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Profound Suppression of Hepatitis B Virus Replication With Lamivudine

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The therapeutic goals in chronic hepatitis B are to prevent or decrease cirrhosis and hepatocellular carcinoma in patients with pre-cirrhotic or early cirrhotic disease and to stabilise patients with end-stage cirrhosis. Lamivudine is an oral nucleoside analogue that suppresses hepatitis B virus (HBV) replication, and so may achieve both these treatment objectives. The active 5′-triphosphate metabolite of lamivudine has two modes of viral suppression. First, it mimics deoxycytidine triphosphate and is incorporated into newly synthesised HBV DNA to cause chain termination. Second, it demonstrates competitive inhibition of viral DNA-dependent and RNA-dependent DNA polymerase activity (i.e., reverse transcriptase activity). Lamivudine may, therefore, act at four possible stages during HBV replication: reverse transcription of pre-genomic mRNA into nascent minus-strand DNA; formation of plus strand DNA from nascent minus-strand DNA; completion of double-stranded DNA; and formation of covalently closed circular DNA. In clinical studies, lamivudine therapy reduced serum HBV DNA and this was associated with significant improvements in liver histology and significant and sustained enhancement of the proliferative CD4-mediated response to HBeAg and hepatitis B core antigen (HBcAg), and an increased frequency of HBeAg-specific T cells. HBV DNA concentrations often returned to pre-treatment values when therapy ended prior to the loss of hepatitis B e antigen (HBeAg). Although the emergence of HBV variants with a mutation in the YMDD (tyrosine-methionine-aspartate-aspartate) motif has been observed, such variants show reduced susceptibility to lamivudine due to limited replication competence, and their emergence is not a signal to cease lamivudine therapy. In conclusion, viral suppression with lamivudine offers a means of disease improvement and immunological control in chronic hepatitis B. ***J. Med. Virol. 61:367–373, 2000.*** © 2000 Wiley-Liss, Inc.

KEY WORDS: chronic hepatitis B, YMDD HBV variant; treatment; nucleoside analogue

INTRODUCTION

Hepatitis B is a major public health problem worldwide, associated with a broad spectrum of acute and chronic liver disease. An estimated 350 million people worldwide are chronic carriers of hepatitis B virus (HBV) [Davey, 1996], with 75% of these residing in Asia and the Western Pacific regions [Gust, 1996]. Chronic HBV carriage is associated with the development of potentially fatal long-term sequelae, and around one-quarter of carriers are expected to develop serious, progressive liver disease [Zuckerman, 1999]. Indeed, the overall mortality rate in HBV carriers is around 5.2-fold higher than that of the general population [Di Marco et al., 1999].

The World Health Organisation has specified target groups for vaccination against HBV. Ensuring that the vaccine is accessible to all at-risk individuals, however, is an enormous undertaking and, as a result, there have been reports that vaccination has not yet significantly decreased the global incidence of infection [Massari et al., 1998; McQuillan et al., 1999]. In individuals already infected with HBV, therapeutic intervention is the only option for the control of liver disease.

Previously, the standard treatment for hepatitis B was interferon (IFN) alpha, although influenza-like symptoms and the need for regular subcutaneous injections complicate its use. A reduced response is also observed in Asian patients, who have the highest HBV carriage rate. This is thought to be due to immune tolerance to HBV after infection at birth or in early childhood [Lai et al., 1987; Lok et al., 1992]. A newer therapeutic option is lamivudine, an oral antiviral agent with clinical activity in chronic hepatitis B. Lamivudine shows potent inhibition of HBV DNA synthesis and rapidly suppresses serum HBV DNA levels [Lai et al., 1997, 1998]. Clinical studies have shown lamivu-

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dine to have a tolerability profile that is superior to that of IFN alpha and to have a safety profile similar to that of placebo. Treatment with lamivudine may also be more cost-effective than IFN alpha [Lacey et al., 1999; Haiderali et al., 1999].

The aims of treatment in chronic hepatitis B are essentially two-fold. In individuals with pre-cirrhotic or early cirrhotic diseases, decreasing or preventing the complications of cirrhosis and hepatocellular carcinoma is of primary clinical importance. In end-stage cirrhosis, the goals are to stabilise the patient to prevent a fatal outcome and, if transplanted, to prevent, or treat, any HBV reinfection of the transplanted liver. This paper will consider the activity of lamivudine against HBV and discuss how, by suppressing HBV replication, this agent can help to achieve these therapeutic goals.

CLINICAL PHARMACOLOGY OF LAMIVUDINE

Lamivudine is the (–) enantiomer of the deoxycytidine analogue 2′-deoxy-3′-thiacytidine. Following administration, it is metabolised inside HBV-infected hepatocytes, where stepwise addition of phosphate groups yields the active 5′-triphosphate form [Cammack et al., 1992]. Unlike some nucleoside analogues, lamivudine has minimal activity against mammalian DNA polymerase-gamma and is not incorporated into mitochondrial DNA [Chang et al., 1992]. As a result, it has no effects on bone marrow, hepatocytes, nerves and muscle tissue.

Lamivudine is rapidly following absorbed oral dosing, reaching peak serum concentrations within 0.5–1.5 hr [Yuen et al., 1995]. The extent of absorption is unaffected by food [Angel et al., 1993]. The absolute bioavailability of lamivudine is 80–85% [Yuen et al., 1995], and it has a serum terminal elimination half-life of 5–7 hr [Johnson et al., 1998a]. In addition, the active anabolite of lamivudine, lamivudine 5′-triphosphate, has an intracellular half-life of 17–19 hr in HBV cell lines [Johnson et al., 1999]. Over 70% of the dose is excreted unchanged in the urine within 24 hr, with 5–10% undergoing hepatic metabolism to the transsulphoxide metabolite, followed by elimination in the urine [Johnson et al., 1998a, 1999]. As excretion is primarily via the renal route, dose reduction may be necessary in patients with renal impairment [Johnson et al., 1998b]. Due to the low rate of hepatic metabolism, however, the pharmacokinetics of lamivudine are not affected by moderate-to-severe hepatic impairment or the presence of ascites [Johnson et al., 1998a]. As such, lamivudine is well-tolerated in patients with end-stage cirrhosis awaiting liver transplantation.

As lamivudine exhibits low metabolic clearance, a lack of hepatic metabolism and limited protein binding, significant drug interactions are theoretically unlikely. Clinical studies have found no clinically significant interactions between lamivudine and other anti-human immunodeficiency virus (HIV) drugs, trimethoprim-sulphamethoxazole or IFN alpha [Moore et al., 1996; Johnson et al., 1999].

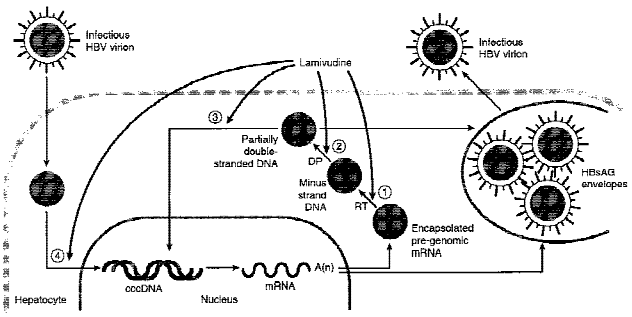

Fig. 1. Replication of hepatitis B virus (HBV) and proposed sites of action of lamivudine. cccDNA, covalently closed circular DNA; HBsAg, hepatitis B surface antigen; DP, DNA polymerase; RT, reverse transcriptase.

HBV REPLICATION

HBV replication involves a mechanism related to that of retroviruses, such as HIV, in which the reverse transcription of RNA into DNA is a critical step. Infectious HBV particles bind to and enter host hepatocytes via cell-surface receptors. Within the hepatocyte, the virion loses its surface coat, releasing the relaxed, circular double-stranded HBV DNA (Fig. 1). The minus strand of the HBV DNA is full length while the plus strand is around 50–80% of the full-length strand. Within the host cell cytoplasm the plus strand is completed by the viral DNA polymerase, and this fully double-stranded DNA is transported to the nucleus, where it undergoes conversion to supercoiled covalently closed circular DNA (cccDNA), a step that is mediated by host cell enzymes.

During viral replication within the nucleus, the minus strand of cccDNA is transcribed from separate promoters to form four mRNA species of variable length. The three smaller HBV mRNA transcripts participate in the synthesis of viral proteins, whereas the larger pre-genomic mRNA is transported to the cytoplasm where it acts as the template for the synthesis of new HBV DNA (Fig. 1). It also directs the synthesis of several viral proteins, such as the hepatitis B surface antigen (HBsAg), the viral core protein and, at low frequency, the DNA polymerase. After DNA polymerase is formed it binds to the pre-genomic mRNA and directs packaging of the mRNA/polymerase complex into viral nucleocapsids localised in the cytoplasm. Within these nucleocapsids, pre-genomic mRNA is reverse transcribed to form full-length minus strand DNA, while the template mRNA is degraded simultaneously [Summers and Mason, 1982; Büscher et al., 1985]. A partial-length plus strand is then formed from the minus strand by the viral DNA polymerase, resulting in a partially double-stranded relaxed circular DNA (Fig. 1). Some of the nucleocapsids migrate to the endoplasm reticulum, where they acquire viral envelopes to form complete virions ready for release into the bloodstream. Partially double-stranded DNA may also migrate to the nucleus, where it forms further copies of cccDNA, so amplifying and replenishing the intra-

nuclear store of this species. It is estimated that there are 10–50 copies of cccDNA within each hepatocyte [Averett and Mason, 1995], and amplification of cccDNA copy number is regulated by viral envelope proteins. These proteins shut down the pathway by interacting with viral nucleocapsids [Furman et al., 1992], and this regulation is presumed to depend on secretion of the enveloped nucleocapsids from the cell.

LAMIVUDINE: MECHANISM OF ACTION

Viral Suppression

Lamivudine exhibits two modes of viral suppression. First, the active metabolite lamivudine 5′-triphosphate mimics deoxycytidine triphosphate and is incorporated into newly synthesised HBV DNA to cause chain termination. Second, this metabolite demonstrates competitive inhibition of both the viral DNA-dependent and the RNA-dependent DNA polymerase activity (i.e., reverse transcriptase activity). As such, there are four possible sites of action for lamivudine (Fig. 1). The most obvious is during reverse transcription of the pregenomic mRNA into nascent minus-strand DNA (Fig. 1, pathway 1), and this has been demonstrated by in vitro assays [Seifer, 1998; Gaillard et al., 1998]. The second site of action interrupts the formation of plus-strand DNA from the nascent minus-strand DNA, which is dependent on viral DNA polymerase activity (Fig. 1, pathway 2). This inhibition has also been demonstrated by in vitro assays [Xiong et al., 1998; Condreay 1999]. Lamivudine may also exert its effect by inhibiting the completion of double-stranded DNA (Fig. 1, pathway 3) and by interrupting cccDNA formation (Fig. 1, pathway 4), both during the initial infection and at the subsequent amplification steps. These sites of action, although difficult to prove, are highly likely.

Immunomodulatory Effect

T-cell hyporesponsiveness to HBV antigens is well documented in hepatitis B carriers, and may represent a critical pathogenic determinant of viral persistence [Ferrari et al., 1990; Jung et al., 1991]. The mechanism is not known, but may be related to the high viral or antigen load typically observed in these patients.

A recent study has used the reduction of viral and antigen load by lamivudine as an experimental model to investigate the importance of these two factors in the immune response to HBV infection [Boni et al., 1998]. In all, 12 patients with HBeAg-positive chronic active hepatitis received lamivudine, 100 mg/day, for 12 months and were then followed up for 6 months. The T-cell response to HBcAg and HBeAg before treatment initiation was low (Fig. 2), but with lamivudine therapy the responses to both antigens showed a rapid and significant increase that persisted throughout the treatment period. After 7–14 days of lamivudine therapy, significant and sustained enhancement of the proliferative CD4-mediated response to HBeAg and HBcAg was noted in 10 patients (83%). These patients also

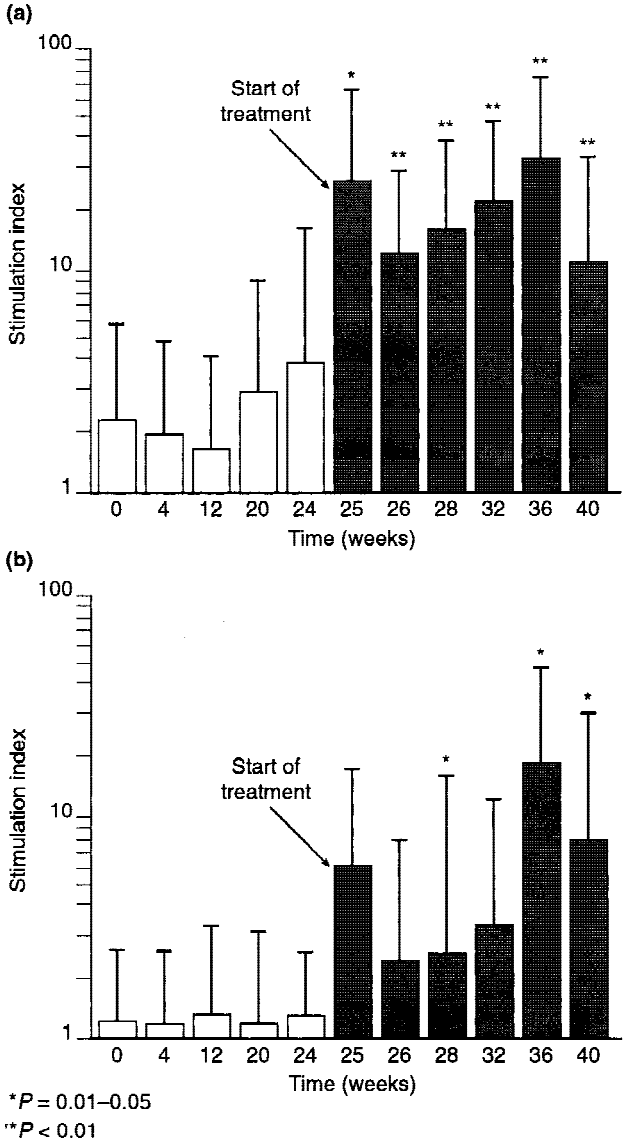

Fig. 2. Immunomodulatory effect of lamivudine. (**a**) T-cell response to hepatitis B core antigen and (**b**) T-cell response to hepatitis B e antigen. Reproduced with permission from Boni et al., 1998.

demonstrated an increased frequency of HBeAg-specific T cells and enhanced responses to mitogens and recall antigens, such as phytohaemagglutinin, tetanus toxoid and mouse anti-human CD3 monoclonal antibody. A clear temporal association was noted between recovery of T-cell reactivity to viral antigens and a reduction in viraemia. The enhanced proliferative responses to HBV proteins and the increased frequencies of circulating HBeAg-reactive T cells were generally preceded by, or temporally associated with, a decline in serum HBV DNA. A similar enhancement of CD8-positive CTL frequency and reactivity was observed [Ferrari et al., 1999]. These findings indicate that lamivudine therapy overcomes T-cell hyporeactivity, and that this effect is probably associated with a reduction in the level of viraemia.

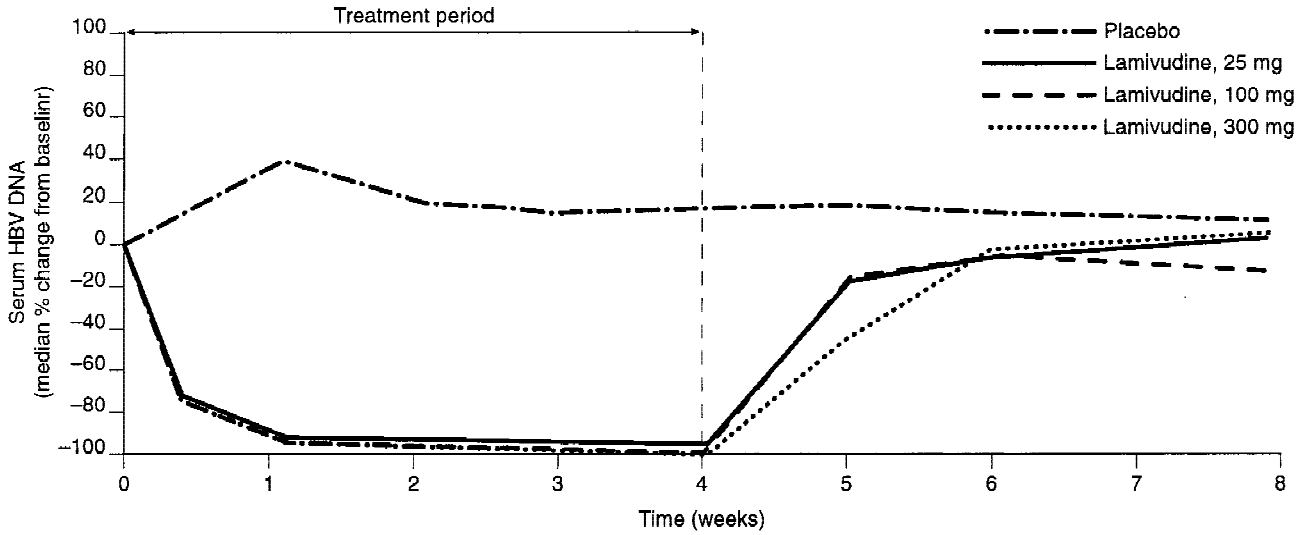

Fig. 3. Suppression of hepatitis B virus (HBV) DNA by lamivudine. Reproduced with permission from Lai et al., 1997a.

LAMIVUDINE: SHORT-TERM CLINICAL TRIALS

Two dose-ranging studies have investigated the use of lamivudine, 5–600 mg/day, for 28 days in patients with chronic hepatitis B. In the first of these two studies [Tyrrell et al., 1993] conducted in Europe and North America, 64 patients received lamivudine in doses of 5 to 600 mg/day for 4 weeks, and were then followed during an 8-week observation period. Reductions in serum HBV DNA were observed at all lamivudine doses, although return of pre-treatment HBV DNA concentrations was seen after treatment completion.

In the second of these studies conducted in Hong Kong, 42 patients received either placebo or lamivudine, 25, 100 or 300 mg/day [Lai et al., 1997]. In all lamivudine treatment groups, a significant reduction from baseline HBV DNA values of 73–78% was observed by Day 3, falling to 94–98% of pre-treatment values by day 8 of treatment (Fig. 3). This decrease persisted for the rest of the treatment period. In contrast, patients receiving placebo showed a slight increase in HBV DNA values. As in the first study [Tyrrell et al., 1993], a rapid return of HBV DNA concentrations back to pre-treatment values was observed within 4 weeks following withdrawal of lamivudine.

From the results of both studies, it can be concluded that lamivudine therapy results in rapid HBV DNA suppression in patients with chronic hepatitis B. Once treatment is withdrawn, however, there is a rapid return to basal HBV DNA values, indicating that lamivudine should be given on a long-term basis, or until sustained HBeAg seroconversion occurs. Although extended therapy may be acceptable based on the tolerability profile and convenient once-daily oral dosing regimen of lamivudine, the possibility of the emergence of escape mutants must be considered.

YMDD VARIANT HBV

Genotypic Resistance

The YMDD (tyrosine-methionine-aspartate-aspartate) motif is a well-conserved viral motif situated centrally in the DNA polymerase gene close to the putative lamivudine binding site [Allen et al., 1998]. Three possible mutations are seen at this site. In the first mutation, valine is substituted for methionine 552 (YVDD) and an upstream substitution of leucine 528 to methionine is seen. In the second mutation, isoleucine is substituted for methionine 552 (YIDD) and, in the third variant, the YIDD mutation is accompanied by the leucine to methionine substitution at 528.

The YMDD motif has a high affinity for nucleotides and is important for the formation of minus-strand DNA by the viral polymerase. However, lamivudine 5′-triphosphate interacts with this motif and blocks HBV DNA synthesis. The configuration of the wild-type motif is altered in YMDD HBV variants, leading to a marked weakening of the inhibitory effect of lamivudine (genotypic resistance). The mutation, however, also weakens the affinity of the motif for natural nucleotides, such that the minus-strand DNA is formed at a much lower level. Consequently, YMDD HBV variants have reduced replication competence [Melegari et al., 1998] and a slower replication rate [Chayama et al., 1998]. These findings have been confirmed by transient transfection assays of replication competence in which HBV YMDD variants demonstrated significantly reduced encapsidation of pre-genomic RNA and reduced nucleotide substrate affinity [Melegari et al., 1998; Ling and Harrison, 1999].

Clinical Relevance of YMDD Variants

An overview of data from lamivudine Phase III studies [Lai et al., 1998; Dienstag et al.,1999; Schiff et al., 1998; Schalm et al., 2000] showed that about 24% of

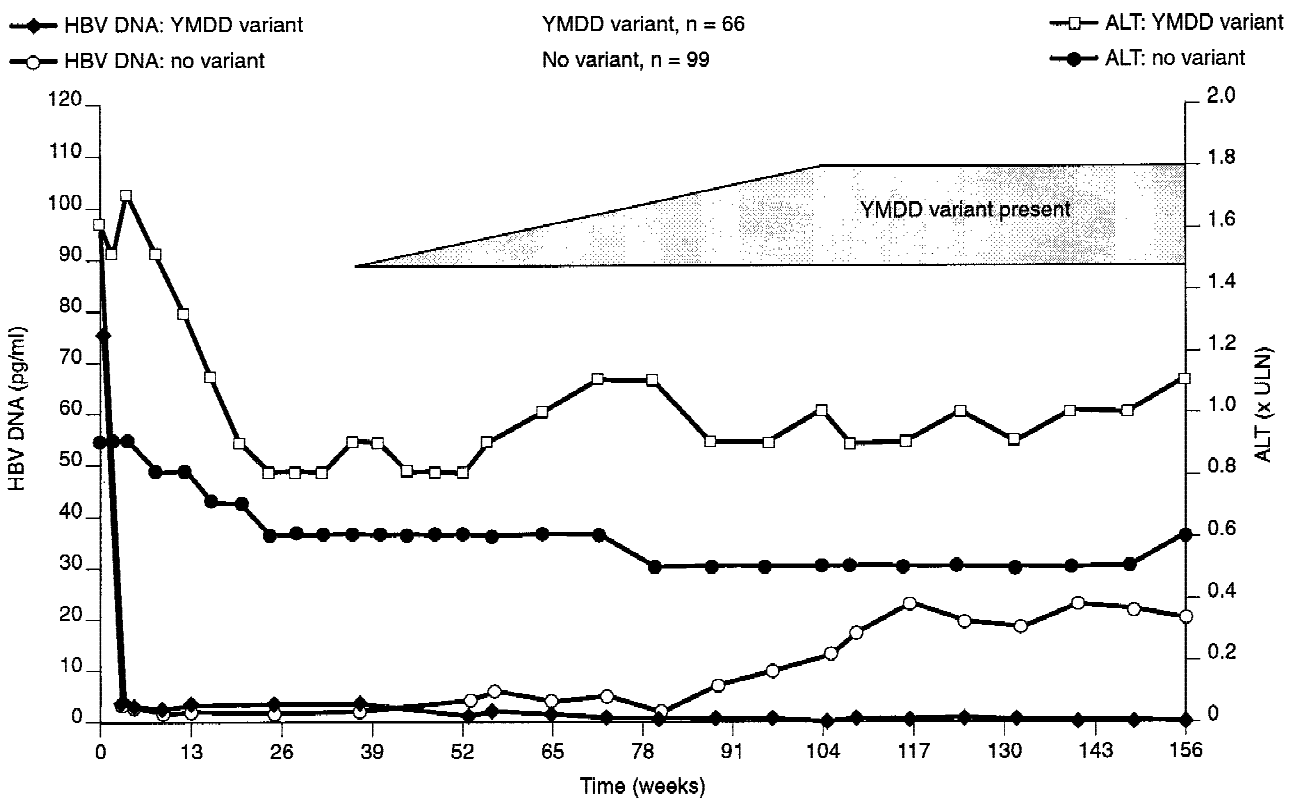

Fig. 4. Median hepatitis B virus (HBV) and serum alanine amino transferase (ALT) values in patients with and without the YMDD variant.

patients develop YMDD variants after 1 year of treatment with lamivudine 100 mg daily. Recent clinical studies [Lai et al., 1998; Liaw et al, 1998; Leung et al., 1999], however, have shown that patients who develop YMDD variant HBV continue to receive benefit on prolonged lamivudine therapy. In the long-term study by Lai et al [1998] 66 patients had detectable YMDD variants that started to appear after 36 weeks of lamivudine therapy, whereas 99 patients remained free of YMDD variant (Fig. 4). This analysis included patients with variants detected up to the end of the second year (Week 104), with a total follow-up period of three years, enabling the effects of these variants to be studied for at least 1 year. In patients who did not develop YMDD variant, HBV DNA levels fell by the second week of treatment and remained low throughout the 3 years of the study. Even among those patients who developed the variant, HBV DNA levels generally remained below pre-treatment values.

Baseline serum ALT values were somewhat lower in individuals who did not develop YMDD variants compared with those who did. Despite this, lamivudine therapy led to reduction in ALT values regardless of whether variants subsequently emerged. Serum ALT generally fell to normal range values within 6 months after the start of treatment, then remained around this level for the duration of the study. In both groups of patients, serum ALT concentrations at the end of treatment were much lower than pre-treatment values, though median values during the study were higher in the YMDD variant group. Thus, patients with YMDD variant HBV benefit from continued lamivudine therapy, as evident by lower concentrations of HBV DNA levels and improvement in serum ALT concentrations, relative to pre-treatment values.

CONCLUSIONS

HBV DNA replicates in the cytoplasm of hepatocytes while viral gene expression is sustained by the nuclear cccDNA. This species is generated from the DNA produced in the cytoplasm and does not replicate directly. As a consequence, cccDNA is difficult to target, though cell destruction may dilute the amount of cccDNA when its formation in surviving hepatocytes is blocked by the inhibition of viral DNA synthesis [Averett and Mason, 1995].

The mechanism of action of lamivudine leads to inhibition of both HBV DNA-dependent and RNA-dependent DNA polymerase activity. This activity may cause suppression of HBV DNA replication at four sites, and also has the indirect effect of restoring T-cell hyporesponsiveness. Although lamivudine demonstrates rapid and profound suppression of HBV DNA, long-term therapy may be required to perpetuate this activity. Extended treatment is feasible as, in contrast to IFN alpha, lamivudine can be taken orally once daily and is associated with a lower incidence of adverse events (Schalm et al., 2000). Thus, patient compliance is unlikely to be compromised by long-term

therapy. Extended therapy with lamivudine has also been found to produce significant improvements in liver histology [Suzuki et al., 1999]. As with all antiviral agents, however, prolonged treatment is associated with a risk of escape mutants, and the emergence of YMDD variants has been observed during lamivudine therapy. Although these variants have reduced susceptibility to lamivudine, they also have reduced replication competence both in vitro and in the clinical setting. As such, their emergence is not a signal to cease therapy with lamivudine. Moreover, HBeAg seroconversion can still occur in patients with YMDD variant HBV.

As liver disease is a consequence of hepatic replication of HBV, viral suppression with lamivudine offers a means of disease improvement and immunological control. Therapy may also limit viral transmission and lessen immunological damage to the liver, thus reducing the progression to cirrhosis and hepatocellular carcinoma. In addition to improving the long-term prognosis of the patient, a reduction in the symptoms of chronic hepatitis B will improve patients' quality of life, particularly in those with extrahepatic manifestations of disease.

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