Current and Novel Therapies for Hepatitis B Virus Infection

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Abstract: Many therapeutic reagents for hepatitis B virus infection have established efficacy in goals such as alanine aminotransferase normalization, hepatitis B virus DNA suppression, HBeAg seroconversion, histological improvement, and reduce disease progression. However, it is not established that the efficacy of these reagents for the long-term survival and prevention of hepatocellular carcinoma although recent meta-analyses have also shown antiviral therapy to be efficacious. This article reviews the current status and innovative new options for antiviral therapy for hepatitis B and also discusses the various mechanisms of action for each drug, the results of clinical studies for each therapy, and the problems yet to be solved with respect to hepatitis B treatment.

Keywords: Hepatitis B virus, therapy, host cellular target, life cycle, interferon, nucleoside/nucleotide analog, adverse effects, and drug resistance.

INTRODUCTION

With more than 400 million carriers worldwide, hepatitis B virus (HBV) infection is a major public health concern [1]. Of the total population of HBV carriers, Asia and the Western Pacific account for approximately 75% of all carriers, according to reports from the World Health Organization [1], although there has been a marked increase in the number of cases of chronic hepatitis B in Europe and the United States due to changes in immigration patterns [2].

Persistent HBV infection can lead to chronic hepatitis, cirrhosis, hepatic decompensation and hepatocellular carcinoma. These complications cause up to 5000 people each year to die in the United States [3]. Although hepatitis B vaccination has become increasingly more common, the universal vaccination of children and young adults is still lagging [4]. Thus, the development of more effective antiviral drugs is of imperative. However, due to the variety of available drugs and the constantly changing guidelines, the management of HBV can be complex.

Interferon (IFN) or lamivudine has been shown to stop disease progression, reduce the complications of cirrhosis, improve hepatic fibrosis and increase survival [5-8]. Recently, the serum HBV DNA level is identified as a risk factor for liver cirrhosis and hepatocellular carcinoma, regardless of serum alanine aminotransferase (ALT) levels and hepatitis B e antigen (HBeAg) status [9,10]. Thus, reduction or disappearance of the serum HBV DNA level is of great importance in improving prognosis in HBV infection. However, current antiviral therapies are generally insufficient to eradicate HBV infection because of the inability to clear covalently closed circular DNA (cccDNA).

This article summarizes the current treatment options and the various novel antiviral reagents for hepatitis B infection, including HBV life cycle inhibitors and host cellular target-directed antiviral reagents.

HBV Life Cycle and the Potential Drug Targets

The HBV life cycle is schematically illustrated in Fig. (**1**). The HBV life cycle starts when the infectious viral particles, HBV virions attach and enter the host through an interaction of the host cell membrane and viral envelope proteins. The precise targets and mechanisms for viral entry are unclear. A process of receptor-mediated endocytosis rather than membrane fusion is assumed to be involved [11]. After viral entry, HBV virions are internalized, uncoated and viral genome translocates to the hepatocyte nucleus by mechanisms that have not yet been completely clarified [12,13]. When the viral genome enters the nucleus, the partially double-stranded relaxed circular viral DNA (rcDNA) is converted into cccDNA by an incompletely elucidated mechanism [12].

HBV cccDNA is a unique episomal replicative intermediate responsible for persistent infection of hepatocytes. The nucleus based cccDNA enables stable production of progeny that is not lost during cell division [12,14]. It continues throughout the course of chronic hepatitis B, even in patients with a loss of serum HBV DNA [15]. cccDNA functions as the transcriptional template for host RNA polymerase Ⅱ. This results in the formation of the viral minichromosome, the major template of HBV that is used for the transcription of all the viral messenger RNAs (mRNAs). All the viral mRNAs are transported to the cytoplasm and translated to produce the viral proteins, namely hepatitis B core antigen (HBcAg) or nucleocapsid protein; the soluble and secreted HBeAg; the polymerase protein; the viral envelope proteins,

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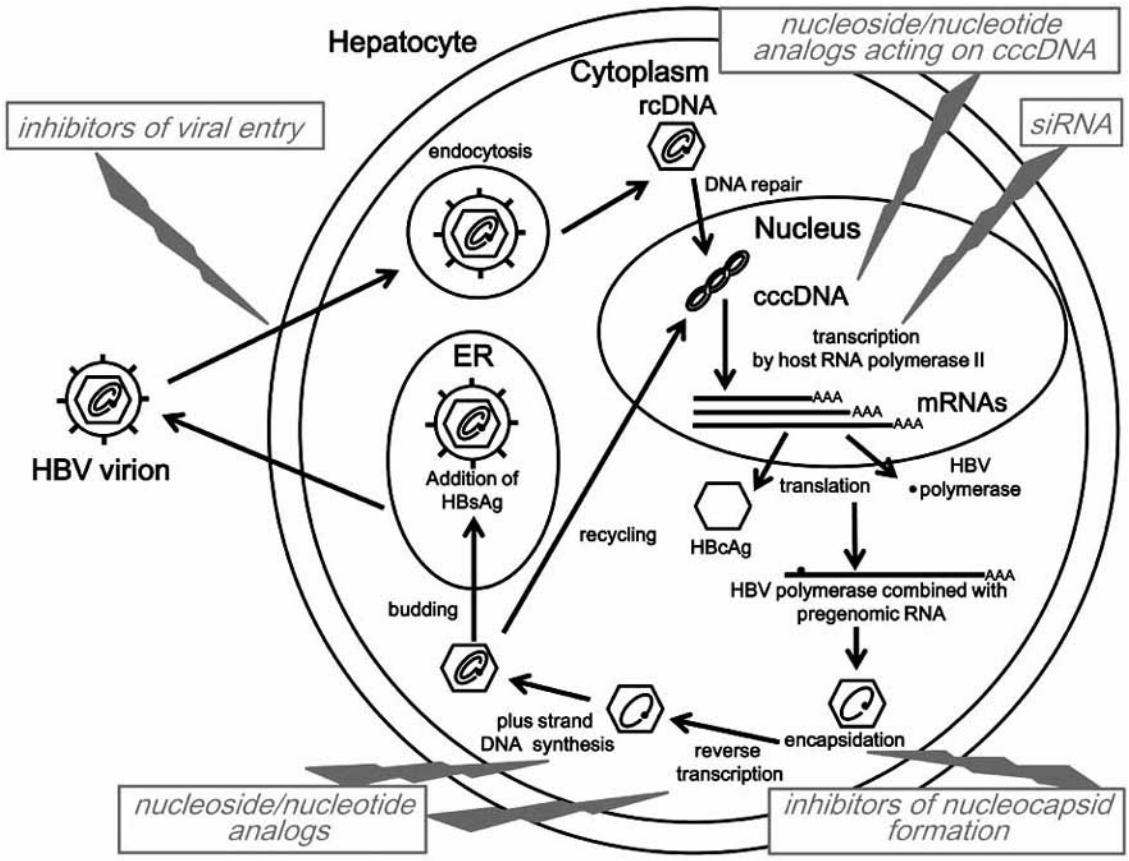


Fig. (1). HBV life cycle, the potential drug targets, and the novel antiviral approaches.
The genome of HBV consists of partially double-stranded relaxed circular DNA (rcDNA) with 4 partially overlapping open reading frames. After HBV virions enter the hepatocyte, it is uncoated and migrates to the hepatocyte nucleus, where their genomes are repaired to form a cccDNA that is the template for viral mRNA transcription. The viral mRNAs are translated in the cytoplasm to generate the viral envelope proteins, hepatitis B core antigen (HBcAg), polymerase proteins, and X proteins. The pregenomic RNA is encapsidated after binding of the polymerase and HBcAg in the cytoplasm. This RNA is reverse-transcribed into viral DNA. The resulting nucleocapsids can either bud into the endoplasmic reticulum (ER) to be enveloped by hepatitis B surface antigen (HBsAg) and exported from the cell or recycle their genomes into the nucleus for conversion to cccDNA.

The potential drug targets and the novel antiviral approaches include inhibitors of viral entry, small interfering double-stranded RNA (siRNA), inhibitors of nucleocapsid formation, or a variety of nucleoside/nucleotide analogs including adefovir dipivoxil which also reduces the intracellular cccDNA content.

which express hepatitis B surface antigen (HBsAg); and hepatitis B X protein. The pregenomic RNAs are bifunctional, serving both as the template for reverse transcription of the viral genome and for translation of HBcAg and the polymerase protein [11]. During nucleocapsids are assembled in the cytosol, a single molecule of pregenomic RNA is incorporated into the assembling viral core [16]. Once the viral RNA is encapsidated, reverse transcription starts [16]. The two viral DNA strands are sequentially synthesized. During or after the first DNA strand is synthesized from the encapsidated RNA template, the RNA template is degraded and the second DNA strand is synthesized from the newly synthesized first DNA strand as a template [17-19]. In parallel with DNA synthesis, the nucleocapsid grows mature and interacts with the S protein synthesized in endoplasmic reticulum (ER) to begin viral assembly in the ER. Subsequently, the envelope proteins bud into the lumen as lipoprotein HBsAg particles which then suffer further glycosylation in the ER and Golgi apparatus [20,21]. Some nucleocapsids bearing the mature genome are transported back to the nucleus and recycled to maintain cccDNA [22]. However, most nucleocapsids bearing the mature genome bud into regions of intracellular membranes bearing the viral envelope proteins and get lipoprotein envelopes containing the viral L, M, and S surface antigens and are then exported from the cell [21].

The potential targets of representative new antiviral approaches that are currently evaluated *in vitro*, *in vivo*, or in clinical trials are shown in Fig. (**1**). This article, however, focuses on the reagents that are currently evaluated in clinical trials.

CURRENT ANTIVIRAL REAGENTS

Standard and pegylated IFNs, which are host cellular target-directed antiviral reagents, and nucleoside/nucleotide

analogs, which are HBV life cycle inhibitors, have been approved for use in chronic hepatitis B. Since lamivudine became available and licensed for chronic hepatitis B, the use of nucleoside/nucleotide analogs has been preferred, likely due to the convenience of oral medication and the decreased risk of adverse events, as compared to IFNs.

Host Cellular Target-Directed Antiviral Reagents

Standard IFNs

IFNs are naturally occurring cytokines that act as immunomodulatory, antiproliferative and antiviral reagents [23,24]. IFNs bind to their receptors on cell membranes, activating a cascade of secondary messengers to induce and express intracellular genes, with a subsequent production of several cellular proteins that are essential components in producing an effective immune response to viral invasion [25]. IFNs suppress viral protein synthesis and prevent the viral infection of cells through the phosphorylation of eukaryotic initiation factor 2 (EIF2) protein after its activation *via* serine/threonine and tyrosine phosphorylation of the most pivotal IFN-dependent signal transduction molecules, such as signal transducer and activator of transcription 1 and 2 (STAT1 and STAT2, respectively) [26]. Moreover, IFNs exert immunomodulatory effects by boosting the antigen presenting function of HLA, increasing the activation of natural killer (NK) cells and other immune cells, and enhancing cytokine production [15], resulting in an acceleration of the body's ability to clear the infecting virus.

IFN alpha-2a, alpha-2b and beta, as well as pegylated IFN alpha-2a and alpha-2b are currently available in many countries. In Japan, pegylated IFN alpha is not available for chronic hepatitis B at present. Standard IFN alpha therapy results in a loss of HBeAg in 25 to 40% of patients and some patients show a durable response, while ALT flares occur in 10 to 20% of patients for which HBeAg remains positive [5,27-30]. Standard IFN alpha therapy induces a less durable response, with sustained response at 12-18 months after the end of therapy seen in 10 to 47% of HBeAg-negative patients [31,32]. Loss of HBsAg, which reflects a greater loss of cccDNA, occurs in 3-5% of IFN-treated patients within one year of follow-up but has been shown to increase with time in sustained virological responders [5,33]. Meta-analyses of randomized controlled studies showed that the loss of viral replication happened approximately 20% more often in IFN-treated patients than in controls (37% compared with 17% for the loss of HBV DNA) [29]. In a recent long-term follow-up study of chronic hepatitis B patients treated with standard IFN, 29% of them achieved HBsAg seroconversion at the end of follow-up [34]. Besides, the reduction of hepatocellular carcinoma development was also noted in an IFN-treated group after long-term follow-up [35]. The response to standard IFN therapy has been reported to be associated with HBV genotype. Accumulating evidence shows a better sustained response to standard IFN therapy in chronic hepatitis B patients with genotype B than those with C, and, in chronic hepatitis B patients with genotype A than those with D [36-38]. However, there are conflicting results regarding the response to long-term standard IFN therapy [39-41]. A recent report [42] increasing study subjects with HBeAg positive demonstrated that genotype B is associated with better response to standard IFN therapy assessed by HBeAg loss, that is contradictory to the prior result [41] in the same institution.

Pegylated IFNs

Pegylated IFNs, the addition of a polyethylene glycol (PEG) molecule to IFNs, extend the half-life of drug and, thus, enable prolonged IFN activity. Pegylated IFNs include a branched 40-kDa PEG linked to IFN alpha-2a (PEG-IFN alpha-2a) and a linear 12-kDa PEG linked to IFN alpha-2b (PEG-IFN alpha-2b) [43]. The PEG-IFNs have dual immunomodulatory and antiviral activity similar to the standard IFNs. However, the PEG-IFNs result in improved pharmacokinetic characteristics that allow for administration by injection on a weekly basis and maintain more effective IFN concentrations throughout the dosing period, as compared to the standard IFNs [44].

Several large randomized studies of PEG-IFNs therapy in patients with chronic hepatitis B [45-50] have demonstrated that PEG-IFNs are superior to lamivudine in their ability to increase the incidence of HBeAg seroconversion and HBsAg seroconversion and also to decrease serum HBV DNA in patients with HBeAg-positive and -negative chronic hepatitis B, especially in those with higher baseline ALT levels and lower baseline HBV DNA levels. In addition, approximately 25% of HBeAg-negative patients who showed biochemical and virologic responses maintained this for at least 3 years in an extended follow-up study [51]. Based on the data reported on adverse events, tolerance of PEG-IFNs seems to be similar to the tolerance observed in trials for chronic hepatitis C [44,52]. There are no significant differences in adverse effects developed during therapy between PEG-IFNs and standard IFNs. Representative frequent adverse effects, which include influenza-like symptoms, fatigue, myalgia, nausea, headache, alopecia, and erythema at injection site, are rarely associated with discontinuation of IFN therapies but do result in an increased preference for nucleoside/nucleotide analogs as therapeutic reagents. Discontinuation or dose reduction was caused by more serious adverse effects such as myelosuppression and psychoneurotic symptoms, the frequency of which are similar between PEG-IFNs and standard IFNs [53]. To date, there are no studies that demonstrate that PEG-IFNs therapy is associated with the development of drug resistance. Most evidence shows the response to PEG-IFNs therapy assessed by HBeAg loss is associated with HBV genotype, similar to standard IFN therapy [45,54], although a prior study [47] shows that HBeAg seroconversion is generally consistent across all genotypes.

HBV Life Cycle Inhibitors

Nucleoside/Nucleotide Analogs

Nucleoside/nucleotide analogs mimic natural nucleotides and inhibit activity of viral polymerase by competing with endogenous nucleotides during the incorporation of such nucleotides into newly replicated viral DNA. They are then able to terminate HBV-DNA synthesis by blocking the incorporation of the subsequent nucleotide into the viral DNA, resulting in a decrease in the production of infectious viral particles.

Nucleoside/nucleotide analogs can be divided into three groups, which are as follows: 1) L-Nucleoside derivatives such as lamivudine, 2) Cyclopentene/Cyclopentane derivatives such as entecavir, 3) Acyclic phosphate derivatives such as adefovir dipivoxil. The drug targets are assumed to be the priming of reverse transcription, RNA dependent DNA polymerase activity, reverse transcription in viral minus strand DNA synthesis, or DNA dependent DNA polymerase in viral plus strand DNA synthesis [55]. Neither nucleoside/nucleotide analogs target RNAseH activity.

Licensed Nucleoside/Nucleotide Analogs

Lamivudine

Lamivudine (3-thiacytidine: Epivir-HBV™, GlaxoSmithKline) is the deoxy-cytidine analog S-enantiomer 2',3'-dideoxy-3'-thiacytidine (Fig. (**2**)), which competes for cytosine during HBV-DNA synthesis. Lamivudine competitively inhibits the viral polymerase/reverse transcriptase activity, terminating viral DNA synthesis [56]. It is the first L-nucleoside analog licensed for the treatment of chronic hepatitis B. Lamivudine is able to exert antiviral activity through a phosphorylation process that occurs after absorption by oral administration [57]. The phosphodiester bond formation that induces chain elongation is inhibited based on its structural characteristics in which a carbon atom is replaced by sulfur at the 3' position of the sugar ring in absence of the normal 3' hydroxyl group. Lamivudine exerts antiviral activity equally on both the wild-type virus and the precore/core promotor mutants as well. This occurs regardless of the race of the patients [58,59]. Lamivudine treatment can restore cytotoxic T lymphocyte reactivity, which may be impaired in patients with chronic hepatitis B, although its effect is transient [60,61].

Although lamivudine has a superb safety profile and is the least expensive of the licensed nucleoside analogs, it is no longer recommended as first-line therapy due to the high rate of drug resistance, especially in those who have used the drug for long periods of time [62,63]. Drug resistance is caused by a substitution of isoleucine or valine for methionine within the tyrosine-methionine-aspartate-aspartate (YMDD) motif in the C domain of the HBV gene encoding for polymerase. Steric hindrance caused by the side chain group of isoleucine or valine of the YMDD mutants hampers the configuring of lamivudine into the nucleotide binding site of the reverse transcriptase. YMDD mutants reduce reverse transcriptase activity and replication capacity, which is restored by the compensatory mutations in the B domain [64,65]. The cumulative incidence of lamivudine resistance is about 70% at year 4-5 after administration in nucleoside/nucleotide analogs-naïve patients [62,63]. High viral load, high histological activity index (HAI), and high body mass index have been identified to be predictors of lamivudine resistance [62,66]. Although the high resistance rate of prolonged lamivudine therapy, lamivudine is the only drug that has been documented to decrease risk of hepatocellular carcinoma development in patients of advanced fibrosis or cirrhosis [6], which is the most important and solid endpoint of antiviral therapy.

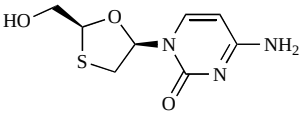
Adefovir Dipivoxil

Adefovir dipivoxil (bis-pivaloyloxymethyl-9-(2-phosphonyl-methoxyethyl) adenine, PMEA: Hepsera™, GlaxoSmithKline) is the orally-bioavailable prodrug of adefovir, a phosphonate acyclic nucleotide analog of adenosine monophosphate (Fig. (**2**)). Adefovir dipivoxil includes a phosphate group that becomes active through an additional phosphorylation phase after the removal of the bis-pivaloyloxymethyl moiety [67]. An elastase in the serum or the intestine promptly converts adefovir dipivoxil to adefovir. Adefovir selectively and competitively inhibits the reverse polymerase/transcriptase of HBV for its endogeneous substrate, deoxyadenosine triphosphate, and causes the termination of the DNA chain [68]. In addition, adefovir dipivoxil is estimated to stimulate natural killer cell activity and to induce endogeneous IFN production [69].

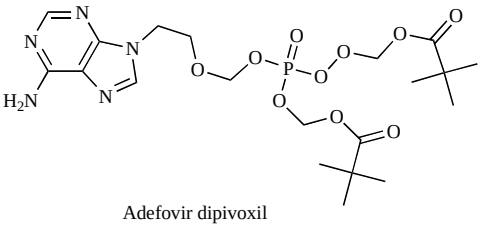
Adefovir dipivoxil demonstrates effectiveness in both HBeAg-positive and HBeAg-negative patients [70,71]. It suppresses the replication of not only wild-type virus, but also lamivudine-resistant mutants, both *in vitro* in tissue culture models and *in vivo* in patients. However, in comparison to other nucleoside analogs, it has moderate antiviral activity, especially for HBeAg-positive patients with high viral load [2]. Adefovir dipivoxil may be substituted in the near future by tenofovir, which has superior antiviral efficacy with a similar safety profile as adefovir dipivoxil [72]. Two mutations, N236T in the D domain of viral polymerase and A181V in the B domain have been identified and result in a 5- to 10-fold reduced susceptibility to adefovir dipivoxil *in vitro* [55]. Adefovir-resistant mutants are uncommon during the first 2 years after therapy, but are detected by population sequencing in about 29% of patients after 5 years of therapy [73]. Thus, long-term adefovir dipivoxil monotherapy is suitable for HBeAg-negative patients because of their lower baseline viral load and relatively low incidence rate of adefovir dipivoxil-resistant mutants [2]. In fact, a recent clinical trial for HBeAg-negative patients showed that treatment with adefovir dipivoxil for up to 240 weeks was well tolerated and achieved significant and increasing improvements in hepatic fibrosis, durable inhibition of HBV replication, normalization of ALT, and delayed development of resistance [73]. Of note, lamivudine is usually effective for adefovir dipivoxil-resistant mutants and vice versa [2]. Higher dose (30 mg) of adefovir dipivoxil has more antiviral activity against HBV, but is often associated with nephrotoxicity [15] and is not an approved dose.

Entecavir

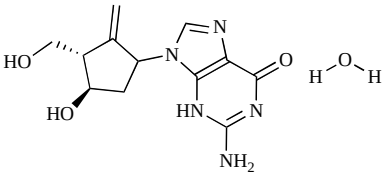
Entecavir (Baraclude™, Bristol-Myers Squibb Company) is a potent cyclopentyl guanosine analog with antiviral activity specific for hepadnaviruses and is a very potent inhibitor of HBV DNA polymerase activity (Fig. (**2**)). Entecavir triphosphate, the active intracellular form of entecavir, suppresses HBV DNA polymerase more efficiently than lamivudine or adefovir dipivoxil [74]. Entecavir triphosphate shows superior binding affinity for HBV DNA polymerase than the natural guanosine triphosphate substrate [74]. Of



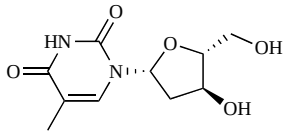
Lamivudine



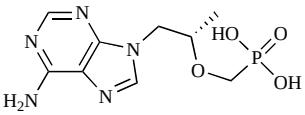
Adefovir dipivoxil



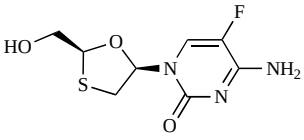
Entecavir



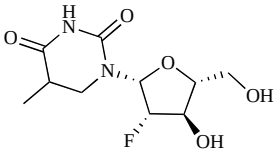
Telbivudine



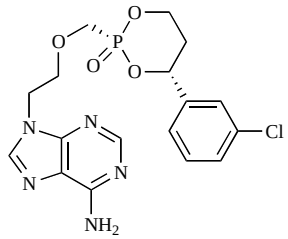
Tenofovir



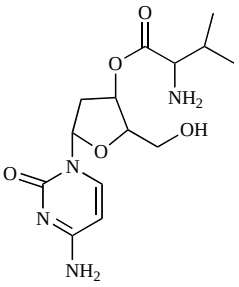
Emtricitabine



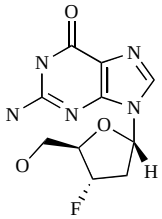
Clevudine



Pradefovir



Valtorcitabine



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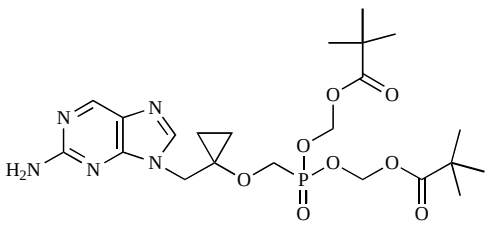

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Fig. (2). HBV life cycle inhibitors.

note, entecavir inhibits all three synthetic activities of HBV DNA polymerase: the priming of HBV DNA polymerase (a step relating covalent linkage with guanosine triphosphate), the reverse transcription of the negative-strand HBV DNA from the pregenomic mRNA and the synthesis of the positive strand of HBV DNA [74]. Although entecavir demonstrates potent effectiveness in both HBeAg-positive and HBeAg-negative patients [75,76], the rate of HBeAg seroconversion is relatively low and comparable to that achieved by other nucleoside analogs in the first 2 years of therapy.

Recent large phase III studies [47,75,77,78] showed that entecavir surpasses lamivudine in its ability to reduce HBV DNA to undetectable levels, normalize ALT, and improve liver histology in nucleoside-naïve HBeAg-positive and -negative patients and in lamivudine-refractory HBeAg-positive patients after 48 weeks of treatment. Entecavir exerted its virological and biochemical efficacy in a 96-week prolonged therapy trial [78]. Entecavir is comparable to lamivudine in regards to safety profiles.

Contrary to lamivudine, the rate of entecavir resistance is very low. The rate of entecavir resistance remained at only 1.2% in nucleoside-naïve patients, after up to 6 years of treatment (Tenney DJ *et al*. EASL April 22-26, 2009, Abstract 20). However, the rate of entecavir resistance increased to 57% of patients after 6 years of treatment in lamivudine-refractory patients (Tenney DJ *et al*. EASL April 22-26, 2009, Abstract 20). This phenomenon is due to the two-step mechanism of the emergence of entecavir resistance: primary resistance conferring lamivudine resistance and the emergence of additional mutations, T184, S202, and/or M250 [79]. Thus, entecavir monotherapy is a good strategy for nucleoside-naïve patients, while it should not be used for lamivudine-refractory patients even at a double recommended dose of 1 mg.

Telbivudine

Telbivudine (L-deoxythymidine, LdT: Tyzeka™, Idenix Pharmaceuticals) is a synthetic thymidine nucleoside analog (L-nucleoside) and belongs to a new class of β-L-configuration nucleoside analogs with specific activity against hepadnaviruses [80,81]. Telbivudine is structurally related to lamivudine [82]. The chemical name is 1-(2-deoxy-β-Lribofuranosyl)-5-methyluracil [82]. The molecular formula and weight of telbivudine are C10H14N2O5 and 242.09, respectively. The chemical structure of telbivudine is shown in Fig. (**2**) [82].

Human cellular kinases efficiently phosphorylate LdT to the active triphosphate form [83]. Telbivudine inhibits viral reverse transcriptase activity, resulting in an inability to reverse transcribe HBV pregenomic mRNA and a subsequent inhibition of DNA-dependent DNA synthesis, which terminates HBV-DNA synthesis [82,84]. Although telbivudine inhibits both first-strand and second-strand HBV DNA replication, second plus strand synthesis seems to be inhibited preferentially by telbivudine [82,84]. As with lamivudine, however, telbivudine does not inhibit HBV DNA priming [84]. Importantly, telbivudine is a highly specific and selective inhibitor of HBV-DNA polymerase and, thus, the active moiety of telbivudine triphosphate does not affect human DNA polymerase (α, β, γ), mitochondrial function, or morphology in an *in vitro* model [80].

The multinational GLOBE trial was a major Phase III trial evaluating the efficacy and safety profile of telbivudine 600 mg/day (n = 680) in comparison to lamivudine 100 mg/day (n = 687) in the management of treatment-naive patients with chronic hepatitis B [85]. The GLOBE trial showed that the rates of therapeutic and histologic response at 1 year were significantly higher in patients treated with telbivudine than in patients treated with lamivudine for patients with HBeAg-positive [85]. The trial also showed that telbivudine demonstrated greater HBV DNA suppression with less resistance than did lamivudine in both patients with HBeAg-positive and HBeAg-negative [85]. The final 2-year GLOBE trial also showed the superior efficacy of telbivudine over lamivudine with a similar frequency of adverse events [86].

Telbivudine mainly selects for the M204I mutation, which leads to cross resistance to lamivudine and entecavir, but retains sensitivity to adefovir dipivoxil and tenofovir [2]. The early antiviral effect of telbivudine seems similar to that of entecavir and is greater than that reported with adefovir and lamivudine [70,76,78,85,87]. In addition, telbivudine is much less expensive than adefovir dipivoxil, tenofovir and entecavir, although telbivudine is more expensive than lamivudine [2]. However, telbivudine monotherapy may lead to limited use due to the low rates of resistance with entecavir and tenofovir [2].

Tenofovir

Tenofovir disoproxil fumarate is the oral prodrug of tenofovir (Fig. (**2**)). Tenofovir disoproxil fumarate (Viread™, Gilead Sciences) is an acyclic nucleotide analog and belongs to the same family of nucleotide analogs as adefovir dipivoxil. Tenofovir inhibits HBV DNA polymerases by direct binding and termination of the DNA chain due to the absence of a requisite 3' hydroxyl on the tenofovir molecule after incorporation into DNA [88].

Tenofovir has potent inhibitory activity against the wild-type, lamivudine-resistant mutants and entecavir-resistant mutants [89,90]. Tenofovir is superior to adefovir dipivoxil and entecavir in patients with lamivudine-resistant HBV and has much lower renal toxicity than adefovir dipivoxil [90]. Tenofovir is currently approved in Japan, the United States and more than 50 other countries as therapy for human immunodeficiency virus-1 (HIV-1) and is also approved for therapy in chronic hepatitis B in several countries. Thus, tenofovir has been used primarily in HIV-HBV co-infected patients [91,92].

In two recent double-blind, phase III studies, tenofovir or adefovir dipivoxil were assigned to patients with HBeAg-negative or hepatitis B e antibody (HBeAb)-positive chronic hepatitis B once daily for 48 weeks [72]. Tenofovir was superior to adefovir dipivoxil in regards to viral suppression and normalization of ALT levels in both studies [72]. The rate of HBsAg loss was significantly higher in patients receiving tenofovir than in patients receiving adefovir dipivoxil [72]. There has been no definite observation of HBV resistance to tenofovir with 48 weeks of treatment [72].

Thus, tenofovir may be recommended for use in not only treatment-naïve patients but also in patients with drug-resistant mutants. Actually, tenofovir is reported to be active against lamivudine-, entecavir-, telbivudine- and even adefovir dipivoxil-resistant mutants [2]. Further studies are warranted to determine the appropriate indication for its use.

ANTIVIRAL REAGENTS IN CLINICAL DEVELOPMENT

Nucleoside/Nucleotide Analogs

Emtricitabine

Emtricitabine (2'3'-dideoxy-5' fluoro-3'thiacytidine: Emtriva™, Gilead Sciences) (Fig. (**2**)) is a 5-fluoro-oxathiolane derivative, structurally related to lamivudine; it differs only by a fluorine at the 5-position of the nucleic acid. Emtricitabine is a cytosine analog and is converted to triphosphate by cellular enzymes. It competes with deoxycytidine triphosphate (dCTP) as a substrate for HBV polymerase and inhibits HBV DNA replication [93]. Originally, emtricitabine was approved as part of a regimen of combined therapy for HIV infection in the United States and Europe. In a recent randomized, double-blind study, patients with HBeAg-positive and HBeAb-negative were assigned to receive 25, 100, or 200 mg of emtricitabine once a day for 48 weeks [94]. The ratio of patients with loss of serum HBV DNA was 3.8%, 42%, or 61% for three groups receiving 25, 100, or 200 mg of emtricitabine at 48 weeks, respectively [94]. The loss of HBeAg was observed in 32% to 50% of HBeAg-positive patients [94]. Serum ALT levels became normal in 95% of patients at week 48 [94]. Histological improvement by emtricitabine monotherapy was confirmed by another randomized, double-blind study [95]. However, emtricitabine has been shown to be cross-resistant to lamivudine-resistant mutants, a reflection of the structural similarity of emtricitabine to lamivudine. The implication of this finding is that the use of emtricitabine as monotherapy in HBV may be limited [94]. Phase III clinical trials of emtricitabine in combination with other nucleoside/nucleotide analogs are currently ongoing.

Clevudine

Clevudine, 1-(2'-deoxy-2'-fluoro-β-L-arabinofuranosyl)-5-methyluracil, 1-(2'-deoxy-2'-fluoro-β-L-arabinofuranosyl)thymine (L-FMAU™, Gilead Sciences), is a pyrimidine nucleoside analog of the unnatural β-L configuration (Fig. (**2**)). It has marked *in vitro* and *in vivo* anti-HBV activity that is distinct from other nucleoside analogs [96-98]. Clevudine acts through preferential inhibition of the DNA-dependent activity of the HBV DNA polymerase by clevudine 5'-triphosphate [99]. Thus, clevudine binds to the catalytic site of HBV polymerase and inhibits viral plus strand DNA synthesis as a competitive inhibitor [100]. Deoxycytidine kinase, cytosolic thymidine kinase, and mitochondrial deoxypyrimidine kinase are mainly responsible for phosphorylating clevudine to the 5'-monophosphate. Then thymidylate kinase and 3-phosphoglycerate kinase catalyze phosphorylation to the di- and triphosphate forms, respectively [99]. Thus, the active triphosphate inhibits HBV DNA polymerase by being an obligate chain terminator.

In a randomized controlled study, a total of 98 patients with HBeAg-positive chronic hepatitis B were treated with placebo, clevudine at a daily dose of 30 mg, or clevudine at a daily dose of 50 mg [101]. Patients were followed up for 24 weeks after a 12-week course of therapy. Median HBV DNA reductions from baseline at week 12 were 0.2, 4.49, and 4.45 $\log_{10}$ copies/mL in the patients receiving placebo, 30 mg clevudine, and 50 mg clevudine, respectively. At the end of the follow-up period, median HBV DNA reduction from baseline was decreased to 2.28 and 1.40 $\log_{10}$ copies/mL in patients receiving 30 mg clevudine and 50 mg clevudine, respectively [101]. Median serum ALT levels decreased and remained below the upper limit of normal for the testing period [101]. The rates of adverse events were similar among the three groups [101]. Also, clevudine has been shown to be cross-resistant to lamivudine-resistant mutants [102]. Recently, long-term clevudine therapy was found to cause depletion of mitochondrial DNA and to result in mitochondrial myopathy associated with myonecrosis [103]. Clinical studies of clevudine were terminated due to the side effects of myopathy.

Pradefovir

Pradefovir, a cyclic 1-aryl-1,3-propanyl prodrug of adefovir dipivoxil (Fig. (**2**)) has been developed in order to avoid much of the renal toxicity of adefovir dipivoxil [104]. Pradefovir belongs to HepDirect prodrugs and represents a new class of cytochrome pigment-activated prodrugs that direct drugs to the liver [105]. The HepDirect prodrug is chemically modified to form an acyclic 1,3-propanyl ester prodrug of phosphonate or phosphate, which is activated after the modification is cleaved off by a liver specific enzyme [104]. HepDirect prodrugs are resistant to esterase cleavage and, as a result, they are stable in most tissues, including blood, except the liver [104]. These prodrug approaches have the capability of increasing the efficacy and safety of certain drugs [104]. Therefore, contrary to other prodrug approaches [106], pradefovir was intended to improve adefovir dipivoxil delivery to the liver, while minimizing systemic exposure. In fact, tissue distribution studies using experimental animal models showed that pradefovir increased the delivery of adefovir dipivoxil and its metabolites to the liver, with pradefovir achieving a 12-fold improvement in the liver/kidney ratio over adefovir dipivoxil [107].

An open-label phase II study, pradefovir (5, 10, 20, or 30 mg) was compared with adefovir dipivoxil (10 mg). Median serum HBV DNA reductions from baseline at week 48 were 4.09, 4.84, 4.89, or 5.54 $\log_{10}$ copies/mL in patients receiving the respective pradefovir doses, compared with 4.19 $\log_{10}$ copies/mL in the patients receiving 10 mg of adefovir dipivoxil. Patients who received pradefovir did not demonstrate an increase in adverse events, as compared to those who received adefovir dipivoxil therapy. No obvious dose relationship for any adverse effects was observed [108].

Valtorcitabine

Valtorcitabine is a valine ester prodrug of 20-deoxy-β-L-cytidine (L-dC or torcitabine) (Fig. (**2**)) [104]. L-dC, which has an unnatural L-configuration of the natural deoxynucleo-

sides. It is a powerful and selective HBV DNA polymerase inhibitor [81]. L-dC is readily phosphorylated in cells to the corresponding active triphosphate metabolite [104]. L-dC had low oral bioavailability, like other nucleoside analogs [81], thus, several derivatives including valtorcitabine were synthesized to improve oral bioavailability of the parent drug [109-111]. Valtorcitabine significantly increased oral absorption of L-dC with a bioavailability of 84% in monkeys, as compared to that of 16% for L-dC [111] most likely due to the involvement of PepT1 mediated transport [104]. Valtorcitabine has the 30-monovaline ester replaced more easily with the synthesized 30,50-divaline ester of L-dC due to superior stability and similar bioavailability to 30,50-divaline ester of L-dC [104].

Valtorcitabine 900 mg/day for 28 days achieved an average decrease in serum HBV-DNA of 3.04 $\log_{10}$ copies/mL, showing a 99.9% decrease in viral load. Valtorcitabine was well tolerated in all subjects, with safety profiles similar to that of placebo [74].

MIV-210

MIV-210 (Medivir AB) is the 3'-fluoro-2',3'-dideoxyguanosine prodrug and is one of the nucleoside reverse transcriptase inhibitors that have antiviral activity specific for HIV and HBV (Fig. (**2**)) [112]. MIV-210 targets the reverse transcriptase of HIV and HBV and acts as a chain terminator in the reverse transcriptase reaction. Thus, incorporation of MIV-210 into nascent DNA results in chain termination of viral DNA. In phase I development trials, MIV-210 was shown to have satisfactory oral uptake and was well tolerated [113]. Currently, phase II studies are ongoing.

LB80380

LB80380 (Fig. (**2**)) is a potent oral nucleotide prodrug that is chemically similar to adefovir dipivoxil and tenofovir disoproxil fumarate. LB80380 is rapidly absorbed and converted to its parent drug LB80381 in the liver and intestine by the removal of the two pivaloyl groups (deacetylation) [114]. LB80381 is further metabolized to LB80317, a nucleotide analog of guanosine monophosphate by oxidation of the nucleoside base at the 6 position through oxidases such as aldehyde oxidase and xanthione oxidase [114]. LB80317 is the active metabolite with the antiviral activity. It inhibits viral replication following incorporation into viral DNA after phosphorylation to the di- and triphosphate forms [114]. LB80317 has a long half-life at steady-state, supporting the use of a one-daily dosing regimen [114].

An *in vitro* study showed that LB80380 was effective against HBV strains resistant to lamivudine, adefovir dipivoxil, entecavir, and telbivudine [114]. LB80380 is worthy of attention because it can be used as a rescue therapy for possible multidrug resistance in long-term use of current antiviral reagents. LB80380 has been shown to be effective against not only wild-type HBV but also YMDD mutants in both *in vitro* and *in vivo* studies [115]. A randomized placebo-controlled phase I/II clinical study showed that LB80380 was well tolerated and induced a marked suppression of serum HBV DNA levels in HBeAg-positive patients [116]. Currently, phase II studies are ongoing.

CONCLUSIONS

The primary goal of therapy for patients with chronic HBV infection is to avoid progression of liver disease to liver cirrhosis, liver failure or hepatocellular carcinoma through robust and rapid viral suppression and the long lasting continuation of undetectable levels of serum HBV DNA. To date, we have several antiviral reagents that are licensed or are soon to be licensed for use in chronic hepatitis B. However, the choice of a first-line therapy must be selected with utmost care. We must consider efficacy, safety, drug resistance, and method/period of administration. For instance, PEG-IFNs lack drug resistance and show long lasting effects in certain patient populations, but are expensive, require administration by injection, and can cause several adverse effects. Among nucleoside/nucleotide analogs, entecavir, and tenofovir, are promising and should be considered as first-line therapy due to their superior efficacy to comparable reagents and their low rates of drug resistance. However, drugs with higher rates of resistance such as lamivudine and telbivudine can be used as first-line therapy, provided the use of on-treatment early viral suppression is adopted.

Combination therapy of antiviral reagents may improve therapeutic efficacy, in comparison to monotherapy, and may decrease or delay the incidence of drug resistance and virological breakthrough. Combination therapy of two powerful nucleoside/nucleotide analogs with different resistance characteristics, such as entecavir and tenofovir, may be promising. In addition, in view of convenience, combination therapy of oral nucleoside/nucleotide analogs may be preferable. However, combination therapy is expensive and may increase the risk of adverse events and robust multidrug resistance in long-term use. In fact, combination therapy of first-line reagents is not to be any benefit in the near future as the new potent antiviral drug, entecavir has very low resistance rate (1.2% after 6 years) in nucleoside-naïve patients as mentioned above (Tenney DJ *et al.* EASL April 22-26, 2009, Abstract 20). Thus adding a second-line reagent is unlikely to be of any benefit as compared with monotherapy in nucleoside-naïve patients. However, combination therapy of first-line reagents may target lamivudine-reflactory patients because combination therapy of adefovir dipivoxil and lamivudine reduces the rate of resistance to adefovir dipivoxil better than switching to adefovir dipivoxil monotherapy for patients with lamivudine-reflactory patients [117-119].

In addition, we should keep developing novel reagents with different mechanism of action and drug targets for complementary coverage against possible multidrug resistance to current antiviral reagents on more long-term basis. If a reagent was developed that can clear cccDNA or have higher rates of HBsAg clearance, the drug will be recommended as first-line therapy.

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