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

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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 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).

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 (TFV) for 30 min at 4°C 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 Cyc A protein levels in HepAD38 cells, as shown by Western blot.

CRV431 treatment reduces HBV replication in HepAD38 cells

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). IC50 = 42 nM

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REFERENCES

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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.

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