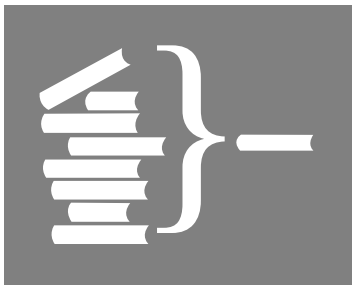

Lamivudine Treatment of Chronic Hepatitis B

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SUMMARY

Several new nucleoside analogues have been developed which can inhibit hepatitis B replication by at least two logs. Lamivudine is the most widely studied of these new agents. Extensive phase II and III studies in patients with chronic hepatitis B have been completed. The sustained HBeAg seroconversion rate in patients who have received 100 mg lamivudine increases from 17% after a year of treatment to 27% after 2 years of treatment. Histological improvement has been noted in 38%–52% of lamivudine-treated patients, exceeding the improvement seen in placebo recipients. Similar histological improvement has been noted in anti-HBe-positive, DNA- positive patients. Lamivudine can prevent recurrence of hepatitis B after liver transplantation. It is likely that in the absence of immune clearance to accelerate elimination of infected hepatocytes, inhibitors of virus replication such as lamivudine will need to be administered for a long period to reduce the burden of infected hepatocytes in the liver, and to prevent relapse.

The drug is generally well tolerated with few direct adverse events. Genotypic mutations have been observed in 23% (range 13–32%). In a study in Asian patients treated for two years the incidence of these mutants increased to 38% (as detected by PCR). Loss of susceptibility to lamivudine has been found to be due to reverse transcriptase amino acid substitutions. Lamivudine is likely to be reserved for patients with replicative hepatitis B infection with active chronic hepatitis, and/or active cirrhosis. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

Recently, several new antiviral agents have been developed which can inhibit hepatitis B replication to a greater degree than seen with alpha interferon. The possibility exists that these antiviral therapies could improve the outcome of chronic hepatitis B (reviewed in Terrault[1]). HBV DNA becomes undetectable (by dot-hybridisation) in serum during treatment in a high proportion of patients treated with, for example, lamivudine, famciclovir, ganciclovir, adefovir dipivoxil or lobucavir. Several other new nucleoside analogues are in development.

Lamivudine is the most widely studied of these new agents. This antiviral drug is active *in vitro* and *in vivo* against human hepatitis B and HIV. Initial data suggest that lamivudine could prove useful in improving hepatic histology in patients with chronic hepatitis B, and preventing recurrence of hepatitis B after liver transplantation. Larger, phase II and III trials assessing the efficacy of this drug have now been completed.

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Abbreviations used: ALT, alanine aminotransferases; anti-HBe, antibody to hepatitis B e antigen; AUC, area under the curve, C_{max}, maximal concentrations; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBIG, hepatitis B immune globulin; HCC, hepatacellular carcinoma; 3TC, 3′ dideoxy thiacytidine.

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LAMIVUDINE

Lamivudine (3TC, epivir) is a cytidine dideoxynucleoside analogue; the drug is a negative enantiomer of 3′-thiacytidine and is a potent inhibitor of HBV and HIV replication. Hepadnavirus DNA polymerases, from human woodchuck and duck HBV possess reverse transcriptase activity. Lamivudine acts as a reverse transcriptase inhibitor to decrease HBV DNA synthesis through chain termination of the nascent proviral DNA. At the time of writing, the drug has not been approved for treatment of hepatitis B, but has been approved for the treatment of HIV infection (in combination with other nucleoside analogues) in several countries, including in the European Union, the United States and Canada.

Pharmacology

Lamivudine is presently available as 75, 100, 150 and 300 mg tablets, and as 5 and 10 mg/mL oral solution. Inhibition of HBV replication in hepatocellular carcinoma

(HCC) cell lines occurs at an IC_{50} of 0·02 μM. Lamivudine is phosphorylated within the cell to the triphosphate derivative. Incorporation of the monophosphate form into viral DNA polymerase results in chain termination of HBV DNA synthesis. Lamivudine 5′-triphosphate has an intracellular half-life of 17–19 h in HBV transfected HCC cell lines. Lamivudine triphosphate is fortunately only a weak inhibitor of cellular enzymes including DNA polymerase gamma, which is required for mitochondrial DNA replication.

Lamivudine is rapidly and well absorbed after oral administration and is generally well tolerated with few direct adverse events. The drug is cleared almost entirely by renal elimination, mainly as unchanged lamivudine, via glomerular filtration and active tubular secretion.[2] Body weight may significantly influence lamivudine clearance. Bioavailability is reduced in children compared with adults, resulting in lower C_{max} and AUC concentrations. Systemic clearance is increased in patients under the age of 12 years. An 8 mg/kg/day dose in children under the age of 12 years gives similar plasma drug concentrations to adult doses of 4 mg/kg/day.

The oral and intravenous pharmacokinetics of lamivudine have been compared in human patients with HIV infection. The mean plasma half-life ranged from 8·4 to 9·1 hours after comparison of 100 mg of lamivudine administered i.v. over 1 h, and 100 mg of oral lamivudine given as solution (1 mg/mL), 100 mg capsule, or 100 mg tablet. The mean absolute bioavailability was in the range of 86% for all formulations of lamivudine.[2]

Preliminary pharmacokinetic data have also been obtained in HIV positive patients with abnormal renal function, given 300 mg of lamivudine. In these analyses, in which lamivudine was assayed in serum and urine by reverse-phase high-performance liquid chromatography, peak concentrations of lamivudine increased with decreasing renal function. The terminal half-life also increased with decreasing renal function; because of the linear relationship between creatinine clearance and lamivudine clearance, dosage adjustment, by either dose reduction or lengthening of the dosing interval, is necessary and a formula has been produced to guide therapy.[3]

The pharmacokinetics of lamivudine have also been evaluated in woodchucks infected with woodchuck HBV, following intravenous and oral administration of 20 mg of lamivudine per kg. Following intravenous administration, the concentrations of 3TC in plasma declined with a terminal half-life of 2·84 ± 0·85 h.[4]

Lamivudine pharmacokinetics have also been studied in patients with moderate hepatic impairment and, fortunately, no major changes were identified.

Lamivudine crosses the placenta, and is also detectable in breast milk. Although not teratogenic in the rat and mouse, the drug has shown some potential teratogenicity in rabbits, at relatively low doses.

Pharmacodynamics

Doses of lamivudine ranging from 25–300 mg daily are effective in reducing HBV DNA levels by two logs within 4 weeks of therapy. However, doses of greater than 100 mg per day are not more efficacious for hepatitis B. Estimates of the half-life of hepatitis B in patients treated with lamivudine have been made by comparing the rate of viral replication before and after treatment. Although preliminary data suggest that viral resistance does not usually occur within 24 weeks of treatment, such estimates will require further *in-vitro* data.

Lamivudine treatment of hepatitis B

Extensive phase II and III studies in patients with chronic hepatitis B are in progress, following phase I (1 month) assessments which identified the efficacy of this drug. Treatment has gradually been extended to 12 weeks, 6 months and now 12 months or longer in dose-ranging studies with 5, 25, 100, 300 or 600 mg per day. In an early pilot study, 32 HBeAg-positive patients were treated with 25, 100 or 300 mg daily for 12 weeks and then followed for 24 weeks off treatment. Seventy percent of the patients treated with 25 mg lamivudine and 100% of those treated with 100–300 mg became HBV DNA-negative by molecular hybridisation. However, in most patients HBV DNA soon reappeared after treatment was stopped but six patients (19%) had sustained suppression of HBV DNA and normal ALT levels and 12% lost HBeAg. Treatment with lamivudine was restarted in the HBeAg-positive patients, and continued for a year in 24 patients, of whom nine (almost 40%) patients lost HBeAg.

A brief treatment trial of lamivudine in 42 Chinese HBsAg carriers who received placebo or lamivudine in dosages of 25, 100 or 300 mg daily for 4 weeks has been completed. All 36 patients who received lamivudine had a decrease in HBV DNA values of more than 90%. HBV DNA returned to pre-treatment concentrations within 4 weeks of stopping therapy, however, and there was no change in HBeAg status or aminotransferases levels after this short course of treatment.[5]

A recently published study details the results in a similar, somewhat longer European study. In this study, 51 patients were treated with 25, 100, or 300 mg of lamivudine orally once daily for 24 weeks, with 24 weeks of follow-up. Serum hepatitis B DNA (by liquid hybridisation) decreased in all patients and was undetectable at the end of the treatment in between 58% and 93% of patients depending upon the dose.[6] Two patients (4%) were anti-HBe positive at the end of the study—indicating only low rates of seroconversion after relatively short courses of therapy.

A large, extended treatment study is ongoing in Asia. To date, it has been reported that HBeAg has been lost in 13% and 16% of Chinese HBeAg positive patients after 1 year of treatment with 25 mg (n=142) and 100 mg (n=143) respectively, compared with 4% of 73 patients receiving placebo. The 2 year treatment results have also been summarised in abstract form.[8] The investigators previously reported that 1 year of lamivudine 100 mg produced improvement in necro-inflammatory activity in the liver in 56% of treated patients versus 25% of placebo recipients. Of the 358 patients, 334 continued treatment for a further year. Patients who received lamivudine

25 mg in year 1 were randomised to receive lamivudine 25 mg or placebo in year 2. Patients receiving placebo in year 1 were randomised to receive lamivudine 100 mg in year 2. Patients receiving 100 mg lamivudine in year 1 were randomised to 100 mg or placebo in year 2. Using an intent to treat analysis, the sustained HBV DNA response after the second year of treatment was found to be maximal in patients treated with the lamivudine 100 mg for 2 years (47/90, 52%). However, in patients treated with lamivudine 100 mg for 1 year followed by placebo 2/41, 5% were HBV DNA negative. The sustained HBeAg seroconversion rate with lamivudine 100 mg daily increased from 16/93 (17%) at year 1 to 25/93 (27%) at year 2. 50% of patients treated with lamivudine 100 mg for 2 years had a sustained ALT response through to the second year.

The combined effect of lamivudine and alpha-2b interferon (in previously untreated patients) has been assessed and the results have been published in an interim analysis.[9] In this trial, the efficacy of lamivudine has been compared with alpha-2b treatment or to lamivudine, used concurrently with alpha-2b interferon, (after initiating treatment with lamivudine). Two hundred and twenty six patients HBeAg positive patients were randomised to receive lamivudine 100 mg for 52 weeks, lamivudine for 8 weeks and then concurrently with alpha 2b interferon, (to 24 weeks) or alpha 2b interferon alone (for 24 weeks). Of the patients, 20%, 25% and 22% respectively seroconverted from HBeAg to anti-HBe at 64 weeks. A second liver biopsy was done at 52 weeks to assess the efficacy of therapy: It should be noted that the liver biopsy was done at the end of lamivudine therapy in the lamivudine treated patients, but, in contrast, after therapy had been stopped for patients in the other two treatment arms.

In this study, histological improvement was noted in 38% of lamivudine-treated patients, 36% of the interferon treated patients and 28% of the lamivudine and alpha interferon patients. The major improvement was noted in portal and bridging necrosis. Some improvement in hepatic fibrosis in the lamivudine treated patients was also noted. Relatively few adverse events were documented in this trial.

Lamivudine has also been assessed in anti-HBe positive, HBV DNA positive patients: the major form of chronic hepatitis B in several parts of the world and the results have been reported in abstract form.[10] In this protocol, patients were treated either with placebo or lamivudine. 60 patients were randomised to each group, and treated initially for 26 weeks. At week 24, patients with detectable HBV DNA were continued with lamivudine. The preliminary results of this study show that by week 52, 65% of the lamivudine recipients were HBV DNA negative and had normal serum aminotransferases compared with 9% of the placebo recipients. 60% of the lamivudine treated group showed some improvement in histology (36% showed no change). Relapse rates after discontinuing lamivudine are not yet available.

A controlled, phase III trial in American HBeAg positive patients, in which patients were randomised to lamivudine 100 mg per day or placebo for 52 weeks, with 16 weeks of post-treatment follow-up has recently been published in abstract form.[11] HBeAg to anti-HBe seroconversion was noted in 17% of lamivudine treated patients versus 6% of the placebo recipients. An interim analysis of these data suggest that approximately 52% of the 66 lamivudine treated patients show histological improvement compared with 23% of 71 placebo treated patients.

Adverse events

The drug appears to be well tolerated, and relatively few serious side effects have been reported. A toxic effect against bone marrow progenitor and stromal cells has not been observed; no direct cytotoxicity against liver cells was observed. Dosage reduction is necessary in patients with renal impairment, and creatinine clearance of less than 50 mL/min. Of more concern is the clinical toxicity that can result during and after treatment. Exacerbations of hepatitis accompanied by jaundice have been reported in patients whose HBV DNA became positive after stopping treatment. In a few reported cases, these exacerbations have been severe. Fortunately, the available evidence suggests that the drug has an acceptable tolerance in patients with advanced cirrhosis who require liver transplantation and can be cautiously administered in these patients. However, as with all treatments the risk should be considered greater in patients with chronic hepatitis B cirrhosis who have jaundice, ascites, septicaemia and hepatorenal syndrome.

The possibility of sub-clinical mitochondrial toxicity has been investigated by studying the morphology and function of mitochondria in patients treated with lamivudine. No electron microscopic signs of toxicity were observed. Mitochondrial function, assessed by 2- keto[1-C-14] isocaproic acid decarboxylation and by measuring the activity in liver biopsy specimens of mitochondrial enzymes encoded by mitochondrial DNA remained normal. Breath testing did not reveal any differences in treated patients, untreated patients with hepatitis B, or healthy controls.[12]

Pre-liver transplant prophylaxis

In an initial pilot study we analysed the efficacy and safety of prophylaxis both before and after transplantation of lamivudine (without HBIG), in patients undergoing liver transplantation. Seventeen HBsAg-positive patients with decompensated cirrhosis and previous evidence of viral replication were enrolled. Twelve were HBV-DNA-positive by a signal amplification assay. Patients were treated with oral lamivudine (100 mg daily) for at least 4 weeks before transplantation and followed up for 18–90 weeks after transplantation. HBV DNA became undetectable in this group in serum before transplantation in all HBV-DNA-positive patients. Four died before transplantation from complications of cirrhosis; one patient was withdrawn from the study because of a cerebrovascular accident. The remaining 12 patients underwent transplantation. Two patients died

after transplantation (one at 3 days and one [suicide] at 20 weeks). HBV DNA reappeared in one patient with histological evidence of recurrent hepatitis (72 weeks). By week 24 the nine remaining patients had lost HBsAg and remained negative for HBV DNA. These first data suggest that lamivudine could prove useful in preventing recurrence of hepatitis B after liver transplantation.[13]

Other smaller studies have shown similar results.[14] A multicentre trial is underway in North America to access the efficacy of pre-transplantation prevention of hepatitis B. Again, only interim analysis data are available. 77 patients have been enrolled and 45 have been transplanted. At 52 weeks 17% of the analysable patients are HBsAg positive, 17% are HBeAg positive, and 18% are HBV DNA positive.

Treatment of post-liver transplant recurrence

Post-transplant recurrence of hepatitis B after failed HBIG immunoprophylaxis also responds to lamivudine therapy, and the poor outlook from fibrosing cholestatic hepatitis can be avoided.[15,16] A larger study in the USA for patients treated for post–transplant recurrence, has shown that although the majority of patients are still HBsAg positive, HBV DNA has remained negative by hybridisation in 75%. Histological improvement has been noted.

This agent has also been successfully used to treatment hepatitis B in renal transplant recipients, and there is reason to believe that similar efficacy would be observed in bone marrow or heart transplant patients.[17]

VIRAL RESISTANCE

The optimism engendered by the efficacy of new antiviral agents is being tempered by the realisation that resistance complicates the treatment of hepatitis B, and may compromise the outcome.[18] Extraordinarily high rates of hepatitis B replication, and high viral loads in some patients, particularly after immunosuppression, may favour the development of mutations. Generally, loss of susceptibility to nucleoside analogue RT inhibitors has been found to be due to reverse transcriptase amino acid substitutions that map near the active site of the enzyme, and which result in diminished ability of the enzyme–template–primer complex to recognise inhibitor.

We have described mutations in the hepatitis B polymerase that were seen in patients who reactivated hepatitis B during therapy with lamivudine and following orthotopic liver transplantation for chronic hepatitis B. In these cases resistance to lamivudine was associated with mutations which lead to amino acid site situations in the highly conserved YMDD motif, part of the active site of the polymerase. Substitution of valine and isoleucine for methionine were observed.[19] Lamivudine resistant HBV therefore is similar to resistance conferred by the change from methionine to valine or isoleucine at codon 184 of HIV reverse transcriptase.

In patients examined before treatment, the polymerase sequence encoded YMDD, while the lamivudine-resistant mutant HBV coded for YLDD or YIDD. These are similar to the mutations seen in resistant human immunodeficiency virus, suggesting common mechanisms of resistance after critical point mutations in the polymerase/transcriptase. Other mutations have been observed but their significance requires further study.[20]

Genotypic mutations were observed in 14% of Chinese patients treated with 25 mg and 100 mg respectively after 1 year of treatment. YMDD mutations were observed in 38% of patients treated with lamivudine continuously for 2 years. For patients with YMDD mutations, the median ALT at week 104 was 0·85 times the upper limit of normal and the median HBV DNA was 7 pg/ml compared to median baseline ALT values of 1·64 times the upper limit of normal and HBV DNA of 78 pg/ml. Twenty-seven percent of anti-HBe positive patients were noted to develop either YMDD or mixed mutations after 52 weeks of treatment.

Resistance was found in 4/14 immunocompetent patients who received lamivudine monotherapy for a period over 26 weeks with an actuarial cumulative incidence of 39%.[21] YMDD mutations in the polymerase gene have been observed in 22% of patients treated for recurrent hepatitis B post-transplant.

The susceptibility to lamivudine of YMDD mutant HBV has been assessed by infecting primary human hepatocytes with serum taken before treatment and after recurrence. When using wild type virus preparations, HBV DNA levels were markedly reduced (to less than 6% of those in control cultures) by addition of lamivudine in concentrations as low as 0·03 μmol/L. In contrast HBV DNA concentrations were not reduced by lamivudine at 30 μmol/L[22] in experiments employing mutant virus.

Experimental studies using engineered site-directed mutations have confirmed the importance of YMDD mutations in conferring resistance of both retroviruses[23] and hepadnoviruses[24] to lamivudine.

CLINICAL USE OF LAMIVUDINE

The available data suggest that lamivudine may prove to be an important new drug for the treatment of hepatitis B infection. The overwhelming majority of patients have a two log decline in HBV DNA from baseline which is accompanied by an improvement in serum aminotransferases and liver histology. This effect is seen in both HBeAg positive and anti-HBe positive patients, as well as in immunocompromised liver transplant patients.

The optimum duration of treatment remains undefined. It is likely that in the absence of immune clearance to accelerate elimination of infected hepatocytes, inhibitors of virus replication would have to be administered for a long period to substantially reduce the burden of infected hepatocytes in the liver, and prevent relapse. Further understanding of the quantum dynamics of hepatitis B

replication may provide insights into the optimal duration of lamivudine treatment in chronic hepatitis B.[25,26]

Supercoiled covantly closed circular (ccc) DNA is the most resistant to antiviral therapy, and importantly hepatic transplantation, may significantly and rapidly reduce this viral replicative intermediate. The necessity for treating recurrent hepatitis B after transplantation could reduce as pre-transplant prophylaxis improves.

In most of the studies completed to date, HBV DNA reappears in the majority of patients after therapy is stopped after 1 year. Longer duration of treatment will be required to achieve greater than 25% HBeAg seroconversion. Reactivation of HBV replication almost certainly occurs from residual replicative intermediates, in long lived hepatocytes. It has been shown that ccc DNA levels persist in woodchuck hepatocyte cultures, and that treatment for up to 36 days with several reverse transcriptase inhibitors, including lamivudine, does not markedly reduce the amount of ccc DNA in the cultures after treatment.[27]

Unfortunately despite the profound reduction in HBV DNA levels, an obvious HLA class II restricted T helper response does not typically occur during lamivudine or lamivudine plus interferon alpha therapy, whereas delayed T cell activation occurs with the rebound in serum HBV DNA concentrations after therapy is stopped.[28] Thus despite rapid reduction in HBV DNA levels in serum in patients treated with either lamivudine alone or in a combination with alpha interferon, the inhibition of HBV replication by this potent nucleoside analogue does not immediately restore a virus-specific proliferative T cell response to hepatitis B. It may be important to potentiate the antiviral effect with an immunomodulatory effect.

The drug is likely to be reserved for patients with discernable replicative hepatitis B infection with active chronic hepatitis, and or active cirrhosis. Both HBeAg and anti-HBe positive patients benefit, but treatment of the latter group will need careful judgement, as fluctuating levels of viral replication are not infrequently seen in these patients. The place of the drug for patients with mild chronic hepatitis, or in children with mild chronic hepatitis is uncertain, and more safety data, particularly longer term, are required.

It remains difficult to extrapolate whether the initial antiviral effect and effect upon the histological chronic hepatitis will translate into modification of the disease outcome, given the long natural history of chronic hepatitis B. However, the promising results seen in patients with hepatic cirrhosis requiring transplantation, and the overall safety of the drug in this group suggest that the drug may be usefully used in patients with advanced disease, to either prevent recurrence of disease after transplantation, or to prevent progress of the disease to hepatic failure and/or hepatocellular carcinoma. Future trials in patients with cirrhosis will be necessary but the outcome of treatment in this group is more easily ascertained in the short term.

A relatively small number of patients with extrahepatic manifestations of chronic hepatitis B, including glomerulonephritis have been treated and the effect on renal disease of suppressing hepatitis B replication needs further study. Similarly, the role of lamivudine for acute or fulminant hepatitis B is uncertain. The majority of adult patients with acute hepatitis B will recover; it is theoretically possible that lamivudine could inhibit HBV replication in the acute phase of the illness to ameliorate or shorten the clinical illness. However, early treatment with lamivudine could, in theory circumvent the immune response to hepatitis B virus, without eradicating replicative intermediates that result in reactivation of the virus when the drug is stopped.

The cost effectiveness and cost benefit of long term lamivudine in patients with chronic hepatitis or cirrhosis has not yet been ascertained, and the affordability of the drug in developing countries where hepatitis B remains endemic is an open question.

There is also a potential therapeutic use for this agent in HIV positive patients co-infected with HBV, but because of the rapid emergence of resistance in HIV infection, lamivudine must be used with additional antiviral agents.[29] High levels of HBV DNA seen in HIV and HBV positive patients are rapidly suppressed during treatment.

Patients with decompensated chronic hepatitis B may benefit from antiviral therapy, and there is anecdotal evidence of improvement in liver function in responders, which may enable the postponement of treatment by liver transplant, or even, perhaps, forestall the necessity for transplantation. However, the exact timing of antiviral treatment must be weighed against the risk of recurrence before transplantation.

Disease activity in chronic hepatitis B correlates with levels of replication of around 10^5 copies/mL (together with an active immune response). However, a fuller understanding of the mechanism of antiviral agents will require application of more sensitive quantitative assays capable of detecting and quantitating levels of virus of less than 10^3 copies/mL.

The appropriate management of patients who develop resistant mutations after lamivudine treatment has not been resolved, and the clinical implications of resistance remain uncertain. Preliminary evidence suggests that HBV DNA concentrations do not reach pre-treatment levels and, so that resistant genotypes and could be less pathogenic and less likely to lead to severe disease. Preliminary evidence suggests that patients may not develop severe hepatitis or in particular fibrosing cholestatic hepatitis if lamivudine is continued despite the emergence of resistance. However, the wisdom and logic of this policy will need careful appraisal in the future, when more data become available.

The most successful paradigm for the management of patients with end-stage chronic hepatitis B remains to be determined through clinical trials. Such trials would require sufficient power to provide comparisons between treatments, and their sample size, feasibility and ethical standards will require careful examination. The degree of illness and consequences of treatment failure in these patients does not allow a large margin of failure.

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