INTRODUCTION

The small/short heterodimer partner (SHP, NR0B2) is a nuclear receptor corepressor lacking a DNA binding domain. SHP is induced by bile acid-activated farnesoid X receptor (FXR) resulting in CYP7A1 gene suppression. In contrast, Pregnane X receptor (PXR) activation by its ligands was recently suggested to inhibit SHP gene transactivation to maximize the induction of PXR target genes. However, there are also conflicting reports in literature whether PXR or rodent Pxr activation down-regulates SHP/Shp expression. Moreover, the PXR-mediated regulation of the SHP gene has been studied only at the SHP mRNA and transactivation (gene reporter assay) levels. In this study, we studied the effect of rifampicin, a prototype PXR ligand, on SHP mRNA, and protein expression in three primary human hepatocyte cultures. We found that SHP mRNA is not systematically down-regulated in hepatocyte in culture after 24 h treatment with rifampicin. Consistently, we did not observe down-regulation of SHP protein in primary human hepatocytes after 24 and 48 h of incubation with rifampicin. We can conclude that although we observed slight down-regulation of SHP mRNA and protein in several hepatocyte preparations, the phenomenon is unlikely critical for PXR-mediated induction of its target genes.

Keywords: SHP, cytochrome P450, induction, PXR, CYP3A4
PXR (Ourlin et al., 2003). In addition, SHP blocks PXR interaction with HNF4α but does not affect PXR and PGC-1α interaction (Li and Chiang, 2006). In addition, SHP blocks PXR binding to CYP3A4 promoter DNA (Ourlin et al., 2003; Li and Chiang, 2006). In a positive feedback loop, PXR was supposed to inhibit SHP gene transactivation and SHP mRNA expression to maximize the PXR induction of the CYP3A4 gene in human hepatocytes (Li and Chiang, 2006).

The aim of this study was to examine in detail the effect of rifampicin on SHP expression in PXR-mediated transactivation of the main drug-metabolizing enzyme, CYP3A4, in primary human hepatocytes. In particular, we examined the effect of a prototype PXR ligand rifampicin on SHP protein expression in several primary human hepatocyte preparations.

MATERIALS AND METHODS

CELL LINES AND PRIMARY HUMAN HEPATOCYTES

The human MZ-Hep1 hepatocarcinoma cell line (kindly donated by Dr. Ramiro Jover, Hospital La Fe, Valencia, Spain) was maintained in antibiotic-free DMEM supplemented with 10% Fetal bovine serum (FBS) and 1 mM sodium pyruvate. FBS was purchased from PAA (Pasching, Austria). Other chemicals and cell culture media were purchased from Sigma-Aldrich (St. Louis, MO, USA). The final concentration of DMSO in the culture media was 0.1% (v/v) in all experiments.

Long-term human hepatocytes in monolayer were purchased from Biopredic International, Rennes, France, and were maintained according to the protocols provided by Biopredic. The medium was exchanged for serum-free medium the day after delivery, and the culture was allowed to stabilize for an additional 6–24 h prior to treatments.

LH28 and LH29 primary human hepatocyte preparations were isolated, cultivated, and treated as described in our previous papers (Bachleda et al., 2009; Dvorak et al., 2010). Our tissue preparation protocol was designed in accordance with the requirements issued by local ethical commissions in the Czech Republic. In addition, we used three commercial preparations of Long-term human hepatocytes in monolayer: Batch HEP220466 (75-year-old female suffering from hepatocellular carcinoma), Batch HEP220465 (63-year-old male with liver metastases), and Batch HEP220492 (66-year-old female with hepatic metastases; Biopredic International, Rennes, France).

DNA CONSTRUCTS

A chimeric p3A4–luc reporter construct containing the basal promoter (−362/+53) and the distal xenobiotic responsive enhancer module (−7836/−7208) of the CYP3A4 gene 5’-flanking region was described elsewhere (Cerveny et al., 2007). The expression plasmids for PXR and SHP receptors, pSG5–hPXR, and pSG5–hSHP, were kindly provided by Dr. S. Kliewer (University of Texas, TX, USA). pRL–TK was purchased from Promega and the empty pSG5 vector was purchased from Stratagene.

TRANSIENT TRANSFECTION AND LUCIFERASE GENE REPORTER ASSAYS

All transient transfection assays were carried out in MZ-Hep1 cells, as described previously (Pavek et al., 2010).

QUANTITATIVE REAL-TIME RT-PCR

Total RNA isolation and quantitative real-time RT-PCR (qRT-PCR) analyses of CYP3A4 (hCYP3A4_Q2) and SHP (hNR0B2_Q2) mRNA expression in primary human hepatocytes were performed by employing commercial assays from Gene- Biotech (Hradec Kralove, Czech Republic) as described elsewhere (Pavek et al., 2007, 2010; Svecova et al., 2008). HPRT (hHPRT_Q3) housekeeping gene expression levels were used as normalization controls. Experiments were performed in triplicates (i.e., three individual cell samples) and each cDNA sample has been analyzed as triplicate in one RT-PCR run.

IMMUNOBLOTTING

The relative abundance of each specific protein in 25–50 μg of whole cell lysate was determined by Western blot analysis, as described previously (Pospechova et al., 2009). Anti-SHP (H-160: sc-30169; dilution 1:250), anti-human CYP3A4 (HL3: sc53850; 1:2000), and goat polyclonal anti-actin antibodies (clone I-19: 1616; a loading control) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, USA). Densitometric analyses have been performed to semi-quantify the expression of the tested proteins.

STATISTICAL ANALYSES

All data are expressed as the mean ± SD. Differences between the groups were compared with Student’s paired two-tailed t-test. Kruskal–Wallis test with Dunn’s Multiple Comparison post hoc test and One-way analysis of variance (ANOVA) with Tukey’s Multiple Comparison test were used to analyze data in Figure 3. All statistical analyses were performed using GraphPad Prism 4 Software. p Value <0.05 was considered statistically significant.

RESULTS

RIFAMPICIN DOES NOT SYSTEMATICALLY SUPPRESS SHP mRNA GENE EXPRESSION IN PRIMARY HUMAN HEPATOCYTES

First, we examined whether rifampicin, a prototype PXR ligand, affects the expression of SHP mRNA in primary human hepatocytes. We found that rifampicin (10 μM) had no statistically significant effect on SHP mRNA expression on three primary human hepatocyte cultures (analyzed with Student’s paired t-test, Figure 1A), although in individual preparations we observed the effect of rifampicin on SHP mRNA expression. At the same time, we evaluated the expression of the main PXR target gene, CYP3A4. We found that rifampicin (10 μM) significantly (p < 0.01) induced expression of CYP3A4 mRNA after 24 h in the same primary human hepatocyte preparations (analyzed with Student’s paired t-test, Figure 1B). The effect of rifampicin on CYP3A4 mRNA expression reflects high inter-individual variation in response to PXR inducers in various hepatocyte preparations and in human population.

RIFAMPICIN DOES NOT SYSTEMATICALLY AFFECT SHP PROTEIN EXPRESSION IN PRIMARY HUMAN HEPATOCYTES

In next experiments, we examined the effect of rifampicin (10 μM) on SHP protein expression in hepatocytes treated with rifampicin employing Western blotting analysis. Rifampicin did not significantly decrease SHP protein expression after 24 or 48 h treatment in either human hepatocyte preparation (Figures 2A,B).
THE EFFECTS OF SHP OVEREXPRESSION ON CYP3A4 TRANSACTIVATION IN GENE REPORTER ASSAYS

In next transient transfection gene reporter experiments with p3A4–luc construct, we examined whether SHP suppresses CYP3A4 in gene reporter assays in hepatocellular carcinoma cell line MZ-Hep1 cells. The MZ-Hep1 cell line was confirmed to express SHP protein (Figure 2B). Consistently with reported data in other liver-derived cell lines (Ourlin et al., 2003; Li and Chiang, 2006), we observed that overexpression of SHP significantly abolished rifampicin-mediated activation of the CYP3A4 construct (p < 0.01, ANOVA) with Tukey’s Multiple Comparison test; Figure 3).

DISCUSSION

Recently, Li and Chiang proposed an elegant model of PXR-mediated transactivation of CYP3A4 in human hepatocytes (Li and Chiang, 2006). This model supposes that activated PXR trans-represses SHP expression and since SHP competes with HNF4α and SRC1 coactivators for binding to PXR, this feedback loop maximizes PXR-mediated induction of the CYP3A4 gene.
However, earlier reports did not find any statistically significant effects of PXR and rodent ortholog Pxr activation, either by specific ligands or using engineered animal models, on SHP or Shp mRNA expression (Ourlin et al., 2003; Rosenfeld et al., 2003; Hartley et al., 2004; Guzelian et al., 2006; Bailey et al., 2011) or on SHP binding to PXR target genes promoters employing chromatin immunoprecipitation (ChIP; Hariparsad et al., 2009). In primary human hepatocytes, Ourlin et al. (2003) first reported no effect of rifampicin on SHP mRNA expression.

In our experiments, we did not observe significant effect of rifampicin on SHP expression, although slight decrease was observed in some experiments. Notably, we observed hepatocyte preparation-dependent effect of rifampicin on SHP mRNA expression (Figure 1A). These data might indicate inter-individual effect of PXR activation on SHP expression in hepatocytes. We can also consider some unspecified dietary, physiological, or environmental aspects of inter-individual response to PXR activation in terms of SHP expression.

Indeed, in population, CYP3A4 displays high inter-individual variability in expression and catalytic activity in the liver. To date, however, no single nucleotide polymorphisms of the CYP3A4 gene coding region or its promoter region have been found as the direct cause of the variability (Martinez-Jimenez et al., 2007). It is therefore supposed that transcription activity of nuclear receptors including SHP might be the cause of CYP3A4 expression variability in the liver and in the intestine.

We conclude that the physiological role of SHP in regulation of CYP3A4 induction needs to be further studied in a larger population of human hepatocyte preparations. In addition, our results again confirm that SHP is an important negative regulator of CYP3A4 transactivation that may contribute to inter-individual variability in CYP3A4 hepatic expression.

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