# Evaluation of Chronic Hepatitis B Virus (HBV) Infection in Coinfected Patients Receiving Lamivudine as a Component of Anti–Human Immunodeficiency Virus Regimens

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**The effect of lamivudine on chronic coinfection with hepatitis B virus (HBV) in human immunodeficiency virus (HIV)–infected patients was studied prospectively. Nineteen patients with HIV infection, who were receiving an anti-HIV regimen containing lamivudine (150 mg twice daily), and who had replicative chronic HBV infection, were followed for a median of 14 months. Twelve patients' regimens contained protease inhibitors. Serum HBV DNA became undetectable, by means of molecular hybridization, in 14. Seroconversion of hepatitis B e antigen to antibody occurred in 6 of 17 patients, and seroconversion of hepatitis B surface antigen to antibody occurred in 1 of 19. The median serum alanine aminotransferase concentration had decreased by the time of the final evaluation. The median CD4 cell count increased and plasma HIV RNA was undetectable in 10 of 19 patients. Five patients had recurrence of detectable serum HBV DNA despite good compliance with treatment, and 2 mutations related to the resistance of HBV were detected. These patients had a significantly longer duration of treatment (21 versus 13 months; $P < .05$). In conclusion, resistant strains of HBV emerge at high detectable levels while patients receive anti-HIV regimens containing lamivudine.**

Coinfection with hepatitis B virus (HBV) and HIV is frequent because the modes of transmission are common to both infections [1]. HIV infection has a significant effect on the natural history of HBV infection. Persistent HBV infection is likely to develop in HIV-infected patients, and reactivation may occur despite seroconversion to antibody to HBV surface antigen (HBsAg), particularly if the CD4 cell count is low [2, 3]. Such infections, compared with those that occur in immunocompetent patients, are characterized by high levels of viral replication and low rates of loss of serum hepatitis B e antigen (HBeAg): a 3% loss of serum HBV

e antigen in HIV-coinfected patients versus an 11% loss in patients without HIV infection at 2 years [4]. Elevation of alanine aminotransferase (ALT) concentrations is moderate [5], most probably because cytolysis is induced by the immune response against HBV-infected hepatocytes [6]. Histological lesions are mild, according to some authors [7], and they do not differ from typical lesions, according to others [8]. Fatal fibrosing cholestatic hepatitis may also occur [9]. Interferon, the classic treatment for HBV infection, produces a 38% lower decrease in HBeAg in HIV-infected patients than it does in patients who are not infected with HIV [10], although the degree of immune deficiency may be a confounding factor. The current use of highly active antiretroviral treatments that include protease inhibitors has changed the prognosis of HIV infection, increasing the likelihood of survival and inducing a sustained immune restoration [11]. Although increased life expectancy may allow HBV-related lesions more time to man-

ifest, the immune restoration could either promote clearance of the virus [2] or lead to an exacerbation of cytolysis [12].

Lamivudine, the (−)enantiomer of 2′-deoxy-3′-thiacytidine, is a well-tolerated nucleoside reverse transcriptase inhibitor used in anti-HIV therapy, although resistance is liable to emerge rapidly when it is administered as monotherapy for HIV [13]. Because the replication of HBV includes a phase of reverse transcription, lamivudine is also an inhibitor of HBV polymerase [14], and it has a dose-related inhibitory effect on HBV replication in patients who have chronic HBV infection despite prior failure of therapy with interferon [15]. Lamivudine therapy in patients who have chronic infection with HBV induces significant decreases in serum HBV DNA and seroconversion to antibody of HBeAg (HBeAg seroconversion) [16]. Despite an initial virological response, however, resistant HBV strains may emerge at high plasma levels after 1 year in immunocompromised [17–19] or immunocompetent patients [16, 20, 21].

In HBV- and HIV-coinfected patients who are given lamivudine at standard HIV treatment dosages (300–600 mg/day), alone or combined with zidovudine, high rates of decrease in HBV DNA serum levels below the detection limit, as assessed by means of molecular hybridization (96.3%) or by means of PCR (88.5%), and of loss of HBeAg (18.5%) have been reported at 1 year [22].

The aim of our study was to evaluate the response of patients with chronic HBV infection to lamivudine when it is given as a component of current anti-HIV regimens and to assess the rate and pattern of the emergence of resistance.

## PATIENTS, MATERIALS, AND METHODS

*Patients.* Patients were followed from September 1996 through May 1998 in our university hospital department of care for ambulatory HIV-infected patients. Inclusion criteria were the following: HIV infection, confirmed by means of immunoblot assay; replicative chronic HBV infection; and receipt of an anti-HIV regimen that contains lamivudine. Replicative chronic HBV infection was defined by the detection of HBsAg for >6 months, combined with the detection of HBV DNA in serum by means of molecular hybridization and/or of HBeAg [23]. For patients who had already started treatment with lamivudine, existing data were retrospectively collected and missing virological data were tested on frozen samples.

*Treatment.* Lamivudine (Epivir; Glaxo-Wellcome) was administered at standard anti-HIV dosages, that is, 150 mg b.i.d. Follow-up was every 2–8 weeks and included clinical examination and treatment, which was recorded on standardized forms, and performance of biological tests. Serological tests for hepatitis C and D viruses (HCV, HDV) was done for all patients at baseline. HIV infection markers (CD4 cell count, plasma HIV RNA level) and HBV replication markers (HBeAg and

serum HBV DNA level as assessed by means of molecular hybridization) were monitored. Liver biopsy was not done except when clinically indicated. Histological lesions were classified according to standard criteria [24].

*Laboratory assays.* The usual biochemical test included measurement of serum alanine transferase (ALT) concentration (reference range, 1–30 IU/L). Serological tests for HBV and HCV was done by use of microparticular EIA (Axsym System; Abbott Diagnostics). If positive results were obtained, they were confirmed by means of a second serological test (Ortho HCV ELISA; Ortho Diagnostic Systems), and PCR (Amplicor HCV test; Roche Diagnostic Systems) was done. EIA was used to detect HDV antigen and antibody (Eti-Deltak/Eti-Ab-Deltak; DiaSorin). Plasma HIV RNA load was quantified by use of nucleic acid sequence–based amplification assay (Nasba Amplification System; Organon-Teknika).

Serum HBV DNA was detected by use of a molecular hybridization test (Murex Diagnostics), which allows quantification ranging from 5 pg/mL ($10^6$ copies/mL) to 2000 pg/mL. Qualitative detection of serum HBV DNA was assessed by means of a PCR directed at the C gene with primers HBVCA1 (nt 1893–1913) and HBVCA2B (nt 2302–2325 [Digene Sharp Signal System; Digene Diagnostics]; all nucleotide positions refer to Ono adw strain [25]). PCR was done only if serum HBV DNA testing yielded negative results for 2 months. DNA was extracted from 200 $\mu$L of serum by use of the QIAamp blood kit (Qiagen). Five microliters of DNA extract was amplified in a 50-$\mu$L reaction volume that contained 1 U of Taq DNA polymerase, 5 $\mu$L of 10× buffer, 1 m$M$ of MgCl$_2$, and H$_2$O with commercial reagents (Gibco BRL, Life Technologies). The amplification was run on a thermocycler (Gene Amp PCR System 9600; Perkin Elmer Cetus) for 5 min at 94°C; followed by 40 cycles of 30 s at 94°C, 30 s at 55°C, and 30 s at 72°C; and, finally, for 7 min at 72°C. Positive and negative samples were used as controls. PCR products were hybridized with a total HBV RNA probe (Digene) in a colorimetric microwell assay that allowed for a detection level of <30 copies/mL.

For direct sequencing, PCR analysis was performed for a 386-bp segment coding for part of the S gene and of the overlapping P gene, with use of the same reaction conditions. PCR and sequencing primers were BCS4 (nt 459–479) [26] and MD13 (nt 822–853) [27]. Sequencing of purified PCR products was done according to the Sanger method with use of a kit (Abi Prism Dye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems). Nucleotide sequences were obtained by use of an automatic DNA sequencing system (model 373; Applied Biosystems) and analyzed by use of Sequence Analysis and Sequence Navigator (Applied Biosystems).

*Statistical analysis.* Because of the small size of the cohort, quantitative data were analyzed by use of the nonparametric Mann-Whitney test. $P \leq .05$ was considered significant.

## RESULTS

From September 1996 through May 1998, 19 patients who were followed at our hospital for HIV infection with associated chronic HBV infection received an anti-HIV regimen that included lamivudine (table 1). Six had started lamivudine treatment before September 1996, for periods of 3–15 months, and prior data were available. HIV transmission risk was engaging in homosexual sex in 7 patients, injection drug abuse in 2, and unknown in 10 (but sexual transmission was suspected because of epidemiological history, lack of injection drug use or transfusion, and negative results of serological tests for HCV).

All patients were men. No patient had end-stage cirrhosis or jaundice. Ten patients underwent liver biopsy; examination of specimens showed that 3 patients had moderate chronic hepatitis, 4 had mild chronic hepatitis, and 3 had minimal chronic hepatitis. Serum HBV DNA had been detectable, by use of molecular hybridization, in 16 patients for >6 months before they began lamivudine treatment. Of these 16 patients, 14 tested positive for HBeAg and 2 tested positive for antibody to HBeAg. Among the 3 other patients, serum HBV DNA was undetectable by means of molecular hybridization in 2, and baseline data were unavailable for 1. All 3 of these patients tested positive for HBeAg. In 2 patients, including 1 drug addict, HCV serological test results were positive but HCV-specific PCR results were negative. No patients tested positive for markers of HDV infection. Of the 19 patients, 6 had undergone 6-month courses of interferon ($3–5 \times 10^6$ U, given 3 times per week) during the last 4 years, all of which had resulted in treatment failure. The median plasma HIV RNA level was 4.8 $\log_{10}$ copies/mL (range, <2.6–5.8 $\log_{10}$ copies/mL), and the median CD4 cell count was 157 cells/mL (range, 5–597 cells/mL). Only 2 patients had serum ALT levels that were below the upper limit of normal.

The other anti-HIV drugs given in combination with lamivudine were nucleoside reverse transcriptase inhibitors for all 19 patients (zidovudine for 17 patients, zalcitabine for 1, and stavudine for 1) and protease inhibitors for 12 (ritonavir for 5 patients, indinavir for 5, and saquinavir for 2); 4 of the 12 also received nonnucleoside reverse transcriptase inhibitors (nevirapine for 2 patients and delavirdine, for 2). Of the 19 patients, 8 were naïve of antiretroviral treatment, and 11 had previously received antiretroviral treatment for a mean duration of 4 years. The antiviral regimen consisted of 2 drugs for 7 patients, 3 drugs for 8, and 4 drugs for 4. During follow-up, 1 patient received a 21-day course of iv foscavir (10 g/day) for suspected cytomegalovirus infection.

Median duration of follow-up was 14 months (range, 6–27 months). One patient was lost to follow-up at 6 months, having moved. One patient at an advanced stage of AIDS died of Castelman disease and Kaposi's sarcoma. No patients developed liver failure or clinical ascites. One patient developed a hepatocarcinoma, which was detected on an ultrasound and confirmed

**Table 1.** Clinical and biological characteristics of patients who are coinfected with HIV and hepatitis B virus (HBV) at baseline.

| Characteristic | Value |
|---|---|
| No. of patients[a] | 19 |
| Age, y | 34 (23–45) |
| HIV infection | |
|   Duration, y | 5 (0.7–12) |
|   CDC stage | |
|     A | 5 (A1, 2; A2, 3) |
|     B | 7 (B2, 6; B3, 1) |
|     C | 7 (C3, 7) |
|   CD4 cell count, cells/mL | 157 (5–597) |
|   Plasma HIV RNA level, $\log_{10}$ copies/mL | 4.8 (<2.6–5.8) |
| HBV infection | |
|   Duration, y | 4 (0.5–12) |
|   HBsAg positive | 19/19 |
|   Serum HBV DNA by molecular hybridization | |
|     >5 pg/mL | 16/19 |
|     Quantification, pg/mL | 4140 (301–14,700) |
|   HBeAg positive | 17/19 |
| HCV positive by serological testing | 2/19 |
| Serum ALT concentration, IU/L | 61 (23–140) |
| Knodell score >10 | 5/10[b] |

**NOTE.** Data are median (range) or no. of patients/total. ALT, alanine aminotransferase; CDC, Centers for Disease Control and Prevention; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HCV, hepatitis C virus.

[a] All patients were men.
[b] Only 10 patients had biopsies of liver specimens performed.

by means of histological analysis after surgical resection. Seventeen patients had cytolysis before they underwent treatment, but ALT concentrations were <3 times the upper limit of normal in 13 patients and <5 times the upper limit of normal in 4 patients. Seven of these 17 patients had increases in ALT levels to >5 times the upper limit of normal starting in the first month, with a peak after a median of 5 months of lamivudine therapy (range, 1–6 months). Transaminase elevation was >10 times the upper limit of normal in 4 patients. Ritonavir was withdrawn for 1 month for 1 patient. Increased ALT concentrations were accompanied by decreases in serum HBV DNA levels in 6 of these 7 patients and by HBe seroconversion in 1. Two patients had initially normal ALT concentrations but elevated levels at the end of follow-up (<2 times the upper limit of normal). In 1, increased ALT concentration (<5 times the upper limit of normal) was associated with a significantly increased plasma bilirubin level; ritonavir was withdrawn for 1 month and delavirdine was stopped altogether. At the end of the follow-up period, 10 of 19 patients had normal ALT concentrations, and the median variation from baseline was −30 IU/L (range, −87 to +402).

Plasma HIV RNA fell below the detection limit (<400 copies/

**Table 2.** Factors related to high-level recurrence of infection with a strain of hepatitis B virus (HBV) that is resistant to lamivudine and of sustained seroconversion from HBe antigen to antibody for 19 patients who are coinfected with HIV.

| | Mean values for patients with or without | | | | | |
| | High-level recurrence of HBV | | | Sustained HBe seroconversion | | |
| Factor | Yes ($n = 5$) | No ($n = 14$) | $P$ | Yes ($n = 5$) | No ($n = 12$) | $P$ |
|---|---|---|---|---|---|---|
| Duration of lamivudine treatment, mo. | 21 | 13 | <.05 | 13 | 16 | NS |
| CD4 count, cells/mL | | | | | | |
|   At baseline | 96 | 254 | NS | 377 | 169 | <.05 |
|   At final follow-up | 348 | 376 | NS | 467 | 338 | NS |
|     Change in CD4 count, cells/mL | +251 | +122 | NS | +90 | +169 | NS |
| HBV DNA in serum at baseline, pg/mL | 5369 | 3519 | NS | 3178 | 4013 | NS |
| HIV RNA level in plasma, $\log_{10}$ copies/mL | | | | | | |
|   At baseline | 4.8 | 4.5 | NS | 4.5 | 4.8 | .05 |
|   At follow-up | 3.1 | 3.3 | NS | 3.2 | 3.4 | NS |
| ALT concentration, IU/L | | | | | | |
|   At baseline | 78 | 30 | NS | 63 | 30 | NS |
|   At follow-up | 51 | 75 | NS | 15 | 42 | <.01 |

**NOTE.** Mean values were determined by use of the Mann-Whitney test. ALT, alanine aminotransferase; HBe, HBV e antigen; NS, not significant.

[a] Determined by use of molecular hybridization.

mL) in 10 patients, with a median variation of $-1.4 \log_{10}$ copies/mL (range, $-3.2$ to $+0.3 \log_{10}$ copies/mL). The median variation in CD4 cell count was 117 cells/mL (range, $-110$ to $+869$ cells/mL). No new opportunistic infections were detected in any of the patients.

Serum HBV DNA fell below the limit of detection (as determined by means of molecular hybridization) in 14 of 16 patients after a median of 4 months of treatment (range, 1–12 months), and in 12 of 14, HBV DNA was also undetectable by use of sensitive PCR. In 3 of these 14 patients, HBeAg became undetectable and antibody to HBeAg became detectable; this was also true of the 3 patients who had no detectable serum HBV DNA at the start of lamivudine treatment. Only 5 of these 6 seroconversions were sustained until the end of the follow-up period. The median time to HBe seroconversion was 8 months (range, 4–15 months). Serum HBV DNA became undetectable in the 2 patients who were initially positive for antibody to HBeAg, and 1 underwent HBs seroconversion at protective levels of antibody to HBsAg. Two patients had results that remained persistently positive for serum HBV DNA, by use of molecular hybridization, but both admitted to poor compliance with the anti-HIV treatment.

Recurrence of serum HBV DNA detectable by means of molecular hybridization occurred in 7 of 14 patients. One case was due to poor compliance, and serum HBV DNA levels decreased again after the reintroduction of lamivudine. A second patient developed jaundice after lamivudine had been with-drawn in a modification of his anti-HIV regimen. The reappearance of detectable serum HBV DNA was associated with raised plasma ALT (<5 times the upper limit of normal) and bilirubin levels. On resumption of lamivudine therapy, detectable serum HBV DNA and biochemical signs of hepatitis disappeared. No compliance problem was noted among the other 5 patients. For 4 of these 5, recurrence occurred from the twelfth month through the fifteenth month of treatment, with no symptoms or rise in ALT concentrations. None had undergone HBe seroconversion. The fifth patient had tested negative for serum HBV DNA for at least 2 years and underwent HBe seroconversion during lamivudine therapy. The recurrence of detectable HBeAg and serum HBV DNA, together with a limited flare-up of ALT, followed a decrease in CD4 cell count and worsening of AIDS before death. In these 5 patients, it was suspected that resistant HBV strains might have emerged. Direct sequencing of PCR products, which were amplified before treatment and after recurrence, revealed the emergence of the common nucleotide mutations A739G and T667G, which resulted in substitution of a methionine by a valine at codon 550 (M550V) in the YMDD motif of the polymerase and in substitution of a leucine by a methionine at codon 526 (L526M). Other occasional mutations were seen. In 9 responsive patients, amplification and direct sequencing of serum HBV DNA before treatment showed neither of these 2 main mutations.

The 5 patients in whom resistant HBV strains emerged (table 2) had significantly longer durations of lamivudine treatment

(21 vs. 13 months; $P < .05$) but did not differ with regard to mean levels of initial serum HBV DNA or of initial plasma HIV RNA nor in mean initial or final CD4 cell count or variation in CD4 cell count. Patients with sustained HBe seroconversion (table 2) had a significantly higher initial CD4 cell count (377 cells/mL vs. 169 cells/mL; $P < .05$) and lower initial plasma HIV RNA level (4.5 $\log_{10}$ copies/mL vs. 4.8 $\log_{10}$ copies/mL; $P = .05$).

## DISCUSSION

In our cohort, lamivudine was given at standard HIV treatment dosages (150 mg b.i.d.) combined with current anti-HIV drugs, as either bitherapy, with a nucleoside reverse transcriptase inhibitor, or as multitherapy, with a protease inhibitor and a nonnucleoside reverse transcriptase inhibitor. As is usually reported [28], lamivudine was well tolerated in these patients with HBV coinfection and treatment never had to be stopped because of side effects. Transient increases in ALT concentrations in 37% of the patients followed modifications of markers of HBV infection, as is usual when HBV-infected patients successfully respond to interferon [29]. The effect of lamivudine is often only a suspension of infection, and recurrence of virus with symptoms can occur [30]; this happened in 1 of our patients.

The good initial response of HBV replication after the patients started lamivudine therapy (87% suppression of serum HBV DNA detectable by molecular hybridization in the first months) was similar to that observed in studies of other cohorts. Benhamou et al. [22] reported 96% suppression of serum HBV DNA at 1 year in 27 patients with HBV-HIV coinfection who were treated with 300–600 mg per day. In a controlled study in 143 patients who were not infected with HIV and who were treated with 100 mg of lamivudine per day, Lai et al. [16] reported that, during the first year of treatment, 96% had at least 1 undetectable sample, compared with only 23% of the patients in the placebo group. Initial suppression of serum HBV DNA, as detected by use of PCR—a much more sensitive method than molecular hybridization—was high in our cohort (12 [75%] of 16 patients), as it was in the study reported by Benhamou et al. (89%) [22]. These results are difficult to compare with those in patients who were not infected with HIV, because most studies have used only the molecular hybridization assay [31], or PCR was done at shorter duration of treatment: Honkoop et al. [32] reported that 37% of patients with serum HBV DNA that was undetectable at 24 weeks, according to the results of PCR, with a detection limit of 500 copies/mL. A different kinetic of HBV DNA negativation in HIV-infected patients might be related to the immune restoration induced by the anti-HIV treatment.

Our 20% rate of HBe seroconversion at the end of the follow-up period is close to the rate of 16% that was found for the 100-mg dose group of patients who were not infected with HIV in the study of Lai et al. [16] and to the rate of 11% among HIV-infected patients who were treated mostly with lamivudine monotherapy in the cohort described by Benhamou et al. [22]. Although, in HIV-infected patients, a decrease in mean ALT concentration may only partially reflect the impact of HBV infection [8], the low final ALT concentrations in the group with HBe seroconversion does suggest some reduction in hepatic inflammation.

As in studies that were reported elsewhere, there is little doubt that the overall results in our cohort are partly due to the use of lamivudine as a component of the anti-HIV regimen, because those patients who failed to respond to lamivudine therapy admitted to poor compliance. Immunity may also have played its part, contributing particularly to the durability of the serological response rather than to the initial suppression of virus, sustained HBe seroconversion being associated with higher initial CD4 cell count and lower initial plasma HIV RNA level. The single HBs seroconversion occurred in a patient whose CD4 cell count increased from 490 cells/mL to 782 cells/mL during the first 6 months of treatment with ritonavir. Resolution of this kind, which is associated with a transient rise in ALT concentration during the immune restoration, has been described in patients who are receiving ritonavir monotherapy, despite this drug's lack of any demonstrated anti-HBV activity in vitro [12]. Although initial serum ALT concentration and serum HBV DNA level are among the classic predictors of poor response to interferon [29], we were unable to identify these factors as influences on the response to lamivudine in our patients. On the contrary, 6 patients responded to treatment despite prior interferon failure.

The time that it took for resistant strains to emerge at high serum HBV DNA levels in 4 of the patients in our study was the same as it was in patients who were not infected with HIV [17–19, 21]. The emergence of resistant strains that followed the worsening of AIDS in 1 patient, despite 2 years of HBe seroconversion and undetectable levels of circulating HBV DNA, confirms the fact that HBe seroconversion is only 1 step on the way to control of HBV infection [33]. Duration of treatment was the only factor significantly related to the emergence of resistance; however, it is still <2 years since most of the responsive patients started treatment, and prolonged follow-up may reveal further recurrences.

We did not observe the YIDD mutation [20] by use of direct sequencing; perhaps this was because of the small size of the cohort or the expression in a minority strain [34], because we did not clone resistant strains. The mutation M550V in the YMDD motif of the reverse transcriptase site of HBV polymerase is analogous to the mutation that confers resistance to lamivudine in HIV at codon 184 [13]. It modifies the ligation site for lamivudine, and in vitro studies have shown that it also

induces defective viral replication [35]. The second, associated mutation at codon 223 has been shown to partly restore the levels of viral replication [35], and it is also responsible for cross-resistance to famciclovir [36].

Lamivudine resistance was not associated with clinical worsening of hepatitis, but the period of observation was too short for any definite conclusion with regard to this point. In the patient who died, there was first a deterioration due to AIDS-related diseases. In our study, control liver biopsies were not performed because they would not have led to any direct therapeutic consequences. In patients who undergo liver transplantation, recurrence seems to induce milder injuries than does infection with wild type virus [37], but fatal graft loss has recently been reported [38]. In the controlled study by Lai et al. [16], hepatic lesions were milder in the treatment group than they were in the placebo group, despite resistant strains being detected; however, follow-up was short in their study.

Rapid and complete suppression of virus, combined with the restoration of immunity, may be necessary to prevent the emergence of resistance before all infected cells have been completely cleared [33, 39]. A high initial dosage of lamivudine may be useful during the first months of therapy: Honkoop et al. [32] found a significant difference in the decrease in serum HBV DNA levels from week 12 through week 24 only in the group of patients who received 300-mg and not in the groups of patients who received 25- or 100-mg. Nevertheless, because the 300-mg dosage did not prevent recurrences in our study, initial combinations of other anti-HBV drugs with no common genetic resistance sites need to be evaluated [33]. The use of drugs with activity against both HBV and HIV infections, such as adefovir dipivoxil [40], deserve further evaluation. Combination therapies that include interferon and strategies for enhancing immunity should also be considered in the future.

## Acknowledgments

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