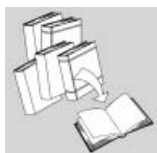


REVIEW



Antiviral treatment of chronic hepatitis B virus infections: the past, the present and the future

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SUMMARY

A decade ago, standard therapy against chronic hepatitis B virus infections only consisted of lamivudine or IFN- α . Treatment with lamivudine and IFN has been compounded by, respectively, the emergence of drug-resistant virus strains and the appearance of serious side effects. In the last 10 years, hepatitis B treatment has made much progress. Several treatments are now licensed for the treatment of patients with chronic hepatitis B and others are under development. Here, we provide an overview of the potential and mode of action of anti-HBV agents that are currently available, and/or may become available in the near future. Foremost among these newer compounds are adefovir dipivoxil, entecavir and telbivudine. Copyright © 2007 John Wiley & Sons, Ltd.

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INTRODUCTION

Despite the existence of an effective vaccination program, up to 400 million people worldwide are chronically infected with HBV currently. Moreover, the World Health Organisation estimates a death rate of 1 million people annually [1]. In a minority of the cases, an HBV infection can lead to acute fulminant hepatitis. Chronic hepatitis B (CHB) can lead to life-threatening conditions like cirrhosis and hepatocellular carcinoma (HCC). Cirrhosis develops in approximately 20% of

chronically infected patients, subsequently leading to hepatic insufficiency and portal hypertension [2]. Moreover, these patients have a 100 times higher risk of developing HCC than non-carriers [3–5]. Hepatitis B excreted antigen (HBeAg) represents an important marker for HCC, since HBeAg-positive subjects are at highest risk of developing HCC [6]. In late stages of cirrhosis or HCC, liver transplantation is the only option left. Therefore, detection of HBV infection at an early stage and prompt treatment are of crucial importance.

Indicators for a sustained virological response are clearance of HBeAg, seroconversion from HBeAg to corresponding anti-HBe antibodies, and a drop in circulating HBV DNA below detection level [2,7].

At present, six drugs are licensed by the United States Food and Drug Administration (FDA) for the treatment of CHB: IFN- α and pegylated IFN- α , three nucleoside analogues (lamivudine, entecavir and, since October 2006, telbivudine) and one nucleotide analogue (adefovir dipivoxil). Because of high rates of resistance (as in the case of lamivudine), poor tolerability (as in the case of IFN) and possible side effects (i.e. nephrotoxicity with adefovir if used at supra-optimal doses), new therapeutic options are warranted. HBV DNA polymerase is the main target for the nucleoside or nucleotide analogues. Future therapy may lie

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Abbreviations used

ALT, alanine aminotransferase; 3TC, lamivudine [(β -L-2',3'-dideoxy-3'-thiacytidine)]; 3TC-TP, 3TC-triphosphate; cccDNA, covalently closed circular DNA; CHB, chronic hepatitis B; dCTP, deoxycytidine triphosphate; DDDP, viral DNA-dependent DNA polymerase; DHBV, duck hepatitis B virus; DR, direct repeat; dsl, double stranded linear; EC₅₀, 50% effective (inhibitory) concentration; ECB, elvucitabine; FDA, food and drug administration; FLG, 2',3'-dideoxy-3'-fluoroguanosine; (-)FTC, emtricitabine; HAPs, heteroaryl-dihydropyrimidines; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; L-dC, β -L-deoxycytidine; L-dT, β -L-2'-deoxythymidine; L-FMAU, clevudine; L-FMAU-TP, 2'-fluoro-5-methyl- β -L-arabinofuranosyluracil-triphosphate; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PEG-IFN, pegylated interferon α -2a; pgRNA, pregenomic RNA; PMEO-DAPy, 2,4-diamino-6-[(2-phosphonomethoxy)ethoxy]pyrimidine; RC, relaxed circular; siRNAs, small interfering RNAs; TDF, tenofovir disoproxil fumarate; Val-L-dC, valtorcitabine; WHV, woodchuck hepatitis virus.

in (i) drug combinations of existing anti-HBV drugs, (ii) non-nucleoside reverse transcriptase inhibitors (NNRTIs), (iii) small interfering RNAs (siRNAs) [as was shown for many other viruses, siRNAs can be used to inhibit HBV replication *in vitro* [8,9] and *in vivo* [10]], (iv) helioxanthin and related molecules, which inhibit viral nucleic acid and viral protein expression [11] or (v) the prevention of nucleocapsid formation by heteroaryldihydropyrimidines (HAPs), such as BAY 41-4109 [12].

HBV REPLICATION

During an HBV infection, three types of particles are produced: (i) Dane particles (infectious virions) [13], (ii) 20 nm hepatitis B surface antigen (HBsAg) spheres and (iii) variable length HBsAg filaments [14]. The latter two particles are non-infectious and highly immunogenic and, besides HBsAg, also contain host-derived lipids [15–17].

The virions consist of a 3.2 kb relaxed circular (RC) DNA with four overlapping ORFs, namely P, C, S and X.

The S gene encodes for three surface proteins: large, medium and small HBsAg [18]. The polymerase is translated from the P ORF. The polymer-

ase protein consists of four domains: terminal protein, spacer, RT and RNase H [19–21]. The RT domain can be divided into seven subdomains (A–G), of which domain C harbours the highly conserved YMDD motif, that is, the catalytic site of the enzyme [22,23]. Infected hepatocytes also contain the hepatitis B core antigen (HBcAg). HBcAg and the HBeAg are encoded from the C ORF [24,25]. HBeAg and anti-HBe could be used as a marker of viral replication and recent findings suggest that HBeAg (i) acts as an immunoregulator [26], (ii) functions as a promoter of viral persistence and (iii) could play a role in chronicity and carcinogenesis [27]. Yang *et al.* showed that HBeAg is an activator of the IL-1 pathway [27]. Finally, the X gene encodes the protein X (a transactivator), which has been linked to the pathogenesis of HCC [28].

After interaction with (an) unknown receptor(s) (Figure 1: step 1), RC DNA from the virion is transported to the nucleus and converted into covalently closed circular DNA (cccDNA), while the nucleocapsids remain in the cytoplasm (step 2) [29]. This DNA intermediate plays an important role in viral persistence and serves as a template

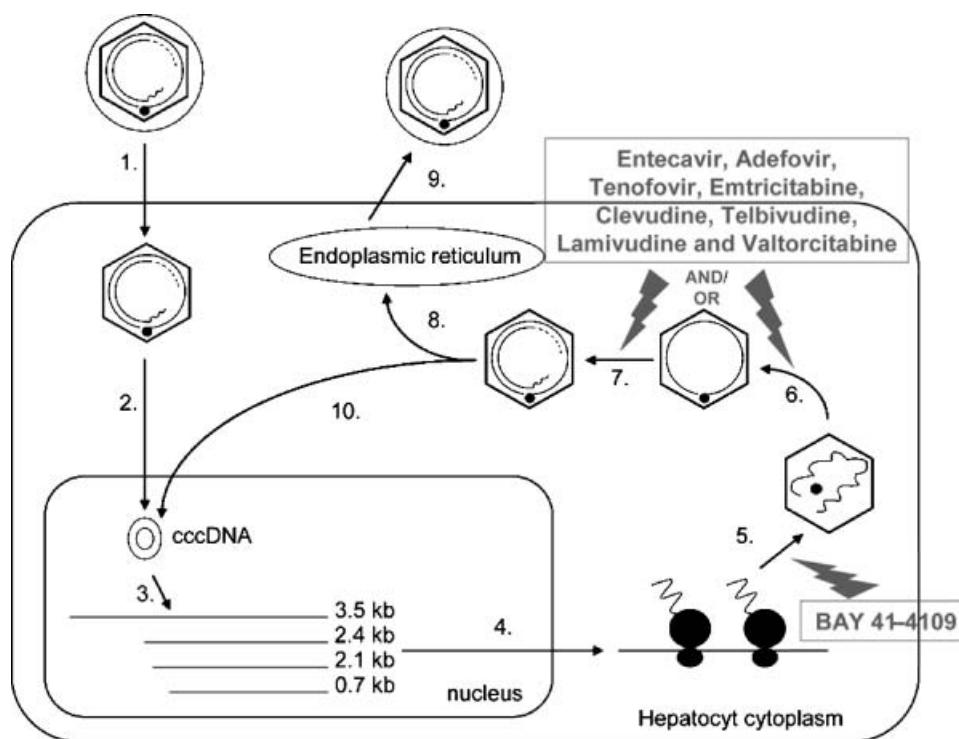


Figure 1. HBV replication cycle and site of action of several anti-HBV agents. The numbered steps are discussed in the text

for transcription by host RNA polymerase II to produce four mRNA transcripts of 3.5, 2.4, 2.1 and 0.7 kb, respectively (step 3) [30]. Importantly, cccDNA may be indicative of risk for HCC development, as Wong *et al.* have shown that tumour tissues had higher levels of cccDNA compared to non-tumour tissues [31].

The mRNA transcripts are transported to the cytoplasm (step 4), where translation to proteins occurs (step 5). The 3.5-kb pregenomic RNA (pgRNA) contains an ϵ -stemloop, which serves as an encapsidation signal. During core particle formation, encapsidation of the pgRNA takes place and reverse transcription starts (step 5) [32]. The polymerase binds to the ϵ -stemloop, together with chaperones (i.e. p23 and Hsp90) at the 5'-end of the pgRNA [33,34]. After this so-called protein priming reaction, a 3- or 4- nucleotide DNA oligomer is synthesised and transferred to the 3' copy of direct repeat 1 (DR1) and synthesis of the (–) DNA strand continues (step 6). Simultaneously, the RNA template is degraded by RNase H, except for a small RNA oligomer. Once (–) DNA synthesis is complete, a second translocation (the RNA primer translocation) to the 5' end of DR2 occurs and (+) DNA synthesis starts using (–) DNA as template (step 7). Finally, circularisation and extension of (variable length) (+) DNA strand terminates the DNA synthesis [34,35].

Alternatively, in a minority of the virions (+) DNA synthesis starts from DR1 (this process is called *in situ priming*), leading to the formation of double stranded linear (dsl) DNA [36]. Dsl DNA can either be (i) converted into cccDNA through illegitimate replication [37,38] or be (ii) integrated into chromosomal DNA [39–41]. However, unlike for retroviruses, the integration of hepadnaviral DNA into chromosomal DNA is not essential for viral replication. After maturation, the nucleocapsids are enclosed by envelope glycoproteins after budding into the endoplasmic reticulum and Golgi apparatus [42,43]. Finally, mature virions are released through the secretory pathway (steps 8 and 9). Alternatively, nucleocapsids may also recycle back to the nucleus to maintain cccDNA levels (a process referred to as recycling pathway) (step 10) [29]. A more detailed description of HBV replication and gene function is given by Ganem and Schneider [44].

Of all the steps in the HBV replication cycle (Figure 1), the reverse transcription represents

the most attractive target in current anti-HBV chemotherapy.

CURRENTLY APPROVED ANTI-HBV DRUGS FOR THE TREATMENT OF CHB

Lamivudine

Lamivudine [(-) β -L-2',3'-dideoxy-3'-thiacytidine (3TC)] (Figure 2A) is an L-nucleoside analogue with anti-HIV and anti-HBV properties. It was approved in 1998 by the FDA for the treatment of CHB infections. Lamivudine is available as Epi-vir-HBV[®] or Zeffix[®] and administered daily at an oral dose of 100 mg.

After cell penetration, 3TC is converted to its active form 3TC-triphosphate (3TC-TP) [45]. Subsequently, 3TC-TP can work as (i) a chain terminator, after incorporation into the growing HBV DNA chain or (ii) a competitive inhibitor of deoxycytidine triphosphate (dCTP). Thus, 3TC-TP inhibits viral DNA synthesis, but not mitochondrial DNA synthesis [46]. More specifically, lamivudine acts as an inhibitor of the viral DNA-dependent DNA polymerase (DDDP) and RNA-dependent DNA polymerase (RT). In addition, it may interrupt the recycling of virions to the nucleus and suppress the formation of cccDNA from RC DNA [47].

Lamivudine treatment generally results in a 3–4 log drop in circulating HBV DNA levels, at least during the first months of treatment [48,49]. Concomitantly, HBeAg is cleared more rapidly from the circulation, anti-HBe antibodies become detectable in the blood, and serum alanine aminotransferase (ALT) levels tend to normalise [48,49]. In general, a sustained response can be achieved after 2 years of treatment in patients with HBeAg-negative CHB as demonstrated by swift virological (i.e. serum HBV) and biochemical responses (i.e. ALT level). The virological and biochemical response may show a reduction of up to 74 and 66%, respectively [51]. In addition, the drug is usually well tolerated, although side effects may include malaise and fatigue [7,50].

However, lamivudine monotherapy leads to high rates of resistance. Approximately 20% of HBeAg-positive patients develop resistance after 1 year, which increases up to 65% after 5 years [52]. The most common mutation is observed in the catalytic YMDD (tyrosine-methionine-aspar-

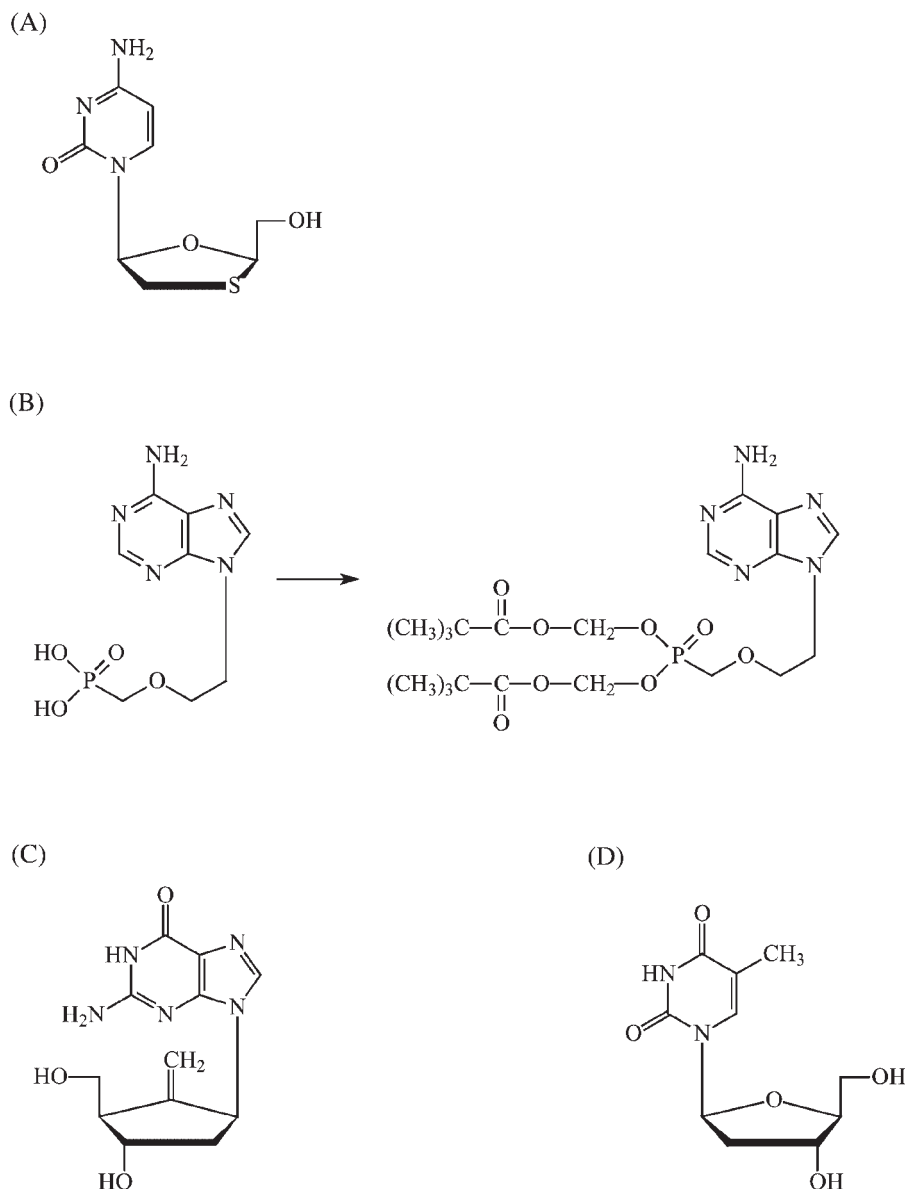


Figure 2. Currently licensed anti-HBV drugs. (A) The L-nucleoside analogue lamivudine, (B) the acyclic nucleoside phosphonate adefovir (PMEA) licensed as its prodrug adefovir dipivoxil [bis(pivaloyloxymethyl)ester of 9-(2-phosphonylmethoxyethyl)adenine or bis-(POM)PMEA], (C) the carbocyclic D-nucleoside analogue entecavir and (D) the L-nucleoside analogue β -L-2'-deoxythymidine (L-dT) or telbivudine

tate-aspartate) motif of the viral polymerase. The primary cause of lamivudine resistance is substitution of methionine for valine or isoleucine (rtM204V/I, in which rt refers to the viral RT polymerase) in the highly conserved YMDD motif (rtM204I/S has also been described), often combined with a double mutation (predominantly rtL180M). More specifically, these mutations are

found in sub-domain B and C of the polymerase. Based on molecular model interactions, this mutation leads to steric hindrance for lamivudine [53]. Recently, Yatsuji *et al.* demonstrated that lamivudine-resistant mutations can also occur outside the YMDD motif [54].

Although lamivudine-resistant HBV mutants remain highly sensitive to adefovir and tenofovir,

cross-resistance has been observed towards other L-nucleoside analogues such as emtricitabine or (-)FTC and telbivudine [55].

Adefovir dipivoxil

Adefovir (or PME A [9-(2-phosphonylmethoxyethyl)adenine]) is a member of the acyclic nucleoside phosphonates. Together with HPMPA, PME A is the prototype of this class of compounds [56]. To increase its oral availability, PME A has been esterified to its prodrug bis(POM)PME A (Figure 2B). Since September 2002, Adefovir dipivoxil (licensed as Hepsera[®]) is available for the treatment of CHB. It is administered orally at a dose of 10 mg daily.

When PME A enters the cell, it is phosphorylated twice by AMP kinase [57] to its active form PMEApp. Inside the cell, it is incorporated into the growing HBV DNA chain, where it acts as (i) an obligatory chain terminator [see also reference 58] and/or (ii) a competitive inhibitor of the natural substrate dATP. In addition to its anti-HBV activity, PMEApp has also demonstrated activity against other viruses, like herpesviruses and retroviruses as well as bacteria producing adenylate cyclase toxins (e.g. *B. anthracis*, *B. pertussis*, *P. aeruginosa*) [59].

In contrast to HBeAg-negative patients, HBeAg-positive CHB patients have higher median levels of cccDNA and total intrahepatic viral DNA. Forty-eight weeks of adefovir dipivoxil therapy led to a decrease of both cccDNA and HBeAg levels, but it may take approximately 14.5 years to clear infected cells of cccDNA [60].

Although treatment with 10 mg adefovir dipivoxil for 48 weeks showed a good anti-HBV response in CHB patients who were either negative or positive for HBeAg [61,62], efficacy can be improved if treatment is extended to 144 weeks in HBeAg-negative patients [63]. Adefovir used at a dose of 10 mg/day has very little adverse effects; at higher doses, that is 30 or 60 mg/day (or 125 mg/day as initially used when the compound was developed for HIV treatment) the compound may be nephrotoxic [2,7,62].

In contrast to lamivudine monotherapy, the rate of development of adefovir resistance is much lower: not more than 6% after 3 years [63], and up to 18% after 4 years [64]. After 5 years of therapy, 29% of the treated patients will harbour adefovir-resistant HBV strains (70% for lamivudine) [65].

The main adefovir resistance is associated with the rtN236T and rtA181V/T mutations [66,67]. In lamivudine-resistant patients, emergence of the rtN236T and rtA181V/T mutations is more common, compared to nucleoside-naïve patients [68]. Recently, three patients with primary adefovir resistance were described, which remained sensitive to tenofovir. The HBV variant already had a mutation before adefovir therapy was even initiated. When investigated more thoroughly, results showed that an rtI233V mutation was responsible for the adefovir resistance [69]. In general, adefovir-resistant HBV mutants remain susceptible to L-nucleoside analogues like lamivudine, entecavir, etc. [70]. Adefovir resistance can be associated with viral rebound, hepatic flares and hepatic decompensation [71]. To prevent the emergence of adefovir resistance, lamivudine should be combined with adefovir, even in lamivudine-resistant patients [72]. As described earlier, adefovir is still active against lamivudine-resistant HBV strains (in liver transplant patients) [73].

Entecavir

Entecavir (Figure 2C) has been available in the United States for the treatment of CHB virus infections since April 2005 (licensed as Baraclude[®]). Its metabolism is comparable with that of the other nucleoside analogues. ETV is phosphorylated three times by human cellular kinases to its active form ETV-TP. Intracellular accumulation occurs rapidly, its half-life is approximately 15 h (as for lamivudine) [74] and it interferes with the HBV polymerase at different steps: (i) inhibits the priming of the polymerase, (ii) has a high affinity for the HBV polymerase, (iii) acts as a competitive inhibitor of dGTP (natural substrate) and (iv) acts as a chain terminator two or three nucleotides downstream from its incorporation [35,75]. Mycophenolic acid and ribavirin enhance the antiviral effect of entecavir against wild-type HBV and the rtM204V lamivudine-resistant HBV *in vitro* [76]. Ribavirin, being part of standard therapy for the treatment of HCV infections, markedly increases the *in vitro* anti-HBV activity of entecavir. The mechanism of this potentiation is assumed to be the result of depletion of intracellular dGTP pools by ribavirin and thus less competition of the 5' triphosphate metabolite of entecavir with dGTP at

the level of the viral polymerase. This observation may be important when treating HBV/HCV co-infected patients [76].

In vivo studies with woodchucks, chronically infected with woodchuck hepatitis virus (WHV), demonstrated the pronounced antiviral activity of ETV. Woodchucks were treated with 0.5 mg/kg ETV daily for 8 weeks and showed decreased viremia levels. Long-term therapy with ETV once a week was also effective in maintaining low levels of viral load, decreasing cccDNA levels and viral antigens, expanding the life of the animals and delaying the onset of HCC [77].

Two double-blind phase III studies with 715 HBeAg-positive and 648 HBeAg-negative nucleoside-naïve CHB patients showed that entecavir therapy led to higher improved histological and virological values (like reduction in viral load, HBeAg loss and seroconversion) and reduced alanine transaminase levels compared with lamivudine [78,79].

ETV showed good anti-HBV activity, and no resistance in nucleoside-naïve CHB patients was noted after 2 years of therapy. For patients with lamivudine failure, higher doses of ETV are recommended as 10% of these patients might develop ETV resistance after 2 years [35].

So far, four mutations have been described that are associated with ETV resistance: rtS184G, rtI169T, rtS202I and rtM250V, but only in the presence of lamivudine resistance mutations [80]. Additionally, several studies have also shown activity of ETV against adefovir-resistant HBV strains *in vitro* by site directed mutagenesis and *in vivo* (decreased HBV DNA levels of 4.3–5.5log₁₀ copies/ml) [81,82].

Telbivudine

The FDA has recently approved the L-nucleoside analogue telbivudine for the treatment of CHB virus infections (Figure 2D). It will be marketed as Tyzeka[®] (in the US) or Sebivo[®] (outside the US, presently available in Switzerland) and given orally once daily as a single tablet of 600 mg.

The L-nucleoside telbivudine (β -L-2'-deoxythymidine or L-dT) is a specific anti-HBV agent *in vitro* and *in vivo* with no effect on human DNA polymerases. Structure-activity relationship studies have revealed that the 3'OH group is essential for anti-HBV activity, and removal or substitution results in the loss of activity [83].

In vitro studies with HepG2 cells and primary human hepatocytes have shown high phosphorylation rates of L-dT and β -L-deoxycytidine (L-dC) [84]. Its active form L-dT-TP prefers to inhibit (+)-strand DNA synthesis and acts as a chain terminator [85]. Data from phase I and phase II clinical trials have shown that different concentrations of telbivudine give considerable reductions in HBV DNA levels after 4 weeks of treatment. Upon withdrawal of telbivudine the viral load increased [86]. A 1 year trial has shown that telbivudine decreased HBV DNA levels with >6log₁₀ compared with lamivudine (\sim 4.5log₁₀) [87]. In HBeAg-positive, compensated CHB patients, L-dT gave an HBV DNA reduction of 6.30 log₁₀ (44 patients) after 24 weeks as compared to 4.97 log₁₀ for adefovir dipivoxil (89 patients) [88].

Until now, one mutation, rtM204I, has been observed in patients who received telbivudine [87]. Data from Yang *et al.* showed that lamivudine-resistant strains show cross-resistance towards several L-nucleosides such as L-dT, L-dC and (-)FTC (see later) [55]. Therefore, telbivudine cannot be used to treat patients with lamivudine-resistant HBV. The phase III clinical GLOBE trial showed that telbivudine compared with lamivudine in HBeAg-positive and -negative patients gave a higher antiviral and clinical efficacy after 2 years of treatment [89]. From a pharmacokinetic point of view, telbivudine could be combined with lamivudine or adefovir, because no drug interaction was observed [90].

IFN- α

IFN- α is the first substance licensed to treat CHB virus infections. Only 30% of the patients showed a successful response with loss of HBeAg, HBV DNA and normalisation of ALT levels. Numerous side effects were observed, including influenza-like symptoms.

The mechanism of action for IFN is twofold [91]: (i) it shows antiviral activity (e.g. induction of 2', 5'-oligoadenylate synthetase) as well as (ii) immunomodulatory activity (e.g. increased expression of MHC I, and stimulation of CTLs). The recommended regimen of IFN- α is 5 \times 10⁶ units administered daily or 1 \times 10⁷ units given three times a week subcutaneously for a period of 4 to 6 months [92].

At present, two types of IFN are approved for CHB treatment: IFN- α -2b (Intron A; Schering-

Plough) and pegylated interferon α -2a (PEG-IFN; Pegasys; Roche labs). The pegylated form has a longer half-life and, thus, at a lower concentration it has the same efficacy as normal IFN.

Lau *et al.* investigated the antiviral activity and safety of PEG-IFN- α in the presence or absence of lamivudine for HBeAg-positive CHB. During 48 weeks, 814 patients received either PEG-IFN plus placebo, PEG-IFN plus lamivudine or lamivudine alone and were followed-up for 24 weeks. This study demonstrated that PEG-IFN with or without lamivudine treatment led to higher percentages of HBeAg seroconversion, HBV DNA suppression, and HBsAg seroconversion (this was not observed with lamivudine monotherapy) and, thus, PEG-IFN 2 α provides a significant improved efficiency over lamivudine [93].

PEG-IFN α -2b is effective against HBeAg-positive CHB, but combinations with lamivudine [94] or ribavirin [95] gave no additional benefit. In contrast, a recent combination treatment study in CHB patients by Wursthorn *et al.* using PEG-IFN α -2b and adefovir showed a strong reduction in HBV DNA, cccDNA and HBsAg levels [96].

NOVEL ANTI-HBV AGENTS IN CLINICAL AND PRECLINICAL TRIALS

Tenofovir disoproxil fumarate

Tenofovir disoproxil fumarate (TDF) (Viread[®]) has been approved for the treatment of AIDS in HIV and HIV/HBV co-infected persons. In addition, analysis of anti-retroviral therapy in co-infected patients has demonstrated the efficacy of TDF against wild-type as well as lamivudine-resistant HBV strains [97] and adefovir-resistant strains. However, a 3- to 4.2-fold resistance increasing shift in effective concentration (EC_{50}) values was observed in *in vitro* assays [98,99]. *In vitro* studies revealed that the combination of tenofovir with (-)FTC gave additive to synergistic effects. Combinations with lamivudine, entecavir or telbivudine resulted in additive effects [100]. The antiviral activity of tenofovir and adefovir are similar. Tenofovir undergoes two efficient phosphorylations to its active form PMPApp that has a long half-life (95 h) [98]. It functions as a chain terminator and represents a poor substrate for cellular DNA polymerases α , β and ϵ [101]. To increase absorption, tenofovir is esterified to its bis(isopro-

pyloxy-carbonyloxymethyl)ester [tenofovir disoproxil, bis(POC)PMPA] (Figure 3A).

Woodchuck studies using different concentrations of TDF administered once a day for 4 weeks showed a good safety profile and reductions in viremia levels. Withdrawal of TDF therapy increased WHV DNA levels to the original (elevated) values [102]. Fewer side effects (i.e. nephrotoxicity) have been reported with TDF given orally at 300 mg a day [92].

The rtA194T mutation *in vitro* and in HBV/HIV co-infected patients showed TDF resistance in the presence of lamivudine mutations rtL180M and rtM204V [103]. Adequate monitoring will be required to investigate TDF resistance in CHB infections [98,103]. TDF offers an important alternative for patients with low lamivudine or adefovir dipivoxil responses [104].

Emtricitabine

Emtricitabine or (-)FTC (Figure 3B) has been licensed for the treatment of HIV infections and is currently undergoing phase III clinical trials for CHB infections. The mechanism of action is similar to that of lamivudine. After three phosphorylations, (-)FTC acts as (i) a chain terminator in the nascent HBV DNA chain and/or (ii) as a competitive inhibitor of its natural substrate dCTP. Additionally, (-)FTC-TP is a weak inhibitor of cellular and mitochondrial DNA polymerases. At different doses, (-)FTC when given to chronically WHV-infected woodchucks caused reduced viremia levels [105].

Studies in humans have shown that 200 mg (-)FTC (the optimal dose) daily for 2 years gave a safe antiviral profile and a resistance rate of 18% [106] (vs. 20% for lamivudine after 1 year). The (-)FTC resistance mutations observed were rtM204I/V \pm rtL180M and rtV173L.

A study comparing the combination of the standard dose (-)FTC and 10 mg clevudine (L-FMAU) (see later) and (-)FTC alone for 24 weeks showed no significant difference between both groups, but the combination group had a significant greater virological and biochemical response at 24 weeks post-treatment. The prolonged anti-HBV activity of L-FMAU was also observed in the combination group, as well as in phase II clinical trials where L-FMAU monotherapy was administered [107,108].

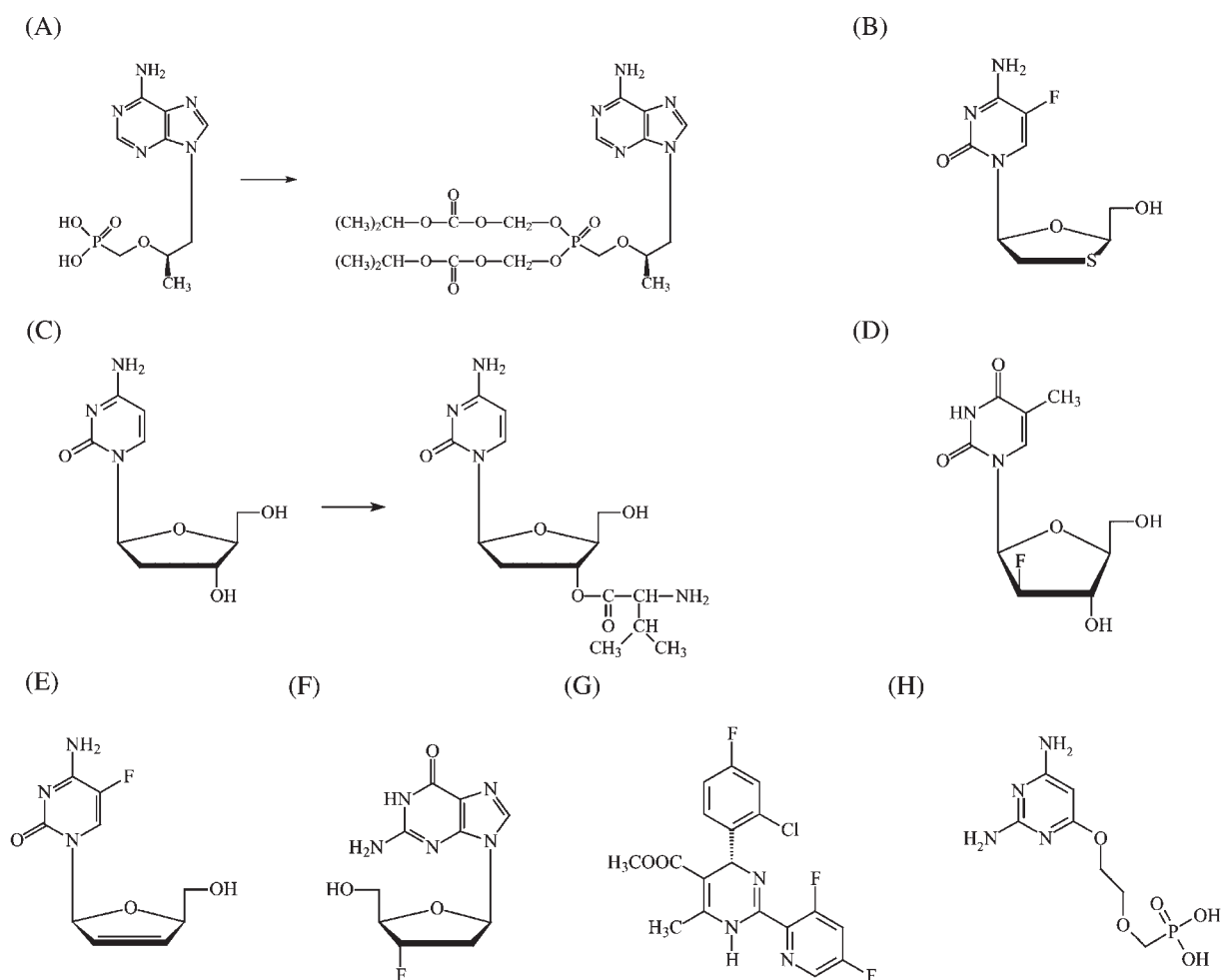


Figure 3. Anti-HBV agents under investigation. (A) The acyclic nucleoside phosphonate tenofovir and its prodrug tenofovir disoproxil fumarate (TDF), (B) the L-nucleoside analogue emtricitabine, (C) the L-nucleoside analogue β-L-deoxycytidine (L-dC) and its 3'-valine ester (valtorcitabine), (D) the L-nucleoside analogue elvucitabine (L-FMAU), (E) the L-nucleoside analogue elvucitabine (β-L-Fd4C), (F) 2',3'-dideoxy-3'-fluoroguanosine (FLG), (G) BAY 41-4109 and (H) the acyclic nucleoside phosphonate analogue PMEO-DAPy

Since they are both active against HBV, it is likely that the combination of TDF with (-)FTC may be further pursued, that is as Truvada[®] is used for HIV in the treatment of CHB.

Possible side effects observed with (-)FTC include lactic acidosis and hepatotoxicity [109]. In addition, flare-ups of HBV infection after withdrawal of (-)FTC have also been reported [109].

Valtorcitabine

Valtorcitabine (Val-L-dC) is the valine ester prodrug of L-dC (Figure 3C). *In vivo*, val-L-dC is converted to L-dC and subsequently phosphorylated three times to its active form L-dC-TP. *In vitro*

results showed an efficient metabolism of L-dC and L-dT in HepG2 cells and primary human hepatocytes [84]. Akin to L-dT, L-dC is a specific anti-HBV agent, with no activity against HIV. Their mode of action, however, is not identical since L-dT inhibits (+) strand DNA synthesis, whereas L-dC inhibits both (-) and (+) DNA synthesis [110].

Studies in woodchucks have demonstrated that the combination of L-dT and L-dC is more active in inhibiting WHV DNA synthesis than L-dC monotherapy or the combination of L-dT and lamivudine. Woodchucks treated for 4 weeks with L-dT or L-dC showed a WHV DNA level reduction

Table 1. Currently licensed anti-HBV drugs as well as anti-HBV agents that are in clinical or preclinical development. In addition, the table contains the mutations these agents can generate. Also indicated are the cross-resistance and sensitivity profiles of the drug-resistant variants

Agent	Mutation(s)	Cross-resistant to	Sensitive to
Lamivudine	rtM204V/I or rtM204I/S ± rtL180M ± rtV173C	Other L-nucleoside analogues (emtricitabine, telbivudine, clevudine, etc.)	Adefovir, tenofovir, FLG, LB80380/ANA380, PMEO-DAPy, entecavir ± MPA/ ribavirin (reduced sensitivity)
Adefovir	rtA181V/T and/ or rtN236T		Lamivudine, entecavir, emtricitabine, tenofovir, elvucitabine, FLG
Adefovir	rtI233V		Tenofovir
Entecavir	rtS184G or rtI169T or rtS202I or rtM250V rtM204I	Lamivudine	Adefovir
Telbivudine	rtA194T + Lamivudine resistance mutations (rtM204V + rtL180M)		
Tenofovir	rtM204V/I ± rtL180M and rtV173C	Adefovir	
Emtricitabine	rtM204V/I ± rtL180M	Lamivudine	
Valtorcitabine	rtM204V/I ± rtL180M	Lamivudine	
Clevudine	rtM204V/I ± rtL180M	Lamivudine	
Elvucitabine	rtM204V/I ± rtL180M	Lamivudine	
FLG	Not known		
LB80380/ANA380	Not known		
HAP	Not known		
PMEO-DAPy	Not known		

of respectively 8 logs and 2 logs [83], whereas woodchucks treated with the combination of both L-nucleoside analogues had undetectable WHV DNA levels after treatment without obvious toxicity.

Both L-dT and L-dC exhibited an excellent safety profile and showed a pronounced antiviral activity in clinical trials. In a placebo-controlled study, patients who received 200–400 mg/day of val-L-dC, HBV DNA levels decreased by $2\log_{10}$ copies/ml after 4 weeks [110].

Clevudine

The L-thymidine analogue clevudine (2'-fluoro-5-methyl- β -L-arabinofuranosyluracil) or L-FMAU (Figure 3D) is a potent antiviral agent active against HBV as well as EBV [111,112], showing a long half-life, low toxicity and a minimal effect on mitochondrial DNA [113]. Unlike other nucleoside analogues (e.g. lamivudine), L-FMAU has no effect on HIV.

After passing the cell membrane, L-FMAU is phosphorylated three times by cellular kinases to its active form 2'-fluoro-5-methyl- β -L-arabinofuranosyluracil-triphosphate (L-FMAU-TP) that can serve as a substrate for cytosolic thymidine kinase, deoxycytidine kinase and mitochondrial deoxypyrimidine kinase [114]. Molecular dynamic studies have shown that L-FMAU has a different mode of action compared to other anti-HBV nucleoside analogues. Because of conformational changes that are essential for its antiviral activity, L-FMAU-TP is unable to act as a substrate [115]. Additionally, it inhibits HBV DNA synthesis in a dose-dependent manner [113].

Woodchuck studies showed that the combination of L-FMAU and vaccine therapy inhibits the progression of chronic hepatitis and delays the onset of HCC in chronically WHV-infected woodchucks [116]. The first clinical trials have shown that the levels of viral DNA remain suppressed for up to 6 months after 28 days of treatment [108]. Significant antiviral and biochemical efficacy was observed after 6 months of therapy in a phase III trial with either HBeAg-positive or -negative patients receiving 30 mg L-FMAU daily [117]. A Study by Yoo *et al.* showed a sustained response after withdrawal of L-FMAU in CHB patients [118,119]. However, lamivudine-resistant HBV mutants (L180M + M204V; V173L + L180M + M204V; M204V; M204I; L180M + M204I) proved highly

resistant to treatment with L-FMAU, as was reported by Yang *et al.* [55].

Elvucitabine

Elvucitabine (β -L-2',3'-dideoxy-2',3'-didehydro-5-fluorocytidine) (β -L-Fd4C or ACH-126443); (ECB) (Figure 3E) is a nucleoside type of reverse transcriptase inhibitor with antiviral activity against both HIV and HBV.

Like other nucleoside analogues, it is phosphorylated three times to its active 5'-triphosphate form. However, phosphorylation of ECB is more efficient than that of lamivudine. Moreover, the half-life of ECB is higher than that of lamivudine [120]. Incorporation of ECB into the growing HBV DNA chain leads to chain termination and ECB-TP also acts as a competitive inhibitor of its natural substrate dCTP [121].

Studies in the duck HBV (DHBV) model showed a prolonged antiviral activity on viral DNA synthesis, although cccDNA levels could not be cleared [121]. Also, when its anti-viral effect on WHV in chronically infected woodchucks was compared with that of lamivudine, ECB showed a higher antiviral activity (i.e. on HBV DNA serum levels), although cccDNA levels were unaffected [122]. Finally, ECB does not seem to be active against lamivudine-resistant strains, but it is capable of inhibiting the replication of adefovir-resistant mutants [123].

FLG

The anti-HIV drug 2',3'-dideoxy-3'-fluoroguanosine (FLG) (Figure 3F) also showed anti-HBV and anti-DHBV activity *in vitro* [124]. *In vivo* studies revealed a good safety profile and anti-DHBV activity in infected ducks [125]. FLG proved equally effective against wild-type, lamivudine-resistant and/or adefovir-resistant HBV strains [126].

LB80380/ANA380

This prodrug of ANA317 is an orally available guanosine phosphonate analogue with anti-HBV activity against wild-type and lamivudine resistant strains. *In vitro* tests and *in vivo* woodchuck studies showed a pronounced antiviral response and safety toxicity profile [127]. Preliminary data from clinical trials showed a good antiviral activity and safety profile after 28 days of treatment at a dose of up to 240 mg once daily in HBeAg-positive

CHB patients. After withdrawal of ANA380, the viral load increased back to pre-treatment levels [127]. Other studies revealed that different doses for 12 weeks, up to 240 mg once daily, were safe and well-tolerated against HBeAg-positive lamivudine-resistant HBV strains [128]. Additional studies for long-term therapy and other target groups are underway.

Heteroaryldihydropyrimidines

BAY 41-4109 (or (methyl(R)-4-(2-chloro-4-fluorophenyl)-2-(3,5-difluoro-2-pyridinyl)-6-methyl-1,4-dihydro-pyrimidine-5-carboxylate)) (Figure 3G) belongs to the HAP-family, and has been described as a highly potent non-nucleoside HBV inhibitor [129]. Unlike other anti-HBV molecules, HAPs prevent nucleocapsid assembly by binding to the core particles (nucleocapsids) (Figure 1) in an enantioselective and reversible fashion. Studies also revealed that HAP acts specifically on HBV, since no interaction between HAP and DHBV core particles was observed [12].

Because of its fast absorption, good oral availability, highly specific antiviral effect, safety and pharmacokinetic and toxicological profile, this molecule might represent a new anti-HBV candidate for mono- or combination therapy [12,129].

PMEO-DAPY

Recently, 2,4-diamino-6-[(2-phosphonmethoxy)ethoxy]pyrimidine (PMEO-DAPy or also referred to as PMEO-DAPym) (Figure 3H), an acyclic pyrimidine nucleoside analog phosphonate, was shown to inhibit HBV replication with a potency comparable to that of adefovir on tenofovir. Moreover, PMEO-DAPy proved to have equipotent activity against wt, lamivudine-resistant and multidrug-resistant variants [130,131].

CONCLUSION

Nowadays, CHB virus infections can be treated with several antiviral agents. Resistance is likely to remain a problem in permanently reducing the viral load. A variety of promising candidate compounds is undergoing clinical trials, so that the armamentarium of drugs to treat CHB patients will likely expand in the near future. Apart from monotherapy and combination therapy, preventive measures whether based on vaccination or chemoprophylaxis, should play a more prominent role in reducing HBV infections in the near future.

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