CRV431 Blocks NTCP-Mediated Uptake of HBV and HDV Independently of Effects on Bile Acid Transport

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BACKGROUND

Hepatitis D virus (HDV) co-presents with hepatitis B virus (HBV) infection because HDV requires HBsAg for the formation of enveloped HDV virions and entry into hepatocytes. Virion entry occurs by binding to sodium taurocholate co-transporting polypeptide (NTCP). Blocking NTCP-mediated uptake of HBV and HDV is a novel antiviral strategy being developed for these viruses. Cyclosporine analogs and preS1-HBsAg peptide mimics are two classes of pharmacological agents that bind to NTCP and block NTCP-mediated HBV and HDV uptake. However, many of these compounds also block the primary physiological function of NTCP, bile acid transport, especially uptake by hepatocytes. For example, Myrcludex B was found to elevate plasma levels of conjugated bile acids 100-fold in patients (see references). Inhibiting virus uptake without blocking bile acid transport would be preferred.

CRV431

- CRV431 is a novel, non-immunosuppressive cyclophilin inhibitor with sub-micromolar anti-HBV activity., presumably the result of blocking the interaction of host cyclophilins and the HBV life cycle at multiple steps. CRV431 suppresses HBV DNA, HBsAg and HBeAg and has been demonstrated to block the binding of cyclophilin A to HBsAg and HBx. Its mechanisms of actions are being elucidated. Previous studies showed that CRV431 blocks NTCP-mediated HBV uptake with an IC50 ≈ 0.5 µM.

PURPOSE

The purpose of this study was to investigate whether the cyclosporine analog, CRV431, inhibits HDV and HBV infection *in vitro* and to evaluate whether CRV431 inhibited NTCP-mediated bile salt transport.

METHODS

MODEL#1: HBV and HDV co-infection of stably transfected NTCP-HepG2 cells

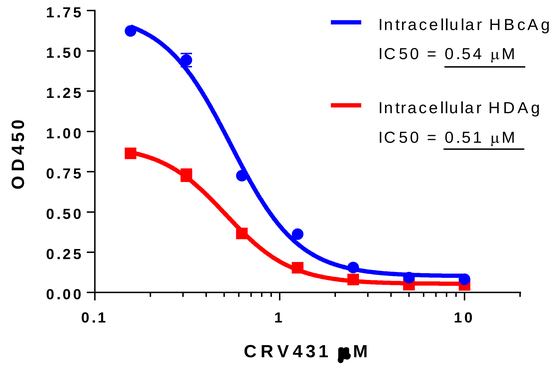
MODEL #2: [^{3}H]Taurocholate (bile acid) uptake by stably transfected NTCP-HEK293 cells (performed by Sekisui XenoTech, LLC)

HBV + HDV CO-INFECTION

- **CRV431 inhibited production of HBV core antigen and HDAg with similar IC50 values (≈ 0.5 µM)**
- **These findings are consistent with CRV431 inhibiting HBV and HDV uptake via NTCP.**

MODEL: HBV and HDV co-infection of stably transfected NTCP-HepG2 cells

- HBV virions were produced from HepAD38 cells. HDV virions were produced by co-transfection of Huh-7 cells with one plasmid containing 1.03 overlength HDV and one containing truncated HBV expressing HBsAg.
- HBV and HDV virions were administered together to NTCP-HepG2 cells for 4 h ± CRV431 (0.2 – 10 µM). Virus inoculum was then removed, and CRV431 treatments re-applied.
- After 6 days of culture, cells were harvested and assayed for intracellular HBV core antigen (HBV) and HDAg (HDV) by ELISAs.

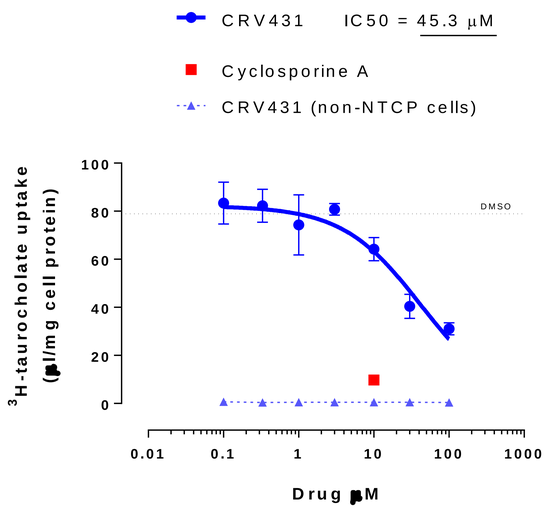


BILE SALT TRANSPORT

- **CRV431 inhibited taurocholate uptake with an IC50 = 45.3 µM, which represented significantly lower inhibitory potency than cyclosporine A.**

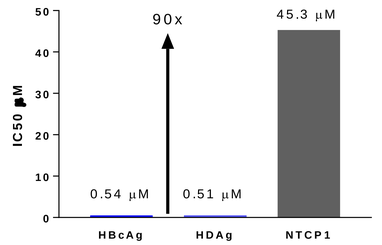
MODEL: [^{3}H]Taurocholate uptake by stably transfected NTCP1-HEK293 cells

- DMSO (0.2%), cyclosporine A (10 µM), or CRV431 (0.1 – 100 µM) were applied to NTCP1-HEK293 cells or non-transfected cells for 15 min (pre-incubation).
- After pre-incubation, the treatments were re-applied but in the presence of the radiolabeled bile salt [^{3}H]taurocholate for 2 min.
- After 2 min incubation, cells were rinsed and harvested for scintillation counting of [^{3}H]taurocholate taken up by the cells.



Selective Index

- **NTCP inhibition IC50 = 90x higher than HBcAg and HDAg IC50s**



CONCLUSIONS

- CRV431 blocked infection of HBV and HDV with similar potencies, consistent with an inhibitory effect on NTCP-mediated virion uptake.
- A 90-fold selective index (functional separation) exists between inhibition of bile salt uptake and virion uptake, suggesting that efficacious CRV431 dosing in patients should not increase bile acid concentrations in plasma.
- Together with previous data showing additional, independent anti-HBV effects of CRV431, these findings support the therapeutic potential of CRV431 for both hepatitis B and D.

References

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