Drug Design, Development and Therapy | Dovepress

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# The Culprit Behind HBV-Infected Hepatocytes: NTCP

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**Abstract:** Hepatitis B virus (HBV) is a globally prevalent human DNA virus responsible for over 250 million cases of chronic liver infections, leading to conditions such as liver inflammation, cirrhosis and hepatocellular carcinoma (HCC). Sodium taurocholate co-transporting polypeptide (NTCP) is a transmembrane protein highly expressed in human hepatocytes and functions as a bile acid (BA) transporter. NTCP has been identified as the receptor that HBV and its satellite virus, hepatitis delta virus (HDV), use to enter hepatocytes. HBV entry into hepatocytes is tightly regulated by various signaling pathways, and NTCP plays an important role as the initial stage of HBV infection. NTCP acts as an initiation signal, causing metabolic changes in hepatocytes and facilitating the entry of HBV into hepatocytes. Thus, a comprehensive understanding of NTCP's role is crucial. In this review, we will examine the regulatory mechanisms governing HBV pre-S1 binding to liver membrane NTCP, the role of NTCP in HBV internalization, and the transcriptional and translational regulation of NTCP expression. Additionally, we will discuss clinical drugs targeting NTCP, including combination therapies involving NTCP inhibitors, and consider the safety of NTCP as a therapeutic target.

**Keywords:** NTCP, HBV, HBV entry, Bulevirtide

## Introduction

Hepatitis B virus (HBV) infection is a major global public health challenge. According to data from the World Health Organization (WHO) showed that in 2019, 290 million people were living with chronic hepatitis B (CHB) infection, and 1.5 million new infections were diagnosed each year.[1] As a prototype virus of the *Hepadnaviridae* family, HBV has partial double-linked ring DNA and encodes a variety of functional proteins.[2] Chronic HBV infection is a major cause of cirrhosis and hepatocellular carcinoma (HCC).[3]

Currently, the primary treatments for HBV infection are nucleotide analogues (NAs) and interferons (IFNs).[4] Nucleotides and NAs inhibit the reverse transcription of pre-genomic RNA (pgRNA) into HBV DNA but are associated with the risk of drug resistance and often fail to achieve hepatitis B surface antigen (HBsAg) clearance.[5,6] Some cccDNA degradation can be achieved by immune-mediated degradation, such as interferon alpha, lymphosin-beta receptor agonists, tumor necrosis factor alpha, and interferon gamma, by upregating apolipoprotein B mRNA editing enzymes to catalyse cellular effectors such as apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3A/3B (also known as APOBEC3A/3B cytidine deaminase). The cccDNA is deammoniated in the nucleus. In addition, IFNs therapy can regulate innate and adaptive immunity and also affect the activity of HBV covalently closed circular DNA (cccDNA). However, it has the disadvantages of poor tolerance and serious side effects. While these treatments can help control HBV infection, long-term or even lifelong therapy is often required. Patients must also contend with high treatment costs, frequent disease monitoring, and strict adherence to their treatment regimen. Despite treatment, the risk

Received: 27 May 2024
Accepted: 16 October 2024
Published: 28 October 2024

of cirrhosis and liver cancer remains elevated. Therefore, the discovery of new anti-HBV drugs is very important and urgent.

Given the current inability to completely eradicate cccDNA from infected liver nuclei, therapies have been developed to inhibit the entry, assembly and secretion of HBV and to clear cccDNA from infected liver cells.[7] In particular, in 2012, the cellular entry receptor for HBV, sodium taurocholate co-transporting polypeptide (NTCP), was discovered. NTCP is a transmembrane bile acid (BAs) transporter expressed in hepatocytes and also serves as the entry receptor for HBV's satellite virus, HDV.[7] This provides new clues to potential treatments for HBV infection.

NTCP plays a crucial role in the entry of HBV into hepatocytes. It serves as a key mediator of the liver's innate antiviral immune response, and its involvement in various viral infections has been demonstrated through multiple mechanisms.[8] During hepatocyte proliferation, the down-regulation of membrane-localized NTCP expression renders newly formed hepatocytes resistant to HBV reinfection. This process may accelerate viral clearance during immune-mediated cell death and the compensatory proliferation of surviving hepatocytes.[9] Inhibition of Ntcp has been shown to prevent the spread of HBV-infected hepatocytes in mice.[9] NTCP-targeted therapy has potential for regulating HBV infection in patients with CHB.[10] Post-translational glycosylation of NTCP is critical for its proper localization to the hepatocyte membrane, which may help explain physiological cholestasis in newborns and also play a significant role in HBV infection after birth.[11] In addition, as an important target of HBV entry, NTCP is not only related to new HBV infection, but also closely related to the addition of cccDNA library in HBV-infected hepatocytes.[12,13]

In this review, we review the complex regulatory mechanisms of NTCP. However, the role of these mechanisms in different conditions remains largely unknown, and further elucidation may lead to the identification of other anti-HBV drugs with novel modes of action.

## NTCP and HBV

### The Mechanism of HBV Enters Cells

HBV is a pathogen responsible for acute and chronic hepatitis, as well as HCC. It has a circular, partially double stranded DNA genome that selectively enters liver cells and transmits the genome, thus initiating various processes of virus replication.[14] The HBV envelope contains three forms of HBsAg, namely large (L), middle (M), and small (S) proteins[15] (Figure 1). Notably, the pre-S1 domain of the large envelope protein plays a crucial role in HBV attachment and entry into hepatocytes. And NTCP is the key to pre-S1 binding and HBV infection.[16]

HBV entry into hepatocytes occurs in several stages. It begins with HBV's low-affinity interactions with heparan sulfate proteoglycan (HSPG), including glypican-5 (GPC5), on the surface of hepatocytes.[17,18] HSPG exists in the extracellular matrix or cell surface of almost all cells[19] Studies have shown that highly sulfated inhibitors are more effective at blocking HBV binding to cells.[20] Previous studies have found that HSPG is related with HBV cell attachment.[20,21] This attachment is facilitated by the interactions of HSPG with the Arg122 and Lys141 residues in the S-domain antigenic ring of all HBV envelope proteins, creating electrostatic interactions with negatively charged HSPG.[22] This low-affinity binding stabilizes HBV on the cell surface, allowing the virus to engage with its high-affinity receptors[23] After this initial interaction, HBV binds to NTCP, which oligomerizes before the virus enters the cell, following its attachment to HSPG.[24] HBV is attached by HSPG, but the expression of NTCP significantly affects HBV internalization. GPC5 is an HBV entry factor, promoting HBV infection. Studies have shown that GPC5 adheres to the surface of HBV particles and facilitates the entry of host cells.[18] HBV's preferential binding to GPC5 may partly explain its strong hepatotropism.[18] Once attached to the high-affinity NTCP receptor,[25] HBV undergoes endocytosis, leading to further viral infection.[26] NTCP is an important receptor in HBV binding and entry (Figure 2). These studies suggest that the initial interaction between HBV and NTCP occurs before the virus enters the cell.[27]

### An Overview of NTCP

NTCP is a member of the SLC10 transporter family and a sodium-dependent transporter of BAs, also known as solute carrier family 10A1 (*SLC10A1*). Human NTCP (hNTCP) is a protein with a molecular weight of 56kDa and consists of 349 amino acids.[28,29] Of course, NTCP is also responsible for transporting BAs to enter hepatocytes. Therefore,

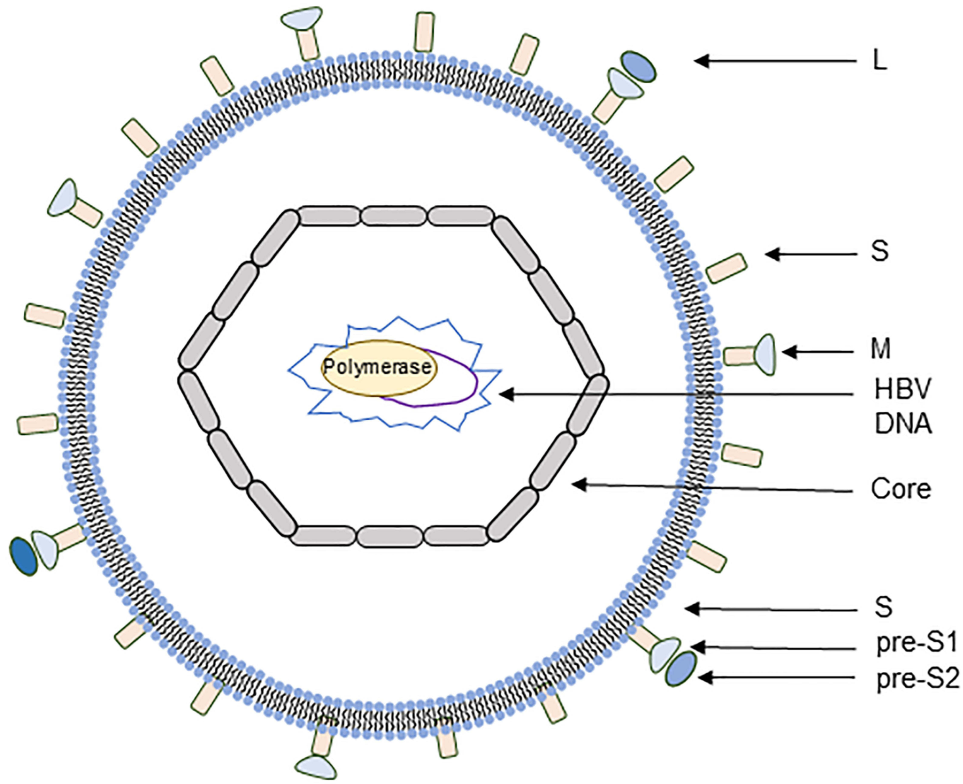


**Figure 1** Schematic diagram of hepatitis B virus. HBV is a partially double-stranded DNA genome of approximately 3.2 kilobases(kb). HBV contains three envelope proteins: large (L), middle (M), and small (S). The C-terminal S domain is common to all three envelope proteins. The preS1, preS2, and S domains are indicated. The viral polymerase is covalently attached to the partially double-stranded DNA genome. The core protein (HBc) forms the capsid of viral particles.

competition between BAs and HBV for NTCP binding is an important consideration in future research on NTCP inhibitors. The effect of NTCP inhibitors on Bas is a key factor in evaluating their safety, as significant alterations in BAs levels could lead to hepatotoxicity or other adverse effects. Therefore, careful monitoring of BAs homeostasis is essential to ensure the safe use of NTCP inhibitors. NTCP's mainly function is to uptake conjugated BAs from plasma into hepatocytes in a sodium-dependent manner, which makes a vital part in the enterohepatic circulation of BAs.[30] Moreover, certain hormones, drugs, and combinations of drugs and BAs are also substrates for NTCP uptake.[31–33] Some studies have shown that the HBV envelope protein pre-S1 regulates HBV invasion and cell infection by specifically binding to NTCP.[34] There are five different papers showing different structures of NTCP in different conformations that give hinds for different proposed transport modes.

The protein NTCP has the following characteristics. In terms of structural characteristics, its overall structure consists of nine transmembrane helices but lacks the first transmembrane helix. The N-terminus is exposed on the extracellular surface and shows similarities and differences compared with bacterial bile acid transporters. It has a panel domain composed of TM1, TM5, and TM6 and a core domain composed of TM2-TM4 and TM7-TM9. Regarding transport mode hints, when binding with Fab YN69083, the formed complex structure provides atomic-level details for studying the interactions between NTCP, HBV, and bile acids, indicating specific conformational changes in the N-terminal region of NTCP upon antibody binding. For bile acid transport, through mutant studies, key sites related to bile acid transport such as the cross-region of TM3 and TM8 and amino acid residues like Leu27, Leu31, and Leu35 have been identified. A structure-based transport mechanism is proposed where bile acid molecules are co-transported with two sodium ions, and conformational changes of NTCP may play an important role. In terms of interaction with HBV, it interacts with the myristoylated N-terminal preS1 domain of the large surface protein of HBV. Amino acid residues at positions 9–18 of this domain are crucial for binding to NTCP. The infection mechanism is that HBV induces viral internalization and infection through the binding of preS1 to NTCP, while compounds such as Bulevirtide can inhibit this process.[35]

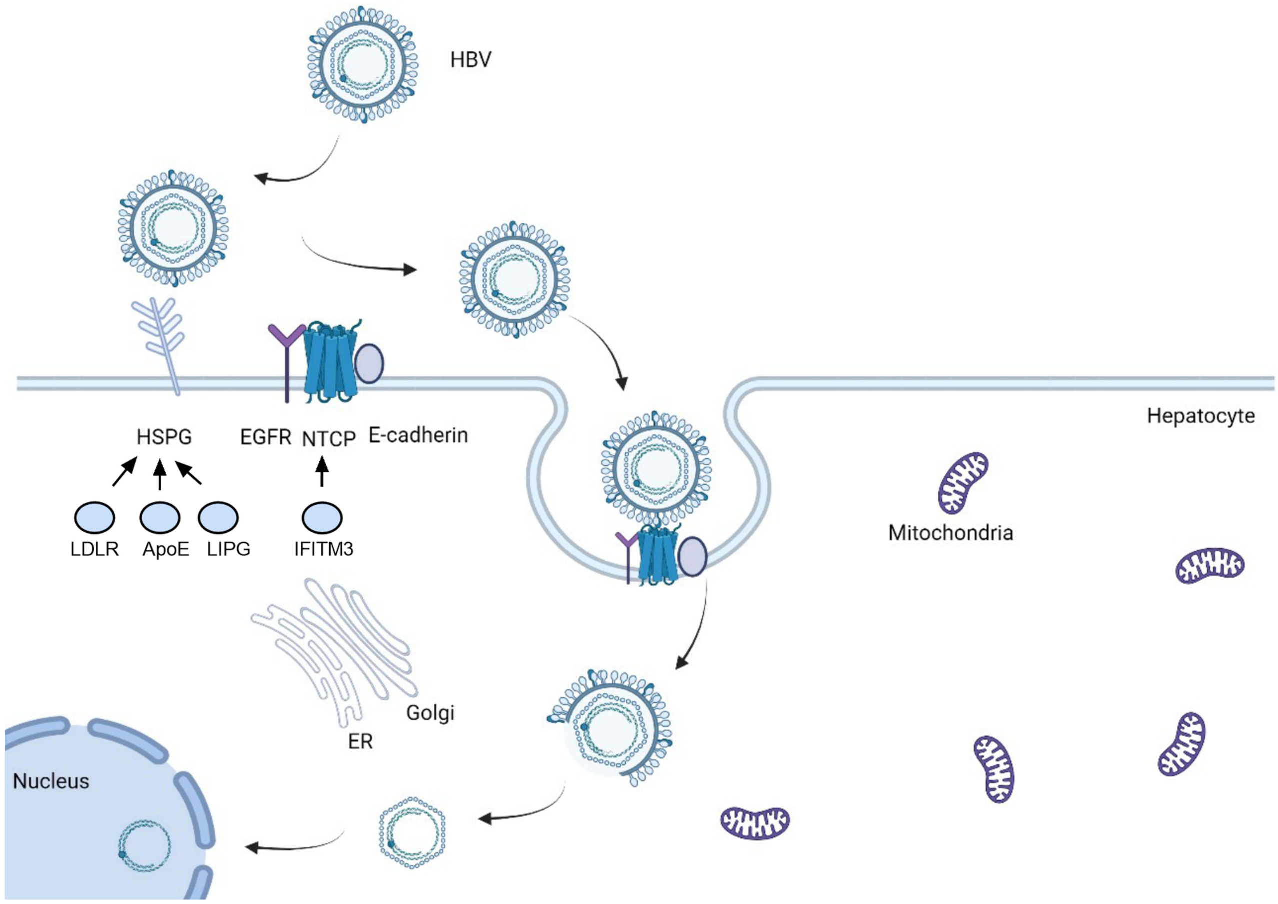


**Figure 2** Diagram of viral entry of hepatitis B virus. HBV first enters the liver with the blood, then enters the lesion space and reaches the sinuses of hepatocytes. HBV virus first binds to HSPG with low affinity on the cell surface. Subsequently, HBV pre-S1 binds to NTCP with high specificity. HBV binding to NTCP can lead to endocytosis of the receptor, and eventually the HBV nucleocapsid enters the nucleus and establishes infection. Created with BioRender.com.

The protein NTCP exhibits the following characteristics. In terms of structural features, it adopts the SLC10 fold structure, comprising nine transmembrane α-helices and having two domains, core and panel. When binding with nanobodies, NTCP undergoes conformational changes to form a wide transmembrane pore that serves as the transport route for bile salts. The important sodium binding sites (Na1 and Na2) and substrate binding-related sites are structurally conserved, and some mutations can affect the transport function of NTCP. Regarding the transport mode hints, in the transport process, NTCP undergoes a conformational change from an inward-facing state to an open pore state, which is caused by the relative movement of the core and panel domains. The open pore structure indicates that NTCP can transport various amphipathic bulky substrates including bile salts, sulfated steroids, and statins, and the substrate interacts with amino acid residues within the transmembrane pore during transport. In terms of receptor recognition for HBV/HDV, the receptor binding domain preS1 of HBV/HDV interacts with amino acid residues within the transmembrane pore of NTCP. The open pore state of NTCP is conducive to the binding of preS1, while the inward-facing state inhibits the binding of preS1. This mechanism explains why myr-preS1 binding inhibits bile salt transport and why certain mutations of NTCP can affect HBV infection and bile acid transport.[36]

The overall structure of hNTCP in complex with Fab fragments, nanobodies and substrates was resolved by cryo-electron microscopy. It has a tunnel structure connecting the extracellular environment and cytoplasm. The external part of the tunnel is partially open to the outer leaflet of the membrane, while the internal part is open to the inner leaflet of the membrane and cytoplasm. Substrate molecules (such as glycochenodeoxycholic acid) are arranged in parallel in the tunnel, and their hydrophilic tails can move in the hydrophilic part of the tunnel. In terms of transport mechanism, NTCP co-transports bile acid molecules with two sodium ions. Based on conformational changes during transportation, a transport mechanism is proposed, explaining the stoichiometry of sodium ions and bile acid transportation. The broad specificity of NTCP for bile salts can be explained by the binding mode and conformational changes of substrate molecules in the tunnel. The disorder of the glycine part enables NTCP to bind different bile acid molecules. The research results provide clues for understanding the transport mechanisms of human transport proteins ASBT and SOAT, and suggest that NTCP, ASBT and SOAT may share a common transport mechanism.[37]

The cryo-electron microscopy structures of human, bovine and rat NTCPs in the absence of substrates and the structure of hNTCP in the presence of the myristoylated preS1 domain of hepatitis B virus envelope protein LHBs are reported. NTCP has nine transmembrane helices. The panel domain is composed of TM1, TM5 and TM6, and the core domain is composed of TM2-TM4 and TM7-TM9. There are some special regions between transmembrane helices, such as the crossover region of TM3 and TM8, which is of great significance for sodium binding and substrate transportation. Substrate transportation occurs through transmembrane tunnels. Lipid-like densities in the tunnel suggest that this region may be part of substrate transport. PreS1 and substrate compete for the extracellular opening of the NTCP tunnel. This competitive binding mode explains why there is mutual interference between HBV infection and bile acid transport. The key binding sites for HBV preS1 are determined, such as amino acid residues in regions such as the TM2-TM3 loop and TM5. The binding of preS1 to NTCP forms a lasso-like structure, in which the N-terminal region folds into the tunnel and forms extensive interactions with amino acid residues in the tunnel, and the C-terminal region binds to the extracellular surface region.[38]

The complex structure of hNTCP and myristoylated preS1 (2–48) peptide segment was analyzed. In this complex, NTCP and preS1 form a ternary complex with a ratio of 1:1:1. After NTCP binds to preS1, the overall conformation is similar to that in the ligand-free state, but conformational changes occur in some regions. For example, the conformational change of TM5 is closely related to the binding of preS1 and virus infection. The N-terminal region (amino acids 2–34) of preS1 fills the extracellular opening of the NTCP tunnel and forms multiple hydrogen bonds and hydrophobic interactions with amino acid residues in the tunnel. The C-terminal region (amino acids 35–48) of preS1 interacts with the extracellular surface region of NTCP (such as the TM2-TM3 loop), including hydrogen bonds and hydrophobic interactions. This study reveals the mechanism of NTCP as an HBV receptor. That is, preS1 induces the attachment and infection of the virus to the host cell through specific binding to NTCP. It also provides a structural basis for the development of anti-HBV drugs targeting the HBV entry step and identifies some potential drug targets, such as amino acid residues Q264, T268, and V272 on TM8b.[39]

## The Regulatory Factors of HBV Entry into Hepatocytes and Its Relationship with NTCP

However, NTCP alone may not be sufficient for the effective internalization of HBV into hepatocytes. Other regulatory factors likely contribute to HBV susceptibility, suggesting a complex, multi-step entry process (Table 1).

The entry of HBV into liver cells begins with the low-affinity binding of the pre-S1 domain on HBV's surface to HSPG on hepatocyte membranes.[18,21,22] Low density lipoprotein receptor (LDLR) is a known apoE-binding receptor like HSPGs and plays an important role in HBV infection.[51] LDLR plays a key role in lipid and cholesterol metabolism by binding to very low-density lipoprotein (VLDL), apolipoprotein E (ApoE), and high-density lipoprotein (HDL) particles

**Table 1** The Regulatory Factors of HBV Entry into Hepatocytes

| Name | Mechanism | References |
|---|---|---|
| LDLR | Acts as an HBV cell attachment receptor by binding to HBV-associated ApoE | [40] |
| ApoE | Binds to cell surface receptors such as LDLR and HSPG | [41,42] |
| beta2-GPI | Facilitates the transfer of viral particles to the NTCP | [43] |
| LIPG | Increases HBV adherence to NTCP | [44] |
| EGFR | Promotes NTCP | [45–47] |
| IFITM3 | Promotes NTCP | [8,48,49] |
| ATP5B | Interacts with pre-S1 | [59] |

on ApoB100 on LDL and chylomicrons. Studies have shown that knocking down of *LDLR* significantly reduces HBV infection.[40] The enrichment of human ApoE on the HBV envelope is vital for the effective infection and production of HBV.[52,53] In fact, strong evidence links HBV to ApoE, as specific ApoE antibodies can block HBV infection and capture HBV particles.[52] Interestingly, patients infected with HBV who carry the *ApoE3* allele have lower rates of HBsAg clearance.[41,42] Viral envelope proteins play a key role in mediating cell entry.

The high expression of beta2-glycoprotein I (beta2-GPI) has been shown to promote the transfer of viral particles to NTCP, and its interaction with annexin II facilitates viral membrane fusion.[43] Endothelial lipase (LIPG) has been reported to enhance HBV adherence to HSPG or promotes HBV adherence to NTCP.[44] The introduction of *SLC10A1* by mRNA transfection increases the sensitivity of hepatoma cells to HBV. Conversely, transfection of *SLC10A1* mRNA into non-hepatocytes can support the uptake of BAs, but still does not make the cells susceptible to HBV, suggesting the need for additional host factors.[54]

Next, the pre-S1 structural region of the HBV envelope binds to NTCP.[16,45,55] Knocking down of EGFR has been shown to reduce both HBV internalization and NTCP expression.[45] Additionally, activation of EGFR triggers time-dependent relocalization of HBV preS1 to early and late endosomes and lysosomes in coordination with EGFR trafficking. Inhibition of EGFR ubiquitination by site-directed mutagenesis or by knockdown of two EGFR sorting molecules, the signal-transducing adaptor molecule (STAM) and lysosomal protein transmembrane 4β (LAPTM4B), indicates that EGFR trafficking to late endosomes is crucial for efficient HBV infection.[46] Studies have shown that knocking out of EGFR significantly impairs NTCP transport to the cell surface.[47]

Interferon-induced transmembrane protein 1–3 (IFITM1-3) is a limiting factor for the entry of many viruses.[48] The upregulation of IFITM3 may be an adaptive upregulation during the process of HBV infecting hepatocytes. Knocking out of *IFITM3* significantly inhibits HBV infection in NTCP-expressing in HuH7 and PHH cells, which proves from the side that IFITM3 is conducive to HBV infection.[48] IFITM expression is regulated by interferon stimulated response elements (ISRE).[56,57] A proteomic study has shown that FITM3 is one of the most upregulated proteins during HBV infection.[49] In addition, IFITM3 expression increases when hepatocytes are exposed to myr-pre-S1 lipopeptides that mimic HBV/HDV binding to NTCP.[8]

ATP5B, the beta subunit of the F1 component of F1F0 ATP synthase, is responsible for most ATP synthesis in many organisms.[58] Establish ATP5B knockdown cells to test the importance of ATP5B for the life cycle of HBV. Lower ATP5B expression is associated with lower production of HBeAg and HBsAg. Total HBV DNA and cccDNA are significantly reduced. Studies have shown that knocking out of ATP5B reduces HBV infectivity.[59]

## SLC10A1 Mutation

HBV only infects humans and chimpanzees, posing major challenges for modeling HBV infection and chronic viral hepatitis.[50] Studies have shown that the expression of NTCP in liver organs is significantly increased, and infection with HBV in vitro differentiated organoids leads to higher levels of infection and replication. Because high expression of NTCP alone does not result in more effective HBV infection, NTCP by itself may not be sufficient to facilitate optimal viral entry.[60] The natural course of hepatitis virus infection in woodchucks closely resembles that of HBV infection in humans. However, woodchuck hepatocytes expressing hNTCP are only susceptible to HDV infection, indicating that other key factors mediate HBV infection, which are subject to strict species-specific limitations. This suggests that while hNTCP is essential for HDV entry, HBV infection likely requires additional human-specific factors, suggesting that while hNTCP is crucial for HDV entry, HBV infection likely requires additional human-specific factors. These factors may include specific co-receptors, host proteins involved in viral replication, or immune evasion mechanisms that are absent or function differently in woodchucks, leading to species-specific limitations on HBV infection.[61] Studies have shown that exogenous expression of hNTCP has established a robust model of HBV infection in rhesus monkeys.[62] *SLC10A1* gene mutations also affected the efficiency of HBV infection. Detection of the primary protein sequence of human NTCP shows that 139YIYSRGIY146 is a highly conserved tyrosine-rich motif. To study the roles of amino acids Y139, Y141, and Y146 in NTCP biology, site-directed mutagenesis was used to replace the above residues with alanine, phenylalanine, or glutamic acid (simulating phosphorylation). Only Y141E has a transport defect, most likely due to the accumulation of mutant proteins intracellularly. Most importantly, the Y146A and Y146E mutations completely eliminate the binding of the viral preS1 peptide to NTCP, while the Y146F mutant of NTCP shows some residual binding ability to preS1. Therefore, tyrosine 146 and tyrosine 141 both belong to the tyrosine-rich motif

139YIYSRGIY146 in human NTCP to a certain extent. They are newly discovered amino acid residues that play an important role in the interaction between HBV and its receptor NTCP, and thus play an important role in the process of virus entry into hepatocytes.[63] The *NTCP* S267F variant of the *SLC10A1* gene provides protection against both HBV and HDV infection, significantly reducing the risk of developing cirrhosis and HCC[64,65] In patients with CHB, the p.Ser267Phe variant serves as a protective factor, lowering the risk of liver failure, cirrhosis, and HCC.[66] The rs4646287 G > A genotype of the *SLC10A1* gene may be linked to a reduced risk of HBV vaccine failure in children born to highly infectious mothers.[67] The *SLC10A1* p. Ser267Phe variant has also been associated with a faster anti-HBV response to first-line nucleoside analogues.[68] Additionally, species-specific differences and mutations in the *SLC10A1* gene influence the efficiency of HBV infection.

## Regulation of NTCP

NTCP plays a critical role in BAs transport and HBV binding, making its regulation at both the transcriptional and translational levels crucial for BA metabolism and HBV-related diseases. NTCP expression is regulated by many factors (Figure 3).

### Transcriptional Regulation of NTCP

The transcriptional regulation of NTCP is mediated by BAs, nuclear receptors and nuclear factors. Other factors, such as hormones and cytokines, also regulate the expression of NTCP. NTCP mRNA regulation is primarily associated with BA concentration. Farnesoid X receptor (FXR), a liver-enriched nuclear receptor activated by BAs, forms a heterodimer with

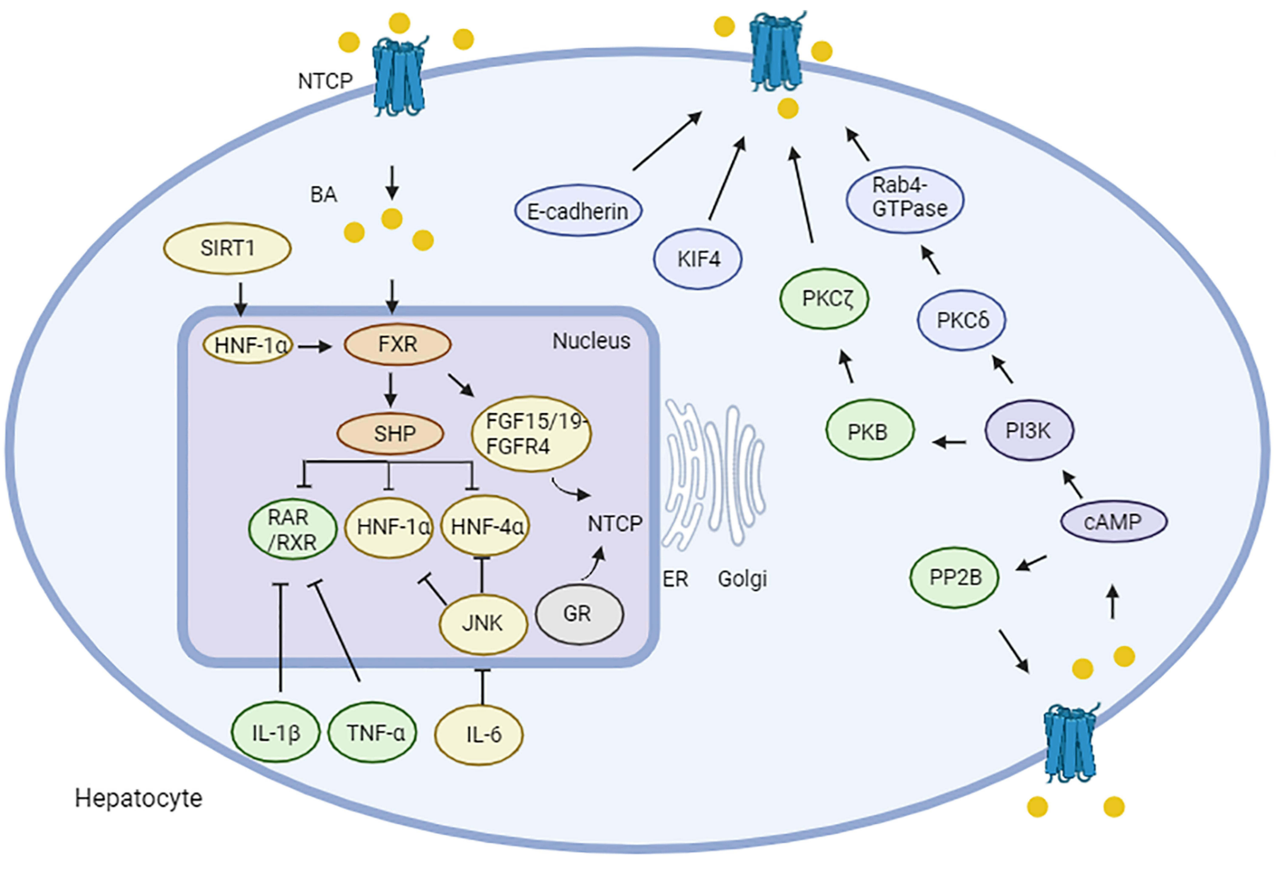


**Figure 3** Regulation of sodium taurocholate co-transporting polypeptide expression. BA-induced, FXR-mediated induction of nuclear suppressor SHP is a key mechanism to reduce NTCP expression by interfering with RXR, RAR, HNF-1α and HNF-4α with binding sites within the NTCP promoter. SIRT1 regulates FXR expression through HNF-1α. FXR further regulates the expression of NTCP mRNA through the FGF15/19 signaling pathway inhibits the down-regulation of NTCP by HNF1α and HNF4α via JNK-dependent pathway. And IL-1β and TNF-α down-regulated the expression of NTCP through inhibition of RAR/RXR complex. cAMP stimulates $Ca^{2+}$ or PP2B to dephosphorylate NTCP. After cAMP activation, the PI3K/PKB/PKC isomers jointly promote the intracellular movement of NTCP into the plasma membrane. Other factors, including KIF4, E-cadherin, that promote the transport of NTCP to the membrane. Among them, yellow represents mouse, green represents rat, blue represents human, Orange represents mouse and rat, and purple represents rat and human, showing the effect. Created with BioRender.com.

**Abbreviations**: BA, bile acid; FXR, farnesoid X receptor; SHP, small heterodimer partner; HNF-1α, hepatocyte nuclear factor 1 alpha; SIRT1, hepatic sirtuin 1; RXR, retinoid X receptor; RAR, RXR-retinoic acid receptor; PP2B, protein phosphatase 2B; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha.


https://doi.org/10.2147/DDDT.S480151

retinoid X receptor alpha (RXR-α) to recognize response elements.[69] FXR activation by chenodeoxycholic acid (CDCA) increases the synthesis of pgRNA and DNA replication intermediates in the HBV genome.[70,71] FXR binds to two response elements in the HBV core promoter region, which are activated by ligands to modulate HBV core promoter.[70,71] However, in HBV transgenic models, FXR only participates to a limited extent in HBV biosynthesis.[72] Although FXR activation regulates *SLC10A1* expression, FXR does not directly interact with the *SLC10A1* promoter. Instead, it induces the expression of small heterodimer partner (SHP), which inhibits the activation of *SLC10A1* gene by retinoic acid receptor (RAR) and retinoid X receptor (RXR) response elements in rat.[73] FXR also modulates Ntcp through the FGF15/19 signaling pathway in mice.[74] Other key regulators of BA metabolism include HNF-1α, HNF-4α and HNF-3β. HNF-1α binds to and activates Ntcp promoters in rat, while HNF-3β regulates transcriptional inhibition of NTCP/Ntcp promoters by directly binding to response elements in all three species.[75] HNF-1α regulates the expression of FXR in mice,[76] and the absence of *Sirt1* has been shown to reduce HNF-1α binding to the FXR promoter, disrupting BA metabolism.[77] HNF-4α directly binds to *Slc10a1* promoters via HNF-4α response elements in mouse, with HNF-4α-induced *Slc10a1* activation further enhanced by proliferator-activated receptor-γ coactivator-1α (PGC-1α).[78] Over-expression of NTCP has been shown to inhibit adenosine production and HIF1α expression.[79]

As a ligand-activated transcription factor, the glucocorticoid receptor (GR) binds to *Slc10a1* promoter gene, down-regulating its expression and reducing NTCP-mediated BAs transport in GR-deficient mice.[80] Other hormones, including growth hormone, prolactin, thyroid hormone and estrogen, also play significant roles in regulating the expression of Ntcp and maintaining its homeostasis. The maximum secretion rate of taurocholate also increased by 10%, 31%, and 24% on days 2, 14, and 21 postpartum, respectively, which is associated with increased mRNA and protein expression of Ntcp and Bsep. Infusion of ovine prolactin (oPRL) to ovariectomized rats increases the mRNA and protein expression of Ntcp and Bsep. These data indicate that the coordinated expression of bile salt transporters postpartum and when affected by prolactin is increased.[81] Prolactin promotes the binding of Stat5 to *Slc10a1*-GLEs through the long form prolactin receptor PRLRL, thereby transcriptionally regulating *Slc10a1*.[82] In addition, studies have confirmed that prolactin (PRL) and placental prolactin can induce *Slc10a1* expression through the prolactin receptor and the signal transducer and activator of transcription (Stat) 5a pathway. However, in pregnant rats, elevated levels of placental prolactin do not increase the expression of *Slc10a1*. Since plasma estradiol ($E_2$) levels also increase during pregnancy, there is an inhibitory effect of $E_2$ on PRL-induced *Slc10a1* activation. $E_2$ treatment can inhibit the increase in liver *Slc10a1* induced by PRL to the same level as in rats treated with $E_2$ alone. In HepG2 cells, in the presence of co-transfected ERα, $E_2$ inhibits PRL-induced *SLC10A1* reporter gene expression in a dose-dependent manner.[83] Studies have shown that both thyroid hormones and glucocorticoids can directly induce the level of *Slc10a1* mRNA without being affected by the secretion pattern of growth hormone. However, the effect of estrogen in reducing *Slc10a1* mRNA is not due to a direct effect on the liver but requires the stable secretion of pituitary hormones, especially growth hormone.[84] Estradiol inhibits the expression of NTCP, thereby inhibiting viral entry, limiting infection, and spreading in the liver. In some cases, women may have a stronger immune response to HBV than men,[85] which may have a potential impact on the progression of HBV from acute to chronic infection. Cytokines play a crucial role in the immune response to HBV. During acute infection, their balance is important for virus clearance. Pro - inflammatory cell factors such as IFN-γ and tumor necrosis factor-α (TNF-α) can activate immune cells to clear infected cells. However, if the balance is disrupted, the immune response may be ineffective.[86] High levels of interleukin-6 (IL-6) during chronic inflammation or due to other factors may lead to immune dysregulation. Chronic elevation of cytokines can cause immune exhaustion, making acute infection likely to turn into chronic infection.[87]

During cholestasis, the chronic pathologic elevation of BAs concentrations triggers the release of cytokines such as IL-6, IL-1β, and TNF-α, which inhibit *SLC10A1* transcription. IL-6 is an important cytokine that plays a role in inflammation and liver regeneration.[88] The expression of NTCP is regulated by IL-6.[89] The regulation of NTCP expression by IL-6 has been mainly studied in rodents. These studies show that the down - regulation of *Slc10a1* can be mediated by activating c-Jun-N-terminal kinase (JNK), resulting in the decreased expression and binding activity of multiple nuclear factors such as HNF1α, HNF4α and nuclear receptor dimer RXR.[90] The transcriptional regulation of the human and mouse NTCP/Ntcp genes was compared with that of rats. HNF1α, HNF4α as well as the retinol X receptor/retinoic acid receptor dimer (RXRα/RARα) bind and activate the rat NTCP/Ntcp promoter but do not bind and activate the human or mouse NTCP/Ntcp promoters. Conversely, the activation of CCAAT/enhancer-binding protein-β is specific to human and mouse NTCP/Ntcp. The only common motif present in these three species is HNF3β. In electrophoretic mobility shift assays, HNF3β forms specific DNA-protein complexes and inhibits NTCP/Ntcp promoter activity in co-transfection analyses.[91] In rats, inactivation of TNF-α completely prevents the downregulation of Ntcp and reduces the binding activity of HNF-1. In endotoxemia, the downregulation of Mrp2 and partial downregulation of Ntcp can be prevented by blocking IL-1β but not by blocking TNF-α.[92] In summary, the expression of NTCP/Ntcp is regulated by BAs, hormones and cytokines.

There are studies on whether HBV infection is partially determined by diet. Research on HBV transgenic mice fed different levels of casein diets showed that reducing casein to 6% significantly inhibited HBV expression. Serum HBsAg concentrations differed among different casein diets. Low casein diet is an inhibitor of HBV transgene and liver injury. This suggests that a low casein diet is an effective inhibitor of HBV transgene and HBV-induced liver injury, indicating that diet management could be a practical way to help control HBV infection.[93] Epidemiological observations show chronic alcoholics have more HBV markers. In HBV transgenic SCID mice, ethanol diet increased HBsAg and viral DNA levels. Changes were observed in liver antigens. However, no such changes were observed in transgenic mice on an isocaloric diet.[91]

## Translational Regulation of NTCP

NTCP localization and membrane expression are regulated by various factors, including cyclic adenosine monophosphate (cAMP), phosphoinositide-3-kinase (PI3K), KIF4 and E-cadherin.[94] It has been found that cAMP promotes protein phosphatase 2B (PP2B) or $Ca^{2+}$ to dephosphorylate Ntcp in rat.[95] cAMP leads to dephosphorylation of Ntcp and translocation to the plasma membrane through the Ser-226 site of Ntcp cytoplasm in rat.[96] This finding raises questions about the precise localization of NTCP on the basolateral membrane. Tyr-321 and Tyr-307 in the C-terminal cytoplasmic domain of NTCP have been implicated in sorting NTCP to basolateral membrane.[97] Moreover, two Asn residues (N5, N11) are believed to be crucial for NTCP expression on the plasma membrane, though there is some controversy surrounding this claim in HepG2.[98] While the complete molecular mechanisms governing NTCP localization remain unclear, these Tyr and Asn residues likely play a role in basolateral membrane sorting via interaction with specific host signaling pathways.

The PI3K signaling pathway, including three downstream effectors (P70 S6 kinase [$p70^{S6K}$], PKC and protein kinase B [PKB]), also influences cAMP-mediated Ntcp translocation in isolated rat hepatocytes.[99] Activation of PKB, for example, has been shown to promote Ntcp translocation into the plasma membrane in rat.[100] However, only a subset of PKC isoforms affect this process. Specifically, PKCζ promotes cAMP-induced Ntcp plasma membrane localization,[101,102] while PKCδ mediates Ntcp transport by activating Rab4 and an endosomal marker in HuH-Ntcp cells.[103] The activation of PKCδ stimulates Ntcp localization to the plasma membrane.[103] Upon cAMP activation, the PI3K/PKB/PKC pathways work together to facilitate the intracellular movement of Ntcp to the membrane. Additionally, elevated BA concentrations in liver cells expedite Ntcp recovery on the basolateral membrane, involving PKC, Src family kinases (Yes and Fyn), and reactive oxygen species and reactive oxygen species.[104–106] Furthermore, phosphodiesterase-induced cAMP degradation restricts NTCP expression on cells, thereby limiting HBV infection.[107]

Kinesin family member 4 (KIF4), a highly conserved member of the kinesin family,[108–110] plays a critical role in NTCP expression. KIF4 is elevated in HBV-related liver malignancies, and its loss reduces surface NTCP levels in HepG2-hNTCP.[111,112] Interestingly, RXR agonists like bexarotene and alitretinoin have been found to reduce KIF4 expression.[112]

E-cadherin is vital for desmosomes and adhesive junctions[113,114], and it has implications for pathogen infections, including HBV. Overexpression of E-cadherin has been shown to promote viral transmission.[115,116] Adhesion junction proteins (nectins and cadherins) are involved in host-pathogen interactions. Nectins are entry receptors for several major human viruses such as herpes simplex virus, measles virus and poliovirus.[117] Many pathogens also target cadherins to enter host cells and survive in them. A study involving siRNA-mediated E-cadherin silencing in Huh7.5.1 cells (a liver cell-derived cancer cell line) showed that the entry of HCV pseudoparticles was inhibited, indicating that E-cadherin plays an important role in mediating host-HCV interaction and regulating virus entry.[117] In addition, viral protein U (a viral pore protein important for the viral transmission and pathogenesis of human immunodeficiency virus type 1 and simian immunodeficiency virus) is reported to interfere with the interaction between E-cadherin and β-catenin in addition to down-regulating E-cadherin expression.[118] This leads to the destruction of the E-cadherin-β-catenin complex and thus the release of viral particles. It also facilitates HBV infection by promoting the localization of glycosylated NTCP on the cell surface.[119] E-cadherin may interact with the cellular surface NTCP-HBV complex,[119] and silencing of E-cadherin reduces HBV pre-S1 binding and internalization in primary human hepatocytes and HepG2-NTCP cells.[119]

# NTCP as a Target of Drug Development

Viral entry is the first step in viral infection and is a potential target for the development of anti-viral drugs. NTCP has the potential to be a therapeutic target for HBV. Currently, there are many drugs that target NTCP, such as Bulevirtide, cyclosporin and its derivatives, Thiazolidinediones and their derivatives, BAs derivatives, antibodies and natural products (Table 2).

Clinical study

## Bulevirtide

Bulevirtide (Myrcludex B) has been marketed in Europe since 2020 as a treatment for HDV.[156,157] As a drug to treat HBV, bulevirtide still in the Phase II clinical trial stage,[156] is a potential drug for Phase III clinical trials.[158] Bulevirtide is a nutmeg acylating peptide synthesized from 2–48 amino acids of pre-S1.[159] Bulevirtide has demonstrated significant efficacy in reducing HBV infection in humanized mice.[160] In Phase I/II clinical trials, it exhibited strong anti-viral activity against HDV in humans.[120,121] Bulevirtide analogs (2a, 2b, and 2d) have also effectively reduced HBV infection

**Table 2** Examples of Drugs and Natural Products Targeting NTCP

| Drugs | Mechanism | References |
|---|---|---|
| Myrcludex-B | Blocks the NTCP receptor | [120–127] |
| Cyclosporin and its derivatives | Blocks the NTCP receptor | [128–133] |
| Thiazolidinediones and their derivatives | Prevents the dimerization of NTCP | [24,55,134] |
| Bile acids and their derivatives | | |
| Bile acids | Inhibits NTCP-mediated HBV entry | [135] |
| Bile acid derivatives: OCA, INT-767, DBA-41 | Blocks HBV entry by inhibiting NTCP | [136,137] |
| TLC | Inhibits NTCP receptor | [138] |
| Antibodies | | |
| N6HB426-20 | Anti-NTCP | [139] |
| MA18/7 | Anti-pre-S1 | [140] |
| 2H5-A14 | Anti-pre-S1 | [141] |
| Other drugs | | |
| Ezetimibe | Inhibits myr-pre-S1 peptide binding | [142,143] |
| Fasiglifam | Inhibits NTCP receptor | [144] |
| Bexarotene | Inhibits NTCP receptor | [112] |
| Irbesartan | Inhibits NTCP receptor | [145,146] |
| Ritonavir | Interrupts NTCP function | [143] |
| Everolimus | Inhibits NTCP receptor | [147] |
| Oxy185 | Inhibits NTCP receptor | [148] |
| Natural products | | |
| Epigallocatechin-3-gallate | Down-regulates NTCP | [149] |
| Ergosterol peroxide | Down-regulates NTCP | [150] |
| Curcumin | Down-regulates NTCP | [151] |
| Proanthocyanidin | Blocks the NTCP receptor | [152] |
| Vanitaracin A | Inhibits NTCP receptor | [153] |
| Exophillic acid | Inhibits NTCP receptor | [154] |
| Betulin derivatives | Inhibits NTCP receptor | [155] |

in HepG2-NTCP cells.[122] Recent advancements in structural biology have provided detailed cryo-EM structures of NTCP bound to either preS1 or Bulevirtide, offering new insights into how NTCP functions as an HBV receptor and how Bulevirtide inhibits both BAs transport and HBV particle entry.[161] Bulevirtide interacts with NTCP in a highly specific manner, forming two distinct domains: a plug that occupies the bile salt transport tunnel of NTCP and a string that covers its extracellular surface. The N-terminally attached myristoyl group of Bulevirtide interacts with the lipid-exposed surface of NTCP. These structures reveal how Bulevirtide inhibits bile salt transport, rationalizes NTCP mutations that decrease the risk of HBV/HDV infection, and provides a basis for understanding the host specificity of HBV/HDV. Such insights not only elucidate the molecular mechanisms by which Bulevirtide works but also pave the way for novel HBV-targeted drug development.[35] To disrupt the HBV-NTCP interaction without impairing BAs uptake, targeting the exposed hydrophobic patches between Asn87 and Glu277 could be effective. These specific residues, such as Ile88, Pro281, and Leu282, were chosen because they are located within the critical regions responsible for HBV binding, while maintaining NTCP's bile acid transport function. Their hydrophobic nature makes them ideal candidates for selective disruption of HBV attachment without compromising BAs transport.[35] Approximately 950 Å² of the NTCP surface area is buried in the formation of the Fab complex. There are two interaction clusters centered on Glu277 of the receptor and Tyr104 of the Fab heavy chain. Glu277 of NTCP is located on the loop between TM8 and TM9. It forms a hydrogen bond with the side chain of light chain Arg95, while its carbonyl oxygen obtains a hydrogen bond from the side chain of Trp90. At the same time, the indole ring of tryptophan is located on the side chain of NTCP Glu277. At about 15 Å, Tyr104 of the heavy chain is located near Asp24 and Asn209 and forms hydrophobic interactions with the side chains of Lys20, Phe283 and Phe284. By filling the pocket between the N-terminal regions of TM1 and TM9, Tyr104 of the heavy chain completely prevents the movement of the panel domain relative to the core. As mentioned above, because this closes the pocket. Therefore, this antibody seems to have strict specificity for the outward-open form of NTCP.[35]

Clinical studies have confirmed the efficacy of Bulevirtide both as a monotherapy and in combination with other antiviral drugs for treating HBV and/or HDV infections.[123,124] As a satellite virus of HBV, HDV relies on the envelope protein of HBV for assembly. In fact, HDV and HBV share the envelope protein and use the same cell entry factors. Bulevirtide has shown a differentiated impact on BAs transport and viral entry: while a high dose (47 nM) affects bile salt transport, a much lower dose (IC50 of 80 pM) is sufficient to inhibit HBV and HDV entry.[125] Blocking NTCP as a receptor for HBV and/or HDV can be achieved without blocking the function of the NTCP transporter.[126] However, this is a mechanistic view based on in vitro data, which is not the case in patients receiving Bulevirtide, and more animal or human studies are needed to confirm this conclusion. The IC50 of Bulevirtide inhibiting NTCP was 4 nM. Notably, the IC50 for inhibiting HBV infection (about 100 pM) and bile salt transport (about 5 nM) varied widely, which is related to the observed binding saturation that does not require NTCP to inhibit infection.[162] A long-term clinical study involving the administration of high-dose Bulevirtide (10 mg) over 48 weeks demonstrated its safety and efficacy. In this study, HBV DNA and HBV RNA levels became undetectable, and HDV RNA levels gradually decreased, with two patients achieving complete viral clearance, and a third patient showing a significant reduction in HDV RNA (from 6.8 log10 cp/mL to 500 cp/mL). Despite a significant increase in BAs, the patients remained asymptomatic, further supporting the safety profile of high-dose Bulevirtide.[127] In addition, *Slc10a1*$^{-/-}$ mice are alive and show few NTCP-specific abnormalities.[163] In a case study, a person with high bile salt levels was diagnosed with functional knockdown of NTCP but did not show obvious clinical symptoms.[164] Moreover, Bulevirtide in combination with pegylated interferon alpha (Peg-IFN-alpha) has shown enhanced synergistic effects on HDV and HBV. This suggests that Peg-IFN-alpha and Bulevirtide have a synergistic effect. However, clinical follow-ups have revealed a common issue of viral rebound upon discontinuation of treatment.[120] Given the persistent global burden of chronic HBV/HDV co-infections, these results suggest that Bulevirtide holds significant potential as a therapeutic option for HBV and HDV treatment, though strategies to manage viral rebound will be essential for sustained long-term outcomes. However, more clinical data are needed to demonstrate the safety and efficacy of Bulevirtide.

Basic study

## Cyclosporin and Its Derivatives

Cyclosporin A, derived from fungal metabolism, is used as a very effective immunosuppressant. Cyclosporin compounds block the interaction between HBV preS1 and NTCP, inhibiting HBV entry. Studies have shown that Cyclosporin A blocks BAs uptake through NTCP transport,[128] and blocks the binding of HBV pre-S to NTCP.[129,130] In order to eliminate the negative effects of immunosuppression and BAs uptake inhibition, a series of Cyclosporin derivatives have been screened.[131] Cyclosporin A derivatives SCYX827830 and SCYX11454139 not only have no immunosuppressive activity, but also have stronger anti-HBV activity in HepG2-NTCP cells.[130] Another Cyclosporin A derivative 27A also inhibits HBV infection without causing immunosuppression.[132] In addition, the Cyclosporin derivatives SCY450 or SCY995 inhibit HBV entry without effect on NTCP transporter activity. SCY446, on the other hand, was able to inhibit both HBV entry and NTCP transporter function.[131] It has also been confirmed that Cyclosporin B is a stronger HBV entry inhibitor.[133] However, the immunosuppressive nature of Cyclosporin A may limit its utility as an anti-HBV therapeutic.

## Thiazolidinediones and Their Derivatives

Troglitazone is an insulin sensitizer for the treatment of insulin resistance in type 2 diabetes.[165] Recently, troglitazone has been identified as a potential inhibitor of HBV entry by targeting NTCP. Troglitazone inhibits HBV internalization and NTCP oligomerization.[24,55,134] Although troglitazone disrupts NTCP oligomerization, it does not impair the interaction between NTCP and EGFR. After HBV attachment, NTCP first binds to EGFR, followed by its oligomerization. Troglitazone prevents NTCP oligomerization, and phenylalanine at NTCP 274 is essential for this process. The formation of a complex involving HBV virions, EGFR, and NTCP oligomers, followed by EGFR autophosphorylation, enables HBV internalization via endocytosis. Therefore, NTCP-EGFR interaction is required for NTCP oligomer formation, and NTCP oligomerization downstream of this association is essential for HBV entry. Administration of troglitazone and pioglitazone significantly reduces the levels of HBsAg without inducing significant cytotoxic effects. Moreover, thiazolidinedione derivatives 1, 2, and 6 inhibit NTCP oligomerization and HBV infection in HepG2-NTCP cells.[24] In conclusion, the discovery of thiazolidinediones and their derivatives provides a better state strategy for inhibiting HBV infection.

## BAs and Their Derivatives

In addition, multiple substrates of NTCP competitively reduce NTCP-mediated HBV entry. BAs such as tauroursodeoxycholate, taurocholate, bromosulfophthalein competitively reduce NTCP-mediated HBV entry.[135] Among BAs derivatives, obeticholic acid (OCA) and INT-767 inhibit HBV entry through reducing the expression of NTCP.[136] It has been found that dimeric BA derivatives (DBADs) synthesized from ursodeoxycholic acid (UDCA), have also been found to effectively inhibit NTCP function. One potent compound, DBA-41, with high selectivity, affinity, and bioavailability for human NTCP, was optimized for better efficacy.[137] Intraperitoneal injection of hNTCP-TG induces an increase in serum total BAs.[137] In addition, secondary BAs taurolithocholic acid (TLC) significantly inhibits the transport function of NTCP, but NTCP protein still exists on the plasma membrane. Preincubation with TLC also significantly reduces HBV/HDV myr-preS1 peptide binding, thereby reducing HDV infection.[138] Therefore, BAs and their derivatives have the potential to become new therapeutic agents against HBV infection.

## Antibodies

Several monoclonal antibodies have been developed that target the pre-S1 region and inhibit HBV entry. The N6HB426-20 antibody, which recognizes the extracellular domain of human NTCP, effectively blocked HBV entry in vitro but had a minimal effect on BAs uptake. It also induced HBV viremia without strongly inhibiting BA absorption.[139] In addition, the MA18/7 antibody blocks HBV infection by targeting the pre-S1 domain of the large HBs protein.[140] Similarly, the 2H5-A14 antibody obstructs the binding of pre-S1 to NTCP, effectively neutralizing both HBV and HDV.[141]

## Other Drugs

As the first selective cholesterol absorption inhibitor, Ezetimibe is primarily used to reduce cholesterol uptake by intestinal cells to lower blood lipids.[166] In addition to its lipid-lowering effects, Ezetimibe has been shown to inhibit HBV infection in HepG2 cells transfected with NTCP,[142] as well as in Huh7 cell lines expressing hNTCP.[143] Fasiglifam, a partial agonist of G-protein-coupled receptor 40 (GPR40), has been used in the treatment of type 2 diabetes.[167] Interestingly, pretreatment with Fasiglifam was found to reduce HBV DNA levels in NTCP-overexpressing HepG2 cells, human liver cell lines, and PXB cells, suggesting its potential as a novel HBV entry inhibitor.[144] Fasiglifam has the potential to be a novel HBV entry inhibitor. Bexarotene is an RXR agonist that has an inhibitory effect on HBV infection.[168,169] KIF4 is a key host factor for HBV infection, and KIF4 controls surface NTCP through anterograde NTCP transport to the cell surface. Bexarotene not only inhibits HBV/HDV entry by targeting KIF4 but also suppresses NTCP-mediated bile salt uptake in primary hepatocytes.[112] Irbesartan is used to fight hypertension in patients with type 2 diabetes.[170] It has also shown some activity in inhibiting liver fibrosis.[171] It was found that irbesartan effectively inhibited HBV by interfering with NTCP activity, stabilized the expression of NTCP, and reduced the formation of HBeAg and cccDNA.[145,146] Ritonavir, a protease inhibitor class of anti-retroviral drugs used to treat HIV infection, has an inhibitory effect on the metabolic function of NTCP.[172] Studies have shown that Ritonavir can block the function of NTCP and inhibit the entry of HBV.[143] Everolimus is a mammalian target of rapamycin inhibitor (mTOR)/proliferation-signal inhibitor with potent immunosuppressive and anti-proliferative effects.[173] Studies have shown that everolimus significantly inhibits NTCP.[147] Oxy185 is a semi-synthetic oxysterol. Studies have shown that Oxy185 interacts with NTCP to inhibit the oligomerization of NTCP, reducing the efficiency of HBV internalization.[148]

## Natural Products

Natural products are an important source for new drug discovery.[174,175] Numerous natural compounds, including terpenoids, alkaloids, and flavonoids, exhibit anti-HBV activity[176] and have been shown to inhibit NTCP expression.

Epigallocatechin-3-gallate (EGCG), a kind of flavonoid in green tea extracts, has a variety of properties.[177] EGCG induces clathrin-dependent endocytosis and protein degradation of NTCP from the plasma membrane, while also reducing clathrin-mediated transferrin endocytosis.[149] Ergosterol peroxide (EP) is a kind of sterol that exists in medicinal mushrooms, sponges, etc. It has anti-viral, inflammatory and oxidation functions.[178] EP directly interferes with the NTCP-LHBsAg interaction by acting on NTCP. EP has the potential to treat HBV infection.[150] Curcumin is a polyphenol derived from herbs and dietary spices that has anti-inflammatory, anti-cancer and anti-viral properties.[179,180] It inhibits HBV attachment and internalization, reducing HBV entry and NTCP density.[151] Proanthocyanidin (PAC), also known as condensed tannins, is the most abundant class of natural phenolic compounds found in a variety of plants.[181] PAC prevents the pre-S1 region in LHBs from attaching to NTCP, targeting HBV particles and impelling their infectiveness without affecting the NTCP-mediated BAs transport activity.[152] Vanitaracin A is isolated from the culture medium of fungus Talaromyces sp,[182] inhibits BAs uptake and blocks HBV and HDV entry by interacting directly with NTCP.[153] Studies have shown that exophillic acid, derived from fungi, also interacts with NTCP, impairing its BAs uptake function.[154] Betulin, a pentacyclic triterpenes, is obtained from natural sources and with a variety of biological activities, such as anti-inflammatory and anti-tumor activities.[183] Betulin derivatives have been shown to effectively and selectively inhibit the NTCP receptor for HBV/HDV, demonstrating a clear structure-activity relationship while preserving NTCP's BAs transport function.[184]

# Safety of NTCP as a Drug Research Target

The inhibition of NTCP activity provides a new approach to the field of anti-HBV drug discovery. The advantage of HBV entry inhibitors that target NTCP is that they remain effective regardless of the presence or absence of viral mutations or subviral particles. Importantly, an important concern with the use of NTCP inhibitors is that these drugs may also block NTCP-mediated BAs transport, interfere with the physiological function of NTCP, and may cause adverse reactions. While studies have shown that some NTCP knockout mice exhibited reduced clearance of conjugated BAs, no significant liver damage was observed.[163] Furthermore, no serious disease that associated with NTCP variants have been reported in humans,[185] and no direct correlation between NTCP inhibition and drug-induced liver injury has been found.[155] One patient with the NTCP R252H homozygous mutation exhibited elevated plasma bile salt levels without any signs of liver damage.[186] Interestingly, 26 of 1828 healthy individuals homozygous for the p.Ser267Phe variant had no functional human NTCP, yet did not experience any related health issues.[160,187] These findings suggest that humans may tolerate some degree of NTCP function loss, potentially compensated by other transporters. Nevertheless, the complete loss of human NTCP function and its long-term consequences remain unclear. Given that many drugs are transported via NTCP in the liver, more information is needed to understand drug-drug interactions when NTCP is inhibited.

## Conclusion and Future Perspectives

Chronic hepatitis, which can progress to cirrhosis and liver cancer, remains a major global public health problem.[188] Human NTCP plays a critical role as a receptor for HBV entry, yet the mechanisms regulating human NTCP during HBV infection remain largely unknown, and many relevant host factors have yet to be identified. Future research should focus not only on NTCP expression at the cell membrane but also on its cytoplasmic activity after binding to HBV. The relationship between NTCP, HBV replication, and HBV DNA warrants further investigation. A deeper understanding of these factors will be instrumental in the development of targeted NTCP inhibitors and the elucidation of HBV infection mechanisms. Given the important role of NTCP in HBV infection and other HBV-related diseases, it is important to find more effective drugs or natural products. Although many molecules and natural products have been tested for their inhibition of NTCP, most show high IC50 values, making it unlikely that they will work clinically or even pharmacologically. Bulevirtide may be a potential drug for the treatment of HBV and HDV, and has no obvious side effects, and has a good application prospect, however has to be daily injected.

While efficacy is paramount, the ideal treatment would involve compounds that are both effective and exhibit minimal side effects. Therefore, future drug development for hepatitis B must carefully balance the inhibition of NTCP's physiological functions with the need to avoid serious adverse effects.

## Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.


## Acknowledgments

The authors acknowledge using Biorender (https://app.biorender.com/user/signin) to create the schemata (Figures 2 and 3).

## Funding

The present study was financially supported by the National Natural Science Foundation of China (No. 81973840 and No. 81273748); National science and Technology major projects of the 13th Five-Year Plan (2018ZX10303502); Science and Technology Program of Hebei (223777156D); Sichuan Provincial Administration of Traditional Chinese Medicine Major science and technology projects (2021XYCZ004).


## Disclosure

The authors declare that they have no competing interests in this work.

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