VIROLOGICA SINICA, August 2010, 25 (4):294-300
DOI 10.1007/s12250-010-3138-9

Development of Novel Therapeutics for Chronic Hepatitis B*

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Abstract: Chronic infection of hepatitis B virus (HBV) presents one of the serious public health challenges worldwide. Current treatment of chronic hepatitis B (CHB) is limited, and is composed of interferon and nucleoside/nucleotide reverse transcriptase inhibitors (NRTI). Interferon is poorly tolerated and is only responsive in a small fraction of CHB patients and NRTIs often face the problem of emergence of drug resistance during long-term treatment. The current treatment of CHB can be improved in several ways including genotyping mutations associated with drug resistance before treatment to guide the choice of NRTIs and suitable combinations among NRTIs and interferon. It is important to continue research in the identification of novel therapeutic targets in the life cycle of HBV or in the host immune system to stimulate the development of new antiviral agents and immunotherapies. Several antiviral agents targeting HBV entry, cccDNA, capsid formation, viral morphogenesis and virion secretion, as well as two therapeutic vaccines are currently being evaluated in preclinical studies or in clinical trials to assess their anti-HBV efficacy.

Key words: Hepatitis B virus (HBV); Treatment; Chronic infection; Antiviral

INTRODUCTION

Hepatitis B Virus (HBV) is the causative agent of viral hepatitis B. Transient infection of HBV may result in acute hepatitis and, in rare cases, fulminant hepatitis. Chronic infection with HBV presents one of the most serious public health challenges worldwide, and often leads to chronic liver injury, cirrhosis and Hepatocellular carcinoma (HCC) [7, 21]. Fortunately, vaccination programs against HBV infection show long-term protection against infection in more than 90% of healthy people and a high efficiency in blocking vertical transmission [21].

In contrast, current treatment for Chronic hepatitis B (CHB) is limited. Two classes of therapeutics are

Received: 2010-03-15, Accepted:2010-05-30
* Foundation items: This work was supported by "973" project (2005CB522902), Grand Science and Technology Special Project (2008ZX10002-010, 015) and Shanghai Municipal Government (8410706800).
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available, immune modulators and nucleoside/nucleotide reverse transcriptase inhibitors (NRTI). The former includes conventional and pegylated interferon that enhances host immune defense against viral infection. However, the response rate of interferon treatment is only about 20%-30% among CHB patients [16]. Furthermore, the development of serious side effects limits its tolerability. The latter is composed of nucleotide and nucleoside analogues that act primarily by blocking reverse transcription of the pre-genomic RNA to HBV DNA. Five drugs that belong to the class of NRTI [Lamivudine (LMV), Adefovir dipivoxil (ADV), Entecavir (ETV), Telbivudine (LdT) and Tenofovir disoproxil fumarate (TDF)] have been approved for the treatment of CHB in many countries and regions of the world [6]. However, a major drawback of NRTIs is the emergence of drug resistance. Several mutations in the polymerase gene of HBV associated with drug resistance have been confirmed and mutations associated with LMV resistance have been found to confer cross-resistance to some of the NRTIs [2].

The current treatment of CHB can be improved in several ways. First, as viral mutations associated with drug resistance have been confirmed, genotyping these mutations before treatment should provide valuable information to guide the choice of NRTIs. Second, suitable combinations among NRTIs should reduce the chance that resistance will develop and thus achieve sustained reductions in viral load[11]. The expected introduction of new NRTIs such as emtricitabine and clevudine will further expand the number of combinations. In addition, combination of pegylated interferon with NRTIs may result in better interferon tolerability and treatment efficacy than monotherapy.

Nevertheless, continued efforts should be paid to elucidating the mechanisms of viral infection, replication and pathogenesis to identify new therapeutic targets. Presently, the life cycle of HBV is only partially understood. HBV gains entry into the cell by binding to an unknown receptor(s) on the cell surface and enters it by endocytosis. The viral genome, in the form of partially double stranded circular DNA, has to be transferred to the cell nucleus and transformed into covalently closed circular DNA (cccDNA) that serves as a template for transcription of four viral mRNAs. The largest mRNA, pregenomic RNA (pgRNA), is used to translate core protein and polymerase. The newly produced polymerase preferentially binds its own pgRNA and is incorporated by core proteins into a capsid where the viral DNA replication proceeds. Upon completion of DNA replication, the capsid is either encapsulated by three viral surface proteins or returns to the nucleus to produce more copies. Progeny virions are secreted from the cell [20]. During HBV infection, the host immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), causes most of the liver injury associated with HBV infection by killing infected cells and by producing antiviral cytokines such as interferon and tumor necrosis factor [18].

As our knowledge pertaining to the life cycle and pathogenesis of HBV advances, novel therapeutic targets for the treatment of CHB will be identified. In this article, we review recent progresses in the development of antiviral agents directed against several targets in the life cycle of HBV as well as a pair of therapeutic vaccines that stimulate antiviral immune response.

TARGETS ANTIVIRAL AGENTS

Entry

The entry of HBV into the cell constitutes a potential target for the inhibition of viral infection and spread. The PreS1 domain of the large envelope protein is known to be crucial for HBV infectivity. In particular, the residues within 21-47 (10-36 in genotype D) amino acids have been considered as the HBV attachment site. The N-terminal myristoylation of PreS1 is also critical for HBV infectivity. With this knowledge, Glebe *et al* and Gripon *et al* designed acylated PreS1-derived peptides to inhibit HBV entry into cultured primary tupaia hepatocytes and HepaRG cells respectively [8, 9]. Petersen *et al* investigated the inhibitory efficacy of PreS1-derived peptides in a mouse model of HBV infection [17]. They showed that the acylated peptides could prevent HBV or WMHBV (wooly monkey HBV) infection of immunodeficient uPA mice repopulated with primary human or tupaia hepatocytes. The acylated peptides could bind to the tupaia hepatocytes and presumably function by addressing the putative HBV receptor. As the HBV receptor may have other physiological functions in the body, whether its occupation by the acylated peptide will result in any adverse effects should be carefully investigated in animal models and clinical studies.

By screening a phage display random peptide library with the PreS protein, we obtained a 5-aa peptide which could bind to PreS1 [21-47] and viral particles [4]. This peptide was able to inhibit the attachment of viral particles to HepG2 cells (unpublished results). In contrast to the acylated PreS1-derived peptides, the PreS1-binding peptide is thought to inhibit virion-binding to the cell by masking the HBV attachment site. The inhibitory efficacy of the PreS1-binding peptide on HBV infectivity *in vitro* and *in vivo* warrants further investigation. Notably, entry inhibitors alone will be of little help for the treatment of CHB though they might be applicable for the prevention of HBV infection in situations such as before liver transplantation or during acute HBV infection. Nevertheless, it is expected that entry inhibitors will reduce the chance of HBV infection of new cells when they are combined with other antivirals such as NRTIs in the treatment of CHB.

cccDNA

cccDNA plays a central role in the life cycle of HBV where it drives viral transcription and progeny virus production. cccDNA is not the direct target of NRTIs and is the source of HBV reemergence when antiviral therapy ceases. cccDNA is stable, and is chemically and maybe structurally, indistinguishable from the host cell chromosome, which makes specifically targeted cccDNA a difficult task.

Using duck HBV (DHBV) as a model, Zimmerman *et al* designed several zinc finger proteins (ZFP) that were used to bind to the DHBV enhancer region [27]. Two of the ZFPs were tested for the inhibition of viral replication in longhorn male hepatoma cells transfected with an over-length replication competent DHBV genome. In the presence of each ZFP, viral transcription, protein levels and progeny virus genomes were significantly reduced. Thus, customized ZFPs that can bind to the DHBV DNA sequence possess potential antiviral activity.

Pregenome encapsulation and capsid formation

Viral pregenome encapsulation and capsid formation also represent potential targets. The phenylpropenamide derivatives AT-61 and AT-130 are non-nucleoside

analogue inhibitors of HBV replication [3, 10]. These compounds were found to be highly selective and potent against HBV replication in human hepatotoma cell lines. They likely interfere with one of the steps between the synthesis of viral RNA and the packaging of pregenomic RNA into immature core particles. There were no significant differences in sensitivity between wild-type and LMV-resistant HBV. Furthermore, AT-61 acted synergistically with LMV to inhibit HBV replication.

Heteroaryldihydropyrimidines (HAP) were discovered as another class of highly potent non-nucleoside inhibitors of HBV nucleocapsid formation. HAPs reduced the half-life of the HBV core protein in HepG2.2.15 cells by specific interaction with this protein [5]. One of the molecules, Bay 41-4109, was shown to prevent HBV infection in a transgenic HBV model by reducing viral DNA in the liver and the plasma with an efficacy comparable to LMV [17].

Viral morphogenesis and secretion

Trimming of the *N*-glycans attached to the envelope proteins of HBV is required in different steps of the viral life cycle. One function of *N*-glycans is to assist in the folding of glycoproteins by mediating interactions of the endoplasmic reticulum (ER) chaperone proteins calnexin and calreticulin with nascent glycoproteins. Inhibition of the host α-glucosidases can prevent glycan modification and results in misfolding of the HBV envelope proteins and prevention of HBV secretion. It has been demonstrated in tissue culture and in the woodchuck model of chronic HBV infection that the imino sugar inhibitors of α-glucosidases, N-butyl-deoxynojirimycin (N-butyl-DNJ) and N-nonyldeoxynojirimycin (N-nonyl-DNJ), can have antiviral activity [1, 12].

Interestingly, N-nonyl-DNJ possesses potent antiviral activity at concentrations that do not inhibit α-glucosidases. A derivative of N-nonyl-DNJ (Nnonyl-deoxygalactojirimycin [N-nonyl-DGJ]) that lacked the ability to inhibit α-glucosidase inhibited HBV secretion, suggesting that the mechanism of the anti-HBV action of the imino sugar involves a mechanism that more complicated than glucosidase inhibition [15].

VACCINES FOR IMMUNE RESPONSE

Innate immune response

HBV is viewed as a stealth virus that does not elicit innate immunity *in vivo* [18]. This assumption has been challenged *in vitro* by several studies, which may lead to the development of novel antiviral agents that modulate the innate response.

Toll-like receptors (TLRs) are key components of the innate immune system and TLR2 has been shown to be involved in the immunopathogenesis of HBV infection *in vivo* [19]. Thompson *et al* used IL-1beta and Pam-2-Cys, a synthetic TLR2 ligand, to stimulate HepG2 cells transduced with recombinant HBV baculoviruses and Huh-7 cells transiently transfected with HBV cDNA. They observed the inhibition of HBV DNA replication in HepG2 cells and the nucleocapsid formation in Huh7 cells [22].

The HepaRG cell line is permissive to HBV infection. HBV infection elicits a strong and specific type I IFN antiviral response in HepaRG cells. Interestingly, viral replication was rescued when IFN-β action was neutralized by antibodies or RNA interference of the type I IFN receptor. Gene expression analyses showed that IFN-β and other IFN-stimulated genes were up-regulated in HepG2 and HepaRG cells transduced with recombinant HBV baculoviruses [13].

CpG oligodesoxynucleotides (CpG ODN) are synthetic agonists of Toll-like receptor 9. Vincent *et al* investigated the antiviral benefit of combining LMV with CpG in HepaRG and HepG2 cells transduced with recombinant HBV baculoviruses and differentiated HepaRG cells infected with HBV. CpG-induced cytokines strongly inhibited HBV replication, as well as HBsAg and HBeAg secretion. Notably, in transduced HepaRG cells, the combination of LMV with CpG dramatically reduced the 50% effective concentration of LMV. Therefore, LMV with CpG represents a promising combination to suppress HBV replication [23].

Adaptive immune response

Immune complexes composed of yeast-derived HBsAg and antibodies (YIC) have been developed as a therapeutic vaccine for the treatment of CHB. The safety of this vaccine has been demonstrated among healthy adults and CHB patients in phase I and phases IIa trials [26]. In a phase IIb trial, Xu *et al* evaluated the efficacy and dosage of this vaccine in 242 HBeAg-positive CHB patients [25]. The patients were immunized with six injections of either 30 μg, 60 μg of YIC or alum adjuvant as placebo at four-week intervals. HBV markers and viral DNA were monitored during immunization and 24 weeks after the completion of immunization. The primary endpoint was defined as loss of HBeAg, or presence of anti-HBe antibody or suppression of HBV DNA, while the secondary endpoint was both HBeAg seroconversion and suppression of HBV DNA. Though statistical differences among the three groups were not reached, a significant difference in group effects was found between 60 μg YIC and the placebo groups in terms of the primary endpoint, and a late HBeAg seroconversion was shown in the 60 μg YIC regimen. The therapeutic efficacy of YIC in CHB patients will need further evaluation in the ongoing phase III trial.

In a phase I clinical trial, Mancini-Bourgine *et al* immunized 10 HBeAg positive patients who had shown a LMV breakthrough with a DNA vaccine expressing HBV small (S) and middle (preS2+S) envelope proteins [14]. Following three injections of the vaccine, IFN-producing T-cells specific for the preS2 antigen were detected in 50% of patients and IFN-producing T-cells for the S antigen were found in all of the patients. Anti-preS2 antibodies and HBeAg seroconversion occurred in two patients. These results suggest that DNA vaccination can restore or activate T-cell responses in CHB patients.

CONCLUSION

Improvement of current treatment through genotyping HBV drug resistant mutants before treatment and combination therapy with available NRTIs and interferon should be able to achieve a better efficacy and reduce drug resistance. On the other hand, identification of novel therapeutic targets in the life cycle of HBV or in the host immune system should stimulate the development of new antiviral agents and immunotherapies. Currently, several antiviral agents and therapeutic vaccines are being evaluated in preclinical studies or in clinical trials to assess their anti-HBV efficacy.

Acknowledgement

YH Xie is supported by “973” project (2005CB 522902), Grand Science and Technology Special Project (2008ZX10002-010, 015) and Shanghai municipal government (8410706800).

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