The cyclophilin inhibitor CRV431 prevents both HBx-cyclophilin complex formation and HBV replication

R. FOSTER[1], D. URE[1], D. TREPANIER[1], P. GALLAY[2]

1 Contravir Pharmaceuticals Inc, Edison, New Jersey, USA and Edmonton, Alberta, Canada
2 The Scripps Research Institute, La Jolla, California, USA

EASL The Home of Hepatology
THE INTERNATIONAL LIVER CONGRESS™ 2017
19-23 APRIL, AMSTERDAM, THE NETHERLANDS

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INTRODUCTION

CRV431 is an anti-HBV drug candidate whose principle action is inhibition of cellular cyclophilins. Cyclophilins are peptidyl-prolyl isomerases that broadly regulate protein structure and function, and they are recruited into the life cycles of several viruses, including HBV, HCV, HIV-1, HPV, and coronaviruses. Previous investigations have highlighted cyclophilin inhibition as a potential anti-HBV strategy, but the mechanism(s) of action is largely unknown. Some studies suggest that one candidate mechanism is interference of the actions of the HBV protein, HBx, However, a molecular interaction of cyclophilins and HBx has not yet been explored.

AIM

The aim of this study is to determine whether CRV431 can demonstrate anti-HBV activity investigating the interaction between cyclophilin and HBx.

METHOD

Cyclophilin A binding to HBx was studied using purified, recombinant GST-human cyclophilin A and His-tagged HBx in pulldown and ELISA assays.

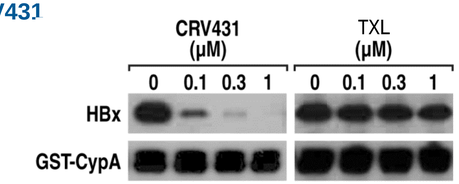
Cyclophilin A-HBx binding specificity was tested by

- site-directed mutagenesis of cyclophilin A isomerase active site (H126Q)
- pharmacological disruption of putative cyclophilin A-HBx interaction with CRV431

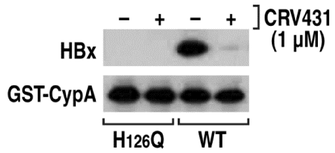
In addition we explored the role of cyclophilin A in HBV replication in AD38 cells using cyclophilin A knockdown with siRNA and using the cyclophilin inhibitor CRV431.

RESULTS

Recombinant HBx binds cyclophilin A at the isomerase active site, and binding is inhibited by CRV431

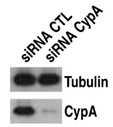


A. Recombinant GST-CypA (500 ng) was mixed with recombinant HBx-His (200 ng) together with DMSO, the cyclophilin inhibitor CRV431 or the polymerase inhibitor tenofovir exalidex (TXL) for 3 h at 4ºC. Glutathione beads were added to the GST-CypA/HBx mixture for 30 min at 4ºC and washed. Bound material was eluted and analyzed by Western blotting using anti-His and anti-GST antibodies.

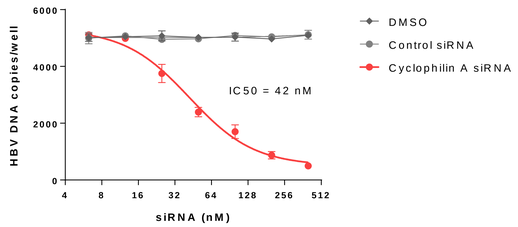


B. Methods as in Part A, except in one instance HBx pull-down was performed with mutant cyclophilin A , deficient in isomerase activity due to histidine-126 conversion to glutamine in the enzyme active site.

Cyclophilin A knockdown reduces HBV replication in HepAD38 cells



CypA siRNA treatment for 24 h significantly lowers Cyp A protein levels in HepAD38 cells, as shown by Western blot.



DMSO, control siRNA, or CypA siRNA were added to HepAD38 cells (triplicate) 24 h after cell plating. After 24 h, cells were treated with increasing concentrations of indicated drugs. After 3 days, medium was removed and replaced with fresh medium and fresh drugs. After 3 days (6 days of drug exposure), intracellular HBV DNA was purified and quantified by quantitative PCR (qPCR).

Recombinant HBx binding to cyclophilin A is inhibited by CRV431

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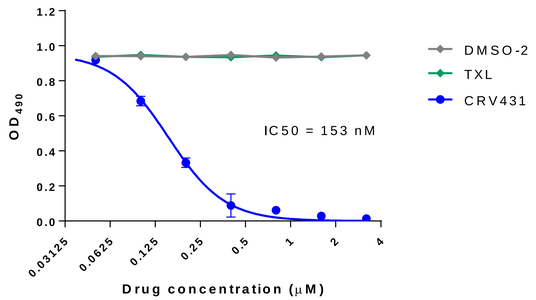

Plates were coated with GST-CypA (10 μg/mL) for 16 h at 4ºC. Recombinant HBx-His (0.1 μg/mL) was added to wells together with DMSO, CRV431, or the HBV polymerase inhibitor, TXL for 16 h at 4ºC. Captured HBx-His was detected using mouse anti-His IgG (1 μg/mL) followed by anti-mouse-HRP IgG (1:1000 dilution). After substrate addition, the reaction was stopped and wells read on a plate reader at OD 490nm.

CRV431 treatment reduces HBV replication in HepAD38 cells

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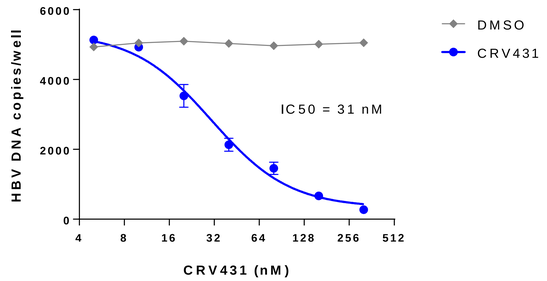

HepAD38 cells (triplicate wells) were incubated for two weeks with tetracycline to suppress HBV replication. Tetracycline was then removed and cells treated with increasing concentrations of indicated drugs. After 3 days, medium was removed and replaced with fresh medium and fresh drugs. After 3 days (6 days of drug exposure), intracellular HBV DNA was purified and quantified by quantitative PCR (qPCR).

CONCLUSIONS

HBx binds to cyclophilin A *in vitro*.

Dependence of binding on an intact cyclophilin isomerase active site suggests that cyclophilin A might enzymatically regulate HBx structure and function.

A role for cyclophilin A in the HBV life cycle is further suggested by siRNA and CRV431 inhibition of HBV replication in HepAD38 cells.

Future studies will investigate the site on HBx that binds to cyclophilin A and the effects on the HBV life cycle of blocking HBx-cyclophilin interaction.

ACKNOWLEDGEMENTS

We greatly thank Dr. Christoph Seeger for HepAD38 cells.

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CONTACT INFORMATION

Dr. Robert Foster
Chief Scientific Officer, Contravir Pharmaceuticals Inc.
399 Thornall Street, 1st Floor, Edison, New Jersey 08837

rfoster@contravir.com
780-909-5041