HEP DART 2017

frontiers in drug development for viral hepatitis

#22

CRV431: Multiple Therapeutic Actions *In Vitro* and *In Vivo*

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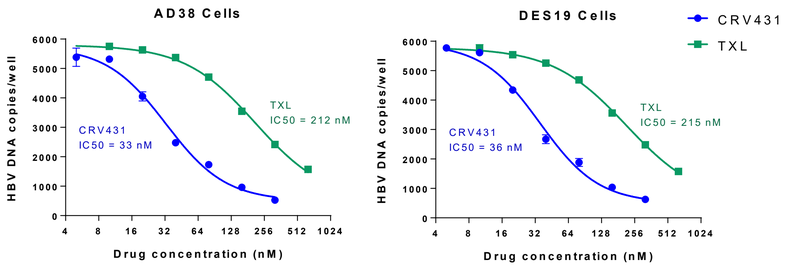
INTRODUCTION

CRV431 is a novel non-immunosuppressive analog of cyclosporine A (CsA) targeting cyclophilins. Cyclophilins are cellular host proteins that participate in the HBV life cycle, and their inhibition has been proposed as a treatment for chronic HBV. The molecular mechanism(s) of action of cyclophilin inhibitors in HBV infection are not well defined. Herein, we describe actions of CRV431 both in vitro and in vivo aimed at elucidating possible mechanisms of action.

CRV431 Reduces HBV DNA, HBsAg, and HBeAg in Cellular Models

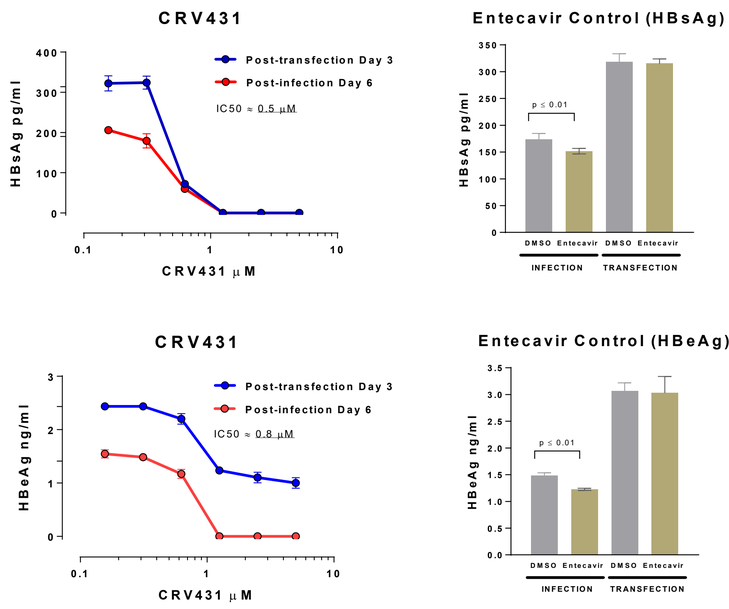
HBV-integrated cell lines: Reduction in HBV DNA

MODEL: AD38 and DES19 cell lines were induced (tetracycline removed) and treated with CRV431 or the tenofovir pro-drug, TXL, for 6 days

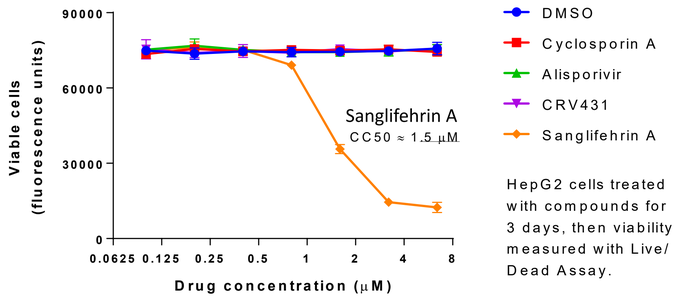


HBV infection and transfection: Reductions in HBsAg + HBeAg

MODEL: CRV431 or entecavir was applied for up to 6 days to NTCP-Huh7 cells infected with HBV or Huh7 cells transfected with HBV plasmid



CRV431 Has a High Selective Index

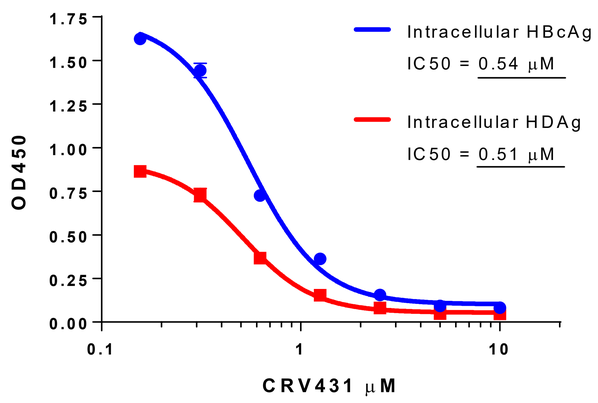


HepG2 cells treated with compounds for 3 days, then viability measured with Live/Dead Assay.

CRV431 Inhibits NTCP in HBV + HDV Co-infection

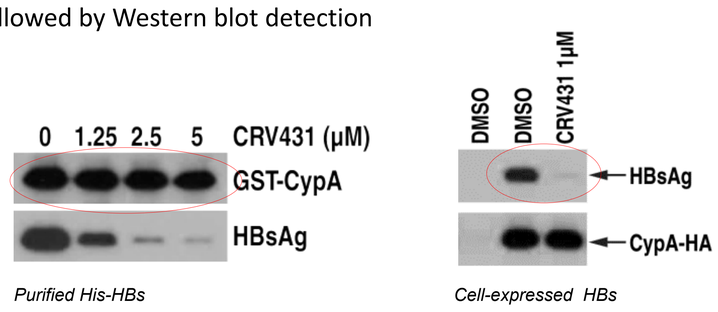
CRV431 inhibited HBV and HDV coinfection *in vitro*, consistent with blocking NTCP-mediated virus uptake

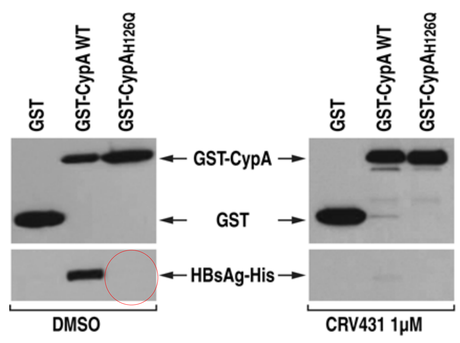
MODEL: HBV and HDV co-infection of stably transfected NTCP-HepG2 cells. CRV431 applied during 4-hr virus inoculation and for 6 days post-inoculation



CRV431 Blocks HBsAg-Cyclophilin Binding

Pull-Down Assays: Tagged wild-type or isomerase-dead (H126Q) cyclophilin A (CypA) was used to capture purified His-HBsAg or native HBsAg in lysates from HBV plasmid-transfected cells, followed by Western blot detection

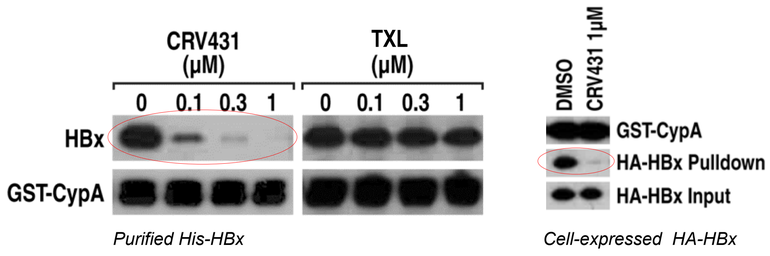


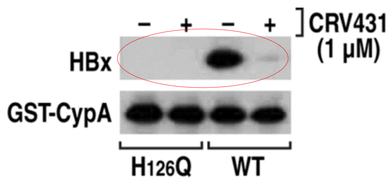


No HBsAg pull-down with Cyp A mutated in the isomerase active site

CRV431 Blocks HBx-Cyclophilin Binding

Pull-Down Assays: GST-CypA was used to capture purified His-HBx or HA-HBx in lysates from HA-HBx plasmid-transfected cells, followed by Western blot detection.

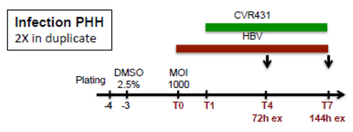


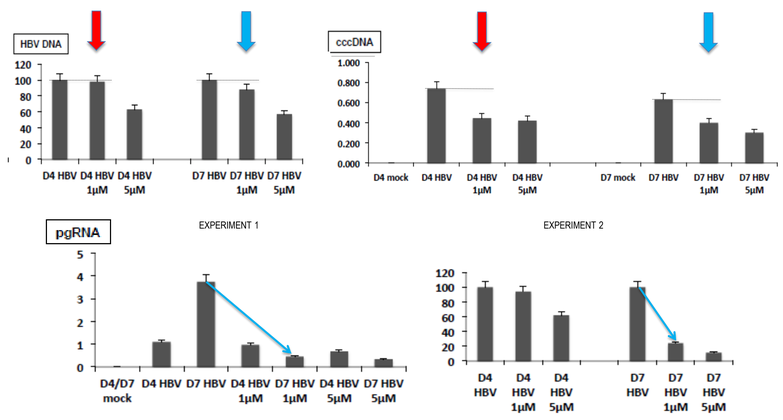


No HBx pull-down with Cyp A mutated in the isomerase active site

CRV431 Reduces cccDNA and pgRNA in Primary Human Hepatocytes

MODEL: PHH treated with CRV431 starting 1 day after the start of HBV infection. Assay HBV DNA, cccDNA, and pgRNA at Days 4 and 7.



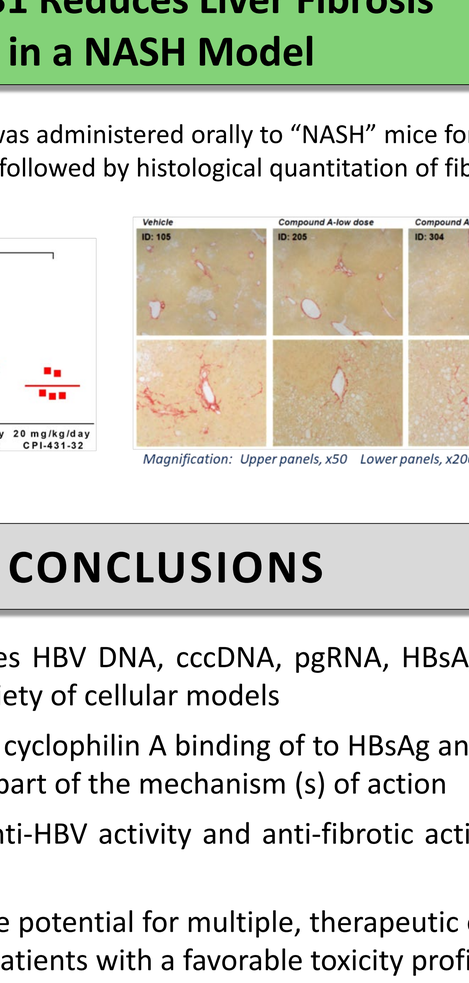


CRV431 Reduces HBV DNA and HBsAg in HBV Transgenic Mice

MODEL: CRV431 and/or the tenofovir pro-drug, TXL, were administered orally to HBV transgenic mice for 16 days, followed by measurement of HBV in livers.

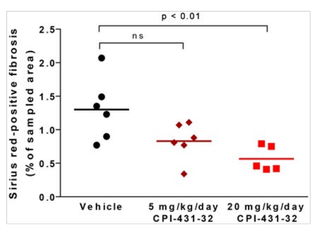
Liver HBV DNA

Serum HBsAg



CRV431 Reduces Liver Fibrosis in a NASH Model

MODEL: CRV431 was administered orally to "NASH" mice for 21 days (Weeks 6-9), followed by histological quantitation of fibrosis.



Magnification: Upper panels, x50 Lower panels, x200

CONCLUSIONS

- ✓ CRV431 reduces HBV DNA, cccDNA, pgRNA, HBsAg, and HBeAg in a variety of cellular models
- ✓ CRV431 blocks cyclophilin A binding of to HBsAg and HBx, which may be part of the mechanism (s) of action
- ✓ CRV431 has anti-HBV activity and anti-fibrotic activity in mouse models
- ✓ CRV431 has the potential for multiple, therapeutic effects in hepatitis B patients with a favorable toxicity profile