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Hepatitis B and D Virus Entry-Inhibitory Activity of Cyclosporin A

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Chronic infection with Hepatitis B - the leading cause of severe progressive liver diseases like cirrhosis and hepatocellular carcinoma - is a major health problem with more than 240 million people chronically infected worldwide. New therapies addressing chronic infection with HBV and HDV are needed. The human Hepatitis B virus (HBV) is a small enveloped hepatotropic DNA virus which causes acute and chronic infection preferentially in hepatocytes. HDV takes advantage of the HBV envelope for viral assembly, release and entry steps. A functional receptor for HBV and HDV entry is the bile salt transporter hNTCP, which is inhibited by CsA. CsA is a strong immunosuppressive drug, which binds to cyclophilin, thereby inhibiting calcineurin and Ca^{2+}-mediated signaling pathways. In the HBV life cycle, after release, the viral nucleocapsid is transported into the host nucleus. By "repair" of viral rcDNA, cccDNA is formed. cccDNA constantly stays in the nucleus to serve as a template for viral replication, thereby preserving chronic infection.
The main part of this work deals with the effect of CsA on HBV and HDV on hNTCP-mediated infection. A second part addresses cccDNA quantification, especially in resting and proliferating host cells. Infection systems comprised PHH, the authentic system of differentiated HepaRG cells and hepatoma cell lines that gained HBV and HDV susceptibility by hNTCP overexpression (HuH7-hNTCP, HepG2-hNTCP, HepaRG-hNTCP).
This work started with demonstrating that physiological substrates of hNTCP - a recently identified functional receptor for HBV and HDV entry - impair HBV and HDV infection at high concentrations. Moreover, they reduce MyrB binding to host cells. CsA is a well established drug, and like other cholestasis-inducing drugs, it is known to inhibit NTCP transport. Most importantly for this work, CsA was identified to block HBV and HDV entry through hNTCP. CsA strongly reduced HBV infection when applied before or during, but not after viral inoculation. The inhibition of infection was dose-dependent and dependent on the hNTCP expression level of the respective infection system. HBV-transfected cells were hardly affected by CsA treatment, revealing that the main effect is based on early infection steps. High concentrations of CsA blocked the interaction between MyrB and hNTCP. Direct binding to hNTCP was demonstrated; this interaction, in return, was competed by MyrB. The mutant hNTCP-KG157-158GR, which is deficient in MyrB binding, was resistant to CsA. This implied identical, or at least overlapping hNTCP binding sites of the two substances. Down-regulation of cyclophilin A, B and C did not reduce the effect of CsA. Structural CsA analogues (Debio025, NIM811) revealed effects on hNTCP that were very similar to those of CsA. In accordance, tacrolimus, a calcineurin inhibitor not related to CsA, hardly inhibited TC uptake and HBV infection.
CsA has been approved for decades with various clinical indications. Besides ist immunosuppressive activity, CsA inhibits HCV infection by binding to multiple cyclophilins which are crucial for HCV replication. CsA also has been reported to indirectly interfere with HBV X protein transactivation through Ca^{2+}-mediated signaling pathways. However, this work revealed that the main effect of CsA inhibiting HBV infection is based on entry inhibition. Taken together, CsA inhibits HBV and HDV entry into host cells by directly binding to hNTCP and thereby impairing transport. The effect is independent from cyclophilin and calcineurin inhibition, which is the classical immunosuppressive pathway of CsA. CsA directly binds to hNTCP, dependent on a site (amino acids 157/158), which is

essential for MyrB binding. hNTCP was much more sensitive to MyrB and CsA with regard to HBV infection inhibition than it was with regard to TC uptake inhibition - indicating a large therapeutic window for entry inhibition without induction of cholestasis. The concentrations of CsA used in this work are higher than those used in patients. The finding that CsA blocks HBV and HDV entry, and the mechanism behind it, can contribute as a basis for the development of further drugs.

In the second part of this work, a method for quantifying cccDNA was established by using a TaqMan polymerase-based qPCR assay. Because cccDNA is formed by "repair" of rcDNA, both molecules share the same size as well as the same nucleotide sequence. Several measures were taken to specifically quantify cccDNA, but not rcDNA, and to confirm specificity of the assay.

Currently, there are no therapies addressing HBV cccDNA, the key molecule maintaining an established infection in the host cell. Elimination of cccDNA can cure a cell from chronic infection. Previous work revealed that after seeding HBV-infected cells in low density, viral markers were strongly reduced. It was hypothesized that during mitosis, when the nuclear envelope disassembles, the cccDNA is mislocated from the nucleus. Because it cannot be reimported into the nucleus, it is then degraded. Indeed, splitting of infected hepatoma cell lines - thereby inducing proliferation - was shown to drastically decrease not only secreted viral markers and HBcAg, but also cccDNA levels (while HDV infection was not reduced by the very same treatment). The factor by which cccDNA was decreased was much higher than the factor by which the cells were diluted - indicating that cccDNA got lost. This way of diminishing cccDNA should be further followed up. It is a promising approach for future therapies, especially in combination with viral entry inhibitors.