Comparison of Induction of CYP1A and CYP3A Gene Expression by Prototypical Drug Inducers in Primary Culture of Human and Monkey Hepatocytes

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Introduction

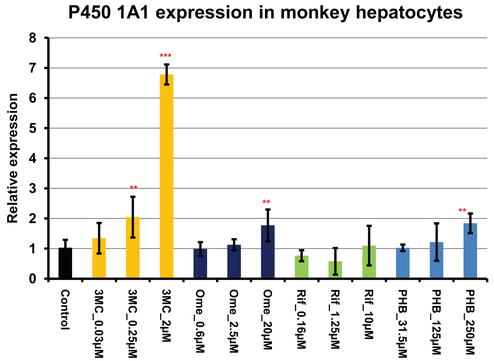
The cynomolgus monkey is an animal species widely used in biomedical research, including drug metabolism and toxicity studies. This study was conducted to compare the inducibility of monkey and human primary hepatocytes to response to prototypical drug inducers such as rifampicin (Rif), omeprazole (Ome), Phenobarbital (PB) and 3-Methylcholanthrene (3-MC) on the CYP1A and CYP3A gene expression. The results showed that compared to human, monkey hepatocytes have similar response to drug inducers on the induction of CYP1A and CYP3A.

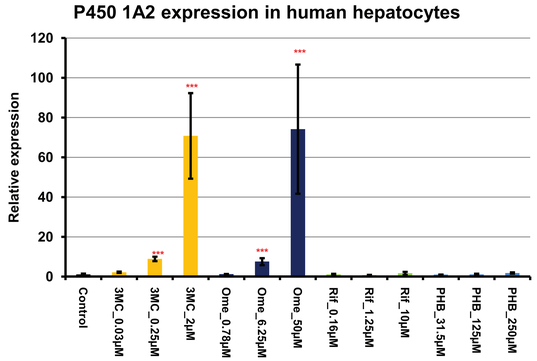
Experimental Approach

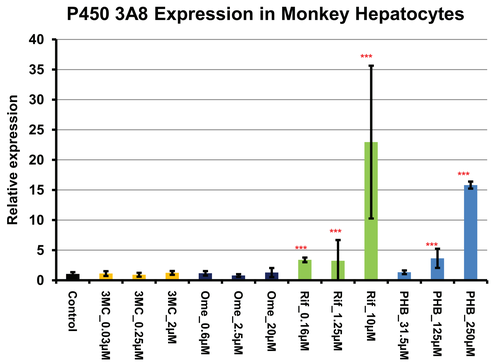
Pooled primary monkey hepatocytes (25 male and 25 female) or pooled primary human hepatocytes (5 male and 5 female) were treated with indicated dose of Rif, Ome, PB, or 3-MC for two days following by q-PCR quantification of mRNA of CYP1A1 and CYP3A8 for monkey, and CYP1A2 and CYP3A4 for human.

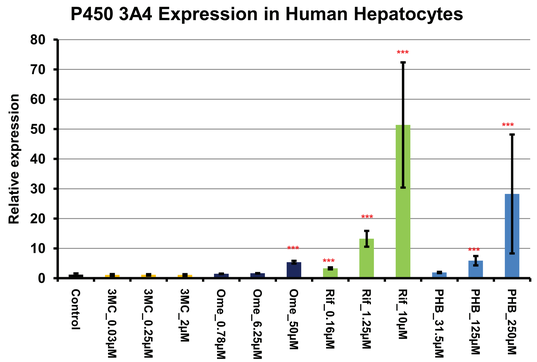
Materials and Methods

Rif, Ome, PB and 3-MC were purchased from Sigma. Cryopreserved monkey hepatocytes (Lot:10365013, Alive laboratory, Albuquerque, NM) or cryopreserved human hepatocytes (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 Rif, PB, Ome or 3-MC were dissolved in 0.1% DMSO. The cells without administration of drugs and only with 0.1% DMSO was used as control.









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

Treatment of Ome and 3-MC caused dose dependent induction of CYP1A1 in monkey and CYP1A2 in human hepatocytes. Induction fold of CYP1A2 in human hepatocytes is much higher than CYP1A1 in monkey hepatocytes. Treatment with Rif and PB caused dose dependent induction of CYP3A8 in monkey and CYP3A4 in monkey hepatocytes. Induction fold of CYP1A2 in human hepatocytes is slightly higher than CYP1A1 in monkey hepatocytes.

Conclusion

The experimental models to use cryopreserved monkey and human hepatocytes have been established in our laboratory for evaluation of drug metabolism and toxicity.

Treatment of monkey hepatocytes with different drug inducers shows similar induction on CYP1A and CYP3A as human hepatocytes although induction fold in monkey hepatocytes is smaller than in human hepatocytes.