The non-clinical profile of FXR agonist EYP001 as a potential therapy of chronic hepatitis B infection.


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INTRODUCTION

Chronic hepatitis B (CHB) is one of the principal causes of the global liver disease burden. Several HBV viral and host targets are being investigated for potential therapies aiming for better tolerance and to avoid the need for a lifelong medication. HBV infection modifies bile acid metabolism and impacts the human nuclear farnesoid X bile acid receptor (FXR) pathway. FXR agonists target hepatocytes and have recently been shown to be active in vitro against HBV (Radreau 2016).

AIM

To present in vitro and in vivo data profiling the synthetic FXR agonist EYP001 supporting its clinical evaluation.

MATERIAL & METHODS

EYP001 was tested in differentiated HepaRG cells (HepaRG) and in primary human hepatocytes (PHH), both supporting the complete HBV replication cycle. Cells were infected with an HBV inoculum prepared from stably transfected HepG2.2.15 cell line (100 genome-equivalent per cell in culture medium).

Viral mRNA, DNA, and proteins, expression of host cell FXR and FXR-regulated genes SHP, APOA1, NTCP, CYP7A1, and CYP8B1 were assayed with standard qPCR techniques and commercial kits.

Beagle dogs and Wistar Hannover rats were gavaged daily up to 4 weeks with increasing doses of EYP001 or vehicle. Animals were checked daily for clinical signs and food consumption.

Toxicology Results: EYP001 was not genotoxic across in vitro assays, nor inducing nervous, cardio-vascular or respiratory system toxicity in appropriate models. Single- and repeat-dose studies in rats and dogs orally administered up to 28 days and during 4 weeks at 10, 40, 120 and 300 mg/kg/day showed no lethal effect. No test item-related clinical signs were observed in any group, except for repeated vomiting at 300 mg/kg/day in dogs. No clinically relevant adverse biochemical or pathology findings were identified. As known from other FXR agonists, lipid profile changes were seen during the 4 weeks exposure: triglycerides increased 57% only in female rats at the highest tested dose of 120 mg/kg/day. A slight dose related increase in total and HDL cholesterol levels was observed in rats at 40 and 120 mg/kg/day and similarly a slight increase in total, HDL and LDL cholesterol was seen in dogs at 120 mg/kg/day.

RESULTS

HBsAg and HBeAg from HBV infected, then treated cells from day 2 to 12, decreased with increasing EYP 001 concentrations, either as an acid form (A) or as a salt form (ethanolamine (B)). HBsAg and HBeAg from cells treated during the first 24h of HBV infection are shown in (C). Data are mean ± SEM of at least three independent experiments (p values: * p<0.01 and * p<0.05 vs. baseline).

EYP001 inhibited in a dose dependent manner the amount of HBV DNA secreted by dHepaRG. Values show day 3 values of a 10 day treatment course with two distinct formulations: acid EYP001 (A) and ethanolamine salt (B). IC50 were in the 1 μM and 4 μM range (acid, salt resp.). Similarly the relative expression of the pre-g and pre-core mRNA (C, D) decreased, as did the level of the intracellular rcDNA (E) and cccDNA (F). A mini-chromosome reduction of about 70% was established based on the cellular cccDNA pool on day 12 (F). Data are mean ± SEM of at least three independent experiments (p value : *p<0.001 and *p<0.01 vs. baseline).

Discussion: EYP001 repressed HBsAg and HBeAg with IC50 in the 1 μM range and reduced HBV RNA and HBV DNA with IC50 in the 1 μM range. Pre-g and pre-core HBV mRNA, intracellular rcDNA were repressed and a reduction up to 70% of quantitative cccDNA was observed. Similar results were obtained in PHH (data not shown). EYP001 and HBV infection had opposite effects on genes under the negative regulation of FXR, in particular SHP and CYP7A1. HBV induced a small increase of FXR mRNA expression and no effect on FXR modulated genes. EYP001 increased expression of SHP and repressed FXR itself and the genes under its negative control.

REFERENCES


Radreau P. et al. Effect of a novel synthetic FXR agonist EYP001 on hepatitis B virus replication in HepaRG cell line and primary human hepatocytes. AASLD LiverResearch®, Nov 16, (2015); 110896


DISCLOSURES

All authors are employees or consultants of Enyo Pharma SA. Patrice André and Jacky Vonderscher are also Enyo Pharma SA founders.

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CONCLUSION

Synthetic specific FXR agonist EYP001 repressed the replication of HBV in two full viral cycle hepatocyte models.

The anti-viral mechanism is probably located at a post-entry level with an impact on the viral transcriptional activity of cccDNA.

DISCUSSION

No effect was observed on NTCP and CYP8B1. We showed previously (Radreau 2015), that EYP001 does not activate GpBAR1 and thus appears as a specific FXR agonist. Overall EYP001 interfered with HBV replication at post-entry steps likely impacting transcriptional activity of cccDNA.