Farnesoid X receptor alpha ligands inhibit *in vitro* HDV replication and virion secretion and infectivity

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Background & Aims

Hepatitis delta virus (HDV) is a satellite of hepatitis B virus (HBV), both using the Sodium Taurocholate Co-Transporting Polypeptide (NTCP), the main transporter of bile acids (BA) in the liver, to enter hepatocytes. Links between BA and HBV infection are not limited to the entry step as we previously showed that some ligands of the farnesoid X receptor alpha (FXR), the nuclear receptor of BA, acted as inhibitors of HBV replication (Radreau *et al.*, FASEB, 2016; Mouzannar *et al.*, FASEB, 2019). Regarding HDV, excepting the role of NTCP in viral entry, putative links between BA metabolism and HDV replication have not been yet explored. We thus wanted to determine whether FXR also played a role in HDV life cycle.

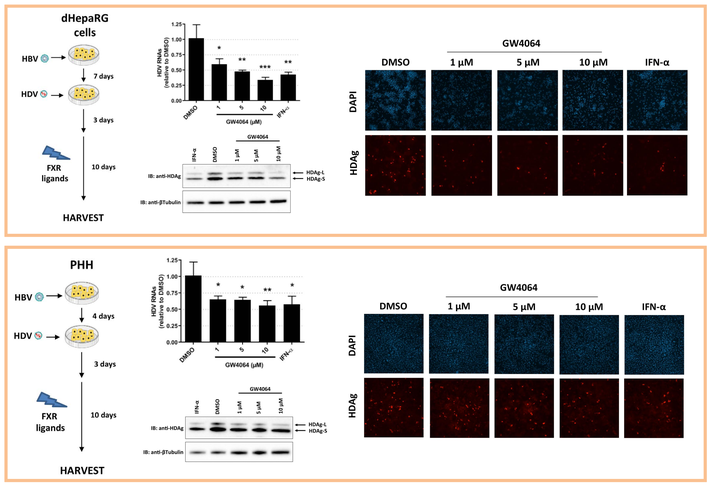
Materials & Methods

In vitro HDV mono-infection or HDV/HBV co-infection and super-infection were performed in differentiated HepaRG cells and primary human hepatocytes (PHH). Cells were treated with several FXR ligands: 6-ECDCA, a BA analog and two synthetic ligands, GW4064 and tropifexor. The impact on distinct forms of HDV RNAs was analysed by quantitative PCR and Northern Blot. Analysis of HDV proteins was performed by immunofluorescence (IF) and Western Blot (WB). Viral secretion was studied by HDV RNA quantification in supernatants and infection of naive Huh7.5^{-NTCP} cells.

Results (1)

FXR ligands inhibit HDV replication in HBV/HDV superinfected dHepaRG cells and PHH.

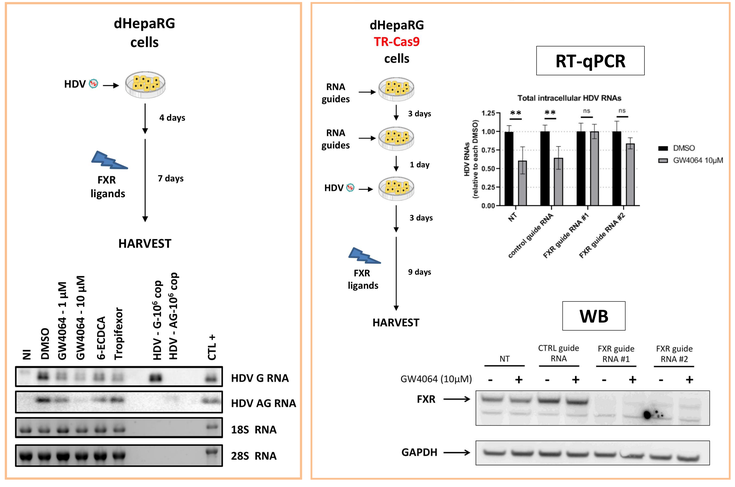
A 10-day treatment with 10µM of FXR ligand GW4064 decreased intracellular HDV RNAs by 60% and 40% in HepaRG cells (upper panel) and PHH (lower panel). IF staining and WB showed that FXR ligands also decreased intracellular amounts of delta antigens.



Results (2)

FXR ligands inhibit HDV replication in monoinfected dHepaRG cells.

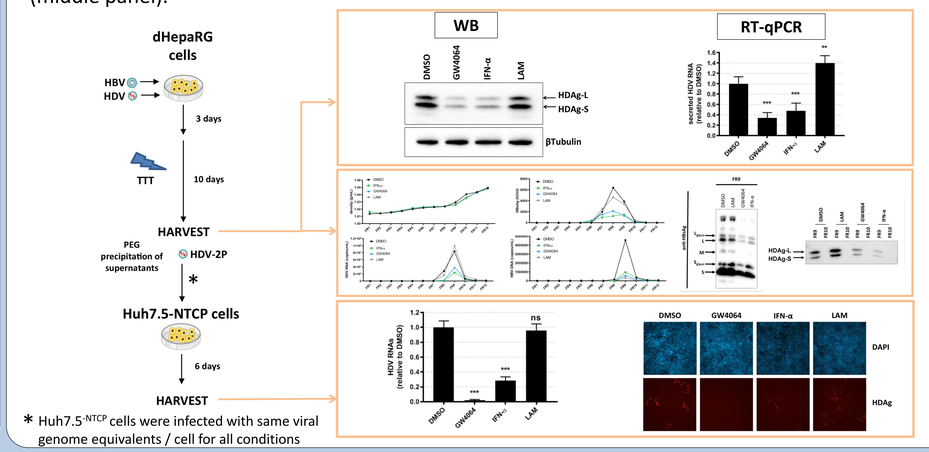
Treatment with different FXR ligands decreased the amount of both genomic and antigenomic HDV RNAs in dHepaRG cells in Northern Blot analysis (left panel).
FXR silencing using a dHepaRG cell line expressing an inducible TR-Cas9 abolished the antiviral effect of FXR ligand GW4064 (right panel).



Results (3)

FXR ligands decrease both secretion and specific infectivity of HDV virions from HBV/HDV coinfected dHepaRG cells.

In HBV/HDV coinfected dHepaRG cells, treatment with FXR ligand GW4064 decreased intracellular HDAg amounts and secretion of HDV RNA in supernatants by 60% (upper panel).
Following infection of naive Huh7.5^{-NTCP} cells, the amount of HDV RNAs was reduced by > 95% using viruses produced in GW4064-treated dHepaRG cells; the number of HDAg-positive cells was reduced in a similar manner in IF analysis (lower panel).
Density gradient analysis did not reveal any obvious modification of HDV virions characteristics (middle panel).



* Huh7.5^{-NTCP} cells were infected with same viral genome equivalents / cell for all conditions

Conclusion

FXR ligands inhibit *in vitro* HDV replication, independently of their previously identified antiviral properties against HBV. The major impact of treatment was observed on virion secretion and specific infectivity, suggesting that FXR ligands may be able to inhibit HDV spreading. Mechanisms underlying this inhibitory effect are under investigation.
Several FXR ligands are either approved or in clinical trials for several liver diseases. As current therapeutic strategies are limited for HDV-infected patients, FXR may thus represent a new and attractive target for HDV antiviral therapy.