Regulation of P450 enzymes and Drug Transporter Expression in Human Hepatocytes Exposed to Cytokine Interleukin-6

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Introduction

Proinflammatory cytokines such as interleukin-6 (IL-6) are known to alter expression of drug metabolism enzymes and transporter in rodent liver. However, their effects on human hepatocytes remain poorly characterized. The current study was to conduct a comprehensive analysis of the effects of Il-6 on the expression of human major P450 enzyme and transporters in human hepatocytes. Our data show that upon exposure to IL-6, expression of both P450 enzymes and drug transporters is altered. In the same experiment, the alterations of P450 and transporters in hepatocytes over a period of 48 hrs were also characterized.

Experimental Approach

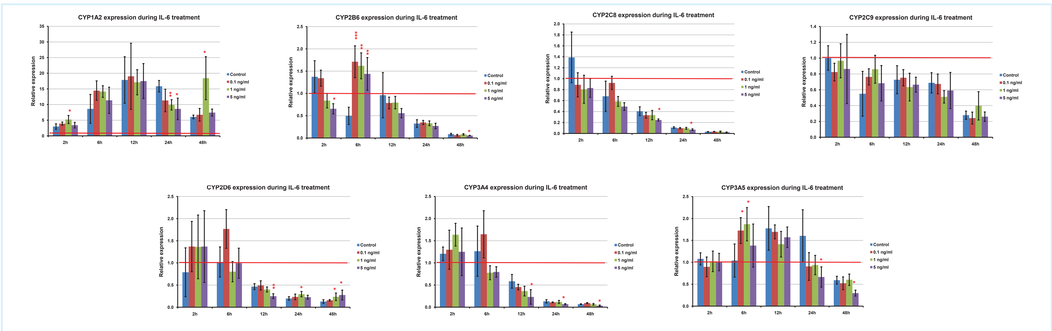
Primary human hepatocytes were treated with indicated dose of IL-6 in indicated time (from 4 hrs to 48 hrs) following by q-PCR quantification of mRNA of IL-6 target genes, P450 enzyme and transporters.

Materials and Methods

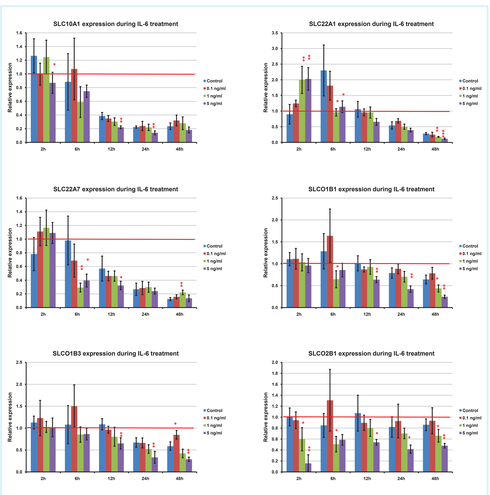
IL-6 were purchased from Sigma.

Cryopreserved human hepatocytes (Hu 4037, Advanced Pharmaceutical Sciences, Columbia, MD) were plated into a 24 well plate at a density of 0.7 × 106 cells/ml (500 μl = 350,000 cells/well) and allowed to attach for 4 hrs. Following attachment plating medium was removed and the cells were overlayed with 0.25 mg/ml Metrigel in plating medium and incubated for an addition 20 hrs. The plate was cultured in an incubator maintained with a humidified atmosphere of 95% air and 5% carbon dioxide. The various doses of IL-6 were diluted in PBS and added to medium. The cells without administration of IL-6 was used as control.

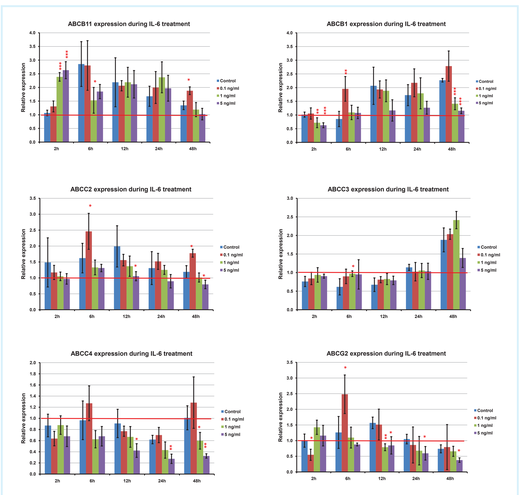
P450 ISOFORMS



UPTAKE TRANSPORTERS



EFFLUX TRANSPORTERS



Materials and Methods (cont'd.)

Total RNA was isolated individually from each well by RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. cDNA was synthesized from each sample by use of High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlslad, CA). Taqman primers were purchased from Applied Biosystems. Real-time reactions were carried out using the ABI 7500 Real-Time PCR System (Applied Biosystems). Each PCR cycle threshold (Ct) was normalized to the average Ct of the endogenous housekeeping control gene GAPDH. The comparative Δ Ct method was used to calculate relative quantification of gene expression. The student t-test was used for analysis for statistical significance. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Summary of Results

In the control hepatocytes, the expression of P450 (except CYP1A2) and uptake transporters was down-regulated with time. In contrast, CYP1A2 expression showed up-regulation. No significant changes on efflux transporters expression were observed.

IL-6 caused down regulation of the expression of P450 enzymes in hepatocytes (especially CYP3A4). In addition, treatment of IL-6 also causes down-regulation of most transporters.

Conclusion

The effects of inflammation especially different cytokines on drug metabolism and transporters in human hepatocytes have been characterized and evaluated in our laboratory.

Treatment of human hepatocytes with IL-6 significantly down-regulated the expression of most P450 enzymes and transporters. These alterations may have profound effects on drug-drug interaction in patients.