Effect of a novel synthetic FXR agonist EYP001 on hepatitis B virus replication in HepaRG and primary human hepatocytes

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Introduction

Treatment of hepatitis B relies on pegylated interferon (pegIFN) and/or on nucleos(t)ide analogs. Only a minority of patients respond to pegIFN with HBsAg seroconversion. Polyamine inhibitors suppress viral replication at the level of a life-long treatment. Indeed such therapies poorly modify the size of the cccDNA reservoir and treatment interruption is followed by viral replication re-emergence with often-deleterious consequences. There is thus an unmet need for new therapy aiming at controlling the expression of the cccDNA through a diminution of the HBV infection.

Materials and Methods

Cells and virus

The HepaRG line derived from a human liver carcinoma cell line obtained from several phenotypic traits of hepatocytes after 4 weeks of culture under defined conditions. After differentiation, these cells are sensitive to inhibition at high concentrations produced by Enyo Pharma (500 μM) under defined conditions. Three differentiated conditions can be observed in the second week post-infection.

Primary human liver hepatocytes (PHLHs) were obtained from Human HepaCell (Paris, France). Upon arrival, cells were plated at a density of 5 × 10^4 cells/cm² in a 6-well plate at 37°C. In Williams medium supplemented with 2% MC355 (Biochir) and 0.1% sodium bicarbonate. At 48 h post-infection, cells were harvested for analysis. EYP001 was purchased from Enyo Pharma (Lyon, France).

Chemicals

The non-sterol, non-covalent reference FXR agonist GW4064 was purchased from Sigma-Aldrich. The liver specific derivative of 6-ethylchenodeoxycholic acid (6-ECDCA) was provided by Enyo Pharma, Lyon, France.

Results

1- Effect of EYP001 on GBP1A and FXR activity

Treatment of HepaRG cells with EYP001 resulted in a dose-dependent increase in the expression of FXR mRNA and a decrease in the expression of GBP1A mRNA. In HepaRG cells, treatment with EYP001 led to a dose-dependent decrease in the expression of GBP1A mRNA and an increase in the expression of FXR mRNA.

2- EYP001 represses HBV replication in dhepaRG

EYP001 represses the secretion of HBs and HBV antigen as well as of viral DNA in a dose-dependent manner after 10 days of treatment.

3- Effects of EYP001 on FXR and HBV replication

EYP001 represses the secretion of viral proteins and cccDNA synthesis.

Conclusions

We showed that EYP001 does not activate GBP1A, the plasma membrane bile acid receptor but instead activates the transcription of genes under the control of FXR. HBV infection reduces the FXR-dependent gene profile and EYP001, as well as two other FXR agonists, can reverse some of the HBV-induced modification of gene expression. EYP001 induces a sustained repression of HBV replication in the HepaRG and PHLH cell culture systems. The decreased expression of the viral mRNA suggests that the inhibition of the viral replication mostly results from the modulation of FXR activation that could perturb the complex FXR network of transcription factors, which is highly targeted and controlled by HBV. These data stress out the importance to exploit drug modulation of metabolic pathways in controlling HBV replication.

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