Comparative Analysis of DILI Compounds on Cytokine Production in Kupffer Cells and Hepatocytes

Rafal P. Witek, Jessica A. Bonzo, Stacy L. Jones, Lauren E. Sangenaro and Mukesh Kumar
Cell Biology, Life Technologies, Thermo Fisher Scientific, Frederick, MD 21704

ABSTRACT

Presence of inflammatory cytokines in the liver is often associated with indirect Drug Induced Liver Injury (DILI) that results in liver parenchymal cell deterioration. This idiosyncratic toxicity is often overlooked due to lack of validated in vitro cell models. Pressuring development of new models that would elevate this problem. One of the recent developments utilizes an organotypic approach of co-culture of human hepatocytes and Kupffer cells to establish a model of liver inflammation. In this model, the presence of inflammatory cytokines modulates metabolic function of co-cultured hepatocytes.

Although this model has been shown to predict Trovafloxacin toxicity that results from cytokine imbalance and accumulation of cytotoxic TNFα, to date there are no published studies examining the predictivity of the system with other drugs known for idiosyncratic toxicity.

For this reason, we performed comparative analysis of cytokine expression by Kupffer cells and co-cultures treated with panel of 20 different toxicants known for inducing cytokine imbalance following their administration. The following list of compounds were used in the analysis: (dose dependent hepatotoxicity) Acetaminophen, Berberine, Amiodarone; (dose independent hepatotoxicity) Fluconazole, Trovafloxacin, Phenacetin, Diclofenac, Quinine, Chlorzoxazone, Methotrexate, Chlorpromazine, Rizikome, Tamoxifen, Mexiteline HCL, Progestrone, Clomipramine, Norfluron, HCL, Halothane; (minimally or not toxic, controls) Levofloxacin, Bupropion HCL, Ramitidine, Preglitzazone, Colchicine, Dexamethasone, Buspirone. Cell cultures (hepatocytes, co-cultures and Kupffer cells) were treated with various concentrations of each of the compounds in presence or absence of LPS. Following 24hr incubation, media was collected and assayed for presence of cytokines using Cytokine 10- and 30-Plex Luminescent Panel.

Obtained data indicates cytokine imbalance affecting hepatic functions that follows administration of various test compounds (Trovafloxacin, Diclofenac, Clomipramine, others) thus results being previously published and observed in vivo. The data, as shown by our comparative analysis, supports use of human hepatocytes co-cultured with Kupffer cells to determine potential imbalance in cytokine production that could result in hepatotoxicity.

RESULTS

Figure 1. Co-culture of hepatocytes and Kupffer cells to recreate liver inflammation and Trovafloxacin (TXV) toxicity. A. Representative image of mononuclear of hepatocytes; B. Representative image of inflammatory co-culture; C. Graph of ATP levels in rat hepatocytes and co-cultures after 24 hours of treatment with LPS (1μg/ml) and/or TXV (0-200 μM) showing toxicity to cultures when treated with LPS and TXV of 50μM or higher; D-E. IL6 and TNFα cytokine response to LPS and TXV treatment. Note that LPS treatment results in increased levels of both cytokines (solid bar), whereas treatment with TXV results in reduced IL6 and induced TNFα (patterned bars); F. Graph of ATP levels in rat hepatocytes and co-cultures after 48 hours of treatment with LPS (1μg/ml) and/or TXV (0-200 μM). ATP levels began decreasing for both cultures at 50μM TXV and exhibited a much sharper dose-dependency drop in ATP levels for co-cultures alone, likely due to the changes shifts in cytokine profiles with increasing TXV dosage.

Figure 2. Comparative screen of Diclofenac, Chlorzoxazone, Amiodarone, Clomipramine, Norfluron and Tamoxifen using inflammatory co-culture of hepatocytes and Kupffer cells to determine levels of toxicities (ATP) as compared to hepatocyte monculture and Kupffer cells. All experiments use compounds at serial dilutions of 3.125μM, 6.25μM, 12.5μM, 25μM, 50μM and 100μM and 200μM. Note changes of EC50 between Co-cultures+LPS and Hepatocytes as those correspond to inflammatory model compared to current hepatic toxicity study workflows. Also, note that at concentrations of 25μM and higher, some toxicants had killing effect on Kupffer cells.

Figure 3. Comparison of IL6, IL10 and TNFα expression in inflammatory co-cultures, hepatocytes and Kupffer cells treated with Amiodarone, Clomipramine, Chlorzoxazone, Diclofenac, Norflurine and Tamoxifen. Only relevant concentrations of compounds and respective cytokine concentrations are shown. Similarly to our TXV data, Amiodarone, Norflurine and Tamoxifen induce TNFα toxicity, whereas Clomipramine, Chlorzoxazone and Diclofenac are independent of TNFα. All the above compounds (except Norflurine and Tamoxifen) induce IL10 response to magnify the effect of IL6 suggesting involvement of Kupffer cells in the natural defense mechanism against those compounds. Data shown here indicates cytokine imbalance plays a role in toxicity of those compounds and induce activity of cytokine-based defense mechanism characteristic to IL6/IL10 modulation of P450 and other liver enzymes. Note that non toxic Diclofenac and Chlorzoxazone display lack of a robust cytokine response. Y-axis: Concentration pg/mL.

CONCLUSIONS

Our data indicate that human hepatocytes co-cultured with Kupffer cells can be used to determine potential imbalance in cytokine production that could result in hepatotoxicity.

This further supports use of co-cultures of hepatocytes and Kupffer cells to predict DILI resulting from indirect adaptive immune reaction due to drug hepatotoxicity.

ACKNOWLEDGEMENTS

The authors would like to thank Tracee Crosett, Wendy Bray, Kristen Vaverek, Alex Vathey and Chelsey Fritz for research and program support.