Effects of prototypical drug inducers on drug metabolizing enzyme and transporter gene expression in human hepatocytes

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Introduction

Drug-drug interactions can occur by induction or inhibition of not only drug metabolizing enzymes, but also the uptake and efflux transporters. We report here a comprehensive evaluation of the effects of prototypical drug inducers such as Rifampin (Rif), Phenobarbital (PB) and Omeprazole (Ome) on the gene expression of major P450 isoforms and transporters in primary human hepatocyte cultures.

Experimental approach

Human primary hepatocytes were treated with different dose of Rif, PB or Ome for two days followed by q-PCR quantification of mRNA for the following genes:

Major human liver P450 isoforms

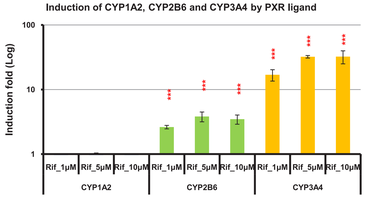
Major human liver P450 uptake transporters

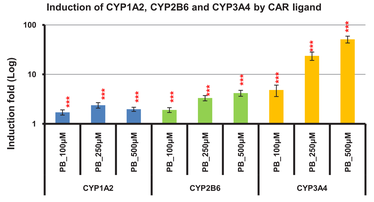
Major human liver P450 efflux transporters

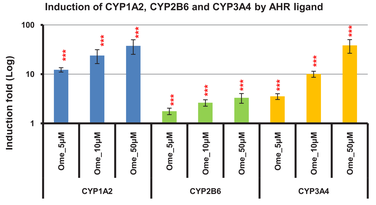
Materials and Methods

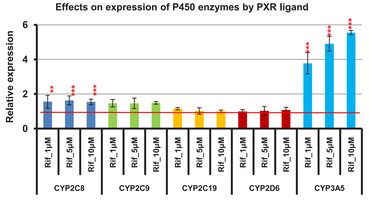
Chemicals
Rif, PB and Ome were purchased from Sigma.

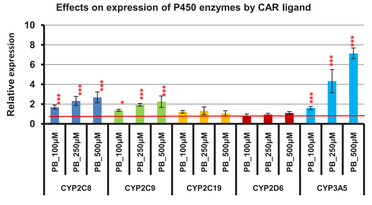
Human Primary Hepatocytes Culture
Cryopreserved human hepatocytes (HU4237, Advanced Pharmaceutical Sciences, Columbia, MD) were plated into a 24 well plate at a density of 0.7 × 106 cells/ml (500 µl = 350,000 cells/well) and allowed to attach for 4 hrs. Following attachment plating medium was removed and the cells were overlayed with 0.25 mg/ml Matrigel in plating medium and incubated for an addition 20 hrs. The plate was cultured in an incubator maintained with a humidified atmosphere of 95% oxygenand 5% carbon dioxide. The various dose of Rif, PB or Ome were dissolved in 0.1% DMSO. The cells without administration of drugs or 0.1% DMSO were used as control.

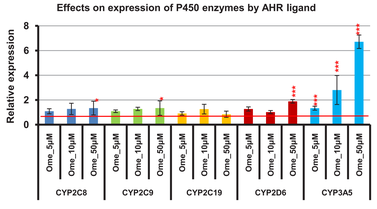


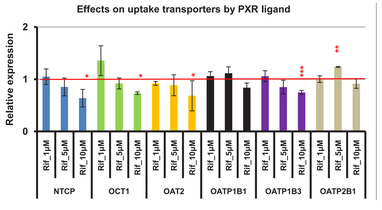


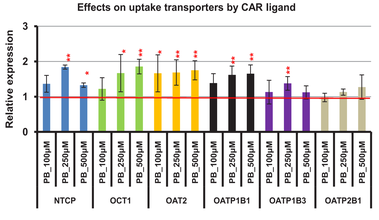


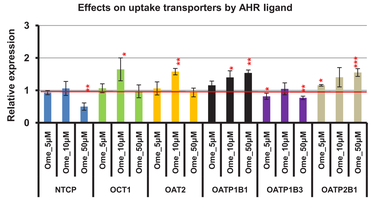


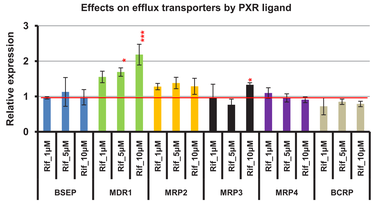


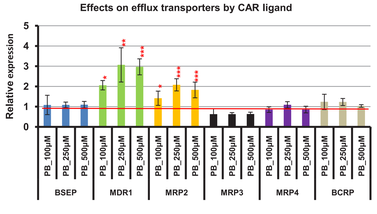


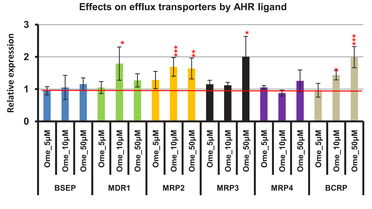












Quantitative real-time PCR
Total RNA was isolated individually from each well by RNeasy kit (Qiagen, Valencia, CA) following the manufacturer's protocol. cDNA was synthesized from each sample by use of High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlslad, CA). Taqman primers were 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 ΔCt 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

For P450 enzymes:
Rif: dose-dependent induction of CYP2B6, 3A4 and 3A5.
PB: dose-dependent induction of CYP1A2, 2B6, 2C8, 2C9, 3A4 and 3A5
Ome: dose-dependent induction of CYP1A2, 2B6, 2C8, 2C9, 3A4 and 3A5
For uptake transporters:
Rif: dose-dependent reduction of NTCP, OCT1, OAT2 and OATP1B3.
PB: dose-dependent induction of OCT1, OATP1B1 and OATP2B1
Ome: dose-dependent induction of OATP1B1, OATP2B1 and reduction of NTCP.
For uptake transporters:
Rif: dose-dependent induction of MDR1.
PB: dose-dependent induction of BSEP, MDR1, MRP2.
Ome: dose-dependent induction of BSEP, MRP2, MRP4 and BCRP

Conclusion

A qPCR-based assay has been developed in our laboratory for a comprehensive evaluation of the effects of drugs on gene expression of drug metabolizing enzymes and transporters.

Treatment of human primary hepatocytes with different drugs led to alterations of the gene expression of a variety of drug metabolizing enzymes and transporters. These alterations may have profound effects on drug-drug interaction in patients.