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Current treatment and new therapies against chronic hepatitis B virus infection

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Abstract

Chronic hepatitis B virus infections are a serious public health problem worldwide. Its consequences can be deadly, and current treatment offers only control of the disease rather than a cure. The main limiting factors for a cure are the difficulty of destroying the cDNA of the virus in the cell nucleus and the presence of viral genetic material integrated into the DNA of the host cell. Multiple fronts of research focus on finding a definitive cure by enhancing the immune response of the host or by acting directly against the virus and its replication cycle. This article presents the main advances that have been made in this field.

Keywords

Hepatitis B virus, Immunotherapy, Antiviral Agents, Chronic Hepatitis, Liver Cirrhosis.

INTRODUCTION

The hepatitis B virus (HBV) is a highly contagious pathogen that has infected humans for more than 1,500 years. (1) Neverthless, only 50 years ago Blumberg et al. discovered the surface antigen (HBsAg) which initially became known as Australia's antigen. (2, 3) Since then, various advances have been made in prevention of transmission and suppression of the virus. It is estimated that one-third of the world's population is infected with this virus, (4) and that of these 240 million continue to have chronic infections. (5) This means that HBV is the leading cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) worldwide. (6) It results in approximately 780,000 annual deaths (5). Due to the high rates of associated morbidity and mortality, HBV infections are a global public health problem.

The lifetime risk of developing HCC for people with chronic hepatitis B (CHB) reaches as high as 40%. (7) This is the most advanced and deadly complication of this disease and, in areas of high endemicity, more than 60% of HCC cases are attributable to CHB. (8)

HBV's highest prevalences, over 8% of the populations, are found in Southeast Asia, China, the Middle East, Greenland, the Amazon basin and most of Africa where 45% of the world's population lives. (9)

Transmission is by parenteral contact with contaminated body fluids including blood, saliva, semen, cervical secretions and exudates from wounds. The outcome of infection and disease varies widely from asymptomatic cases to acute liver failure, cirrhosis and hepatocarcinoma. The latter two are the major causes of mortality associated with this virus (11).

The natural course of disease depends on the host's immune status at the time of infection. Thus, intrauterine, perinatal, or childhood transmission results in chronic infection in 90% of cases, and this is the most common scenario in areas of high prevalence. In contrast, horizontal transmission during adolescence or adulthood results in chronic infection in only 5% of cases. (11)

Chronic infection has several phases that depend on interaction between the virus and the host: immunotolerance, immunoreactivity, inactive carrier and resolution. These vary in duration, are not necessarily sequential, and do not occur in all infected subjects. (12)

Currently, the main limiting factor for treatment of the virus is inability to destroy closed covalent circular DNA (cccDNA) found in the nucleus of hepatocytes and viral DNA that has been integrated into the genome of host cells. (13, 14) This virus's potential to re-replicate once suppression treatment has been discontinued prevents any possibility of a cure. (15) In addition, seroconversion against HBsAg rarely occurs with current treatments.

Currently, there are multiple fronts of research on potential cures for CHB and on a wide variety of drugs with new molecular targets. (16, 17) These are discussed in this article.

VIRAL LIFE CYCLE

HBV is an enveloped virus that belongs to the Hepadnaviridae family. It measures 42 nm and contains partially double-stranded circular DNA formed by 3200 kilobases. (18) Although it infects other primates, its primary hosts are human beings. Ten genotypes (A-J) have been identified. Theywith different geographic distributions, clinical behaviors and responses to available treatments. (11) The genome has four overlapping open reading frames (ORFs) that encode the structural and functional proteins of the virus as shown in Table 1.

Table 1. Open reading frames, proteins and functions

Open reading frame (ORF)	Protein encoded	Function
S	Surface Antigen (HBsAg)	Forms viral envelope and non-infectious viral particles
C	Core antigen polypeptide (HBcAg)	forms the nucleocapsid
	Antigen E (HBeAg)	Immunomodulation of the host
P	Viral Polymerase	Reverse Transcriptase and RNase
X	Protein X (HBx)	Regulates viral and host gene expression, Modulates the transduction of intracellular signals, Participates in hepatic oncogenesis

HBV enters the hepatocyte by endocytosis through the interaction of HBsAg with sodium taurocholate cotransporting polypeptide (NTCP). As a result HBV loses its envelope. (19, 20) The nucleocapsid is transported to the nucleus where its partial DNA is matured by cellular proteins to become complementary DNA (cDNA). This is a very stable minichromosome that serves as a transcriptional model for host RNA polymerase II. (13, 21) This enzyme generates four copies of messenger RNA (mRNA) that are translated by the cellular machinery and thus produce viral proteins. (11, 18)

After translation, the envelope proteins and the HBeAg antigen (HBeAg) target the endoplasmic reticulum for subsequent secretion. The nucleocapsid is assembled in the cytoplasm by interaction of a copy of pregenomic mRNA, core protein and polymerase. Initially, an immature nucleocapsid is formed within which a negative DNA strand complementary to the pregenomic mRNA is synthesized by the activity of the reverse transcriptase of the polymerase. Once the copy is obtained, the pregenomic mRNA is degraded by the activity of the ribonuclease type H (RNase H) of the polymerase. This allows synthesis of the DNA strand which was incomplete at the time of its entry into the endoplasmic reticulum. It acquires its envelope and can subsequently emerge as an infectious viral particle or can be transported back to the nucleus to resume replication and thereby maintain an intranuclear reserve of viral genomes.

CURRENT TREATMENT FOR CHB

The objectives of are virological suppression, biochemical remission, histological improvement and prevention of complications such as cirrhosis, hepatocarcinoma and extrahepatic manifestations. The response to treatment is evaluated in several ways:

- Biochemical: transaminase normalization
- Virological:viral load suppression
- Serological: loss of HBeAg or HBsAg and seroconversion to antibodies against hepatitis B antigen [AntiHBe]/antibody against the surface antigen of viral hepatitis B [antiHBs])
- Histological: improvement of inflammatory activity and fibrosis. (23)

The decision to start treatment is based on the likelihood of sustained response to treatment and the risk of hepatic morbidity and mortality. In general, the indications are based on three criteria:

- Serum HBV DNA levels
- Serum alanine aminotransferase (ALT) levels
- Severity of liver disease

In 2009, the American Association for the Study of Liver Diseases (AASLD) published its guidelines for treating hepatitis B patients. (24) Then, in 2012 both Asia Pacific Association for the Study of the Liver (APASL) and the European Association for the Study of the Liver (EASL) published their own guidelines for hepatitis B patients.

(25, 12) Table 2 compares indications for initiation of treatment.

Table 2. Criteria for initiating treatment in CHB according to international societies

Criteria	EASL 2012	AASLD 2009	APASL 2012
DNA-HBV HBeAg Positive HBeAg Negative	> 2000 IU/mL > 2000 IU/mL	> 20,000 IU/mL > 2000 IU/mL	> 20,000 IU/mL > 2000 IU/mL
ALT	> ULN	> 2 ULN	> 2 ULN
Biopsy	Consider only in selected groups		

ULN: Upper limit of normal

Initiation of therapy is not indicated for patients in the immunotolerance and inactive carrier phase since the disease progresses slowly and treatment may induce unnecessary risks of resistance.

Although determination of when to initiate therapy appears to be simple in the guidelines, in clinical practice the process is more complex since many other variables must be taken into account. These include patient age, gender, genetics, and other drugs the patient is taken. Factors related to the drug itself that must be considered include its efficacy, adverse effects, and resistance to the drug. Inflammation and fibrosis are related to liver disease. There are cofactors such as consumption of alcohol, obesity and diabetes that increase the risk of fibrosis, and there are coinfections including human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis D virus (HDV). Finally, profile of HBV including precore-core mutation, viral load, and genotype must be considered.

Currently, there are two types of therapies for managing CHB: one type enhances the host's immune response to the virus, and the other works directly against the virus and its replication.

1. The two types of pegylated interferon, α-2a and α-2b, have antiviral and immunomodulatory activity but also have many adverse effects. It is administered subcutaneously, may exacerbate autoimmune diseases and is contraindicated in hepatic failure and advanced cirrhosis. For these reasons, its use is restricted to a very small population. It is considered to be a finite therapy for selected patients who are young, do not have cirrhosis, who have low viral loads, and who have marked inflammatory activity. The seroconversion rate of HBeAg is 29% to 32% and the loss of HBsAg is 3% to 7%. (26, 27)
2. Nucleoside/nucleotide analogs inhibit reverse transcription of pregenomic RNA to DNA and have no effect on cDNA. For this reason they suppress replication but do not cure the infection. Treatment is long-term and there is a risk of relapse after suspension. Currently, there are five medicines available: lamivudine, telbivudine, entecavir, adefovir and tenofovir.

Nucleoside and nucleotide analogs are characterized by different patterns of potency and resistance. In general, lamivudine is not currently used as first-line treatment because of its high resistance rate, but it is still used in selected cases where therapy is expected to last for a short time. These include cases of severe acute hepatitis, acute liver failure, vertical transmission prophylaxis during pregnancy and immunosuppression to avoid reactivation. Telbivudine and adefovir are also not used as first line treatments because they have less potency, and there also may be resistance to them. It is now preferred to start treatment with high potency and low resistance nucleoside/nucleotide analogs such as entecavir or tenofovir.

A one-year nucleoside/nucleotide analog course in HBeAg-positive patients results in suppression of viral DNA to undetectable concentrations for 21% to 76% of patients, transaminase normalization for up to 77% of patients, HBeAg seroconversion for 12% to 22 % of patients and HBsAg loss of 0% to 3%. Extension of treatment to five years increases the seroconversion of HBeAg to 48% and the rate of HBsAg loss up to 10%. (28)

NEW THERAPIES FOR POTENTIATING HOST IMMUNE RESPONSE

Low cure rates are probably due to a defect in the reconstitution of a specific adaptive immune response against the virus. (29) For this reason, scientific efforts have focused on finding new therapeutic targets that can enhance immune responses to infection. In recent years, great progress has been made in this field, and this has allowed identification of potential therapies for chronic HBV infection. These include pattern recognition receptor (PRR) agonists, thymosin α- 1 (Tα1), programmed cell death protein (PD-1) blocking antibodies and natural killer (NK) cell based therapy.

PRR Agonists

PRRs are proteins that detect molecular patterns associated with pathogenic microorganisms (PAMP). They are present in various cell groups including hepatocytes and cells of the innate immune system. Key PRRs include Toll-like receptors (TLRs), scavengers, C-type lectins, and NOD-like receptors (NLRs).

HBV was long considered a stealth virus, since it was believed to induce a poor innate immune response during the early phase of infection. However, recent studies have

revealed that HBV can be recognized by PRRs which provoke a cytokine-mediated response that controls viral replication. (30, 31) Evidence of this has been found in Kupffer cells, stellate cells and sinusoidal cells. (32) The aim of PRR agonist therapy is to enhance the antiviral response of cytokines, mainly interferons types I and III to achieve a specific adaptive immune response that lasts over time. (33) Several studies have shown that stimulation of cells expressing TLR 7, TLR 9 and TLR 3 in the liver can produce this innate immune response. (34, 35).

TLR 7 is expressed mainly by dendritic cells and B lymphocytes which can then recognize viral RNA with this receptor. (36) GS 9620 is a TLR 7 agonist in Phase II clinical trials. (37) This drug which is given orally has shown antiviral effects against HBV by suppressing viral DNA in the serum and liver of infected chimpanzees. In the study, three chimpanzees with CHB were treated for eight weeks. The result was decreased persistent viral load and a 50% reduction in serum levels of HBsAg and HBeAg. (38) This study have been replicated in marmots with similar results. (39) Pharmacokinetic and pharmacodynamic studies in healthy subjects suggest that low doses of GS 9620 induce an innate immune response dependent on IFN-I. (40) It has also been reported that one or two low doses administered once a week are safe and well tolerated. However, clinical trials have shown no evidence of clinical efficacy in terms of decreased HBV DNA. (37)

A parenterally administered TLR 3 agonist has been able to amplify immune response and accelerate clearance of HBV in mice by increasing IFN-I and II. (41) Another study using a TLR 9 agonist in mice has resulted in induction of an intrahepatic myeloid cell aggregate that generated an expansion of cytotoxic T lymphocytes in the liver (iMATE) thereby reducing HBV replication in hepatocytes. (42)

The stimulator of interferon genes (STING) transmembrane protein is an adapter protein for multiple cytoplasmic receptors and also acts as a PRR that recognizes bacterial second messengers. (43) STING stimulation has been shown to significantly increase IFN-I with suppression of HBV viral replication in the hepatocytes of infected mice, and this has opened a new field of therapeutic study. (32)

Tα1

Thymosin alpha (Tα) is a protein which is produced by the thymus and which has immunomodulatory activity. In-vitro studies of Zadaxin® (Tα1), a synthetic protein based on natural thymosin, (44) have shown that it increases production and maturation of T lymphocytes which stimulates production of Th1 cytokines like IFN-Y and interleukin type 2 (IL-2). It has also shown an ability to activate NK cells and their cytotoxic activity. (45)

A randomized clinical trial conducted in China has shown that Zadaxin® is safe, well tolerated and effective as an inhibitor of HBV replication. In addition, seroconversion in patients treated with this drug was greater than that in patients treated with IFN-α. (46) Another study has demonstrated that patients who were negative for HBeAg who were treated with Zadaxin plus IFN-α had stronger immune responses than did those treated with a combination of lamivudine plus IFN-α or IFN alone. (47) Similarly, for HBeAg-positive patients, therapy with Zadaxin plus lamivudine was more effective for increasing immune responses and seroconversion of HBeAg than was lamivudine monotherapy. (48) This therapy has also had some poor results. In one study, the combination of Tα1 with Peg-INF α-2a was not superior to this monotherapy in patients with CHB who were HBeAg positive, (49) so more research that supports this type of therapy is required.

Blocking Programmed Death 1 Protein (PD-1) Inhibitory Signals

Specific T lymphocytes mediated responses to HBV have been observed to be lacking in cases of chronic infection. This is due to a phenomenon known as depletion, which is characterized by scarcity or absence of specific T cells against HBV that is associated with poor cytotoxic activity, impaired cytokine production, and increased expression of coinhibitory receptors such as programmed cell death protein 1 (PD-1), cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), lymphocyte activation gene 3 (LAG 3) and others. (50) This phenomenon is maintained by the presence of a microenvironment of immunosuppressive cytokines such as IL-10 and transforming growth factor beta (TGF-β) which are produced by the regulatory T lymphocytes found in large numbers in the liver of patients with CHB. (51, 52)

Recently, blockage of these receptors by means of specific antibodies has been studied as a therapeutic target in cancer treatment. Increased patient survival has been found in those studes. (53) Since cancer and chronic HBV infection have many similarities from the immunological point of view, therapeutic studies of the use of blockage of immunoinhibitory signals, mainly of PD-1, to treat HBV have begun. Zhang et al. have found that 70% of virus-specific T lymphocytes were positive for PD-1 in patients with HBV. (54) Another study, carried out in mice with chronic HBV infections, has shown that anti-PD-1 antibodies were able to reverse immune dysfunction and to a certain extent helped to clear the virus (60% HBsAg negativity compared toh 20% in controls). (55) Currently, anti-PD-1 antibodies are used to treat cancer and are in clinical trials for treatment of chronic HCV hepatitis. (56) To date,

there have been no clinical trials to evaluate the usefulness of anti-PD-1 antibodies in humans, but studies with other HBV-like diseases make it a matter of time before research advances in this field.

NK Cell-Based Therapy

NK cells are part of the innate immune system. A large proportion of them are found in the human liver and are crucial for defense against viral infections. (57) They have at least three defense mechanisms: increasing cytolytic granules that lyse infected cells, apoptosis of cells marked through surface receptors and increasing specific cytokines. These cells play a key role in the early response to HBV by secretion of IFN-γ which helps reduce viral load. (58) In patients with chronic HBV infections, the cytolytic function of NK cells is maintained but activation and secretion of IFN-γ and TNF-α are strongly affected. (59) In one study, restoration of the NK cell's ability to produce cytokines through reduction of viral load succeeded in contributing to virus clearance. (60) Developing a therapy that gives NK cells the ability to produce cytokines may be a strategy for curing patients with chronic HBV.

Therapeutic Vaccines

Various vaccines have been studied. They contain several immune and viral products among which are HBsAg, HBsAg-antiHBs immune complexes, DNA that expresses HBsAg by means of plasmids and T-cell vaccine.

These investigations have shown decreased viremia, seroconversion of HBeAg, and potentiation of specific T cell responses against HBV. However, these changes were insufficient to control chronic HBV infections, so combined anti-viral therapies following antiviral treatment or conomittant with it have been studied for achievement of near-zero viral loads. The results have beenn high vaccine tolerability and potentiation of the specific immune response associated with high viremia suppression levels, but with increased viral DNA upon discontinuation. There was no evidence of a decrease in the risk of relapse or of any increase of HBsAg seroconversion. (61)

DV601 (Dynavax) and GS4774 (Gilead Sciences) are two examples of vaccines which express viral antigen sequences in fusion proteins which are currently in first phase trials. (17)

NEW TREATMENTS DIRECTED AGAINST HEPATITIS B VIRUS AND REPLICATION

In the last few years, a great deal of progress has also been made in the development of new treatments that directly target the virus and its replication. This research has identified potential therapies which inlude entry inhibitors, of the entry, lymphotoxin-β receptor (LTβR) agonists, small interfering RNA (siRNA), outer guiding sequences (EGS) RNA molecules, cDNA cleavage enzymes, and ribonuclease H (RNAse H) inhibitors.

Entry Inhibitors

Inhibition of the entry of viruses into hepatocytes has significant therapeutic implications. It can be achieved by neutralizing proteins on the surface of the viral envelope that inhibit binding mediated by heparan sulfate proteoglycans (PGHS) or by blockage of their receptors.

The first and only currently approved viral entry inhibitor is hepatitis B immunoglobulin (Ig). It significantly lowers the risk of acquiring the infection after exposure. Starting with its use, many monoclonal antibodies with the S or pre-S region as molecular targets have been developed, but so far none have been approved as clinical treatments. (62)

Inhibit of PGHS-mediated binding by heparin and suramin, a derivative of urea, has been described. They bind to the surface proteins of the virus and prevent interaction with PGHS. The evidence is limited to in-vitro studies for both heparin and suramin and an in-vivo study of suramin in ducks. (63)

The sodium-taurocholate cotransporting polypeptide (NTCP) is a bile acid transporter that is only expressed in the basolateral membrane of hepatocytes. It has recently been identified as the receptor for HBV which makes it an ideal molecular target for further therapeutic efforts. Myrcludex B, which is a lipopeptide derived from the pre-S1 domain of the HBV envelope, is being studied because it blocks NTCP. It can bind to NTCP thereby inhibiting binding of HBV and hence its entry. It can prevent viral propagation from infected cells in-vivo and reduces cDNA amplification in newly infected hepatocytes. Petersen et al. have demonstrated that it is capable of preventing both HBV and HDV infections in cultured hepatocytes as well as in humanized mice. Among its adverse effects is inhibition of bile acid transport. (64) Other known inhibitors of NTCP include cyclosporin A and B, ezetimibe, irbesartan, ritonavir, bosentan, and propranolol.

LTβR Agonists

Evidence that specific antibodies to LTβR degrade cDNA by means of IFN-independent mechanisms and without the direct use of other cytokines has been found in cell cultures. Upon binding to the receptor, they initiate a selective process of oxidative deamination of cDNA, and endonucleases from the apolipoprotein B enzyme editing family

of catalytic polypeptide-3 mRNA (APOBEC3) causes it to degrade. Their selective action on cDNA is due to their direct interaction with the core protein, thus explaining why it does not cause damage to the genome of the host hepatocyte. (61)

siRNA

Small interfering RNAs are short sequences that do not encode and are present in cells regulating posttranscriptional expression of certain genes. Because the HBV genome is compact and overlapping, the use of siRNAs is encouraging and some studies have shown that by targeting core transcripts or a viral hepatitis B protein (HBx), they can stop gene expression of HBV as evidenced by significant decreases in HBsAg, HBeAg, RNA and viral DNA. (61, 65, 66). A phase II randomized, double-blind clinical trial is currently recruiting participants with chronic HBV infections for evaluating the efficacy of a drug called ARC-520 which contains two siRNAs against this virus (identifier at clinicaltrials.gov: NCT02065336).

EGS

EGSs are oligonucleotide sequences that are complementary to a specific mRNA. After binding to its complementary RNA, the EGS directs the ribonuclease P to destroy it and thus inhibits the synthesis of proteins (in this case viral proteins). Some studies of cell cultures have demonstrated the ability of these EGS to decrease viral DNA concentrations by up to 200,000 times. (67, 68)

cDNA Cleavage Enzymes

- Zinc finger nucleases (ZFNs) consist of two functional domains: a DNA binding domain and a DNA cleaving domain. Inside the hepatocyte they recognize opposing parts of a viral DNA sequence and cleave the double strand. Theoretically ZFNs designed to attack HBV DNA should have no effect on the host genome. Two in-vitro studies have shown that this mechanism significantly decreases the concentration of pregenomic RNA and cDNA. (69, 70) However, the main challenge is to find an efficient mechanism for delivery into hepatocytes. (66)
- Transcription activator-like effector nucleases (TALEN) have structures similar to those of ZFNs. TALEN have sequences of 33 to 35 amino acids that recognize a specific segment of cDNA at position 12 and 13. Unlike ZFNs, TALENs cleave cDNAs as monomers. In-vitro studies and studies in mice have demonstrated their efficacy, (71) so TALEN could be a definitive cure for HBV infections if they could be delivered into hepatocytes in vivo. (66)
- Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is the basis for CRISPR/cas a bacterial system with nuclease activity that is involved in defense against bacteriophages and plasmids. It is composed of Cas proteins and non-coding RNA that determine the specificity of the system. It has recently been used as a genomic editing method. By synthesizing non-coding guide RNAs it is possible to cause the double stranded DNA to break into the desired sequences. Its effectiveness for reducing in-vitro HBV replication has recently been demonstrated, opening the door for future clinical research on this group of antiviral drugs. (72)

RNAse H Inhibitors

When bound to DNA, retroviral ribonuclease H (retroviral RNase H) cleave RNA, remove primers during DNA synthesis, contribute to the destruction of failed transcription errors and are necessary for reverse transcription of viral genomes such as HBV. Inhibition of this enzyme is a widely known part of HIV treatment, but it has not yet been deeply studied for use as an HBV treatment even though a few in-vitro studies suggest that it could be effective. (43, 73) The aim of drugs with this mechanism of action is to suppress viral replication in order to stop replenishment of cDNA in the liver. This allows the immune system or another drug to eliminate HBV infected cells. (66, 74)

CONCLUSION

It is clear that in the last decades significant advances have been made in the identification of the HBV cell cycle and in treatment of HBV. Despite this, the associated morbidity and mortality rates remain high, and treatment is still little more than measures to control the disease.

There are now multiple fronts of research in the search for new curative therapies for CHB that will allow us to go beyond mere suppression of the disease. Advancement is not limited to discovery and clinical applicability of new therapeutic molecules but also involves identification of enzymes involved in viral and cellular replication and identification of defects of host immune responses that could play key roles in the development of new drugs with healing potential.

With multiple drugs currently under molecular study, in-vitro study, in animal models and in clinical models, it is only a matter of time before the weapons in the therapeutic arsenal for fighting CHB become more and more numerous, and we gradually approach a definitive cure.

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