showed blunted IL-6 response and increased TNFα production that resulted in hepatic necrosis; an observed injury in patients afflicted with TVX induced hepatotoxicity. This data supports use of hepatocytes and Kupffer cells in co-cultures as a powerful in vitro ADMET/Tox tool to evaluate the effects of drug toxicity.

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