International Journal of Molecular Biology and Biochemistry

ISSN Print: 2664-6501
ISSN Online: 2664-651X
Impact Factor: RJIF 5.4
IJMBB 2023; 5(2): 29-34
www.biologyjournals.net
Received: 05-06-2023
Accepted: 11-07-2023

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Novel therapeutic strategies for the treatment of chronic hepatitis-B viral infection

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DOI: https://doi.org/10.33545/26646501.2023.v5.i2a.48

Abstract
Chronic hepatitis B virus infection poses a significant global public health challenge, leading to substantial liver-related illness and death. This infection can be acquired either at birth or through person-to-person transmission later in life. Vaccination has proven to be highly effective in preventing infection of the hepatitis B virus.

Chronic hepatitis B (HBV) infection is prevalent worldwide and a major contributor to liver disease, particularly in Southeast Asia. The use of vaccines and antiviral medications, such as nucleoside or nucleotide drugs, can effectively decrease the occurrence of new infections and the development of liver disease in individuals with HBV who regularly follow long-term suppressive treatment. The primary risk factor for disease progression in individuals with chronic hepatitis B virus infection is a high concentration of hepatitis B virus DNA in the bloodstream. However, various clinical and viral factors also impact the outcomes of the disease. Alongside liver biochemistry, virological markers, and abdominal ultrasonography, non-invasive methods for evaluating liver fibrosis are becoming increasingly significant as an assessment tool. There are ongoing efforts to develop new therapeutic targets and molecules for early clinical trials to cure HBV infection. A promising approach to achieve a lasting and comprehensive cure may involve combining therapies that target various stages of the HBV life cycle, along with immunomodulators. In the pursuit of a cure for hepatitis B infection, it is evident that a combination of new medications will be necessary. The article reflects on curative treatments and explores the compounds currently undergoing clinical trials for hepatitis B. World Health Organization's objective of eliminating the hepatitis B virus as a worldwide health concern by 2030 can be achieved by leveraging existing vaccines, therapies, and a focused effort on enhancing healthcare access.

Keywords: Hepatitis B virus, antiviral therapy, cccDNA, HBV lifecycle

Introduction
Hepatitis B is a liver disease that results from an infection with the Hepatitis B Virus (HBV). It is a viral infection that primarily impacts the liver and can potentially lead to acute and chronic liver diseases. The global incidence of cirrhosis, a condition that characterizes advanced chronic liver disease, is increasing at an alarming rate.

HBV is a member of the Hepadnaviridae family and is categorized into ten genotypes, labelled from A to J (Sekiba K *et al*., 2018) [16]. The virus is transmitted through contact with infected blood or other bodily fluids, such as semen or vaginal secretions, primarily through activities like sexual intercourse. Also, perinatal transmission can occur from an infected mother to her newborn. During the acute phase of the infection, individuals may exhibit symptoms, but it can also be asymptomatic. Acute infections have the potential to resolve spontaneously or progress into chronic infections.

Even though a highly effective preventive vaccine has been available for a considerable period, approximately 10% of the population remains incapable of generating a sufficient antibody response to the hepatitis B surface antigen (Sjogren MH, 2005) [20]. The current clinical treatments for chronic hepatitis B infection involve the use of antiviral therapies such as peg-interferon, standard α-interferon, nucleoside analogues (Including lamivudine, entecavir, tenofovir, and telbivudine), and nucleotide analogues (Such as adefovir). These therapies exhibit strong potency against the hepatitis B virus. Hence, persistent endeavours have been dedicated to investigating distinct immunotherapeutic approaches as potential

substitutes for antiviral medications and α-interferon in individuals with chronic hepatitis B infection. The objective of treating chronic HBV infection is to reduce the likelihood of liver disease advancement and mitigate its negative clinical outcomes. This can be accomplished through long-term suppression of virus replication or, ideally, by achieving a complete cure for the viral infection.

Structure of HBV

HBV infection is a progressive condition characterized by evolving biochemical, histological, and clinical transformations that vary based on the method of acquisition, host factors, and environmental influences. Inside the host, HBV can manifest in three distinct states: the infectious virion and non-infectious particles, including enveloped nucleocapsids containing immature DNA/RNA, subviral particles (such as spheres and filaments lacking nucleocapsid proteins), and naked nucleocapsids (Leoni MC *et al*., 2018) [12].

The HBV virion has the following structure: it consists of a partially double-stranded relaxed circular DNA (rcDNA) measuring 3.2 kb, which is held together by polymerase. The inner nucleocapsid is formed by the core protein (hepatitis B core antigen, HBcAg), while the outer envelope is composed of lipid-embedded small, middle, and large surface proteins (hepatitis B surface antigen, HBsAg). It consists of a complete minus strand and an incomplete plus strand. The viral genome contains four open reading frames (ORFs) that overlap with each other, namely C, P, S, and X. These ORFs are responsible for the production of functional viral proteins. The C ORF gives rise to HBc and related proteins such as E antigen (HBe) and 22-kDa precore protein (p22cr). The P ORF produces the Pol protein. The S ORF is responsible for generating three types of surface antigens: L-HBs, M-HBs, and S-HBs. HBV X protein (HBx) is encoded by X ORF.

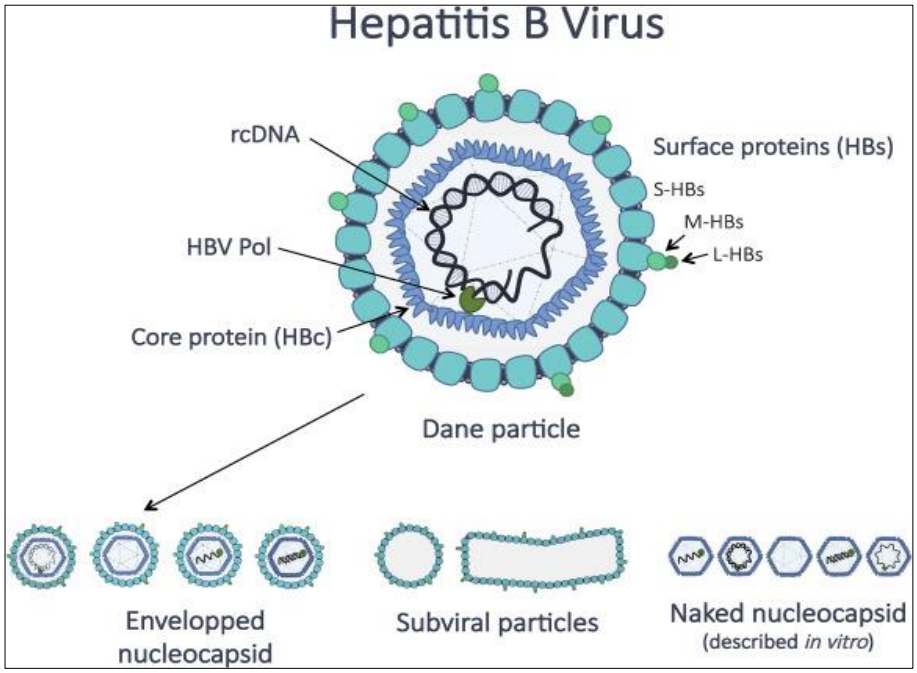

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Fig 1: Schematic representation of HBV particles

Entry of Hepatitis-B Virus

The Hepatitis B virus attaches to the host cell's surface through heparan sulfate proteoglycans (HSPGs) binding factors. Sodium taurocholate plays a role in co-transporting peptides as a receptor for HBV and HDV entry. NTCP is primarily expressed in the liver and is responsible for the uptake of bile salts into hepatocytes. It was discovered that NTCP binds to amino acids 2-48 of the preS1 region, which was already recognized as crucial for receptor binding. Epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, takes part in the internalization of HBV/HDV. This internalization process occurs through a direct interaction between EGFR and NTCP. Many studies have shown that NTCP can oligomerize, and its oligomerization status of NTCP influences its capacity to facilitate viral internalization (Fukano *et al*., 2018) [6]. HBV virions gain entry into the cytoplasm of hepatocytes by specifically interacting with NTCP on the basolateral membrane of the cells. This is followed by a pathway mediated by plasma membrane components, such as clathrin adaptor protein AP-2 and caveolin-1 (Huang HC *et al*., 2012) [12]. Then, HBV is transported within the cytoplasm utilizing the endosomal network. The pH of the endosome progressively decreases facilitating the merging of the outer envelope proteins of HBV with the endosomal membrane. After the disassembly of HBV's inner nucleocapsid by the nuclear pore complex, HBV DNA enters the nucleus (Elkin SR *et al*., 2016) [4].

Formation of cccDNA

The cellular factors in the nucleus modify the genomic DNA of HBV. The Pol-linked terminal at 5' end of DNA minus strand and the RNA oligonucleotide at the 5' end of DNA plus strand are eliminated from the rcDNA. The gaps present in both strands are filled and joined together through ligation, resulting in the formation of cccDNA (Guo *et al*., 2007) [8]. The cccDNA functions as a blueprint for various HBV gene products, such as pgRNA, preS1 mRNA, preS2

mRNA, and X mRNA. These RNAs are subsequently discharged into the cytoplasm. A portion of the pgRNA acts as a template for the production of the core protein and the polymerase through translation. Concurrently, another portion of the pgRNA, in conjunction with the polymerase, assembles a capsid using HBcAgs (Diab A *et al*., 2018) [3]. In infected cells, the relaxed-circular DNA (rcDNA) undergoes a conversion into covalently closed circular DNA (cccDNA). This cccDNA then serves as a template for the production of HBV RNAs of various lengths, primarily 3.5 Kb, 2.4 Kb, 2.1 Kb, and 0.7 Kb. These RNAs are transcribed from different promoters within the HBV genome. The 3.5 Kb RNA is responsible for generating the protein products derived from the C and P regions. The 2.4 Kb RNA gives rise to L-HBs, while the 2.1 Kb RNA synthesizes the other two surface antigens, M-HBs, and S-HBs. Lastly, the 0.7 Kb RNA is responsible for producing HBx. HBx, a versatile protein, facilitates viral production at various stages, encompassing viral transcription and replication. It contributes to the progression of hepatocellular carcinoma associated with HBV.

The cccDNA exists as an episomal entity, it inherently maintains stability and serves as a long-term template for viral replication. A recent study conducted on HepG2 cells overexpressing NTCP revealed a cccDNA half-life of approximately 40 days (Ko *et al*., 2018) [11].

Apart from the formation and preservation of cccDNA, a portion of the HBV DNA introduced into the host genome also integrates. A recent study conducted in cell culture demonstrated that this integration can happen within a week following infection (Tu *et al*., 2018) [22]. Although the integrated HBV DNA lacks replication capabilities, it can serve as a template for generating HBs. This phenomenon is believed to be associated with HBV-specific immune tolerance and the emergence of HBV-related pathogenesis.

Transcription of HBV virus and cccDNA

cccDNA is utilized as a template, to synthesize four distinct lengths of RNAs. The transcription of viral RNAs is governed by four separate promoters for preS1, preS2, core, and X, as well as two enhancers (Enhancer I and Enhancer II). This regulation is facilitated by the host RNA polymerase II machinery-dependent transcription.

Epigenetic modification

cccDNA functions as a compact chromosome that interacts with viral proteins and host factors. The transcriptional activity of cccDNA is influenced by the modification status of histones incorporated into it (Pollicino *et al*., 2006) [17]. A comprehensive analysis called genome-wide ChIP-seq has provided insights into the modifications of histones associated with cccDNA. These modifications include significant levels of trimethylation or acetylation of lysine residues in histone 3 (H3K4me3, H3K27ac, and H3K122ac), known as active transcription markers. These modifications are found at specific locations within the HBV genome. The antiviral effects of interferon (IFN) target the modifications of histones associated with cccDNA. Specifically, IFNα triggers hypoacetylation of histones bound to cccDNA and facilitates the recruitment of transcriptional co-repressors.

Role of transcription factors

The activity of transcription in cccDNA is controlled by the recruitment of cellular transcription factors to the viral promoter/enhancer regions. These regions contain binding sites for multiple transcription factors, including those specific to the liver, such as hepatocyte nuclear factors 3 and 4 (HNF3, HNF4), retinoid X receptor alpha (RXRα), peroxisome proliferator-activated receptor alpha (PPARα), and farnesoid X receptor (FXR), which are classified as nuclear receptors.

Role of HBx in Viral Replication (i.e., HBV Transcription):

HBx's role in HBV replication post-infection has been proven (Lucifora *et al*., 2011) [13]. HBx, a multifunctional protein, has been extensively studied, revealing numerous functions. Studies have indicated that HBx associates with the cccDNA minichromosome, exhibiting a close correlation with cccDNA-bound H3 acetylation kinetics (Belloni *et al*., 2009) [1]. By influencing the recruitment of chromatin-modifying enzymes (p300, HDAC, SIRT1), HBx controls the epigenetic state of histones associated with cccDNA, thereby facilitating active transcription. In the absence of HBx, cccDNA undergoes transcriptional repression characterized by reduced H3 acetylation and H3K4me3, as well as increased H3K9me2/3, leading to the recruitment of heterochromatin protein 1 (HP1) and chromatin condensation. The expression of HBx alleviates this transcriptional repression by restoring increased H3K4me3 and dissociating HP1 recruitment on cccDNA (Riviere *et al*., 2015) [18]. These findings provide evidence supporting the role of HBx in modulating the epigenetic profile of cccDNA-associated histones to regulate HBV transcription.

Life Cycle of HBV

Regarding the elimination of cccDNA, two pathobiological processes are relevant. Firstly, the reduction in cccDNA load within infected hepatocytes is contributed by hepatocyte proliferation itself. In patients with advanced fibrosis or cirrhosis, the burden of cccDNA formation is increased due to hepatocyte replicative senescence (Tang L *et al*., 2019) [21]. A therapeutic approach aimed at reducing cccDNA formation would involve the combination of multiple targeted treatment strategies along with the destruction of infected hepatocytes in the presence of potent replication suppression. cccDNA removal can occur through non-cytolytic clearance of infected hepatocytes stimulated by antiviral cytokines, particularly interferon-α. Studies have shown that higher levels of interferon-α are associated with improved cccDNA clearance by inducing non-cytolytic degradation of cccDNA from infected hepatocytes through the activation of nuclear deaminase A3A or A3B. Recently, it has been discovered that the PASylation technique, which involves adding a polypeptide consisting of Proline, Alanine, and Serine to increase plasma half-life, can enhance the antiviral effect of interferon-α without causing additional toxicity (Mitra *et al*., 2018) [14].

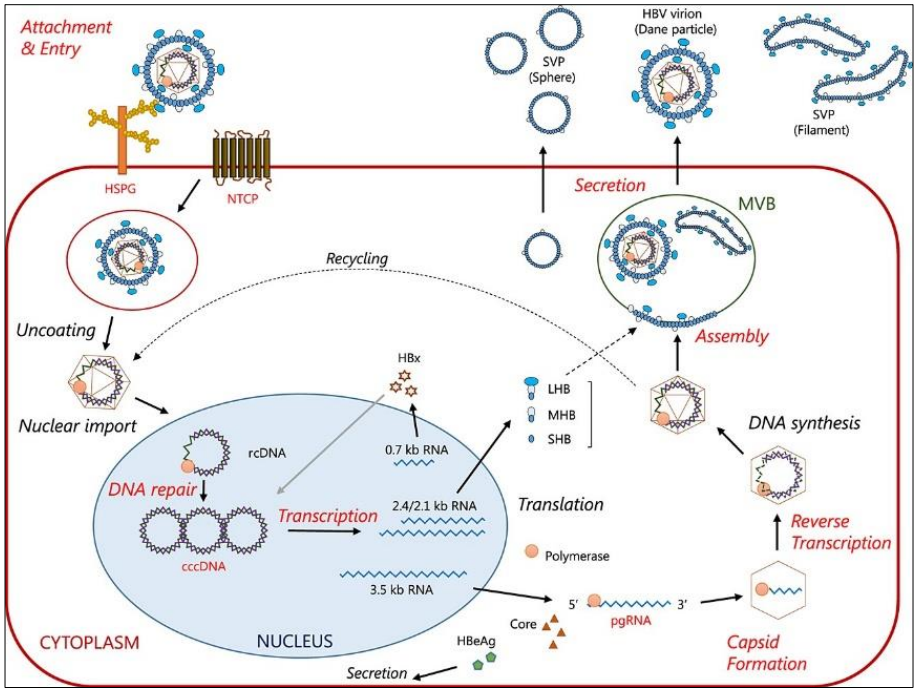

Fig 2: Viral Life Cycle of Hepatitis B Virus https://onlinelibrary.wiley.com/cms/asset/

Vaccination

The primary approach to eliminate hepatitis B and reduce the incidence of liver cancer is through vaccination against HBV at birth. In the United States, the first plasma-derived hepatitis B vaccine was approved in 1981. It was later replaced by recombinant hepatitis B vaccines in 1986 and 1989. The complete hepatitis B vaccine series, which includes a birth dose and two additional booster doses, effectively generates protective levels of anti-HBs antibodies in over 95% of vaccinated infants. The World Health Organization (WHO) recommends administering the first dose of a monovalent hepatitis B vaccine to all infants as soon as possible after birth, preferably within 24 hours, to prevent perinatal HBV transmission. Observational data supports the notion that HBV vaccination is most effective when given within the first 24 hours after birth, but it still retains effectiveness, albeit to a lesser degree, if administered after this time frame. Following the initial dose, it is recommended to administer at least two additional booster doses, either as two doses of monovalent hepatitis B vaccines or as combination childhood vaccines containing hepatitis B.

Goals for Treatment of HBV Infection

Many therapeutic goals have been adopted for the treatment of Chronic Hepatitis Virus.

Partial Cure

Partial cure refers to achieving a continuously undetectable viral load and normalizing ALT levels while still having detectable HBsAg levels after a specific treatment period. This situation, characterized by advancements in virological and biochemical responses, minimizes the advancement of cirrhosis, significantly enhancing patients' quality of life and prolonging their survival. Nevertheless, despite these evident clinical advantages, the risk of developing HCC (Hepatocellular Carcinoma) persists.

Functional Cure

Functional cure is identified by a continual absence of detectable viral presence in the bloodstream, accompanied by a lasting disappearance of HBsAg, either with or without seroconversion to HBsAg antibody. In this state, the covalently closed circular DNA (cccDNA), which serves as the template for viral transcription, remains persistently present. Achieving a functional cure represents the ultimate clinical outcome for the safe discontinuation of nucleos(t)ide analogues (NAs) treatment and serves as the primary objective for several drugs currently undergoing clinical development. This type of cure is associated with the resolution of ongoing liver damage and a further reduction in the risk of HCC to a level comparable to individuals who naturally clear the virus.

Complete cure

Complete cure is linked to the complete absence of both circulating and intrahepatic HBV DNA, accompanied by the disappearance of HBsAg along with the production of HBsAg antibodies, and the elimination of cccDNA. However, it is widely acknowledged that achieving this curative state will be difficult due to the enduring presence of cccDNA.

Sterilizing Cure

Sterilizing cure is characterized by achieving a complete cure along with the eradication of integrated HBV DNA fragments from the chromosomes of the host. This definition of a cure, which resembles the immune state of individuals who have never been exposed to HBV and have been vaccinated, is considered highly unlikely to be achieved.

Novel Therapeutic Approaches

Antiviral Therapy

The primary approach for treating HBV globally is through the administration of oral nucleoside/nucleotide analogue therapy. Among the recommended initial options are entecavir, tenofovir disoproxil fumarate, and tenofovir alafenamide. These three medications are highly effective in suppressing the virus, with over 95% of patients achieving undetectable levels of HBV DNA in their blood. They also

exhibit favourable safety and tolerability characteristics, and their likelihood of developing resistance is very low due to their strong resistance barrier (Chang *et al*., 2010). Prolonged treatment of 5 years, is linked to a decrease in the quantity of cccDNA (covalently closed circular DNA) within the liver. Additionally, extended viral suppression achieved through nucleoside/nucleotide analogue therapy results in substantial histological improvements, with 71% of patients experiencing regression of cirrhosis. Patients with decompensated liver disease experience enhanced liver function as a result of nucleoside/nucleotide analogues (Shim *et al*., 2010) [19].

Prophylactic Therapy

During immunosuppression, the incidence of HBV reactivation has been effectively reduced by administering a nucleotide/nucleoside analogue for a specific period of prophylactic therapy, regardless of the serum HBV DNA concentration (Huang *et al*., 2014) [10]. For individuals with chronic HBsAg carriers who undergo liver transplantation, it is recommended to maintain continuous prophylactic therapy with nucleotide/nucleoside analogues indefinitely. These medications have demonstrated their efficacy in preventing HBV recurrence, even without simultaneous administration of hepatitis B immunoglobulins, for a duration of up to 8 years (Fung *et al*., 2017) [7].

Recent Updates on Diagnosis and Monitoring

The HBV core-related antigen (HBcrAg) is a novel marker that includes a shared amino acid sequence found in both HBeAg and HBcAg, along with the 22-kDa pre-core protein. The presence of HBcrAg is associated with intrahepatic HBV DNA and pregenomic RNA levels in patients undergoing antiviral therapy. The measurement of HBcrAg serves as a reliable serum marker for assessing the liver's active transcriptional activity of cccDNA. Higher levels of HBcrAg are associated with an elevated risk of developing hepatocellular carcinoma (Wang *et al*., 2020) [23]. Another potential viral marker with potential clinical applications is the measurement of HBV RNA. It has demonstrated significant utility in assessing antiviral treatment response and determining the cessation of treatment. HBV RNA measurement aids in identifying functional cures in chronic HBV infection, assessing the risk of HBV-related liver cancer, and evaluating levels of intrahepatic HBV cccDNA. HBV pregenomic RNA reflects viral replication activity and holds substantial value in monitoring treatment efficacy for patients undergoing novel anti-HBV therapies. Viral molecules show promise as surrogate markers for HBV viral activity and, when combined with standard biomarkers, provide a more comprehensive assessment of HBV infection and treatment outcomes (Wei R *et al*., 2018) [24].

Utilizing the artificial intelligence-driven machine learning (ML) technique is an innovative approach to streamlining the HBV diagnostic process. By leveraging machine learning (ML) techniques, a predictive model was developed to determine the inflammation grades of chronic HBV. This model incorporates gene expression data along with three clinical parameters (ALT, AST, HBV DNA). Also, the effectiveness of an artificial neural network (ANN) model in diagnosing the regression of liver fibrosis in HBV patients undergoing therapy has been demonstrated (Nguyen *et al*., 2020) [15].

Current strategy for prevention and treatment of HBV infection

Current treatment options for managing HBV include interferon-α (standard or pegylated) as well as orally administered Nucleos (t) ide Analogs (NAs). As a first-line therapy, it is recommended to use an oral antiviral with a robust genetic barrier against viral resistance. Examples of such antivirals include entecavir, tenofovir disoproxil (TDF), or tenofovir alafenamide (TAF), which is a pro-drug of TDF with a more stable concentration in the bloodstream, resulting in lower doses and reduced systemic exposure (Fanning *et al*., 2019) [5]. Combining TDF and entecavir appears to be a safe and effective rescue option for individuals with chronic HBV hepatitis who have multiple drug-resistant virus strains.

There is also advancement in the immunopathogenesis of HBV, there is ongoing development of various immune-modulating therapeutic agents aimed at promoting the achievement of a functional cure for HBV.

Conclusion

The realm of anti-HBV therapy is embarking on a new era as there is a resurgence of interest from the scientific, medical, and industrial communities to explore novel treatment approaches in the pursuit of achieving a cure for HBV infection. Significant progress has been made in our comprehension of the structure, biology, viral dynamics, and immunopathogenesis associated with chronic HBV-related hepatitis. The introduction of advanced technologies and improved tools, including next-generation sequencing, genome-wide association studies, single-cell RNA sequencing, and gene editing, along with rigorous and well-coordinated collaborative clinical trials, has significantly enhanced our understanding of viral and host-related factors in the development and progression of HBV-related diseases. Novel treatment modalities such as viral RNA interference molecules, capsid assembly blockers, immune checkpoint inhibitors, HBsAg and cccDNA generation blocking molecules, and innate immune system modulators are currently in development. These promising interventions hold the potential to improve outcomes for patients affected by HBV-related conditions in the future.

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