

CLINICAL UPDATE

Treatment of chronic hepatitis B

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SUMMARY. In the last years, marked progress has been made in the treatment of chronic hepatitis B. The efficacy of lamivudine, the first nucleoside analogue available, is limited by the high incidence of resistance. Adefovir, which was recently approved has a comparable efficacy with a very low frequency of resistance. However, adefovir needs to be indefinitely administered as withdrawal of therapy is generally associated with reactivation and sustained response is uncommon.

Recent large randomized controlled trials showed that PEG IFNs induce relatively high sustained response rates both in HBeAg positive and HBeAg negative chronic hepatitis B. So far, the combination of PEG IFN with lamivudine, used simultaneously, is disappointing in terms of short-term efficacy. However, long-term efficacy needs to be assessed and different schedules of combination (for example sequential) need to be evaluated.

A number of nucleoside analogues, with favourable toxicity profiles and a promise of increased effectiveness against HBV, are at various stages of clinical development. Results of phase III trials of entecavir and emtricitabine confirmed their efficacy. However, while entecavir is associated with a low incidences of resistance, emtricitabine is associated with a relatively high incidence of resistance which limits its use as

a monotherapy. The efficacy and safety of new and more potent drugs like telbivudine and clevudine need to be confirmed.

The future of chronic hepatitis B therapy seems to be in the combination of different drugs. Ideally, the optimal drugs to combine would meet the following criteria: they should have different sites of action on HBV DNA replication, a potent antiviral effect, an excellent safety profile and they should induce a sustained response with a limited duration of therapy. Indeed, the concept of combination therapy has been recently developed in order to increase efficacy and to decrease the occurrence of viral resistance. However, so far few combinations have been evaluated. No combination therapy demonstrated a benefit as compared with monotherapy. More potent drugs and new combinations together with the understanding of the mechanisms of resistance to therapy are important challenges to improve the efficacy of treatment and decrease in the future the global burden related to chronic hepatitis B.

Keywords: adefovir, antiviral, cirrhosis, clevudine, emtricitabine, entecavir, hepatitis, hepatocellular carcinoma, interferon, lamivudine, nucleoside analogue, nucleotide analogue, pegylation, telbivudine.

INTRODUCTION

Three agents are currently approved for the treatment of chronic hepatitis B: interferon alpha (IFN), lamivudine and adefovir. Each agent has inherent limitations for use in the treatment of chronic hepatitis B [1–3]. IFN is effective in a minority of patients and has frequent side effects that limit its tolerability [4,5]. The efficacy of lamivudine is limited by the emergence of drug-resistant hepatitis B virus (HBV) mutants, restricting its utility as a long-term therapy for chronic hepatitis B. Adefovir, which has been recently

registered, is well tolerated and is associated with a low incidence of resistance but its antiviral effect is not optimal.

Lamivudine and adefovir have the advantages of oral administration and excellent safety profiles. However, they induce a sustained response after withdrawal of therapy in only a minority of patients and therefore the treatment needs to be indefinitely administered in the majority of patients. As a result, more effective therapy with more potent drugs used alone or in combination are needed and the treatment of chronic hepatitis B remains an open issue.

After a brief summary of the natural history of chronic hepatitis B in order to understand the indications and the objectives of therapy, this review summarizes first the results obtained with current available treatments, IFN, lamivudine and adefovir. The second part of this review summarizes the results obtained recently with pegylated IFN (PEG IFN), combination of PEG IFN with lamivudine, combination of lamivudine with adefovir and newer antiviral agents such as

Abbreviations: ALT, alanine aminotransferase; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; IFN, interferon alpha.

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Table 1 Nucleoside and nucleotide analogues for the treatment of chronic hepatitis B

Lamivudine	Approved
Adefovir dipivoxil	Approved
Entecavir	Phase III
Emtricitabine (FTC)	Phase III
Telbivudine (L-dT)	Phase III
Clevudine (L-FMAU)	Phase II
L-dC, L-dA	Phase II

entecavir, emtricitabine, telbivudine and clevudine. Phase III studies of entecavir and emtricitabine have been achieved but final results have not yet been fully published. The other drugs are still in phase II trials and therefore the information available is limited (Table 1).

NATURAL HISTORY OF CHRONIC HEPATITIS B

Chronic hepatitis B is a dynamic process with an early replicative phase and active liver disease and a late low replicative phase with remission of liver disease [1–3]. The natural history of chronic hepatitis B can be schematically divided in three phases (Fig. 1).

The first ‘immune tolerance’ phase is characterized by hepatitis B e antigen (HBeAg) positivity, high HBV replication level (reflected by high levels of serum HBV-DNA), normal or low levels of aminotransferases, mild liver necroinflammation and no or slow progression of fibrosis. During this phase, the rate of spontaneous HBeAg loss is very low. This first phase is more frequent and more prolonged in subjects infected at birth or during childhood.

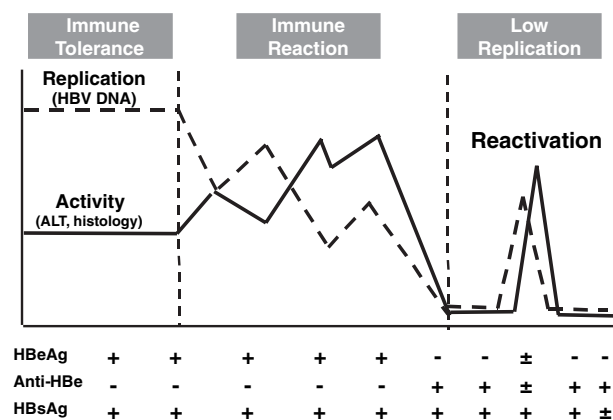


Fig. 1 Natural history of chronic hepatitis B. The natural history of chronic hepatitis B can be schematically divided in three phases: A first ‘immune tolerance’ phase with low activity of the liver disease and high level replication. A second ‘immune reaction’ phase with moderate to severe activity of the liver disease and low level replication. A third phase with very low level of replication and remission of the liver disease.

The second ‘immune reaction’ phase is characterized by a lower level of replication (as reflected by low levels of serum HBV DNA), increased levels of aminotransferases, moderate or severe liver necroinflammation and rapid progression of fibrosis. During this phase, the rate of HBeAg loss is high. This second phase is more frequent in subjects infected during adulthood.

Seroconversion from HBeAg to antibody to HBeAg (anti-HBe) marks the transition from chronic hepatitis B to the inactive hepatitis B surface antigen (HBsAg) carrier state with low or undetectable serum HBV-DNA and normal aminotransferases, and confers favourable long-term outcome with very low risk of cirrhosis or hepatocellular carcinoma (HCC) in the majority of patients. HBsAg loss and seroconversion to antibody to HbsAg (anti-HBs) may occur (resolved hepatitis B).

However a proportion of patients (about 20–30% of cases) continue to have or redevelop high levels of HBV-DNA and active hepatitis despite HBeAg seroconversion; these patients usually have HBV variants (with mutations in the precore or the basal core promoter regions) unable to express HBeAg (HBeAg negative chronic hepatitis B) [6]. HBeAg negative chronic hepatitis B represents a late phase in the natural history of chronic hepatitis B and is associated with a very low rate of spontaneous disease remission. The proportion of patients with this form of chronic hepatitis B has been increasing in the last decades and is majority in the Mediterranean countries [7].

OBJECTIVES OF TREATMENT

The first objective of the treatment is to decrease HBV replication in order to decrease necroinflammation in the liver and therefore prevent the progression of fibrosis. Thus, the interruption of the fibrogenesis process prevents progression to cirrhosis and its complications including HCC and therefore improves survival. The response to treatment can be classified into three phases. The first phase is characterized by the decrease of HBV replication (as reflected by the reduction of serum HBV-DNA level); the liver necroinflammation diminishes, fibrosis is stabilized or may even regress but the risk of reactivation persists. If the antiviral effect is sufficient (<100 000 copies of HBV DNA per mL) and is maintained and accompanied by an effective immune response with clearance of infected hepatocytes, HBe seroconversion may occur; then the risk of reactivation is low. If HBV replication is completely interrupted (as reflected by the absence of detectable HBV DNA in the serum by sensitive assays), with stable HBe seroconversion, loss of detectable HBsAg (with or without HBsAg seroconversion) may occur which is associated with complete disappearance of liver necroinflammation with no risk of reactivation.

With the drugs currently available, one may oppose two different concepts for the treatment of chronic hepatitis B: the first concept is that of sustained response obtained after a

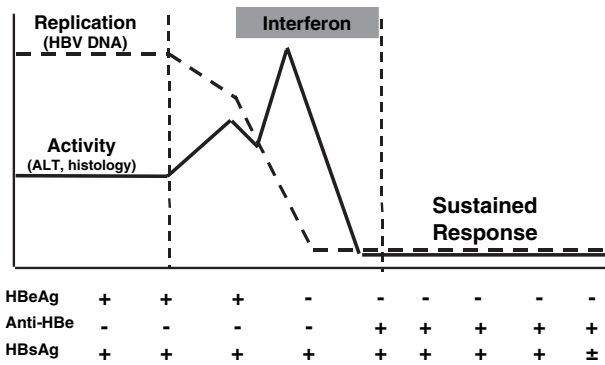


Fig. 2 Treatment of chronic hepatitis B with interferon alpha. During administration of interferon alpha, there is an early antiviral effect with decrease of serum hepatitis B virus (HBV) DNA level, then there is an immune effect with increased cytolysis with elevation of serum alanine aminotransferase (ALT) level. In case of effective antiviral and immune effects, a sustained response is observed after withdrawal of therapy with very low serum HBV DNA level, normal serum ALT level and HBe seroconversion. If HBV replication is completely inhibited with undetectable serum HBV DNA (with a sensitive polymerase chain reaction method), HBs seroconversion may occur within the years following treatment.

limited duration of therapy; the second concept is that of maintained response obtained during administration of therapy. The first strategy is the one usually used with interferon whose duration of therapy is limited by the poor tolerability (Fig. 2). Interferon therapy can induce a sustained response after withdrawal of treatment with a non-negligible chance of HBsAg loss or seroconversion; however, the limitation of this strategy is that the rate of sustained response is relatively low and sustained response is mainly obtained in patients with good predictors of response (i.e. patients in the immune reaction phase with active liver disease and low level replication). The second strategy is the one generally used with nucleoside or nucleotide analogues whose withdrawal is generally accompanied by reactivation. Therefore, therapy needs to be administered indefinitely to obtain a maintained response (Fig. 3). Maintenance therapy is allowed by the good tolerability of these drugs. However, the limitation is the occurrence during therapy of HBV resistant mutants associated with breakthroughs. Furthermore, HBsAg loss with or without HBs seroconversion is rarely observed with maintenance therapy with nucleoside or nucleotide analogues.

CURRENT TREATMENTS

Interferon alpha

Interferon alpha has been used in the treatment of chronic hepatitis B for many years. IFN exerts an antiviral effect on

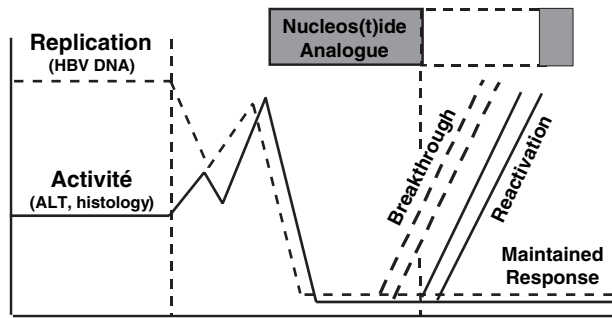


Fig. 3 Treatment of chronic hepatitis B with a nucleoside or a nucleotide analogue. During administration of the analogue, there is an early antiviral effect with decrease of serum hepatitis B virus (HBV) DNA level, however there is no immune effect of the drug and therefore there is no increased cytolysis and no elevation of serum alanine aminotransferase (ALT) level which remains normal. The response is maintained as long as the drug is administered. However, in case of occurrence of a resistant HBV mutant, a breakthrough is observed with a reincrease of serum HBV DNA level then a reincrease of serum ALT level. In the absence of an effective spontaneous immune response with HBe seroconversion, withdrawal of therapy is rapidly followed by a reactivation.

infection with HBV through two mechanisms [8]. First, IFN has a direct antiviral effect by inhibiting synthesis of viral DNA and by activating antiviral enzymes. Secondly, IFN exaggerates the cellular immune response against hepatocytes infected with HBV by increasing the expression of class I histocompatibility antigens and by stimulating the activity of helper T lymphocytes and natural killer lymphocytes. Thus, IFN induces an early diminution of HBV replication (reflected by a diminution of HBV DNA in serum) and a late (about 2 months later) increase in serum alanine aminotransferase (ALT) levels. Many controlled studies of IFN in patients with chronic hepatitis B have been reported. In these studies, with various schedules, mean virological response rate was 37% vs 17%, mean HBeAg loss rate was 33% vs 12% and HBsAg loss rate was 8% vs 2% in the interferon treated groups vs the placebo groups [5] (Fig. 4). A dosage of 5–10 MU thrice a week for 4–6 months allows a good efficacy with a satisfactory tolerance [2].

The discrepancies in the results of the different studies could be due, in part, to the different therapeutic schedules, but are mainly because of the populations of patients included in these trials. A certain number of factors are predictive of poor response to interferon [9] (Table 2). Low serum HBV DNA level and high serum ALT levels are predictors of response. Infection with HBV at birth or early in the patient's life (as it is often the case in countries where HBV infection is hyperendemic, such as South East Asia) is a factor of poor response to IFN.

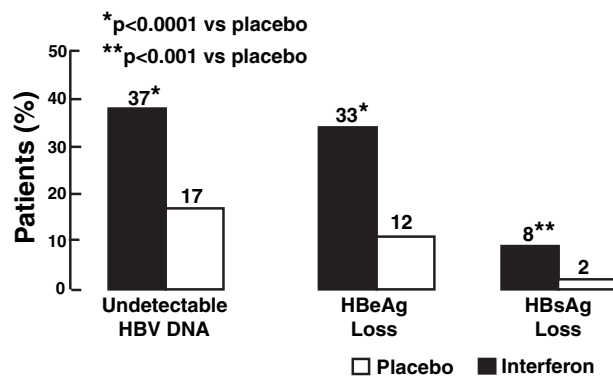


Fig. 4 Meta-analysis of interferon alpha (IFN) trials in hepatitis B e antigen (HBeAg) positive chronic hepatitis B (from Wong *et al. Ann Intern Med* 1993). In this meta-analysis including 15 randomized controlled trials (published between 1986 and 1992) comparing IFN vs placebo, including overall 837 patients with HBeAg positive chronic hepatitis B, the superiority of IFN versus placebo was shown for the rates of undetectable serum HBV DNA (hybridization assays), HBeAg loss and HBsAg loss.

Table 2 Predictors of nonresponse to interferon alpha therapy in chronic hepatitis B

Asian ethnicity
Childhood infection
Male sex
Immunosuppression because of disease (human immunodeficiency virus) or therapy
Coexisting hepatitis D virus infection
Disease caused by core promoter or precore mutant virus (hepatitis B e antigen-negative)
Low serum alanine aminotransferase levels
High serum hepatitis B virus DNA levels
Mild grade of liver necroinflammation

Lamivudine

Lamivudine, a nucleoside analogue which directly inhibits HBV DNA polymerase was first developed as a reverse transcriptase inhibitor for use in human immunodeficiency virus (HIV) infection [8]. It also has activity against HBV at lower concentrations. Lamivudine (2', 3' dideoxy thiacytidine) is a minus enantiomer and it is thought that this may help to explain the very low rates of side-effects noted with this agent. Randomized controlled trials have shown the efficacy of lamivudine in the treatment of HBeAg positive and HBeAg negative chronic hepatitis B.

HBeAg positive chronic hepatitis B

One randomized placebo-controlled trial [10] showed that almost all patients treated with lamivudine (98%) had a reduction of serum HBV DNA levels. Serum HBV DNA levels

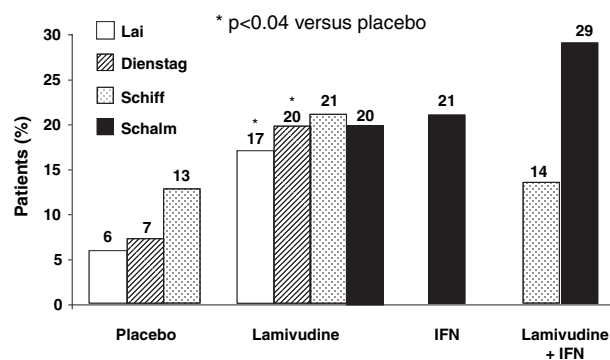


Fig. 5 Rates of HBe seroconversion in four randomized controlled trials of lamivudine (10–13). HBe seroconversion rates ranged from 17 to 21% at 1 year of therapy vs 6 to 13% (spontaneous seroconversion) in the placebo groups. In one study, the rate of HBe seroconversion was comparable with that observed in the interferon alpha (IFN) group; in two studies, the rates of HBe seroconversion were lower or higher in the IFN plus lamivudine combination groups, however, the differences were not significant.

became undetectable under lamivudine (<0.7 meq/mL) in 44% vs 16% in the placebo group. At 1 year of treatment, the HBe seroconversion rates were 17 and 6% in the lamivudine and the placebo group, respectively (Fig. 5). Normalization of serum aminotransferase (ALT) levels was observed in 41% vs 7% in the placebo group. Histologic improvement defined by a decrease of at least 2 points of the Knodell score was observed in 52% vs 23% in those on placebo.

The rates of virological, biochemical and histological response observed in three other randomized controlled trials showed similar results with HBeAg seroconversion rates ranging from 17 to 21% [10–13].

The tolerability and safety of lamivudine are excellent; the incidence of adverse events was similar to that of placebo. Lamivudine therapy seems to be well tolerated for up to 5 years, however data reported on long-term therapy with lamivudine are limited on small series of patients.

Pretreatment factors predictive of response are, likewise with interferon, high serum ALT levels and high degree of histologic necro-inflammation [14]. Pretreatment ALT level seems to be the most important: response rates are low and similar to untreated patients in patients with levels below two times the upper limit of normal. The rate of HBeAg loss was highest among patients with pretreatment ALT levels greater than five times the upper limit of normal.

After withdrawal of lamivudine therapy, ALT flare ups have been reported in 41 individuals treated with lamivudine for a minimum of 3 months and followed for at least 6 months after therapy [15]. Marked flares were observed in 17% and occurred 7–44 weeks after treatment. These flares

were all associated with rising levels of HBV DNA unlike the spontaneous flares associated with HBe seroconversion (with falling levels of HBV DNA). Two of these flares were associated with hepatic decompensation.

The major inconvenient of lamivudine is the high rate of occurrence of viral resistance related to mutations in the YMDD motif. Indeed, even if the HBeAg seroconversion rate may be increased by continuing treatment, the frequency of resistance increases with the time from 24% at 1 year, 38% at 2 years, 50% at 3 years and 67% at 4 years [2,3,16,17].

The most important mutation is a substitution of valine or isoleucine for methionine in the YMDD motif of the HBV polymerase gene (rtM204V/I) [18]. In many patients this is accompanied by a second mutation substituting methionine for leucine in an upstream region (rtL180M). Lamivudine resistance is more likely to occur in patients with high baseline serum HBV DNA levels.

The emergence of lamivudine resistant mutants is usually associated with a breakthrough with moderate increase of serum HBV DNA and ALT levels which may remain lower than at baseline for several months. However, severe cases have been reported in patients with cirrhosis. In patients who develop lamivudine resistant mutant, adefovir is effective and should be initiated rapidly if an increase in ALT is observed, especially in patients with cirrhosis who have a risk of hepatic decompensation. In order to diagnose earlier (before the appearance of detectable serum HBV DNA by standard assays and before the increase of ALT) the emergence of resistance, monitoring of serum HBV DNA level by a sensitive assay is useful. Indeed, an increase of serum HBV DNA of more than one log is generally the reflect of the appearance of a resistant mutant and this allows to switch to adefovir several months before the increase of ALT [18,19].

In case of HBe seroconversion during lamivudine therapy, it is usually recommended to prolong treatment for three to six additional months to decrease the risk of reactivation [1,2]. In the absence of HBe seroconversion, as withdrawal is almost invariably followed by reactivation, it is usually recommended to continue the treatment as long as HBV replication is suppressed and serum ALT levels remain normal, till the appearance of viral resistance.

HBeAg negative chronic hepatitis B

In patients with HBeAg negative chronic hepatitis B, one randomized controlled study [20] showed an efficacy similar to that observed in patients with HBeAg positive chronic hepatitis B with the same rate of resistance.

The HBV DNA was undetectable [nonpolymerase chain reaction (PCR)-based assays] after 12 months of therapy in 90% of patients (70% with PCR-based assays). Serum ALT normalized in 75% of patients. A fall in HBV DNA and normalization of ALT occurred only in 5% of patients on placebo. Histologic response was observed in 60% of lamivudine treated patients. Predictors of response to lamivudine

have not been established in this population. In patients with a virological response at the end of a 12-month course of lamivudine, the sustained response rate 6 months post-treatment was <5%.

In studies with prolonged therapy, response rates peak at 12 months and decrease thereafter [21]. At 30 months, rates of virological and biochemical responses were 30 and 60%, respectively.

The rate of resistance observed in patients with HBeAg negative chronic hepatitis was similar to that observed in HBeAg positive chronic hepatitis. Lamivudine resistant mutants appeared in 10–40% of patients after 1 year of therapy, and in 50–60% of those treated continuously for 3 years [21,22]. Likewise in HBeAg positive patients, the emergence of resistant mutant HBV was accompanied by an increase of HBV DNA and after a few months, by ALT elevation. Flares occurred in 30% of patients and were symptomatic or severe in some cases. Like in HBeAg positive patients, treatment by adefovir is generally effective in patients who develop lamivudine resistance.

Special groups

In patients with decompensated cirrhosis, lamivudine therapy can induce marked improvement in liver disease, impacting favourably on survival [23,24]. But the clinical effects are slow and impaired by the risk of emergence of resistance which can be associated with flares responsible for liver failure and death. Antiviral therapy is required before liver transplantation in patients with detectable HBV DNA in order to decrease the risk of recurrence of HBV infection after transplantation. In addition, patients in whom lamivudine resistance develops before transplant have a higher risk of recurrent hepatitis B post-transplant. In this clinical situation, adefovir should be rapidly initiated if lamivudine resistance is suspected in order to prevent the flare. This explains why in this setting, adefovir might be preferred because of the very low risk of resistance.

In case of recurrence of HBV infection after liver transplantation, lamivudine therapy achieves similar results to those in nontransplant patients with respect to inhibition of HBV replication and normalization of ALT. Breakthrough secondary to lamivudine resistance may lead to severe hepatitis flares, rapidly progressive liver failure and graft loss. For these reasons, after transplantation, the optimal prophylactic regimen seems to be the combination of lamivudine with anti-HBs immunoglobulins (HBIG).

In HIV-HBV coinfecting patients, from the limited data available lamivudine appears to show similar effect in patients on highly active anti retroviral therapy (HAART) in terms of virological and biochemical response, as in patients not coinfecting with HIV [25]. In 40 HIV coinfecting patients, 96% had undetectable serum HBV DNA (<5 pg/mL); serum ALT decreased compared with baseline; HBe seroconversion was observed in 11%. Drug resistant HBV emerged rapidly during monotherapy with

lamivudine: the incidence was 50 and 90% at 2 and 4 years, respectively [26]. Because of the high rate of resistance to lamivudine and because a large proportion of HIV-HBV infected patients have already received lamivudine for the treatment of HIV and/or HBV, adefovir or tenofovir (which is registered for the treatment of HIV) are the drugs generally used in the treatment of chronic hepatitis B in this population.

Adefovir dipivoxil

Adefovir dipivoxil has been recently registered for the treatment of chronic hepatitis B. Adefovir dipivoxil is the oral prodrug of adefovir. Adefovir is a nucleotide analogue of adenosine monophosphate. *In vivo*, adefovir dipivoxil is converted to the parent compound, adefovir, and through two phosphorylation reactions to adefovir diphosphate, the active intracellular metabolite that interacts with HBV polymerase. Adefovir diphosphate acts as a competitive inhibitor and chain terminator of viral replication.

Two large randomized controlled trials have demonstrated that adefovir dipivoxil is effective in patients with HBeAg positive or HBeAg negative chronic hepatitis B. Also, adefovir dipivoxil effectively suppresses lamivudine-resistant HBV in chronic hepatitis B patients postliver transplantation, patients with compensated or decompensated liver disease and patients co-infected with HIV.

HBeAg positive chronic hepatitis B

A large randomized, placebo-controlled study enrolled 515 patients randomized to receive adefovir dipivoxil 10 mg daily ($n = 172$), adefovir 30 mg daily ($n = 173$), or placebo ($n = 170$) for 48 weeks [27]. There was a rapid decrease in the median serum HBV DNA level in patients treated with adefovir dipivoxil, with statistically significant differences compared with placebo. At week 48, the median change from baseline in serum HBV DNA was $-3.5 \log_{10}$ for adefovir (10 mg) compared with $-0.5 \log_{10}$ copies/mL for placebo. Significantly more patients had undetectable serum HBV DNA levels (below 400 copies/mL) (21% vs 0%, $P < 0.001$) (Fig. 6). HBeAg seroconversion was observed in 12% of the patients receiving adefovir 10 mg compared with 6% in the placebo group ($P < 0.05$). ALT normalization was achieved in 48% compared with 16% ($P < 0.001$).

A significantly higher proportion of patients receiving adefovir 10 mg showed improvement in liver histology (improvement of at least 2 points of the Knodell score) at week 48 compared with those receiving placebo (53% vs 25%; $P < 0.001$) (Fig. 6). Results of the blinded ranked assessment of baseline and week 48 biopsies demonstrated that patients treated with adefovir 10 mg had better improvement of necroinflammatory activity ($P < 0.001$) and fibrosis ($P < 0.001$). Using the Ishak fibrosis score, fibrosis improved in 34% compared with 19% and fibrosis progressed in 11% compared with 21% [28].

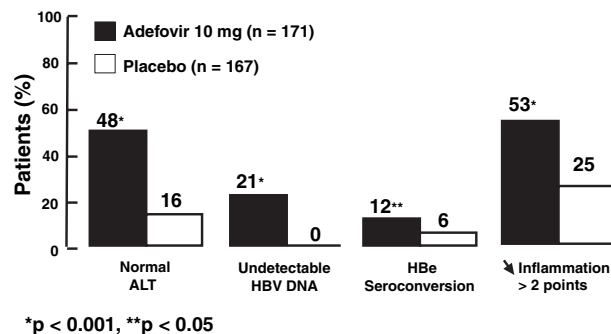


Fig. 6 Adefovir dipivoxil in the treatment of hepatitis B e antigen positive chronic hepatitis B (from Marcellin *et al.* *NEJM* 2003). In this randomized controlled trial, at 48 weeks of therapy, the rates of normal alanine aminotransferase, undetectable hepatitis B virus DNA, HBe seroconversion and histologic improvement were higher in the group of patients receiving adefovir, at the dose of 10 mg daily, as compared with the group receiving a placebo.

The tolerability and safety profile of adefovir at the dose of 10 mg was similar to that of the placebo. Adefovir at the dose of 30 mg was associated with increase in creatinine level in some patients. This increase was moderate, occurred after 24 weeks of treatment and resolved in all cases after withdrawal of the drug. However, this observation conducted to chose the 10 mg dose as the best dose with regard to the benefit/risk ratio and the 10 mg dose is the dose registered.

The durability of the response after withdrawal of treatment is not known as the treatment is maintained according to the design of the trial. However, cessation of therapy in some patients without HBe seroconversion was associated with relapse. Therefore, maintenance therapy is recommended. Preliminary results suggest that the antiviral effect is maintained and the rates of virological response with HBe seroconversion increases with the duration of therapy [29]. However, a longer follow-up is planned (up to 5 years) in order to answer the question of the durability of the response under treatment and of the possible increased efficacy of long-term treatment.

An extensive genotyping study has been carried out in all patients with detectable HBV DNA (by PCR assay). Systematic sequencing did not show any case of emergence of a mutant HBV resistant to adefovir after 48 weeks of treatment with adefovir [30]. Large scale studies of cohorts of patients under adefovir are in progress to assess the incidence of resistance on long term. The explanation for the high threshold for resistance to adefovir dipivoxil may reside in the structural features of adefovir that distinguish it from nucleoside analogues: specifically, its minimal flexible acyclic linker that closely resembles the natural substrate and the fact that it is a nucleotide, containing a phosphorus atom, not a nucleoside.

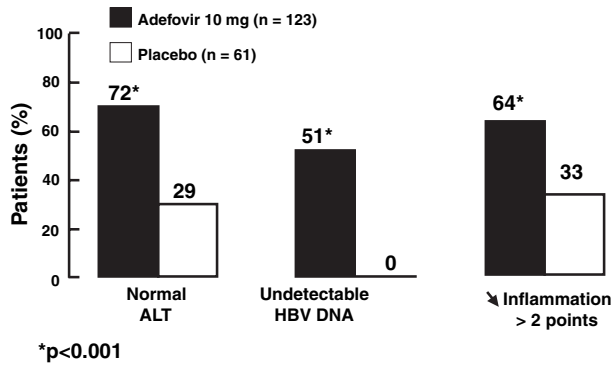


Fig. 7 Adefovir dipivoxil in the treatment of HBeAg negative chronic hepatitis B (from Hadziyannis *et al.* *NEJM* 2003). In this randomized controlled trial, at 48 weeks of therapy, the rates of normal alanine aminotransferase, undetectable hepatitis B virus DNA and histologic improvement were higher in the group of patients receiving adefovir, at the dose of 10 mg daily, as compared with the group receiving a placebo.

HBeAg negative chronic hepatitis B

A large randomized, placebo-controlled study of adefovir dipivoxil has been performed in 185 patients with HBeAg negative chronic hepatitis B [31]. Patients were randomized in a 2:1 ratio to receive either adefovir dipivoxil 10 mg ($n = 123$) or placebo ($n = 62$) for 48 weeks. Adefovir 10 mg once daily resulted in a significant reduction in serum HBV DNA levels at week 48 compared with placebo, with a median decrease of 3.9 \log_{10} copies/mL vs 1.3 \log_{10} copies/mL ($P < 0.001$). Fifty-one per cent of the patients treated with adefovir dipivoxil had no detectable HBV DNA as measured by PCR compared with none in the placebo group ($P < 0.001$) (Fig. 7). ALT normalization was achieved in 72% compared with 29% ($P < 0.001$). Significantly more patients in the adefovir group had histological improvement from baseline to week 48 compared with the placebo group (64% vs 33%, $P < 0.001$).

At 144 weeks of therapy with 10 mg of adefovir daily, the antiviral effect was maintained with, in a subgroup which had repeat liver biopsy at 96 weeks, a maintained histologic improvement. After 144 weeks of therapy, tolerability was good without significant side-effect and without the occurrence of nephrotoxicity [32]. The incidence of resistance related to the occurrence of specific mutations (rt N236T and rt A181V) was low (3% at 96 weeks and 5.9% at 144 weeks). It seems that the HBV strains resistant to adefovir are sensitive to lamivudine.

Special groups

An open label efficacy and safety study of adefovir dipivoxil 10 mg has been performed in 324 chronic hepatitis B patients pre- and postliver transplantation ($n = 128$ and 196, respectively) with clinical evidence of lamivudine-resistant HBV [33,34]. Patients have received adefovir dipivoxil for

varying lengths of time up to a maximum of 72 weeks (preliver transplantation) and 129 weeks (postliver transplantation), respectively. Serum HBV DNA was reduced by approximately 4 \log_{10} copies/mL in both groups, and ALT level normalized in 76 and 49% of patients in the two groups, respectively. Patients also had significant improvement of liver function. Kaplan–Meier survival curves estimates by week 48 were 84 and 93% in the pre- and post-transplantation groups, respectively. These 1-year survival rates are significantly better than survival rates observed in historical groups of patients with no treatment. Noteworthy, in a significant number of patients who improved, the liver transplantation was postponed.

Although many of the patients, all of whom had lamivudine-resistant HBV, were medically compromised as a result of advanced liver disease and co-morbidities, there was a low rate of adverse events leading to drug discontinuation in pre- and postliver transplantation patients (5 and 6%, respectively). A total of 42 deaths (11%) were reported in pre- and post-transplantation patients (24 and 18 patients, respectively). The deaths were considered to be due to complications of progressive liver disease or liver transplantation surgery.

In an open-label pilot study conducted in 35 HIV-HBV coinfecting patients with lamivudine resistant HBV and controlled HIV infection, adefovir therapy, at the dose of 10 mg, induced a 4 log decrease in serum HBV DNA levels at 48 weeks [35]. Two patients underwent HBe seroconversion. A transient increase in serum ALT levels was observed in 15 patients without consequence on liver function. The explanation for this observation is unclear. No HBV DNA breakthrough and no viral resistance was observed through week 48. In addition no significant changes in either HIV RNA or CD4 cell count were observed.

NEW TREATMENTS

Pegylated interferon

More recently, the efficacy of IFN has improved with the replacement of standard interferon by IFN conjugated with polyethylene glycol (PEG IFN). This new form of IFN reduces elimination of interferon by the kidneys, thus significantly increasing its half life and resulting in more stable plasma concentrations of interferon. Moreover, pegylation reduces the immunogenicity of the protein (reduction of the production of anti-interferon antibodies). Finally the number of injections has been reduced from thrice to once weekly, thanks to improved pharmacokinetics, which is obviously more comfortable for the patient.

Two PEG IFNs which differ in the quality and quantity of conjugated PEG to IFN have been produced: 12 kD of linear PEG for IFN 2b and 40 kD branched PEG for IFN 2a. In both cases PEG IFNs have been shown to be twice as effective overall than the corresponding nonpegylated IFNs in

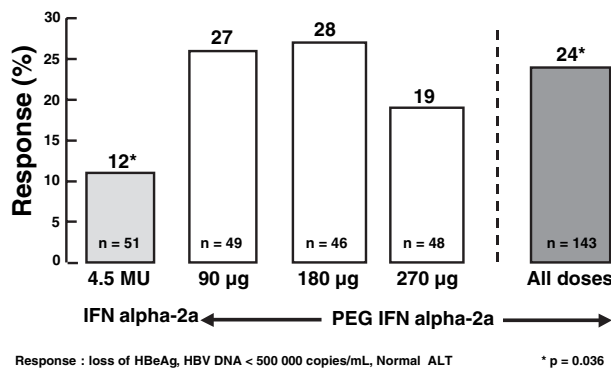


Fig. 8 Pegylated interferon (PEG IFN) alpha 2-a in hepatitis B e antigen positive chronic hepatitis B (from Cooksley *et al. Journal of Viral Hepatitis* 2003). In this randomized controlled trial, 24 weeks after therapy, the rates of response were higher in the three groups which received PEG IFN alpha 2a as compared with the group which received conventional IFN alpha 2a; however, the difference was not significant. When the three PEG IFN groups were pooled retrospectively, there was a significant difference as compared with the IFN group (24% vs 12%; $P = 0.036$).

chronic hepatitis C [36,37]. Therefore, the efficacy of PEG IFNs has been recently assessed in the treatment of chronic hepatitis B.

A first randomized controlled study of PEG IFN alpha 2a has been performed in patients with HBeAg positive chronic hepatitis B [38]. Treatment duration and follow-up were each 24 weeks. At the end of follow-up, treatment response defined by loss of HBeAg with serum HBV DNA level below 500 000 copies/mL with normal ALT was observed in 19–28% of patients receiving PEG IFN alpha 2a (at a dose of 90, 180 or 270 µg/week) vs 12% of patients who received standard IFN 2a (Fig. 8). Side-effects associated with PEG IFN were comparable with those observed with standard interferon. The safety profile of PEG IFN was comparable with that of conventional interferon with the same frequency of adverse events or laboratory abnormalities.

This study does not prove the superiority of PEG IFN alpha 2a as compared with standard IFN 2a because the dose of IFN 2a used was relatively low (4.5 million units, three times a week) and the differences observed between each of the three PEG IFN alpha 2a treatment groups and the standard IFN 2a group were not significant. However, the overall rate of response in patients who received PEG IFN alpha 2a was higher than that observed in the IFN 2a group. A retrospective analysis showed that the rates of response were higher with the PEG IFN among the most difficult to treat patients (with high HBV DNA level or low ALT levels). Therefore this study strongly suggests that PEG IFN alpha 2a is more effective than standard IFN 2a for the treatment of chronic hepatitis B.

Three large randomized controlled trials have confirmed the efficacy of PEG IFNs in HBeAg positive (one trial with PEG

IFN alpha 2b and one trial with PEG IFN alpha 2a) and HBeAg negative chronic hepatitis B (one trial with PEG IFN alpha 2a). These studies which compared PEG IFN monotherapies to the combination of PEG IFN and lamivudine (three studies) and lamivudine (two studies) are detailed in the next chapter.

Combination of pegylated interferon with lamivudine

Previous studies on the combination of IFN and lamivudine suggested that this combination could be more effective than lamivudine monotherapy [39]. However, the results of different studies were discordant which could be due to different treatment regimens which could not be optimal.

HBeAg positive chronic hepatitis

In a randomized controlled study, 307 patients with HBeAg positive chronic hepatitis B were randomized to receive either the combination of PEG IFN alpha 2b 100 µg/week for 32 weeks then 50 µg for 20 weeks and lamivudine 100 mg/day or PEG IFN alpha 2b at the same dose with placebo [40]. At the end of the 26-week post-treatment follow-up, there was no difference in response rates between the two treatment groups: serum HBV DNA was undetectable by PCR (below 400 copies/mL) in 7 and 9%; HBeAg loss was observed in 36 and 35%; normal ALT was obtained in 32 and 35% in the PEG IFN monotherapy and the PEG IFN with lamivudine combination therapy groups. Interestingly, a relatively high rate of HBsAg loss was observed (7% in both groups).

This study shows that in patients with HBeAg positive chronic hepatitis B, 26 weeks after therapy, the combination of PEG IFN alpha 2b with lamivudine (with the simultaneous regimen used) is not superior to PEG IFN alpha 2b used in monotherapy.

Main predictors of response were HBV genotype and pre-treatment ALT level. Response was 34% for those with ALT levels under three times the upper limit of normal and 50% for those with ALT levels above five times the upper limit of normal. Response was 60% for genotype A vs 42% for genotype B, 32% for genotype C and 28% for genotype D.

Another large randomized controlled trial of the combination of PEG IFN alpha 2a with or without lamivudine vs lamivudine showed similar results [41].

Patients were randomized to one of the following treatments: PEG IFN alpha 2a, 180 µg once weekly plus oral placebo once daily for 48 weeks; PEG IFN alpha 2a, 180 µg once weekly plus lamivudine 100 mg once daily for 48 weeks; lamivudine 100 mg once daily for 48 weeks. In total, 814 patients have been enrolled in the study. At the end of the 24-week post treatment follow-up, the two PEG IFN treatment arms (with or without lamivudine) showed the same efficacy which was superior to that observed in the lamivudine treatment arm: HBe seroconversion was observed in 32%, 27% and 19% of the patients, respectively and a virological response (serum HBV DNA below 100 000 copies per mL by quantitative PCR) in 32%, 34% and 22% of

the patients, respectively, HBsAg loss was observed in patients who received PEG IFN alpha 2a (4% and 3% versus 0% in the lamivudine group).

HBsAg negative chronic hepatitis

A phase III, partially double-blinded study has evaluated the efficacy and the safety of PEG IFN alpha 2a alone or in combination with lamivudine vs lamivudine in patients with HBsAg negative chronic hepatitis B [42].

Patients were randomized to one of the following treatments: PEG IFN alpha 2a, 180 µg once weekly plus oral placebo once daily for 48 weeks; PEG IFN alpha 2a, 180 µg once weekly plus lamivudine 100 mg once daily for 48 weeks; lamivudine 100 mg once daily for 48 weeks. In total, 552 patients have been enrolled in the study. At the end of the 24-week post-treatment follow-up, the two PEG IFN treatment arms (with or without lamivudine) showed the same efficacy which was superior to that observed in the lamivudine treatment arm: a biochemical response (normal ALT) was observed in 59, 60 and 44% of the patients, respectively and a virological response (serum HBV DNA below 20 000 copies/mL by quantitative PCR) in 43, 44 and 29% of the patients, respectively (Fig. 9). Interestingly, taking into account that HBsAg loss is rarely observed in HBsAg negative patients, a substantial rate of HBsAg loss was observed in this study in patients who received PEG IFN alpha 2a (4 and 3% vs 0% in the lamivudine group).

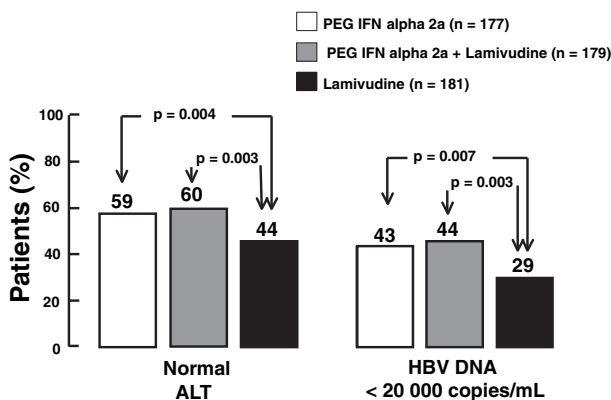


Fig. 9 Pegylated interferon (PEG IFN) alpha 2-a in hepatitis B e antigen negative chronic hepatitis B (from Marcellin *et al.* NEJM 2004). In this randomized controlled trial, 24 weeks after therapy, the rates of response (normal serum alanine aminotransferase level and serum hepatitis B virus DNA below 20 000 copies per mL) were higher in the two groups which received PEG IFN alpha 2a (with or without lamivudine) as compared with the group which received lamivudine. There was no difference in response rates between the group which received PEG IFN alpha 2a alone and the group which received the PEG IFN alpha 2a plus lamivudine combination.

Noteworthy, at the end of the 48-week treatment period, there was a higher incidence of lamivudine resistance in the lamivudine monotherapy group as compared with the PEG IFN alpha 2a plus lamivudine combination group (18% vs < 1%; $P < 0.001$), which confirms previous studies suggesting that IFN decreases the risk of lamivudine resistance [39].

The adverse events associated with PEG IFN alpha 2a therapy were similar to those observed in previous trials in patients with chronic hepatitis C. Interestingly, the frequency of the adverse events was lower than that observed in patients with chronic hepatitis C. In particular, the frequency of depression was much lower: 3–4% as compared with 16–20% in patients with chronic hepatitis C.

This study shows that in patients with HBsAg negative chronic hepatitis B, first, the efficacy, as assessed at 24 weeks post-treatment, of PEG IFN alpha 2a monotherapy is superior to lamivudine monotherapy and, secondly, the combination of PEG IFN alpha 2a with lamivudine (with the simultaneous regimen used) is not superior to PEG IFN alpha 2a used in monotherapy. However, longer follow-up is needed to confirm the similarity of response.

Combination of adefovir with lamivudine

The concept of improving the efficacy by combining two analogues is based on the hypothesis that the combination would maximize the viral suppression and would decrease the occurrence of viral resistance.

One randomized study evaluated the efficacy of the combination of adefovir with lamivudine as compared to lamivudine alone or adefovir alone in 59 patients with HBsAg positive chronic hepatitis B with lamivudine resistant HBV [43]. There was no significant difference in median serum HBV DNA reduction (–3.59 and –4.04 log copies/mL), rates of ALT normalization (53 and 47%) and HBsAg loss (three patients in each group) between the adefovir-lamivudine combination group and the adefovir monotherapy group. Noteworthy, serum HBV DNA level remained stable and there was no significant biochemical or serological change during the study in the patients who remained under lamivudine monotherapy. Therefore, the clinical benefit of continuing lamivudine therapy once resistance develops appears to be questionable. However, it seems reasonable, at least in patients with bridging fibrosis or cirrhosis, to continue lamivudine administration after initiation of adefovir until a significant decrease of serum HBV DNA and serum ALT has been obtained.

Another study compared the efficacy of the combination of adefovir with lamivudine vs lamivudine used in monotherapy in 112 treatment-naïve patients (107 HBsAg positive) [44]. There was no significant difference in median serum HBV DNA reduction (–5.41 and –4.80 log copies/mL), rates of undetectable HBV DNA with PCR (39 and 41%) and HBsAg loss (19 and 20%) between the adefovir-lamivudine combination

group and the adefovir monotherapy group. Interestingly, there was a lower incidence of lamivudine resistance in the combination group (2%) than in the lamivudine monotherapy group (20%) ($P < 0.003$).

These two studies do not answer the question of the benefit of the long-term treatment with the combination of adefovir and lamivudine as compared with adefovir monotherapy. Large randomized controlled trials with a long follow-up are needed to address this issue.

NEW ANTIVIRALS

Many new nucleoside analogues are under evaluation for the treatment of chronic hepatitis B. Entecavir, emtricitabine phase III studies have been achieved and results should be available soon. Other interesting nucleoside analogues like telbivudine and clevudine achieved phase II studies.

Entecavir

Entecavir, a cyclopentyl guanosine analogue, is a potent inhibitor of HBV DNA polymerase, inhibiting both the priming and elongation steps of viral DNA replication. Entecavir is phosphorylated to its triphosphate, the active compound, by cellular kinases. It is a selective inhibitor of HBV DNA and is less effective against lamivudine-resistant mutants than against wild-type HBV [45–47].

In a 24-week, double blind, randomized trial, the safety and efficacy of three different doses of entecavir (0.01, 0.1, or 0.5 mg/day) was compared with lamivudine (100 mg daily). One hundred and sixty-nine patients were included [48]. Compared with lamivudine, entecavir reduced HBV DNA by an additional 0.97 log at the 0.1 mg dose and 1.28 at the 0.5 mg dose ($P < 0.0001$). At 22 weeks of therapy, in patients treated with entecavir 0.5 mg, 83.7% had an HBV DNA level below the lower limit of detection of the assay (0.7 ME/mL) compared with 57.5% treated with lamivudine ($P = 0.008$). However, very few patients achieved HBeAg loss (0 and 6%) in both groups, respectively. A dose–response relationship was observed [48]. Entecavir was well tolerated at all doses; most adverse events were mild-to-moderate and transient with no significant differences observed between the different doses of entecavir and lamivudine. This study indicates that the 0.5 mg dose of entecavir could be the optimal dose.

Entecavir also showed activity in patients with lamivudine-resistant HBV. In one trial including 181 patients with lamivudine-resistant HBV, different doses (0.1, 0.5 and 1 mg daily) of entecavir were tested and compared with lamivudine [49]. At week 24, the percentage of patients with undetectable HBV DNA (<0.7 ME/mL) was 19% with 0.1 mg, 53% with 0.5 mg and 79% with 1 mg of entecavir vs 13% in the group receiving lamivudine ($P < 0.0001$).

Results of phase III studies of entecavir given for 48 weeks as compared with lamivudine in HBeAg positive and HBeAg

negative patients and in patients with lamivudine resistant HBV confirmed the efficacy and safety of entecavir.

In one study, 709 patients with HBeAg positive chronic hepatitis were randomized to receive entecavir or lamivudine. Mean HBV DNA level decreases were 7.0 and 5.5 \log_{10} copies per mL ($P < 0.0001$), percentages of patients with undetectable HBV DNA (PCR) were 91% and 65% ($P < 0.0001$) and histologic improvement was observed in 72% and 65% ($P = 0.008$) of patients, respectively [50].

In another study, 638 patients with HBeAg negative chronic hepatitis were randomized to receive entecavir or lamivudine. Mean HBV DNA level reactions were 5.2 and 4.7 \log_{10} copies per mL ($P < 0.0001$), percentages of patients with undetectable HBV DNA were 91% and 73% ($P < 0.0001$), histologic improvement was observed in 70% and 61% of patients ($P = 0.014$), respectively.

Resistance to entecavir (mutations at positions nt 184, 202 and nt 250) was observed in none of the 432 patients who were not previously treated with lamivudine and in 5.8% of the 172 patients who had previously received lamivudine [51].

Emtricitabine

Emtricitabine (FTC) is a cytosine nucleoside analogue with antiviral activity against both HBV and HIV [45]. It differs from lamivudine by a fluorine at the 5-position of the nucleic acid. In a pilot study, 49 patients with HBeAg positive chronic hepatitis B received five different doses of emtricitabine: 25, 50, 100, 200, or 300 mg daily for 8 weeks. At the end of treatment, serum HBV DNA levels decreased by 2–3 logs in patients receiving the highest doses.

In a randomized, double blind study, 98 Asian patients (77 HBeAg positive and 21 HBeAg negative) were randomized to receive 25, 100, or 200 mg of emtricitabine daily for 48 weeks [52]. At 48 weeks, the median decreases in viral load were 2.6 \log_{10} , 3.1 \log_{10} and 2.9 \log_{10} copies/mL for the three doses respectively. The proportions of patients with undetectable HBV DNA (below 4700 copies/mL) were 38, 42 and 61% for the three doses, respectively. HBeAg loss was observed in a high proportion (40%) of the HBeAg positive patients (ranging from 32 to 50% depending on the dose). Emtricitabine has a comparable efficacy in HBeAg negative chronic hepatitis B [53]. The results of this study suggest that the optimal dose of emtricitabine is 200 mg once daily. Genotypic analysis performed at week 48 showed that 12% of patients treated with 100 mg of emtricitabine and 6% of those treated with 200 mg developed drug-resistant HBV.

Phase III clinical trials are under way to determine the long-term safety and efficacy of emtricitabine. However, the role of emtricitabine as a monotherapy may be limited by its structural similarity to lamivudine with the risk of development of drug resistance.

Telbivudine

The natural nucleosides in the β -L-configuration [β -L-thymidine (LdT), β -L-2-deoxycytidine (L-dC) and β -L-2-deoxyadenosine (L-dA)] represent a newly discovered class of compounds with potent, selective and specific activity against hepadnavirus [54]. 'In vitro' studies have shown that these compounds have marked effects on HBV replication [45]. Telbivudine (LdT) is at the most developed stage of clinical investigation. A phase I study showed safe preclinical toxicology profile with no mitochondrial toxicity and no mutagenic effect [55,56].

A phase II study including 104 HBeAg positive patients compared different therapeutic schedules for 52 weeks: LdT 400 mg daily, LdT 600 mg daily, LdT 400 mg and lamivudine 100 mg daily, LdT 600 mg and lamivudine 100 mg daily or lamivudine 100 mg daily [57]. Median HBV DNA reduction was -6.01, -5.99 and -4.57 in the patients who received telbivudine, the combination of telbivudine and lamivudine and lamivudine, respectively. Percentages with undetectable HBV DNA by PCR were 61, 49 and 32%. Percentages of patients with HBeAg loss were 33, 17 and 28%. YMDD HBV mutants were found in 4.4, 12.2 and 21.1% of the patients. The safety profile of telbivudine appeared similar to placebo. Thus, this study confirms the marked antiviral effect of telbivudine with a safe profile with however a non-negligible 1-year resistance rate (4.4%). The combination of telbivudine with lamivudine was not superior to telbivudine alone. On the basis of these data, phase III studies have been initiated.

Another promising β -L-nucleoside compound is val-LdC. It is in phase II testing and preliminary results indicate interesting antiviral activity with a good safety profile. A combination of these two compounds which act at different levels of HBV replication could be of interest.

Clevudine

Clevudine [L-FMAU;1-(2-fluoro-5methyl- β -L-arabinosyl uracil)] is a pyrimidine analogue with marked 'in vitro' activity against HBV but not HIV [58]. The active triphosphate inhibits HBV DNA polymerase but is not an obligate chain terminator. 'In vitro' studies suggest that it may also be effective against lamivudine-resistant HBV mutants. In the woodchuck model, a daily dose of 10 mg/kg of clevudine resulted in a 9 log₁₀ decrease in viral load. Interestingly, it was observed a delayed reincrease of viral load after cessation of drug administration in a dose-dependent manner [59]. No evidence of drug-related toxicity was observed in treated animals.

An open labelled phase I/II, nonrandomized, dose-escalation study was performed in 35 patients who received clevudine for 28 days at the daily dose of 10 mg ($n = 10$), 50 mg ($n = 10$), 100 mg ($n = 10$) or 200 mg ($n = 5$) followed by a 20 weeks post-treatment period [60]. At the end

of the dosing period, the median reduction in serum HBV DNA was 2.48 log₁₀, 2.74 log₁₀ and 2.95 log₁₀ in the 10, 50 and 100 mg groups, respectively. Interestingly, as in the woodchuck model, there was a slow and delayed reincrease of serum HBV DNA levels with at the end of the post-treatment follow-up, lower median HBV DNA levels as compared with baseline. Clevudine was well tolerated without serious adverse event. An additional trial with clevudine administered for 12 weeks showed comparable results and confirmed safety [61].

These preliminary results show that clevudine might be one of the most potent antivirals available for the treatment of HBV. Further studies are needed to assess the long-term efficacy and safety of this drug.

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