Viral hepatitis B: emerging therapies

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만성 B형간염에서 항바이러스치료의 단기 치료목표는 간염바이러스의 완전제거(clearance of serum HBV DNA/nucleus HBV cccDNA) 및 면역획득(seroconversion of HBsAg to antiHBs)이다. 현재 사용되고 있는 B형간염 바이러스 치료제인 경구용 뉴클레오시(티)드와 주사제 인터페론(페그인터페론) 알파는 그동안 지속적으로 진화하여 바이러스적, 혈청학적, 생화학적, 그리고 조직학적 반응에서 놀랄만한 치료효과를 보여왔으나 상기한 치료목표에는 미치지 못하고 있다. 최근 기존의 치료표적을 달리하여 B형간염 바이러스 복제의 각 단계를 제어할 수 있는 항바이러스제 뿐만 아니라, B형간염 바이러스의 전사 후 과정에 관여하여 바이러스 유전자발현을 차단할 수 있는 RNA (small RNA) 및 인체의 면역체계를 강화할 수 있는 면역조절제 등의 개발을 통해 만성 B형간염을 치료하려는 노력이 활발히 진행되고 있다. 이에 현재 진행되고 있는 만성 B형간염에 대한 항바이러스제 및 면역조절제 등을 알아보고 항바이러스 효과와 함께 향후 B형간염의 치료방향을 전망해 보고자 한다.

색인단어: B형간염 바이러스, 만성 B형간염, 바이러스 복제, 항바이러스제, 치료

Introduction

Management guidelines of chronic hepatitis B virus (HBV) infection have been steadily updated by recommendations from the American Association for the Study of Liver Diseases,[1] European Association for the Study of the Liver,[2] the Asian Pacific Association for the Study of the Liver,[3] and the National Institute for Health and Care Excellence.[4] Current treatment strategies for patients with chronic hepatitis B (CHB) are based on the followings: interferon alpha-based immunomodulation and nucleos(t)ide analogue-based inhibition of viral replication. In the last decade, despite remarkable therapeutic advance in anti-HBV agents based on nucleos(t)ide analogues and interferon-alpha in aspects of virological, biochemical, and histological assessment,[1-4] complete HBV eradication, e.g. clearance of HBV DNA/cccDNA in the serum/liver tissue and seroconversion of

HBsAg to anti-HBs, from the host is still far from the present antiviral era. Therefore, most of hepatologists and virologists feel keenly the necessity of advent of new anti-HBV agents in a different manner than current therapies. So this review focuses on the emerging antiviral therapeutics including novel anti-HBV regimens and their related targets involved in the inhibition of virus replication or the modulation of host immune systems for chronic HBV infection.

Hepatitis B virus replication cycle

HBV is an approximately 3.2 kb-sized partially double-stranded relaxed circular DNA (ds-rcDNA) virus with a hepatotropic property, belonging to the family *Hepadnaviridae*.[5] The replication cycle of HBV has been almost elucidated.[6,7] Briefly, the life cycle of HBV begins when HBV virion, Dane particle, attaches to the cell surface to enter into target hepatocytes, mediated by the binding of the pre-S1 region of viral envelope protein to the cel-

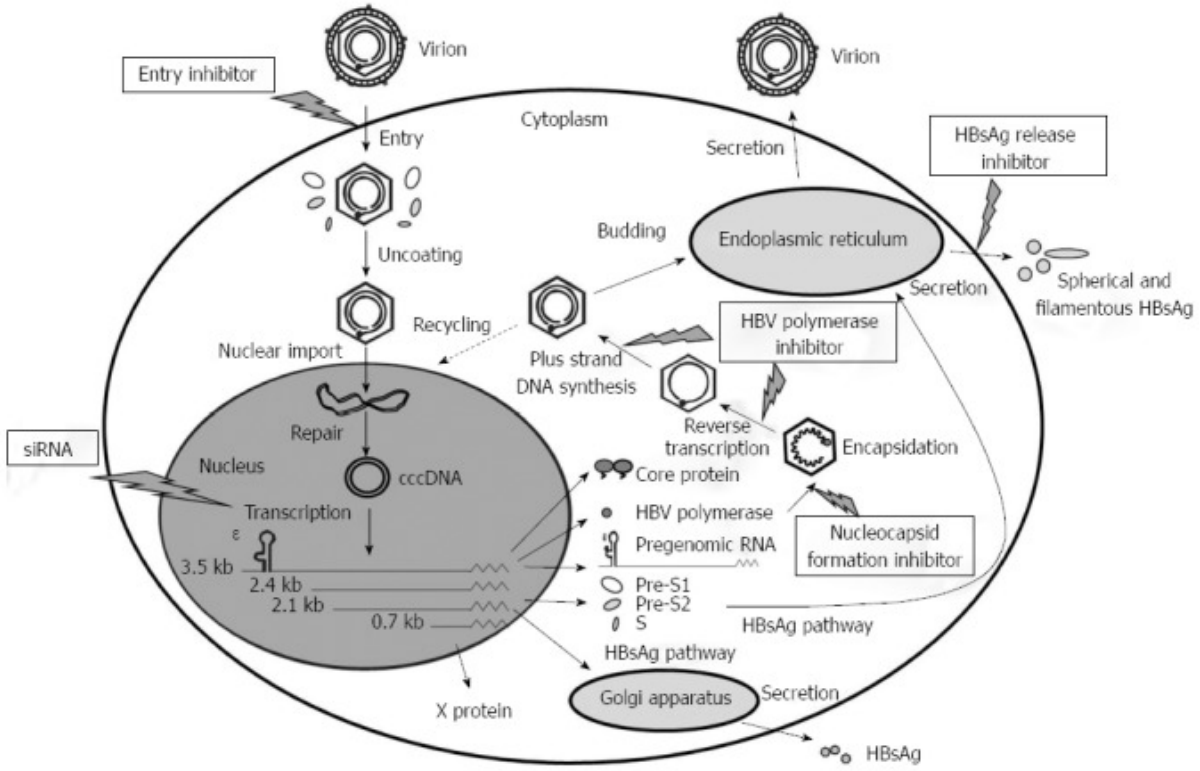

Figure 1. Hepatitis B virus replication cycle and relevant targets.[7]

lular receptor sodium taurocholate cotransporting polypeptide (NTCP),[8] a multiple transmembrane transporter. Following NTCP-mediated viral entry, the viral nucleocapsid is uncoated and transported into the nucleus, where viral rcDNA is transformed into covalently closed circular DNA (cccDNA) via repairing process,[9] followed by transcription of the cccDNA into the four viral RNA transcripts: 3.5 kb precore mRNA and pregenomic RNA (pgRNA); 2.4 kb large surface mRNA; 2.1 kb middle and small surface mRNA; and 0.7 kb X mRNA. Each mRNA is translated to produce a corresponding viral protein: precore protein/e antigen, core protein/c antigen, and polymerase; envelope protein/s antigen (pre-S1 and pre-S2/S); X protein/x antigen. In the cytoplasm, pgRNA serves as the template for the reverse transcription, leading to negative-strand and then positive -strand DNA synthesis within the viral nucleocapsid.[10] After budding into the endoplasmic reticulum and Golgi apparatus, the nucleocapsid with dsDNA acquires an HBsAg-containing envelope and is released to infect neighbouring hepatocytes through the secretory pathway. On the other hand, the nucleocapsid returns back into the nucleus for conversion to cccDNA and its amplification responsible for viral persistence in host cells.[11,12] Figure 1 shows HBV replication cycle and relevant targets .

Virus replication cycle-related therapeutics

The complex replication steps during HBV life cycle of cellular virus entry, uncoating, transformation to cccDNA, transcription, encapsidation, assembly, reverse transcription, and virus particle secretion as progeny virion or recycling to amplify cccDNA may be all potential targets for the development of novel therapeutics in chronic HBV infection. The representative new anti-HBV approaches related to virus replication cycle are currently undergoing in vitro/in vivo preclinical evaluations or early clinical trials (Table 1).

Myrcludex B, a synthetic lipopeptide consisting of the pre-S1 domain structure of the envelope protein, is a prototype of virus entry inhibitor.[13-15] This peptide efficiently blocked the intrahepatic virus spread from HBV-infected mice along with the inhibition of cccDNA amplification in hepatocytes as well.[16,17] A phase I study showed a successful administration of single ascending doses of Myrcludex B with well-tolerated and safety profiles in healthy volunteers. Cyclosporin A is an immunosuppressive drug clinically used after organ transplantation or for treatment of antoimmune diseases. Recently, cyclosporine A inhibited HBV entry by cyclophilin-independent interference with the NTCP receptor, in which the interaction between the drug and the viral receptor may be direct and overlap with a functional binding site of the pre-S1 domain mediating viral entry.[18,19] Thereafter cyclosporine A derivatives with minimal influence to host immune system may provide a new anti-HBV strategy targeting NTCP as a cellular factor.

Table 1. Emerging therapeutics and action mechanisms for chronic hepatitis B virus infection

Therapeutics	Compounds/Targets	Action mechanisms
Replication cycle-related		
Myrcludex B	Synthetic lipopeptide with PreS1 domain	Virus entry inhibition
Cyclosporin A	Immunosuppressant with NTPC blockade	Virus entry inhibition
MC2791/MC3119	Epigenetic modifier of cccDNA	cccDNA inhibition
Zinc finger proteins	DAN cleavage enzymes	cccDNA inhibition
CCC-0975/CCC-0346	Inhibitor of cccDNA formation	cccDNA inhibition
Besifovir (LB80380)	Acyclic nucleotide phosphate	DNA polymerase inhibition
Tenofovir alafenamide	Acyclic nucleotide phosphate	DNA polymerase inhibition
CMX157	Hexadecyloxypropyl conjugate of tenofovir	DNA polymerase inhibition
AGX-1009	Prodrug of tenofovir	DNA polymerase inhibition
Bay 41-4109	Member of HAP family	Nucleocapsid inhibition
GLS4	Member of HAP family	Nucleocapsid inhibition
AT-61/AT-130	Molecule of phenylpropenamide family	Nucleocapsid inhibition
REP 9 AC	Amphipathic DNA polymer	Inhibition of sAg secretion
RNAi-based		
ARC-520	siRNAs targeting HBV transcription	RNAi-based gene silencing
siRNA/ddRNA	siRNA targeting HBV NLS/HBV PRE	RNAi-based gene silencing
Immune-mediated		
GS-9620	TLR-7 agonist	TLR-7 signaling activation
GI013020	Recombinant product with HBV antigens	HBV-specificTcell activation
GS-7446/DV-601	Recombinant HBV antigens	Therapeutic vaccines

NTCP, sodium taurocholate cotransporting polypeptide; HAP, heteroaryldihydropyrimidine; siRNA, small interfering RNA; RNAi, RNA interference; NLS, nuclear localization signal; PRE, post-transcriptional regulatory element; TLR, toll-like receptor

HBV cccDNA seems to be the most important target for the development of antiviral compounds because cccDNA, a main template of virus replication with structurally stable frame, is a principal offender of viral persistence in host cells. The followings are several cccDNA inhibitors: hSirt1/2 activator MC2791 and JMJD2 inhibitor MC3119/epigenetic regulators of cccDNA[20]; zinc finger proteins/DNA cleavage enzymes[21]; CCC-0975 and CCC-0346/cccDNA formation inhibitors as sulfonamide compounds.[22] However, since the studies for the development of these theoretically attractive compounds targeting cccDNA have been performed in the level of cell/tissue culture, further advance of investigational study to preclinical and clinical stage might be necessary.

HBV DNA polymerase is the most popular target for current antiviral agents despite the presence of nucleos(t)ide-related clinical drawbacks such as virus mutation, drug resistance, rebound phenomenon, adverse events, and vague treatment period, etc. besifovir (LB80380),[23-26] tenofovir alafenamide (GS-7340),[27,28] CMX157,[29] and AGX-1009 are new nucleos(t)ide analogues to inhibit viral DNA polymerase, being under clinical trials phase I-III showing favorable safety profiles and clinical outcomes. Lagociclovir valactate (MIV-210), famciclovir, and pradefovir were suspended from the clinical investigations.[30]

Nucleocapsid assembly is initiated by the interaction of the HBV polymerase with the pgRNA in the cytoplasm, triggering encapsidation by the core protein to form the viral nucleocapsid.[10] Heteroaryldihydropyrimidines (HAPs), a family of nucleocapsid assembly effectors, have been identified as potent non-nucleosidic inhibitors of HBV replication in preclinical studies.[31-33] Bay 4104109 and[34-36] GLS4,[37] members of HAP family, inhibits HBV replication by inducing inappropriate assembly. Phenylpropenamide derivatives, AT-61 and AT-130, inhibit HBV replication at the level of viral RNA packaging and also inhibit the replication of wild-type and lamivudine-resistant strains of HBV in vitro.[38-40]

HBsAg secretion is known to contribute to the suppression of host immune system as well as the infection of neighbouring hepatocytes. REP 9 AC,[41] an amphipathic DNA polymer, inhibited HBsAg secretion from infected hepatocytes in patients with chronic HBV infection, resulting in recovery of innate immunity and cytotoxic T cell response in phase I clinical study.

RNA interference-based therapeutics

RNA interference (RNAi) is a cellular gene-silencing mechanism in which double-stranded RNA (dsRNA) induces the post-transcriptional gene-knockout of corresponding homologous mRNA of host genes.[42] Since Elbashir SM et al[43] demonstrated that duplexes of 21-nucleotide RNAs mediate RNAi in cultured mammalian cells, a number of studies have used this gene-silencing mechanism as a tool of gene-knockout techniques for therapeutic application related to inhibiting infectious and genetic diseases as well as the functional study of host genes. There are two classes of small RNAs, e.g. micro RNAs (miRNAs) and small interfering RNAs (siRNAs) or small hairpin RNAs (shRNAs) effective for the regulation of gene expression in a sequence-specific manner.[44]

HBV has been considered as a promising candidate of potentially treatable viruses for RNAi-based therapeutic approach. After targeting HBV nuclear localization signal (NLS), siRNA showed inhibitory effect on viral replication and antigen expression in HBV-transgenic mice injected with siRNA expression vector, especially markedly inhibiting HBV cccDNA amplification.[45] Furthermore, this siRNA-induced inhibitory effects was stronger in combination of siRNAs compared with individual use of each siRNA.[46] Wooddell CI et al[47] presented that siRNAs targeting different sites of HBV resulted in multilog repression of viral RNA, viral DNA, and protein with long duration of effect using a new siRNA delivery system. ARC-520, designed to reduce the expression and release of viral particles by RNAi mechanism, suppressed the expression of HBV DNA, HBsAg, and HBeAg in a HBV-infected chimpanzee with a high viral titer.[48] ARC-520 entered a clinical trial with the subjects of healthy adult volunteers in whom it was intravenously administrated. The results from this phase I trial would

be spread and the further entrance to phase II trial is expected to advance in a moment. Recently, three siRNA target sites were selected on HBV post-transcriptional regulatory element (HBV PRE), a conserved RNA region of HBV, through different siRNA designing programs. Corresponding functional siRNAs to these target sites could drastically decrease the expression of HBV transcripts (core, surface, and X) and surface protein without interferon response and cell cytotoxicity in HepG2 cell lines.[49] The development of anti-HBV agents as novel RNAi-based therapeutics would be anticipated in the future.

Immune-mediated therapeutics

Persistent HBV presence in liver makes the host's innate immune responses weak and dull to sense HBV during infection, resulting in the defect of adaptive immune responses for anti-HBV activity. For examples, HBV inhibits Toll-like receptors (TLRs)-mediated antiviral signaling in hepatocytes.[50] TLRs known to be components of the innate immune system are pathogen recognition receptors serving as a first defence mechanism against invading pathogens.[51] In the chronic HBV infection, the immunostimulative activities, e.g. the production of interferon-ahpha and other cytokines/chemokines, up-regulation of interferon-stimulated genes (ISGs), and activation of natural killer cells (NK cells) and CD8 cytotoxic T lymphocytes, are suppressed, while the immunoinhibitory activities, the expression of programmed death-1 (PD-1)/PD-L1 and cytotoxic T lymphocyte antigen-4 (CTLA-4)/B7-1, and activation of regulatory T lymphocytes (Treg), are up-regulated.[52,53]

GS-9620, an oral selective agonist of TLR-7, activated TLR-7 signaling in immune cells of chimpanzees with chronic HBV infection to induce clearance of HBV-infected hepatocytes, resulting in long-term suppression of serum and liver HBV DNA, the decrease of serum levels of virus proteins (HBsAg and HBeAg) and numbers of HBV antigen-positive hepatocytes, and induction of immunostimulative activities.[54] A Phase I study revealed tolerable safety, pharmacodynamic activity, and favorable antiviral response of GS-9620.[55] Lan P et al[56] presented that HBV-induced hepatocyte-intrinsic immune tolerance was reversed when a dually functional vector containing both an immunostimulating ssRNA and an HBx-silencing shRNA were administered, and the systemic anti-HBV adaptive immune responses, including CD8 T-cell and anti-HBs antibody responses, were efficiently recovered in an interferon-alpha- and TLR-7-dependent manner. GI-13020, a recombinant yeast-based biologic product to express a chimera of HBV X, S, and C antigens, is also immunogenic to induce HBV-specific T cell responses.[7] Further progression of preclinical to clinical stage of this product would be anticipated in the near future, along with combination effects with HBV antivirals to improve HBsAg seroconversion. GS-4774[57] and DV-601[58] are recombinant therapeutic vaccines with HBV antigenicity (X, large S, and Core) to promote

the resolution of chronic HBV infection through the stimulation of specific cytotoxic T-lymphocyte and B-cell antibody response against HBV antigens. In phase I studies, these vaccines appear to be safe and well-tolerated for the treatment of CHB as well as to be evident for virological response.[58)]

Perspectives

Complete HBV eradication and immunity acquisition, clearance of HBV DNA/cccDNA in the serum/liver and seroconversion of HBsAg to anti-HBs are the most important end-point of antiviral therapy for chronic HBV infection. In the present, emerging antivirals focused on virus replication cycle-related targets, RNAi-based targets, and immune-mediated targets should be worth of attention although most of studies are under pre-clinical and early clinical investigation (Table 1). The treatment strategy with these new challengeable agents would be steering the current anti-HBV stream for the prevention of liver disease progression to cirrhosis, hepatic decompensation, and hepatocellular carcinoma, subsequently improving the quality of life and survival in patients with chronic HBV infection.

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