Despite the effective antivirals and vaccines, chronic hepatitis B virus (HBV) infection remains a major public health problem worldwide [1,2]. Due to limited access, late or absent detection and vertical transmission, the prevalence of HBV infection is estimated to be 250–350 million infected individuals [1,3]. HBV infection is a leading cause of hepatocellular carcinoma worldwide. While current therapies, based on pegylated-interferon and nucleos(t)ide analogs effectively decrease viral load, viral elimination is rare. Thus, there is an unmet medical need for novel therapeutic strategies effectively curing HBV infection [1,2,4]. HBV entry inhibitors have been introduced as a novel class of antivirals for viral control and cure [1,5–7]. The discovery of the Na$^+$-taurocholate cotransporting polypeptide (NTCP) as the first receptor of HBV opened the door to understand the molecular mechanisms of viral cell entry and to identify entry inhibitors (reviewed in [8]). In the liver, HBV virions bind to NTCP, a bile acid transporter expressed at the basolateral membrane of hepatocytes [9], triggering viral entry. The preS1 region of HBV envelope interacts with the bile acid pocket site of the transporter, corresponding to the amino acids 157 to 165 of the protein [8], therefore interfering with its transporter function. At the same time NTCP has been explored in great detail as a target for antiviral therapy (reviewed in [5]). Indeed, small molecules interacting with NTCP exhibit antiviral activity, including the immunosuppressive drug cyclosporin A (CsA) [5]. Moreover, a preS1 peptide derived from the HBV envelope and specifically binding to the receptor has been shown to exhibit marked antiviral activity in cell culture, animal models and patients (for reviews, see [5,6]). However, as HBV and bile acids share the same interaction site with NTCP, the current HBV NTCP entry inhibitors are also capable of interfering with bile acid uptake. This may induce putative adverse effects by impairing Na$^+$-taurocholate transport in hepatocytes. Indeed NTCP deficiency in patients leads to hypercholanemia for instance [10] and an increase in glycine-conjugated bile acid concentrations was also observed in some patients treated with myrcludex B, a
preS1 peptide binding to NTCP and currently evaluated in a clinical trial [11]. Although bile acid related adverse effects appeared to be limited or absent [11], the discovery of HBV entry inhibitors not affecting the NTCP transporter function would represent an interesting conceptual advancement addressing a potential safety concern.

In this issue of Journal of Hepatology, Shimura and colleagues [19] addressed this issue, by investigating the ability of CsA derivatives to inhibit HBV infection without interfering with bile acid transport. To do so, they took advantage of an HBV infection model based on NTCP-overexpressing HepG2 cells, named HepG2-hNTCP-C4, which enables HBV infection [12]. The authors synthesized eleven CsA derivatives and characterized their inhibitory activity on virus infection. Observing that five of these drugs were able to inhibit HBV infection in a pan-genotypic manner, they demonstrated that four of them (named SCY806, SCY446, SCY450, and SCY995) have no immunosuppressive activity and were capable of suppressing HBV infection in primary human hepatocytes (PHH). To further characterize the antiviral activity of these compounds, they then tested their inhibitory effect at different stages of viral infection. Using non-susceptible HBV replicating cells, they demonstrated that CsA derivatives had no effect on HBV replication. In contrast, taking advantage of a fluorescent-labeled preS1 peptide, which specifically binds to NTCP [13], the authors showed that these drugs were able to prevent preS1-NTCP interaction, confirming the capacity of CsA derivatives to inhibit HBV entry by interfering with the binding of the virus to its receptor. They validated these findings using surface plasmon resonance analysis, showing that CsA derivatives were capable of interacting with recombinant NTCP protein, such as CsA itself. As CsA is known to target NTCP-mediated bile acid uptake as well, the authors set up a bile acid uptake assay based on HepG2-hNTCP-C4 cells to evaluate the putative effect of CsA derivatives on NTCP transporter function. Interestingly, while SCY806 and SCY446 do inhibit bile acid uptake similarly to CsA, the two other derivatives, SCY450 and SCY995, had no effect on NTCP-mediated bile acid transport in spite of a strong HBV inhibition, suggesting that the bile acid transporter and HBV receptor functions of NTCP can potentially be dissociated. This hypothesis was further confirmed by sulfobromophthalein, a substrate of NTCP interacting with the bile acid pocket site of the protein [14], which was shown to inhibit bile acid uptake without affecting HBV infection. Taken together, the authors conclude that it is possible to develop NTCP-targeting HBV entry inhibitors without affecting the bile acid transporter function of NTCP.

These results have implications for the understanding of HBV entry as well as the development of HBV entry inhibitors as antivirals. First, the results obtained by Shimiura et al. suggest that NTCP domains mediating HBV entry and bile acid transport may not be completely identical. The newly identified compounds could serve as a tool to further elucidate virus-NTCP interactions during HBV cell entry. Furthermore, their findings could advance the further development of HBV entry inhibitors: while host-targeting agents (HTAs) have been shown to be effective against HBV, HDV and HCV infections [7,15,16], target-specific adverse effects need to be carefully addressed [17]. Although it has been suggested that auxiliary transporters may be able to sustain the enterohepatic cycle in the absence of NTCP [10], it cannot be excluded that pharmacological long-term modulation of bile acid transport may result in previously undiscovered adverse effects. Shimura and colleagues addressed this issue by the identification of CsA derivatives which neither
appeared to exhibit immunosuppressive activity, nor interfere with NTCP-mediated bile acid transport. Mechanistically, their study suggests that the SCY995 molecule may target an alternative site of NTCP (putatively overlapping with the bile acid pocket site) compared to native CsA, which may allow to inhibit viral entry without blocking bile acid uptake (Fig. 1). However, it is important to note while no effect was observed in the range of concentrations used in the study, it cannot be excluded that SCY995 interferes with NTCP bile acid transporter function at higher concentrations. Furthermore it would be of interest to investigate whether SCY995 interferes with the recently described antiviral innate immune responses mediated by the NTCP bile acid transporter function [18]. Collectively, the findings of Shimura et al. advance our knowledge by showing: (i) that it appears to be possible to dissect HBV entry and bile acid transporter function; and (ii) providing an opportunity to optimize entry inhibitors by potentially increasing their specificity and decreasing potential adverse effects. However, it would be important to assess whether the compounds exhibit other off-target effects such as interference with other transporters (e.g. the hepatic organic anion transporting polypeptide OATP). Detailed studies of efficacy and safety in animal models and ultimately in the HBV infected patients are needed to evaluate the positioning of the approach introduced by Shimura and colleagues compared to the entry inhibitor myrcludex B [11], other HTAs (for review see [7]) and clinically licensed HBV antivirals.

Acknowledgments

This work was supported by Inserm, the University of Strasbourg, the European Union (InfectERA hepBccc, ERC-2014-AdG-671231-HEPCIR, INTERREG-IV-Rhin Supérieur-FEDER-Hepato-Regio-Net, FP7 HepaMab, EU H2020 Hep-CAR), ANRS (2015/1099), the French Cancer Agency (ARC I 201301187) and the US National Institutes of Health (U19AI123862-01). This work has been published under the framework of the LABEX ANR-10-LAB-28 and benefits from a funding from the state managed by the French National Research Agency as part of the Investments for the future program.

References


Fig. 1. Model of interaction of selected HBV entry inhibitors and Na\textsuperscript{+}-taurocholate cotransporting polypeptide (NTCP) at the hepatocyte basolateral membrane

According to Shimura and colleagues, CSY995, a CsA derivative exhibiting no immunosuppressive activity, contrary to CsA, appears to bind to NTCP via an alternative site (putatively overlapping with the bile acid pocket site of the protein), allowing bile acid transport in spite of a strong anti-HBV activity. Myrcludex B (MyrB) is a myristoylated peptide derived from the HBV envelope specifically binding to NTCP, inhibiting both HBV entry and bile acid transport. CsA, cyclosporine A; HBV, hepatitis B virus.