Bile acids receptor FXR agonists repress HBV replication in HepaRG cell

Pauline Radreau1,2, Marine Porcherot1,2, Christophe Ramière1,2,3, Vincent Lotteau1,2 and Patrice André1,2,3

1 CIRI INSERM U1111, Lyon, France 2 CIRI Université Claude Bernard Lyon1, Lyon, France 3 Virology laboratory, Hospices Civils de Lyon, Lyon, France

Introduction

Treatment of hepatitis B relies on Pegylated interferon or on polymerase inhibitors. Only a minority of patients respond to Peg-IFN with SVR outcome. Polymerase inhibitors suppress viral replication at the cost of a life-long treatment. Indeed such therapies poorly modify the size of the cccDNA reservoir and treatment interruption is followed by viral replication reactivation with often deleterious consequences. There is thus an unmet need for new therapy aiming at controlling the expression and persistence of the cccDNA pool.

cccDNA forms a circular double stranded DNA minichromosome that associates viral proteins HBS and HBC as well as cellular factors including histones, histone modifiers and non-coding RNAs and co-factors or repressors. These factors control both the persistence and expression of the minichromosomes. Among the liver restricted nuclear factors we showed that at least two response elements for the hepatic X receptor (the bile acid nuclear receptor FXR) are induced in vivo and in vitro at the core promoter region (HepaRG) (1). Expression of these FXR target genes (2) is strongly correlated with the repression of the viral RNA expression upon activation by bile acid or FXR agonists. Moreover FXR and SIRT1, two key metabolic factors, form a network of proteins regulating the transcriptional activity of the cccDNA in an FXR dependent manner (3). These bile acids and HBC could for the scaffold histone corepressor corresponding polycomb (4), expressed at the hepatitis B virus-linear plasmid (5), repressed FXR binding to FXR-LP limits its function, inducing a down-regulation of FXR activity with subsequent modifications of the expression of FXR regulated genes including its own over-expression (6). HBV infection is thus characterized by important modifications of the bile acids metabolism. This intimate link between bile acids metabolism and HBV replication suggests that manipulating this pathway might be exploited as a therapeutic approach.

We thus tested the effects of various FXR ligands on HBV replication in differentiated HepaRG cells.

Material and Methods

Cells and virus

The HepaRG cell line derived from a human hepatic carcinoma can differentiate and regain many phenotypic traits of hepatocytes after 4 weeks of culture under defined conditions (2). After differentiation, these cells are susceptible to infection at high MOI of HBV virions produced by HepG2.2.15 line. Under these conditions viral production could be observed in the second week of post-infection.

Chemicals

The reference antagonist 052EDL133 described in patent WO 2007052843; Takeda Chemicals and the selective FXR agonist 6ECDCA were purchased from Sigma-Aldrich. The bile acid derivative UDCA, a non FXR-agonist, was produced by HepG2.2.15 line. Under these conditions viral expression while UDCA and antagonists do not.

Results

1- FXR directly targets FXR and its network

PDC-E2 and SIRT1 effects on the transcriptional activity of HBV Envelope promoter region depend on FXR activation. HBs directly binds to Sirt1 and modulate its activity (7). We then showed that Hbs also binds directly to FXR by a co-immunoprecipitation assay (Fig 1). Our results strongly suggest an interaction between FXR and Hbs.

2- FXR agonists repress HBV replication in HepaRG

The effects of the agonists EDCDDA and GW4064 and of the antagonist 052EDL133 on the FXR ligands mimicked by native bile acid UDCA was tested on a complete viral replication cycle in HepaRG (Fig 2).

In addition to the viral proteins, cells were treated with cyclosporine A (CyA) either during HBV infection in a pre-treatment schedule or 3 days post infection (Fig 3). CyA treatment during HBV infection inhibits viral entry in a dose-dependent manner and does not induce the decrease in HBcAg and HBcAg expression following treatment with FXR agonists. CyA treatment post-infection has no effect on HBV/adenovirus infection whatever the presence or not of FXR agonists. These data indicate that action of FXR agonists on HBV replication occur at post-entry step.

Conclusions

Treatment with Si-deriv 6-Ethyl-cholestadienolic acid (6-EDCDDA) or synthetic non-steroidal 6ECDCA agonists, but not with antagonists or unsaturated bile acids, strongly inhibited the secretion of HBV DNA, HBsAg, HBcAg and of HBVAg in a dose-dependent manner (Fig 9) (8). The inhibition was similar for both agonists suggesting that the effect was not dependent on entry but occurred at a post-entry step. Treatment consistently increased FXR activity as indicated by the increase of the direct heterodimer pathway (DHP) and decreases of the aminopropion-1A mRNA expression. Treatment of FXR agonists increases mainly the effect of HBV inhibition on the bile acid metabolism at the cellular level. We hypothesize that FXR binds to the cccDNA in an in transected state under the control of PGC-1α and Sirt1. Mitigates para 8 and controls the level of FXR expression leading to a global inhibition of the virus replication.

References

5. Kadowaki T, Kadowaki Y, Sano Y, Kuroki Y, Yamanaka T, Nakahara K, et al. FXR activation by bile acids represses the pool of cellular HBV DNA. Cyclosporine A, an NTCP ligand and HBV entry inhibitor, did not affect HBV DNA expression. Treatment consistently increased FXR activity as indicated by the increase of the direct heterodimer pathway (DHP) and decreases of the aminopropion-1A mRNA expression. Treatment of FXR agonists increases mainly the effect of HBV inhibition on the bile acid metabolism at the cellular level. We hypothesize that FXR binds to the cccDNA in an in transected state under the control of PGC-1α and Sirt1. Mitigates para 8 and controls the level of FXR expression leading to a global inhibition of the virus replication.

Acknowledgments and Contact

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