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Journal of Clinical Research and Clinical Case Reports

Open Access

Review Article

Chronic Hepatitis B: Road to Evidenced-based Therapy

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Article Info

Received: April 29, 2021
Accepted: May 10, 2021
Published: May 14, 2021

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Citation: Sheikh Mohammad Fazle Akbar, Mamun Al Mahtab, Md. Sakirul Islam Khan, Ferdousi Begum, et al. "Chronic Hepatitis B: Road to Evidenced-based Therapy". Clinical Research and Clinical Case Reports, 1(2); DOI: http;//doi.org/04.2021/1.1010.

Summary

Hepatitis B virus (HBV) is a DNA virus belonging to the family *Hepadnaviridae*. It is known to infect humans of all ages and exhibits a worldwide distribution, with main prevalence in Asia-Pacific Region and Africa. The virus is an old one and has been traced back to Bronze and Iron ages (1). One landmark observation that led to discovery of HBV was reported in 1885 by Lruman et al. when he observed hepatitis among shipyard workers after small pox vaccination (2). The existence of serum hepatitis was mostly confirmed in 1937 when hepatitis was developed in considerable numbers of population after getting convalescent serum from measles patients (3). However, the nature of the virus remained elusive and most of credits for present day HBV is attributable to Dr. Baruj Blumberg and his team. In 1963, one of Blumberg's coresearcher Harvey Alter reported a new antigen from an Australian Aborigine which reacted with the serum of a New York hemophiliac (4). Thus the term of Australia Antigen (AuAg) entered in scientific community. In course of time, AuAg was confirmed as antigen or HBV and named as hepatitis B surface antigen (HBsAg). Blumberg et al was credited for their discovery and honored by Nobel Prize in Physiology or Medicine in 1976 (5). Extensive researches and clinical investigations have led to develop a potent prophylactic vaccine for HBV. The vaccinated persons are mostly immune from future infection and several countries of the world has adopted HBV vaccination among national Expanded Program of Immunization (EPI) (6,7). Even then, HBV is a non-eradicable pathological entity. If HBV transmission is completely blocked today, we will have to live with HBV for another century of more. The principal causes underlying these are due to presence of millions of chronic HBV-infected persons. These persons act as permanent living reservoir of HBV and transmit the infection to healthy individuals. The virus for hepatitis B has been well characterized. Also, the route of transmission is known and controllable in most cases. Several potent vaccines are available for prophylaxis around the world. Several insights have been developed about molecular and immunological characteristics of the virus and the diseases it causes. Several; drugs have been developed to contain the virus and control chronic hepatitis B (CHB). Even then, remedy from CHB and its complications is out of our reach. Accordingly, the sustainable developmental goal (SGD), although proposed eradication of several communicable diseases, has suggested to control HBV infection until 2030 compared to present level (8). The review that has been formulated here would exhibit a roadmap to have a realistic control of CHB with improvement of quality of life of these patients and reduction of HBV-related mortality.

What we know about the virus

HBV is a small, enveloped hepatotropic virus characterized by the presence of a partly double-stranded DNA (9). It is the prototype member of the *Hepadnaviridae* family and is known to infect humans of all ages. Besides this, animal strains of

Hepadnaviruses are found in woodchucks, ground squirrels, tree squirrels, Peking ducks, and herons (10). HBV is a hepatotropic virus, however, this virus can be found in other tissues as well and its presence has been shown in immunocytes including antigen-presenting dendritic cells by our team via in situ hybridization (11). After entering the body, it mainly moves to the liver and enters hepatocytes. It forms the relaxed circular (RC) DNA that is released at the nucleus and gets converted to covalently closed circular (ccc) DNA (12). The cccDNA acts as a template for the transcription of viral mRNAs using host DNA-dependent RNA polymerase. This is the critical point of HBV pathogenesis. Although it is a DNA virus, it is endowed with a reverse transcriptase activity similar to retrovirus. This extraordinarily complex life cycle of HBV along with the existence of cccDNA make HBV eradication virtually impossible. Although most of the antiviral drugs are capable of destroying replicating HBV DNA, none of the drugs is capable to contain cccDNA (13) and thus new and novel approaches are warranted to control chronic HBV infection.

Magnitude of problem with chronic HBV carriers and public health reaction about the disease

Of the 2 billion individuals infected by hepatitis B virus (HBV) at some points of their life, over 240-300 million are chronic HBV carriers. Considerable numbers of chronic HBV carriers develop CHB, cirrhosis of liver (LC) and hepatocellular carcinoma or liver cancer (HCC) (14). It is estimated that about one million individuals die annually of HBV-related chronic liver diseases.

Although chronic HBV infection represents such a huge international medical burden, CHB has never been considered as a medical emergency, like influenza virus, cholera infection, bird flu virus, Ebola virus or even human immune-deficiency virus or coronavirus disease 2019 (COVID-19). Thus, due attention has never been providing to this deadly pathological lesion.

Another factor is related to the socio-economic and geo-political values and concepts of the modern capitalistic societies. About 90% of chronic HBV carriers reside in developing countries of the world. These countries are yet to develop proper survey methodologies and draw the attention of developed and advanced rich countries regarding diseases those do not create visible medical emergencies. The health care delivery system of these countries is not properly developed. Emerging infectious diseases like bird flu, human immune-deficiency viruses and re-emerging infectious diseases like tuberculosis and malaria have attracted the attention of the government and mass media. However, there are few national programs to find out and tackle chronic HBV infection in most Asian and African countries. HBV infection is detected when the patients become severely ill or develop serious complications or even after death during autopsy. In some cases, their state of infection is detected incidentally when they visit the physicians for different causes. Study about the epidemiology of HBV infection indicates that most chronic HBV carriers become infected with the HBV during their neonatal or perinatal period. This indicates that majority of chronic HBV carriers would remain as living and permanent reservoir of the HBV for decades. In fact, they transmit the virus to healthy and non-infected individuals in most developing countries for several decades. This process is a dynamic one. Accordingly, it is less likely that the numbers of chronic HBV carriers will be reduced in near future. This is in sharp contrast with that what is seen in developed countries of the world. In most of these countries, HBV-infected persons are declining. Most of the patients have been detected by screening or there is an operational system that can detect chronic HBV infection routinely. At least, further transmission of the HBV from HBV carriers has been brought to almost zero by proper screening of donated blood and using clean transfusion apparatuses in many developed countries. These disparities are known to international health organizations, but proper checking of blood for transfusion has not yet been recommended for resource-constrained countries of the developing world. Although we know that HBsAg-negative blood may contain HBV DNA and we and others have provided direct evidence of this feature (15-17), still blood is tested for HBsAg only in several Asian countries according to the advice of WHO. However, transfusable blood are tested by other means in developed and rich countries.

Pathogenesis of Chronic HBV infection

Hepatitis B virus (HBV) can cause a wide variety of pathological processes. In most of the patients, it remains asymptomatic, whereas, in other it may induce inflammation, fibrogenesis, and carcinogenesis of the liver. Thus, acute hepatitis B, acute liver failure due to HBV, HBV-induced acute-on-chronic liver failure, and HBV-related fulminant hepatitis have been defined and these diseases bear various levels of public importance. However, the most intractable problem with HBV infection is the entity of chronic hepatitis B. Usually, when an HBV-infected patient harbor hepatitis B surface antigen (HBsAg) in the blood for more than 6 months, they are regarded as patients with chronic hepatitis B (CHB). These patients also express HBV DNA in the sera with other HBV-related antigens in the sera and the liver. Many of these patients remain asymptomatic, whereas others develop progressive liver diseases like cirrhosis of liver (LC) and hepatocellular carcinoma (HCC) (18-20).

Many CHB patients also reveal a different clinical picture. Some patients with chronic hepatitis B (CHB) may not exhibit HBsAg under certain conditions. Even, they may be negative for HBV DNA in the sera. Several patients with CHB may only express antibody to hepatitis B core antigen (anti-HBc) in the sera. On the other side of the scenario, many patients with CHB express HBsAg, HBV DNA, and other HBV-related virologic and immunological markers in the blood. At the end of the day, it seems that the magnitude of HBV DNA, levels of HBsAg, and hepatitis e antigen (HBeAg)-positivity or negativity are not determinants of severity of HBV-induced liver diseases. Many patients with CHB expressing very high levels of HBV DNA and HBsAg remain asymptomatic, whereas, other expressing low levels of HBV DNA and HBsAg develop progressive liver diseases. Once CHB patients exhibit neuroinflammatory pathologies in the liver, they are prone to develop complications like cirrhosis of liver (LC) and hepatocellular carcinoma (HCC) (21-23).

Antiviral drug for treatment of CHB

Due to lack of curative therapy for CHB, the goal of treatment of CHB patients is to prevent or at least delay progression of CHB to LC and HCC and liver-related deaths. Several antiviral drugs for HBV have been developed since the 1980s (24), a rapid uptake in therapy of CHB started with the availability of oral nucleoside analogs in mid-1990s (25-27). The available antiviral drugs are

safe and well tolerated and can achieve viral suppression. These drugs are used in the global context as per recommendations provided by American, European and Asia-Pacific liver organizations (AASLD, EASL and APASL) (28-30). Based on the recommendations of AASLD, EASL and APASL, most countries and professional liver organizations have also developed their national recommendations for treatment of CHB patients. These drugs are recommended for patients with CHB with high viral loads and evidence of liver damage as indicated by elevation of ALT, a serological marker of liver damage. These drugs are typically endowed with marked capacity to suppress serum HBV DNA to an undetectable level in many patients. Additionally, ALT normalization is achieved in most of the patients on oral antiviral drugs (31). Use of oral antiviral drugs has also been demonstrated to prevent or at least delay progression of CHB to LC and even HCC in some patients. In some emergency conditions, nucleoside analogs rapidly suppress HBV DNA and are thus used to prevent hepatic failure (32). At present, oral antiviral drugs are widely used around the world to treat patients with CHB.

Limitations of nucleoside analogs

Oral antiviral drugs are capable of controlling HBV replication, and accordingly, prolonged usage of oral antiviral drug is essential to maintain the drug's therapeutic effects. Treatment interruptions typically induce the rise of HBV DNA and ALT, and this may lead to hepatic failure in many cases. Thus, prolonged use of oral antiviral therapy (33) is a formidable limitation and a major drawback of using oral antiviral drugs for treatment of CHB in developing and resource-constrained countries. Unfortunately, the majority of CHB patients reside in these countries, where drug compliance is a major problem. If the drug is not regularly used, some patients will develop a marked rebound of HBV DNA and elevation of ALT with severe life-threatening liver decompensation (34-35).

Inability to meet the ultimate treatment goal

Various studies and multicenter clinical trials have revealed that complete elimination of HBV is not a feasible treatment goal with the current available treatment. However, occurrence of LC and HCC may be minimized if HBV DNA can be suppressed for prolonged periods. At present, almost all newly developed antiviral drugs have centered their concentrations on clearance of HBsAg from the sera of CHB patients. Paradoxically, clearance of all forms of HBV DNA, especially covalently closed circular DNA (cccDNA) is achieved in only approximately 1% of patients treated with current available oral antiviral drugs.

Possible causes underlying limitations of antiviral treatment

As we have discussed so far, oral antiviral drugs are capable of suppression of replicating HBV DNA and containment of liver damage to some extent: however, the ultimate treatment outcome remains unsatisfactory. Several factors are related to failure of oral antiviral drugs to treat CHB. From the scientific viewpoint, the major limitations of oral antiviral drugs are their inability to destroy cccDNA that can act as a template for replicative HBV DNA (36,37). In addition, the immune modulatory capacity of oral antiviral drugs is limited, and they are not capable of enhancing the immune response of CHB patients.

New, Novel and Innovative therapeutic approaches for containing chronic hepatitis B

As it became impossible to contain HBV-related complications by antiviral drugs, attention has been focused to development of novel therapy for CHB. Studies have revealed that HBV is a not a direct cytopathic pathological condition. Rather immune system of the host plays cardinal roles during acquisition of HBV, progression of diseases and also during genesis of final complications. Based on these realities, immune therapy represents an innovative and novel method of treatment these patients. Various forms immune therapy has been tried in CHB patients for more than 3 decades, however, most of these are not evidence-based and accordingly could not stand the test of time. Thus, it seems to design a proper immune therapy we should clear understandings about two important variables.

What nucleoside analogs (NAs) can accomplish in CHB patients:

1. NAs can control replication of HBV DNA and may induced negativity of HBV DNA
2. In some cases, it may contain progression to LC for considerable time
3. Induces reduction of liver damage

What NAs cannot do it CHB patients:

1. NAs have almost no role on cccDNA and thus it can never eradicate HBV DNA from the infected hosts.
2. NAs represent an infinite mode of therapy. It should be taken for long period or even for life. Thus, it is not patient-friendly, especially for the patients of developing countries
3. Cessation of taking NAs may lead to development of resistant strain and induce severe forms of hepatitis
4. NA is mostly unable to reduce progression to LC and HCC in most patients
5. NAs have very insignificant immune modulatory capacities and this may be the major constrain of using this drug in CHB

Immune therapy for CHB using polyclonal immune modulators

As HBV is not directly cytopathic, it became evident that host immunity plays cardinal role during acquisition, pathogeneses, progression and therapy of CHB patients. Thus, it has been assumed that immune therapy may be one of the options for treating CHB. With this mind, in 1990s, several investigators applied immune modulators, like cytokine and growth factors for treating CHB. However, those could not stand the test of time. In most cases, either there were dominant adverse effects of the drugs and proper amounts of drugs could not be used. In other cases, although some beneficial effect was recoded, long-term benefit could not be recorded (38). These were found by using various interleukins, GMCSF, thymosin, and other polyclonal immune modulators. Unfortunately, there has been no double-blind controlled trial with polyclonal immune modulators for treatment of CHB. Also, phase III clinical trial with these agents

is mostly unavailable in literatures. Thus, the real potential of polyclonal immune modulators has not been substantiated in CHB patients (39-42).

Concept of HBV antigen-specific immune therapy for CHB

Indirect evidences have shown that one of the main problems of CHB patients is related to their inefficiency of inducing and maintaining proper HBV antigen-specific immunity. It has also been shown presence of proper immunocytes in the liver of CHB patients resulted in better outcome of CHB patients compared to those with absence of proper immunocytes.

However, immune therapy of CHB patients with HBV-related antigens have not been accepted for long time. Two major factors mainly hindered the initiation of the concept of HBV antigen-specific immune therapy in CHB patients. These included "the concept of self/non-self" and "neonatal tolerance" theory those have been originally proposed by Burnett and his group in mid'1950s. Burnett received Nobel in Medicine or Physiology in 1960 (43,44). These theories were accepted by most hepatologists and immunologists as CHB patients have been assumed to be tolerant to HBsAg, as it is regarded as self-antigen with variable properties in CHB patients. Neonatal tolerance concept was also strengthened by the finding that transmission of HBV in new born has been a regular matter when the mother was CHB patients. Thus, treatment of CHB patients by HBsAg or other HBV-related antigens those are potentially tolerogenic have not regarded as inducer of immunity in CHB patients. However, concept contrary to these concepts was also given (45).

Preclinical study to overcome immunological tolerance of HBV TM

Although some questions were always kept to these immunological explanations of not using HBV-specific antigens in CHB patients, during late 1980s and 1990s, we planned to assess the immunogenic tolerance concept in the context of chronic HBV infection. An animal model of chronic HBV carrier state, HBV transgenic mice (HBV TM) (46) that expressed HBV DNA, Dane particle, and HBV-related antigens, like HBsAg expressed were used to solve this immunological puzzle. Administration of HBsAg in HBV TM with high levels of HBsAg in sera produced anti-HBs in the sera and also induced HBsAg-specific cellular immune responses. Eventually, we marked a very specific defect of antigen-presenting dendritic cells (DC) for so-called immunological tolerance of HBV TM. A series of animal experimentation using HBsAg alone or with antigen-presenting dendritic cells resulted in HBV DNA negativity in most HBV TM. These studies pointed that a very specific defects of DC was responsible for inability of HBV TM to respond to HBsAg. However, when HBsAg was provide with adjuvant or DC, HBV TM regained their lost immunological properties (473-53). At the same time other investigators including our laboratory also reported similar findings of immune restoration in various animal models of HBV by manipulating the functions of DC or by adding other adjutant that stimulate antigen presenting cells (54-61).

Immune therapy of CHB patients using HBV-related antigens

From 1994, immune therapy using HBsAg has been accomplished in patients with CHB using various protocols and outcomes (63). In general, it was safe for CHB patients and HBsAg-specific immune therapy also exhibited antiviral properties to some extent. Similar clinical trials were accomplished with different protocols in CHB patients (64-68). The efficacy of HBsAg-based vaccine was limited to transient antiviral potentiality and sustained effects were either not reported. In course of time HBV DNA based vaccine was accomplished in HBV TM various regimens of HBsAg-based vaccine or epitope-based therapeutic vaccination were accomplished in CHB patients (69-73).

New and Novel therapy with a therapeutic vaccine containing HBsAg and HBcAg

In the course of the studies, it became evident HBsAg-based immune induction would have limited and transient efficacy as a therapeutic modality. Also, induction and maintenance of HBcAg-specific immunity would be a main target of immune therapy of CHB patients (74). This led us to develop a therapeutic vaccine that contains both HBsAg and HBcAg.

NASVAC (A combination vaccine of two HBVC-related antigens)

NASVAC is a liquid formulation comprising hepatitis B surface antigens (HBsAg) and the nucleocapsid (core, HBcAg) of the hepatitis B virus (HBV), produced by recombinant DNA technology as virus-like particles (Center for Genetic Engineering and Biotechnology, CIGB, Havana, Cuba). NASVAC contains 100 µg of each antigen. This product was produced as GMP grade product (75).

Preclinical study with NASVAC in HBV TM

We have been working regarding induction of innate immunity, translation of innate immunity to adaptive immunity, and proper functioning of regulatory immunity via antigen-presenting dendritic cells using NASVAC in HBV transgenic mice in Japan (76). NASVAC exhibited a highly potent antiviral effect but did not induce hepatitis or liver damages in HBV TM.

Clinical studies with NASVAC in CHB patients

NASVAC was also safe in normal human volunteers, in a phase I trial accomplished in Cuba (77). A phase I/II clinical trial with NASVAC in Bangladesh in patients with chronic hepatitis B also exhibited production of cytokines of innate immunity (78). Also, usage of NASVAC resulted in both HBV DNA negativity and ALT normalization in more than 50% patients. Finally, a phase III clinical trial with NASVAC in chronic hepatitis B patients with liver damages demonstrated NASVAC was capable to induce HBV DNA negativity for sustained period in more than 75% patients. Also, normalization of ALT was seen in about similar percentage of patients by NASVAC (79). Recently, safety and efficacy of NASVAC has been confirmed in normal individuals and patients with chronic hepatitis B in Japan (80-83). Also, NASVAC has found to have anti-fibrotic potentiality in follow up study of phase III patients with CHB. NASVAC has been found to attain functional cure in some CHB patients by conducting

clinical trial in Japan.

Roadmap to innovative therapy for CHB

CHB is progressive in nature. From public health point of view, all patients of CHB are living and permanent reservoir of virus and they are basically responsible for transmission of the virus to healthy persons. Accordingly, if these huge population of people are not treated properly, "ELIMINATION of HEPATITIS by 2030. WHO goal" would remain a dream for century. Definitely, prevention approaches should be undertaken in full swing for ensuring safe blood and body fluids usage. Vaccination will also be a critical factor to contain further progression of HBV.

However, millions of CHB patients should be treated. The available treatment options are not satisfactory from various point of views that include scientific as well as social and economic spectrums. To treat these patients, we need a finite and safe therapy. It should be cheap and patient-friendly. In order to ensure such a therapy, the nature of therapy should be evidence-based and effective. Thus, all sorts of innovative therapy should be searched for. We have concentrated towards development of immune therapy by using HBV-related antigens and some promising data have been retrieved from phase I/II/and II clinical trials. Various modifications of the protocols are now going on. However, this may not be only innovative approach. We and others have been attempting to explore other areas of development of innovative therapies for CHB by altering dose, duration, nature of antigens, combination of antigens, and manipulating various adjuvants. However, initiating a clinical trial is not an easy job and thus several prospective therapeutic approaches could not be finally accomplished. Several investigators have cited several concepts and proper screening of these proposals for multicenter trial to evolve new and innovative therapy for CHB is a top priority for modern days science (84-110). All sorts of concepts should be properly evaluated form the point of safety and scientific evidence and thus a better regimen of therapy may be developed for CHB.

Acknowledgments:

The study was supported in part by a grant-in-aid from Japan Agency for Medical Research and Development (AMED) to Sheikh Mohammad Fazle Akbar (Grant number 20fk0310103h1904).

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