Introduction

The cytochrome P450 (P450) enzymes have been shown to be responsible for metabolism of the majority of therapeutics and are therefore important for first pass elimination (bioavailability), clearance and drug-drug interactions. The drug-drug interactions involve inhibition and induction of P450s where enzyme induction can lead to decreased exposure to co-administered drugs, with potential loss of efficacy. Since induction is subjected to important species differences, human hepatocytes has so far been the gold standard model for induction studies. Well known problems with human hepatocytes is the scarce availability, interindividual variability and the need to evaluate multiple donors, which makes the model a non high-throughput model. Because of this, work is ongoing deriving cell lines expressing P450s that could be used for induction studies in early drug discovery.

HepaRG cells is a new human hepatoma cell line that resembles human hepatocytes to a high degree. HepaRG cells express P450s, conjugating enzymes, nuclear receptors and hepatic drug transporters and differentiate into a hepatocyte like morphology (Aninat et al., 2006; Le Vee et al., 2006). They have also been shown to respond to prototypical P450 inducers such as 3-methylcholantrene, rifampicin, and isoniazid (Aninat et al., 2006).

In this study we have investigated the effect of culture time on the mRNA expression of a set of 48 genes in the HepaRG cells. The time course of induction of well-known inducers has also been studied by analysis of mRNA for eight P450s and in addition enzyme activities for three P450s.

Expression over time of culture

We examined the gene expression in HepaRG cells cultured for 1-8 weeks. Eight of twelve P450s examined were well expressed during week 3 through 8 and the expression of CYP1A1 and 3A4 was considerably higher than for human liver. A relatively low expression was detected for the conjugating enzymes except glutathione S-transferase A1 (GSTA1). The liver membrane transporters were generally well expressed during week 3 through 6, except for OATP-A, which could not be detected in any sample but human liver. A relatively low expression of the nuclear receptors PXR and CAR were seen whereas the expression for FXR, RREs, RXRα and HNF4α was higher or comparable to human liver. Alpha-fetoprotein (AFP) was highly expressed, but the levels decreased over time in culture.

For induction studies HepaRG cells are cultured without DMSO in the medium for five days. The expression levels in these cells were similar or higher than levels in human liver for 32 genes of 39 genes analysed. The mRNA expression was also measured in HepG2 cells. The only P450s detected in HepG2 cells were low levels of CYP2C6, 3A7 and 1A1 and moderate levels of CYP1A1. Low levels were also detected for the conjugating enzymes. The transport proteins were not detected or expressed in low levels in HepG2 cells except for MRP2. The expression of AFP was extremely high in HepG2 cells, >10 000 times higher than in human liver.

References
