Treatment of HBeAg-Positive Chronic Hepatitis B with Nucleoside/Nucleotide Analogs

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INDICATIONS FOR THERAPY

There is no consensus on which patients should be treated for chronic hepatitis B and current treatments have limited long-term efficacy. In general, treatment of chronic hepatitis B should target patients with active disease and viral replication, preferably before there are the signs and symptoms of cirrhosis or significant injury has occurred [1]. Eradication of infection is only possible in a minority of patients. However, if HBV replication can be suppressed, the accompanying reduction in histological chronic active hepatitis reduces the risk of cirrhosis and hepatocellular carcinoma [2]. Patients with mild chronic hepatitis should be carefully monitored at appropriate intervals. Therapy should only be considered if there is evidence of moderate to severe activity. Hepatitis Be antigen (HBeAg)-positive patients should be followed for a few months to determine their status, and antiviral therapy should be considered if there is active hepatitis B virus (HBV) replication (HBV DNA above $10^5$ copies/mL) and persistent elevation of aminotransferases after 3-6 months of observation. HBeAg-negative patients should be considered for antiviral therapy when serum aminotransferases are raised and if there is active viral replication (HBV DNA above $10^5$ copies/mL). Many clinicians
consider a liver biopsy to be helpful in determining the degree of necroinflammation and fibrosis.

HBV/HIV co-infected patients whose immune status is preserved on highly active antiretroviral therapy (HAART) should be considered for anti-HBV therapy, with appropriate therapy for HIV infection to minimize resistance. If HAART is indicated in a patient with HIV co-infection, lamivudine can be administered since it is active against HIV and HBV. Adefovir dipivoxil (ADV) is also active against both viruses although a lower dose is used for HBV. Although tenofovir is active against HBV and HIV, its efficacy in hepatitis B infection has not been confirmed in large controlled trials.

Hepatitis B surface antigen (HBsAg)-positive patients with extra-hepatic manifestations and active HBV replication may respond to antiviral therapy. Patients with decompensated cirrhosis should be treated in specialist liver units, due to the complexity of antiviral therapy in these cases.

Prophylactic therapy is recommended in all patients undergoing liver transplantation for end-stage hepatitis B to lower HBV DNA levels to less than 10⁵ copies/mL before transplantation. The optimal timing of transplantation has not been established, but the selection of resistant strains should be avoided before surgery. Lamivudine and ADV are suitable. Antiviral therapy for prophylaxis of post-transplantation recurrence will probably require lifelong treatment. The most promising prophylaxis includes lamivudine and lifelong hepatitis B immunoglobulin (HBIG) treatment after transplantation resulting in low rates of reinfection/reactivation. Shorter courses of HBIG and other forms of prophylaxis, including ADV combined with lamivudine, are being studied. The optimal treatments for hepatitis B, including suitable combination therapies, are being evaluated in different studies. Response rates in HBeAg-positive patients are better in patients with higher baseline alanine aminotransferase (ALT) for all currently licensed agents.

Interferon remains a benchmark therapy for chronic hepatitis B. Approximately 35-40% of HBeAg-positive patients are treated effectively by interferon, at a dose of 5-10mIU/three times weekly (5mIU daily in the USA) for 4-6 months. The efficacy of interferon alpha is discussed elsewhere in the present work. The rationale of first-line treatment with alpha interferon is to achieve loss of HBeAg (and even subsequent loss of HBsAg) after a short course of treatment.
Sustained loss of HBsAg is generally associated with a histological reduction in inflammation [3].

There are alternative options and strategies for treatment; new nucleoside analogs which act as chain terminators have influenced the treatment of hepatitis B. Lamivudine can be given (100mg/day for at least one year and maintained for 4-6 months after a virological response (loss of HBeAg) is achieved). Loss of HBsAg has been observed. If a virological response is not achieved within 1 year, the likelihood that continuation of treatment will produce a response is offset by the cumulative risk of developing drug resistance. Therapy remains useful if HBV DNA is suppressed (histological improvement has been documented). ADV 10mg/day is an effective alternative to lamivudine. As with lamivudine, most patients do not have a virological response after one year. ADV can be given at a dose of 10mg/day for at least one year, and maintained for 4-6 months after a virological response occurs (loss of HBeAg is achieved). Unlike lamivudine, the likelihood that continuation of treatment will produce a virological response is not clearly offset by the cumulative risk of developing drug resistance. Therapy also remains useful if HBV DNA is suppressed (histological improvement has been documented). Long-term use of ADV monotherapy (>2 years) will require monitoring for resistance and possible nephrotoxicity.

Patients with decompensated cirrhosis are not candidates for interferon-alpha therapy because of the risk of side-effects, but may be candidates for nucleoside or nucleotide therapy. Patients with moderate to severe chronic hepatitis (HBeAg-positive or -negative) whether treated or not, and patients with advanced liver disease should be monitored for the progression of liver disease and the development of complications, including hepatocellular carcinoma.

LAMIVUDINE

Lamivudine (2',3'-Dideoxy-3'-thiacytidine ((+) -SddC), 3TC or Epivir) is a potent inhibitor of HBV, as well as HIV (Figure 1). The drug acts by inhibiting DNA synthesis through chain termination. The (-)-form ((-)-SddC), which is resistant to deoxycytidine deaminase, is a more active antiviral stereoisomer than the (+)-form. The negative enantiomer (-)-SddC does not appear to affect mitochondrial DNA synthesis. Metabolic studies have shown that the drug is converted to the monophosphate, diphosphate, and triphosphate form. It is rapidly
absorbed after oral administration, with a bioavailability of >80%. Most of the drug is excreted unchanged in the urine. Lamivudine has been used in trials for the treatment of HIV infection since 1990, and this compound has been licensed as a component of HAART. Lamivudine is active *in vitro* against human hepatitis B transfected cell lines and in ducklings affected with duck hepatitis B virus (DHBV), as well as in chimpanzees infected with HBV.

**Figure 1:** Lamivudine (GR109714x) Single (-) enantiomer of the racemic mixture 2’ deoxy-3’ thia cytidine.

Large phase III trials in patients with chronic hepatitis B have been completed. Doses above 25mg reproducibly decrease HBV DNA levels in serum. HBV DNA generally became undetectable (by hybridization assay) in more than 90% of patients who received 25mg-300mg/day. In most patients, HBV DNA reappears after therapy is completed. In large trials in Asia and the Western countries, approximately 15-20% of patients became HBeAg-negative after 12 months of treatment compared to 4% of placebo recipients. Histological improvement was noted after one year of treatment. Lamivudine therapy has consistently been associated with a highly
significant sustained reduction in levels of serum HBV DNA at the end of one year of therapy in up to 98% of patients. Undetectable levels of HBV DNA were sustained in 44% of treated patients compared to 16% on placebo. Loss of the HBe antigen with seroconversion to anti HBe was observed in 17% of patients after a year of treatment with lamivudine versus 6% on placebo. Histological improvement was the main outcome measured in the pivotal trial of lamivudine therapy in adults. Liver biopsies were scored according to the degree of necroinflammation and fibrosis and an improvement of 2 or more Human Awareness Institute (HAI) points. Significant differences in the total HAI score were observed in patients receiving lamivudine compared to those receiving placebo [4]. Lamivudine monotherapy reduces HBV DNA concentrations prior to liver transplant, but may be associated with subsequent resistance. Lamivudine and HBIG prophylaxis have been shown to be effective for the prevention of recurrent hepatitis B post-transplantation.

The drug seems to be well tolerated and relatively few serious side-effects have been reported. Serious side-effects have been observed in about 5% of patients; these include anemia, neutropenia, an increase in liver enzymes, nausea and neuropathy. Increased lipases may occur, but this is uncommon, and serious lactic acidosis has not been observed. Severe exacerbations of hepatitis accompanied by jaundice have been reported in patients whose HBV DNA became positive after stopping treatment, or after the development of resistance. Reactivation of hepatitis was observed in patients who developed a methionine to valine or isoleucine substitution in the highly conserved YMDD motif of the HBV polymerase [5]. This motif is part of the active site of the polymerase, and this mutation parallels the M184 mutation seen in resistant HIV where substitutions of valine and isoleucine for methionine have also been found. Lamivudine-resistance is conferred through acquired selection of HBV with mutations of the YMDD motif of the HBV DNA polymerase gene [5]. Four major patterns have been observed: L180M + M204V; M204I; L180M + M204I; V173L + L180M + M204V; and occasionally L180M + M204V/I. The L180M + M204V pattern occurs most frequently. Although viral “fitness” may be reduced, as lower levels of HBV DNA occur, recent studies have suggested that the disease may progress [6]. These changes cause a marked decrease in sensitivity to lamivudine in vitro. The incidence of lamivudine resistance in chronic hepatitis rises from 24% after one year of
treatment to 66% after 4 years. The incidence increases to 90% in HBV/HIV co-infected patients. The selection of antiviral resistance is a disadvantage of treatment with nucleoside analogs and is a fundamental disadvantage of treatment with long-term lamivudine therapy.

After lamivudine is stopped, HBV replication may reactivate and can sometimes be associated with severe “flares” or exacerbation of hepatitis as HBV DNA increases in serum. The pathogenesis of this injury is not fully understood. It is probably related in part to an immune response. The emergence of resistance could have a similar effect, as viral DNA increases. Combination studies with lamivudine and ADV are in progress.

ADEFOVIR DIPIVOXIL

Pharmacology
Adefovir dipivoxil 9-[[2-[(pivaloyloxy)methoxy]phosphinyl]methoxy]-ethyl]adenine is an orally bioavailable prodrug of adefovir, a phosphonate nucleotide analog of adenosine monophosphate (Figure 2). It requires cellular nucleoside kinases for activation to ADV diphosphate and it then acts as a competitive inhibitor and chain-terminator of HBV replication mediated by HBV DNA polymerase. This drug inhibits viral polymerases and terminates the growing DNA chain by acting as a competitive inhibitor of deoxyadenosine 5’-triphosphate (dATP). Because ADV diphosphate lacks a 3’ hydroxyl group, the compound causes premature termination of viral DNA synthesis upon its incorporation into the nascent DNA chain. ADV is active against HBV, DHBV and woodchuck hepatitis virus (WHV) in cell culture models and against chronically infected animals. This agent also has some immunomodulatory activity and stimulates natural killer activity.
ADV is active *in vitro* against all known lamivudine, emtricitabine, famciclovir and HBIG resistant HBV, using both cell culture and enzyme assays. Resistance to ADV is remarkably delayed in patients with chronic hepatitis B. Recently, a novel N236T mutation was reported in two anti-HBe-positive patients after 96 weeks of treatment, which was not detectable after one year of treatment. This mutant showed lowered susceptibility to adefovir. The mutation does not share cross resistance with lamivudine. Sequencing of the RT domain of the HBV polymerase has suggested that two mutations, i.e. rtN236T and rtA181V confer resistance to ADV. These mutants remain sensitive to lamivudine, emtricitabine, telbuvudine, and entecavir [7, 8]. Life table analysis has suggested a cumulative incidence of 3.9-5.9% (in naive patients) after three years of treatment.

The limited development of resistance with ADV could be related to its close structural relationship with the natural substrate which limits the potential for steric hindrance as a mechanism of resistance. In addition, ADV contains a flexible acyclic linker that may allow it to bind to HBV polymerase with different conformations, and thus, further subvert steric hindrance [9, 10]. ADV also contains a phosphonate bond that is less susceptible to ATP-mediated chain
terminator excision, which has been recognized as a mechanism of HIV resistance.

Following oral administration of single doses of ADV 10mg in patients with chronic hepatitis B or healthy subjects, maximum ADV concentrations in plasma occur a median of 0.76-1.75 hours after administration. ADV may be taken once daily because of the long terminal elimination half-life. In preclinical studies, evidence of renal toxicity, characterized by renal tubular nephropathy, was noted in all species evaluated. The efficacy of ADV has been investigated in patients with compensated liver disease and evidence of HBV replication; in patients who did not respond to lamivudine therapy, including post-transplantation patients, patients with compensated and decompensated liver failure and patients co-infected with HIV.

**Pivotal trials of ADV**

Doses of 5-125mg/day have been assessed in the clinical development program. In Phase I/II clinical studies in both HBeAg-positive and HBeAg-negative patients with chronic hepatitis B, statistically significant decreases in serum HBV DNA concentrations were demonstrated within the first week of treatment, and were maintained for up to 136 weeks. Data from previous studies indicated that daily doses of 30mg/day after 24 weeks is associated with mild, reversible nephrotoxicity (seen at higher doses in HIV studies) after the drug is discontinued. Multinational double blind randomized placebo controlled trials, in both HBeAg-positive and negative patients with liver disease have been performed. The primary endpoint of these studies was the quantitative assessment of histological improvement after 48 weeks of treatment using the Knodell Histologic Activity Index (HAI/Knodell) scoring score. Both necro-inflammatory activity and fibrosis was more improved with ADV 10mg and 30mg than with placebo ($P<0.001$). The pivotal phase III studies examined both ADV 10mg and 30mg to determine the dose with the best risk-benefit profile. These studies were multinational, double blind, randomized, placebo controlled trials, in HBeAg-positive and HBeAg-negative patients with compensated liver disease, with evidence of active HBV replication, who were not undergoing current treatment [11, 12]. In the HBeAg-positive trial, 515 patients were randomized to one of three arms: ADV 30mg/day, ADV 10mg/day or placebo. The primary endpoint of this study was based on the quantitative assessment of
histological improvement after 48 weeks of treatment using the HAI/Knodell score [13]. Histological improvement was defined as a reduction of 2 points or more from baseline in the HAI/Knodell, with no worsening in the fibrosis score. Secondary endpoints in the study were based on established methods to determine the virological response (suppression of HBV replication based on the decrease of serum HBV DNA) and biochemical response (defined by reductions and normalization in ALT during therapy). HBeAg seroconversion, defined as loss of HBeAg and appearance of anti-HBe, was also a key secondary endpoint. Loss of HBeAg has been correlated with long-term clinical improvement [2]. A daily dose of 10mg of ADV was shown to have the best risk-benefit profile for long-term treatment. This dose resulted in significant improvement compared to placebo: improvement in liver histology (53% vs. 25%, \( P < 0.001 \)), reductions in HBV DNA (3.52 vs. 0.55 log copies/mL, \( P < 0.001 \)), normalization of ALT (48% vs. 16%, \( P < 0.001 \)), and HBeAg seroconversion (12% vs. 6%, \( P = 0.049 \)). There were no significant side-effects and no resistance was found. As a result, 10mg of ADV is the recommended and approved daily dose. Improved responses were seen in patients with increased ALT [11]. An effect of ADV on cccDNA was observed in treated patients, but the significance of these findings requires further study. 10mg is the preferred treatment dose because of the favourable risk-benefit ratio. In the large HIV trials an incidence of nephrotoxicity of between 17% and 60% was reported. However, in the two largest hepatitis B phase III trials involving 695 patients, no clinically significant renal toxicity was found at the 10mg dose.

**ADV for lamivudine-resistant hepatitis B**

ADV has been shown *in vitro* to be active against lamivudine-resistant HBV, [9, 14] and there are a number of reports of successful treatment of lamivudine-resistant patients with ADV, particularly for post – transplant recurrence of hepatitis B [15-18]. There does not appear to be an advantage in continuing lamivudine after starting ADV in patients with compensated liver disease if lamivudine resistance develops. Rapid reductions in HBV DNA were observed within 4 weeks in all recipients of ADV, but the median changes from baseline were not greater in those who continued lamivudine. Thus treatment with ADV alone seems to be most effective in these patients, and there is no long-term advantage of continuing lamivudine therapy in
patients with YMDD mutations [19, 20]. Although it is safe to change to ADV in patients with compensated liver disease, an overlapping period before discontinuing lamivudine seems advisable in these patients. However the effect of this strategy on subsequent emergence of ADV resistance requires further study [9, 15, 21].

**ADV in liver transplant recipients**

Recurrent HBV infection in the transplanted liver remains a major problem. A retrospective study of liver transplantation in Europe before lamivudine showed that patients with low levels of hepatitis B replication at transplantation and those given long-term immunoprophylaxis with HBIG had a reduced risk of recurrent HBV infection and reduced mortality [22]. Lamivudine has further improved these outcomes. Pre-transplant treatment with lamivudine resulted in suppression of HBV DNA levels in 12 of 19 treated patients [23, 24]. Currently both HBIG and lamivudine are used prophylactically and recurrent HBV is now rare [25-27]. However, cases associated with lamivudine-resistance are problematic, as patients with recurrent post-transplant hepatitis B may develop fibrosing cholestatic hepatitis, a manifestation of high levels of viral replication in immunosuppressed patients [28, 29]. A study of 10 patients treated with lamivudine pre-liver transplantation for HBV showed that there was a risk of lamivudine-resistant strains following transplant [30]. In a post liver transplant study, lamivudine-resistant patients all developed liver failure with liver dysfunction [31]. ADV has proved to be an important antiviral drug in patients with lamivudine resistance post-transplant. In an open label study 127 liver transplant patients with lamivudine-resistant HBV were treated with ADV 10mg [32]. Treatment resulted in a median 4 log_{10} drop in HBV DNA concentrations at 48 weeks indicating the important role of ADV as second-line therapy in patients who develop lamivudine resistance in the peri-transplant setting. Care should be taken in patients with pre-existing renal damage due to calcineurin inhibitors in liver transplant patients.
NEWER NUCLEOSIDE ANALOGS

Phase I and II trials with several new nucleoside analogs, including entecavir, emtricitabine, clevudine (L-FMAU), and L-dT are in progress.

a) Entecavir is a cyclopentyl guanine analog, which is an inhibitor of all HBV polymerase functions. The drug is readily phosphorylated to the active triphosphate form. It is a potent inhibitor of WHV, and in humans with HBV at doses of 0.05-1mg. In phase II trials, 84% of patients were negative for HBV DNA by bDNA assay after 24 weeks of treatment [33]. The drug is active against lamivudine resistant variants and phase III trials are in progress.

b) Emtricitabine (FTC) is a cytosine nucleoside analog, with fluorine at the 5 position. Pilot studies have shown that the drug causes a 2-3 log reduction in HBV DNA at doses of 300mg in patients treated for 8 weeks. In a 48 week phase II study, 61% of patients had undetectable HBV DNA. Drug resistant mutants were reported in 6% of treated patients. Phase III trials are in progress.

c) Clevudine (L-FMAU) is a pyrimidine nucleoside analog. Patients have been treated in phase II dose escalating studies, and up to 3 log reductions in HBV DNA have been observed [34, 35].

d) Beta-L thymidine (telbivudine), valtorcitabine (Val LdC) and beta-L-2'-deoxyadenosine (LdA) are small molecule inhibitors of HBV DNA polymerase. These agents induce marked viral load reduction in the woodchuck infected with WHV. Telbivudine (LdT) is a specific and potent inhibitor of hepatitis B and is not active against HIV or other viruses. Clinical trials are in progress in HBeAg-positive patients at doses ranging from 25-400mg [36]; phase I studies [37] have shown a dose dependent 2-4 log reduction in HBV DNA after 4 weeks of treatment. A phase II trial testing doses of LdT 400 or 600mg with or without lamivudine in HBeAg-positive patients is in progress. An interim analysis of the results at 24 weeks indicates that LdT 400 and LdT 600mg result in 6 log declines in HBV DNA (compared to a 4 log decline in HBV DNA in lamivudine treated patients). Similarly, a phase III trial of the efficacy of 600mg LdT vs. 100mg lamivudine in HBeAg and anti-HBe-positive patients is in progress.
At this time the long-term efficacy and safety of these new unlicensed drugs and their role in the management of patients with HBeAg-positive chronic hepatitis are being assessed. Patients with chronic type B hepatitis require relatively long courses of treatment, and viral resistance may emerge. The end-points of treatment must be carefully evaluated. Combination treatments may become necessary in some, but not all patients.
REFERENCES


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