Cytokine-Induced Liver Hepatotoxicity of Trovafloxacin In Co-Culture of Hepatocytes and Kupffer Cells

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Introduction

Immune-mediated chemical-induced hepatotoxicity, i.e., indirect hepatocellular toxicity resulting from immune cells activating liver inflammatory responses, is often overlooked as a potential mode of action due to unavailability of appropriate in vitro models. Kupffer cells are the largest population of resident macrophages in the liver and thus play a critical role in immune-mediated hepatotoxicity and liver injury. For this reason, we have established a co-culture system of rat primary hepatocytes and Kupffer cells that can be used to model chemical-induced immune reactions resulting in acute hepatotoxicity. Co-culture of hepatocytes and Kupffer cells may represent a powerful in vitro tool to predict adverse liver effects resulting from indirect adaptive immune reactions during chemical exposure.

Methods

Rat hepatocytes and Kupffer cells were obtained from the Hamner Institutes or Life Technologies. Hepatocytes and hepatic co-cultures were cultured in Advanced DMEM supplemented with Penicillin/Streptomycin, GlutaMax, and 15 mM HEPES. For plating media, an additional 10% FBS was used. Cells were purified for purity using the Attune Acoustic Focusing Cytometer. Kupffer cell monochromal preparations with CD68 and CD163 were performed utilizing standard IHC methodologies. Rat hepatocytes, Kupffer cells, and co-cultures were doped for 24-72 hr with 1 µg/ml E.coli OD1776 (10 µl). TNF and IL-10 were measured using Cell-Tracker Green and LDH levels were measured using cytotoxicity assay. Cytokines were assessed by ELISA or Luminox beads for IL-6 and TNF-α (Life Technologies).

Results

Cytometric analysis of primary Kupffer cells displayed at least 90% purity of two distinct subpopulations of CD68/CD163 cells. Twenty-four, 48, and 72 hr after LP/PS treatment, cytokine analysis of hepatocyte and Kupffer cell co-cultures for IL-6 and TNF-α was performed to analyze the effect of LPS on hepatic metabolism. Cytokine analysis showed down-regulated activity of CYP3A74% and 85% at 48 and 72 hr after LP/PS treatment, respectively. Inhibition of metabolism correlated with IL-6 up-regulation. This response was blunted in co-treatment with LP/PS and TVX showing a reversal of inhibition of CYP3A activity and significantly lower production of IL-6; however, TNF-α production was unaffected. A subsequent study utilizing LPS and increasing amounts of TVX exhibited the same pattern of reversal of inhibition of CYP3A activity and shifting IL-6/TNF-α ratios in a dose-dependent fashion. Activity inhibited by LPS was significantly correlated with observed necrotic cytotoxicity in hepatocytes after LP/PS treatment.

Conclusion

Co-culture of hepatocytes and Kupffer cells can be used to predict chemical-induced immune reactions that result in drug hepatotoxicity. As shown in this work, co-cultures showed blunted IL-6 response and increased TNF-α production that resulted in hepatic necrosis and concurrent increases in LDH and decreases in ATP levels indicating cytotoxicity. This data supports use of hepatocytes and Kupffer cells in co-cultures as a powerful in vitro tool to evaluate the effects of drug toxicity that are not apparent using in vitro models of monocultured hepatocytes.

References

Available upon request.

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