Lamivudine therapy for prevention of immunosuppressive-induced hepatitis B virus reactivation in hepatitis B surface antigen carriers

Oren Shibolet, Yaron Ilan, Shmuel Gillis, Ayala Hubert, Daniel Shouval, and Rifaat Safadi

Viral reactivation in hepatitis B surface antigen (HBsAg) carriers undergoing immunosuppressive therapy is well documented. To evaluate the role of lamivudine prophylaxis in Hepatitis B virus (HBV) carriers treated with immunosuppression for nonhepatic disorders, we reviewed our experience between 1997 and 2000 at Hadassah University Hospital (Jerusalem, Israel). Controls were patients who were HBV carriers and who, between 1990 and 1995, were treated for hematological malignancies but were not treated with lamivudine. Eighteen HBsAg-positive patients were treated with immunosuppression. Fourteen were males, with a mean age of 48 years. Eleven patients had lymphoma; 2 had colonic adenocarcinoma; and 5 had cryoglobulinemia, enophthalmitis, vasculitis, malignant histocytosis, or ulcerative colitis. Fourteen patients were treated with chemotherapy, and 4 with prolonged high-dose corticosteroids. All patients were HBsAg-positive; 4 had hepatitis B e antigen, and 10 had HBV DNA by polymerase chain reaction. Lamivudine was administered to 13 patients in the treatment group 1 to 60 days (mean, 15 days) before immunosuppressive treatment and continued 0.5 to 24 months (mean, 7 months) following initiation of immunosuppression. Mean follow-up after lamivudine administration was 21 months. Three patients died of lymphoma complications and 10 (77%) survived. None of the patients had clinical or serological evidence of HBV reactivation during or after lamivudine prophylaxis. Of 6 patients who presented with liver function test disturbances, 5 improved during combined lamivudine and immunosuppression treatment. At the end of follow-up, HBV DNA became undetectable in 2 of 10 patients. In 2 patients, seroconversion from HBsAg to anti-HBs was observed. In contrast, 2 of 5 control patients had HBV reactivation. Lamivudine prophylaxis in HBsAg carriers receiving immunosuppressive therapy may prevent HBV reactivation and hepatic failure. (Blood. 2002; 100:391-396)

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Introduction

More than 300 million people worldwide suffer from persistent hepatitis B virus (HBV) infection, making the virus a common cause of morbidity and mortality.1 The prevalence of hepatitis B surface antigen (HBsAg) carriers ranges from 0.5% to 10% in some Mediterranean and Middle Eastern populations.2 Therefore, it is not uncommon to encounter HBsAg carriers who are candidates for immunosuppressive treatment, such as chemotherapy and/or corticosteroid treatment for nonhepatic disorders. Viral reactivation in HBV carriers undergoing immunosuppressive therapy ranges from 14% to 50%, associated with 5% to 12% mortality.3,8 HBV reactivation was reported to occur mainly following cessation of such treatment but may also occur during treatment.6,15 The clinical consequences of hepatic injury in HBV-infected patients undergoing reactivation may range from asymptomatic liver function disturbances to massive hepatic necrosis, liver failure, and death.13,15 Withdrawal of immunosuppressive treatment is often not practical because of the underlying condition. Interferon alfa is usually not considered as a therapeutic option in these patients owing to the high rate of nonresponsiveness and its effect on bone marrow suppression.

Lamivudine is a reverse-transcriptase inhibitor of viral DNA polymerase with an excellent profile of safety and tolerability, causing inhibition of viral replication and approved as an antiviral treatment in hepatitis B–infected patients.16,17 Lamivudine suppresses serum HBV DNA values in up to 98% of treated patients within a median period of 4 weeks, leading to aminotransferase normalization, increased hepatitis B e antigen (HBeAg) seroconversion rate, and improvement of histological parameters.18,19 Lamivudine treatment was effective when used in reactivated HBsAg carriers during or after immunosuppression, allowing completion of immunosuppressive courses.20-29 The present study summarizes the course of HBV infection in our group of HBsAg-positive patients treated prophylactically with lamivudine prior to immunosuppression administration.

Patients and methods

Thirteen patients with chronic HBV infection who were candidates for immunosuppressive therapy for nonhepatic disorders were enrolled for lamivudine prophylactic therapy. A carrier state was defined as detectable serum HBsAg for a period of at least 6 months regardless of liver function tests. Patients who were HBsAg-positive but whose length of carrier state was not known were also included provided that serum anti–hepatitis B core (HBc) immunoglobulin M (IgM) was negative. Nine patients fulfilled the former criteria while 4 fulfilled the latter. Five patients who were HBV carriers and were treated with immunosuppressive therapy before lamivudine became available in our institute were used as controls. All control patients fulfilled the first criterion for chronic HBV infection. Reactivation
was defined as new liver function disturbances in the presence of new anti-HBe IgM and/or signs of viral replication with a seroconversion from anti–HBe-positive to HBeAg-positive. Immunosuppressive therapy included a large spectrum of anticancer agents and high doses of corticosteroids. Clinical and laboratory data of patients were retrospectively reviewed at the Liver Unit, Hadassah University Hospital (Jerusalem, Israel).

HBV viral markers
HBsAg and anti–Hbc-IgM were tested by enzyme-linked immunosorbent assay (Axym system; Abbott Laboratories, North Chicago, IL). HBeAg and anti-HBe were tested by radioimmunoassay (EBK; DiaSorin, Saluggia, Italy). Serum was tested for the presence of low-level HBV DNA by quantitative polymerase chain reaction (PCR), by means of the HBV DNA PCR-quantitative assay (AmpliCor Monitor; Roche Diagnostic Systems, Branchburg, NJ). The lower limit of detection of this assay is 500 copies per milliliter.

Lamivudine treatment
Patients were treated with lamivudine (Epivir; Glaxo-Wellcome, Research Triangle Park, NC) at a dose of 150 mg/d as soon as possible prior to initiation of immunosuppression. Lamivudine administration was maintained for 6 months following immunosuppressive withdrawal. (At the time of the study, lamivudine was available only in 150-mg tablets).

Follow-up
Patients were followed both in the Liver Unit’s clinic and by the referring physician according to their underlying disease.

Results
Thirteen patients with chronic HBV infection were treated prophylactically with lamivudine prior to initiation of chemotherapy or immune-suppressive treatment and followed between 1990 and 1995. Ten of them (83%) were men and 3 were women; mean age was 49 years with a range from 38 to 71 years. Five patients with chronic HBV infection treated in the Hematology Department for hematological malignancies between 1990 and 1995 were used as controls. All were men; the mean age was 54 years, with a range from 38 to 71 years. Five patients with chronic HBV infection treated in the Hematology Department for hematological malignancies between 1990 and 1995 were used as controls. All were men; the mean age was 49 years with a range of 18 to 67 years.

Underlying disease and immunosuppressive therapies
Eleven patients (7 of 13 treatment patients and 4 of 5 controls) had lymphoma (61%); 7 patients had B-cell follicular large-cell non-Hodgkin lymphoma (NHL); 2 had B-cell follicular mixed large-and small-cell NHL; another had small-bowel NHL (histological type was not specified); and 1 had Hodgkin lymphoma (histological type was not specified).

Two patients (11%) presented with colonic adenocarcinoma; one of them was classified as duke C and the second as duke D with single liver metastases. One patient (5.5%) had malignant histiocytosis with lung metastases. The remaining 4 patients (22%) suffered from nonmalignant inflammatory conditions: cryoglobulinemia, enophthalmitis, vasculitis, or ulcerative colitis.

Immunosuppressive treatment
All patients with malignant disorders were treated with chemotherapy (Tables 1 and 2). In the treatment group, 6 of 7 of the lymphoma patients received the CHOP (cyclophosphamide, doxorubicin, vincristine [Oncovin], prednisone) regimen. Of these 6 patients, 1 patient received 3 courses of CHOP and 4 patients received 6 courses. Another lymphoma patient (patient no. 4) was

### Table 1. Clinical characteristics of chronic hepatitis B virus patients treated with prophylactic lamivudine

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>Age, y</th>
<th>Basic disease</th>
<th>Immunosuppressive treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>49</td>
<td>Lymphoma</td>
<td>CHOP*</td>
<td>Alive</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>65</td>
<td>Lymphoma</td>
<td>CHOP*</td>
<td>Alive</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>44</td>
<td>Enophthalmitis</td>
<td>Steroids†</td>
<td>Alive</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>57</td>
<td>Lymphoma</td>
<td>Cyclophosphamide, vincristine, prednisone‡</td>
<td>Died</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>57</td>
<td>Cryoglobulinemia</td>
<td>Steroids†</td>
<td>Alive</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>62</td>
<td>Colon carcinoma</td>
<td>5-FU§</td>
<td>Alive</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>38</td>
<td>Lymphoma</td>
<td>CHOP*</td>
<td>Died</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>55</td>
<td>Lymphoma</td>
<td>CHOP*</td>
<td>Alive</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>56</td>
<td>Colon carcinoma</td>
<td>5-FU§</td>
<td>Alive</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>65</td>
<td>Lymphoma</td>
<td>Chlorambucil†</td>
<td>Alive</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>52</td>
<td>Vasculitis</td>
<td>Steroids,† cyclophosphamide¶</td>
<td>Alive</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>42</td>
<td>Lymphoma</td>
<td>CHOP*</td>
<td>Died</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>47</td>
<td>Ulcerative colitis</td>
<td>Steroids†</td>
<td>Alive</td>
</tr>
</tbody>
</table>

*The CHOP regimen was as follows: cyclophosphamide (750 mg/m²); vincristine (Oncovin) (1.4 mg/m²); doxorubicin (Adriamycin) (50 mg/m²) on day 1; and prednisone (100 mg) on days 1-5.
†1 mg/kg prednisone by mouth (PO) and then tapering down.
‡Modified CHOP (the doses of the other medications remain the same as in the regular CHOP).
§900 mg/m² 5-fluorouracil intravenously every 2 weeks.
¶8 mg/day PO for 4 days every month.
§§1 mg/kg every day PO.
Table 4. Serum viral status before and after immunosuppressive therapy in the control group

<table>
<thead>
<tr>
<th>Patient</th>
<th>HBsAg</th>
<th>Anti-HBC IgM</th>
<th>HBeAg</th>
<th>Anti-HBe</th>
<th>HBV DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>B</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>E</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 3.

HBsAg indicates hepatitis B surface antigen; HBc, hepatitis B core; Ig, immunoglobulin; HBeAg, hepatitis Be antigen; HBV, hepatitis C virus; NA, not available.

*Patient died before serological data were obtained.

treated with a modified CHOP regimen that excluded doxorubicin, and one patient was treated with chlorambucil. One lymphoma patient also received 3 courses of fludarabine, mitoxantrone, and 2-chlorodeoxyadenosine. In the control group, 2 lymphoma patients were treated with CHOP, and 1 of those received additional MOPP (mechlorerathamine, vincristine [Oncovin], procarbazine, prednisone). One lymphoma patient was treated with MACOP-B (methotrexate, doxorubicin [Adriamycin], cyclophosphamide, bleomycin), and 1 lymphoma patient with MOPP followed by ABVD (doxorubicin [Adriamycin], bleomycin, vinblastine, and dacarbazine).

One patient in the control group (patient C) who suffered from malignant histiocytosis was treated with 6 courses of CHOP followed by high-dose methotrexate.

The 2 patients with colonic carcinoma received 5-fluorouracil for 6 months.

Treatment for the nonmalignant immune-mediated patients necessitated prolonged high doses of corticosteroid treatment. Prednisone was used at a dose of 1 mg/kg with tapering off during a period of 4 to 6 months; in one case (patient 11), a combination of steroids and cyclophosphamide was used.

Viral and biochemical status prior to lamivudine therapy

On presentation, serum HBsAg was detectable in all cases, HBeAg in 5, and DNA by PCR in 10 patients (Tables 3 and 4).

In the treatment group, viral serology was monitored at a mean of 31 days (range, 3-120 days) prior to lamivudine administration and then at regular intervals every 3 to 6 months. Seven patients (54%) had normal liver function test studies, and 6 presented with liver function test disturbances (Table 5). Three of them manifested mainly with cholestatic dysfunction (mean gamma–glutamyl transpeptidase, 260 U), and the rest had mainly hepatocellular dysfunction (mean alanine aminotransferase [ALT], 145 U). In the control group, all patients had either normal liver function tests or minimal elevations in lactate dehydrogenase or transaminases (Table 6).

Lamivudine treatment

Lamivudine was administered 1 to 60 days (mean, 15 days) prior to immunosuppressive course initiation. It was continued for 0.5 to 24 months (mean, 7 months) following immunosuppression withdrawal.

Outcome

Mean follow-up from the diagnosis of malignancy or immune-mediated disorder until end of follow-up was 21 months (range, 6-38 months) in the treatment group and 55 months in controls (range, 24-120 months). Survival rate at the end of follow-up was 10 of 13 patients (77%) in the treatment group and 2 of 5 (40%) in controls. Three lymphoma patients in the treatment group died during treatment, 2 owing to disseminated disease and multiorgan failure, and the third from Pseudomonas sepsis.

Of the 6 patients presenting with liver function test disturbances, normalization was achieved in 2 patients (patients 2 and 8) and partial improvement in 3 (patients 5, 6, and 12); in 1 patient (patient 3), it did not improve. Patients 3, 5, 6, in whom liver function disturbances remained during lamivudine treatment, died from disseminated lymphoma and sepsis (Table 5). Two of 3 HBeAg patients achieved seroconversion to anti-HBe. Another 2 patients achieved seroconversion of HBsAg to anti-HBs. At the end of follow-up, DNA became undetectable in only 2 of 8 patients by PCR. None of the patients had serological evidence of HBV reactivation during or after immunosuppressive treatment. In contrast, 2 patients in the control group had viral reactivation.
HBV DNA contains a glucocorticoid-responsive element, acting as an enhancer of viral replication. Administration of corticosteroids may therefore lead to accelerated HBV replication associated directly or indirectly with induction of hepatocyte injury.

Until recently, conventional therapeutic modalities for treating HBV reactivation such as interferon alfa and other agents, including ganciclovir, foscarnet, and foscarnet, have been shown to induce a weak antiviral activity against HBV. Lamivudine is a nucleoside analog that competitively inhibits viral reverse transcriptase and terminates proviral DNA chain extension. Like some other antiviral agents, it may also affect the host immune system. It has been reported that lamivudine caused a highly significant enhancement of the CD4-mediated response to HBV nucleocapsid antigens, as well as enhanced response to mitogens and recall antigens. Lamivudine usually induces a rapid reduction in HBV replication within a few weeks of treatment, and the effect is well maintained throughout treatment of up to 1 year. Long-term therapy with lamivudine has been shown to induce resistance in the form of mutations in the Tyr-Met-Asp-Asp locus. Resistance may develop within a few months of treatment and increases progressively with prolonged therapy. Between 16% and 32% of patients develop the Tyr-Met-Asp-Asp mutation after 1 year of lamivudine therapy; however, these patients continue to show partially suppressed hepatocellular injury pattern. Following discontinuation of therapy, the wild-type virus quickly replaces the mutant virus.

Lamivudine treatment following HBV reactivation in immunosuppressed patients was reported as being effective and led to recovery from life-threatening hepatitis. It allows completion of chemotherapy in lymphoma patients with HBV reactivation; recently, there are a few case reports about the effectiveness of

**Table 6. Liver function tests before and after immunosuppressive treatment in the control group**

<table>
<thead>
<tr>
<th>Patient</th>
<th>ALT (6-53 IU)</th>
<th>AST (2-60 IU)</th>
<th>ALP (40-130 IU)</th>
<th>γGT (10-80 IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Before</td>
<td>After</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>A</td>
<td>39</td>
<td>25</td>
<td>67*</td>
<td>43</td>
</tr>
<tr>
<td>B</td>
<td>11</td>
<td>12</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>C</td>
<td>77*</td>
<td>89*</td>
<td>88*</td>
<td>36</td>
</tr>
<tr>
<td>D</td>
<td>46</td>
<td>804*</td>
<td>65*</td>
<td>844*</td>
</tr>
<tr>
<td>E</td>
<td>44</td>
<td>1191*</td>
<td>32</td>
<td>1048*</td>
</tr>
</tbody>
</table>

For abbreviations, see Table 5. *Values that were above the normal limits used in our institute.
prophylactic lamivudine administration to avoid HBV reactivation in patients treated with immunosuppression.\textsuperscript{22,24,28}

In the present study, prophylactic lamivudine treatment was offered to 13 HBsAg-positive patients who were candidates to receive immunosuppressive therapy regardless of transaminase levels or viral load. We tried to administer the drug as closely as possible to initiation of immunosuppression or chemotherapy treatment, \textit{i.e.}, within 1 to 60 days (mean, 15 days).

The optimal duration for administration of lamivudine prophylaxis in patients at risk for lymphoproliferative and malignant disorders is unknown. Previous reports on postimmunosuppression HBV reactivation suggest maintenance of lamivudine therapy for at least 4 to 6 months following chemotherapy. Thus, we treated patients for a total duration of up to 2 years, including maintenance of treatment for 0.5 to 24 months (mean, 7 months) after completion of immunosuppression treatment.

The virologic criteria for selection of patients to receive prophylaxis have not been determined. In the present report, all patients with persistent HBV infection were included regardless of whether they were symptomatic and whether they were with or without evidence for viral replication. The largest group in the present study consisted of patients with NHL, but also included patients with solid tumors and nonmalignant conditions, making results applicable to a larger target population. The need for prophylaxis in cases, such as chemotherapy for solid tumors, where there is low risk for reactivation has been questioned. However, as shown recently,\textsuperscript{8} even these cases carry a risk of reactivation. As reactivation further delays chemotherapy and carries a risk of developing into fulminant hepatitis, for which transplantation is not an option, in these patients, we believe that prophylaxis is recommended for HBsAg carriers in all situations where immunosuppression is planned, especially in view of lamivudine’s excellent safety profile. However, because there were only 2 patients with solid tumors in the treatment group and none in the controls, it is premature to wholeheartedly recommend prophylactic therapy in such patients.

Reports have suggested possible reactivation of hepatitis B in patients who are HBsAg-negative, for example in anti–HBc-positive/HBsAg-negative/anti–HBs-negative patients with or without positive HBV DNA by PCR.\textsuperscript{40,41} Therefore, routine testing for anti–HBc or HBV DNA when anti–HBc is present should be included in the initial workup in patients treated with immunosuppression. Two of 6 patients with elevated transaminases achieved normalization. Three of 4 patients who did achieve normalization of ALT died of disseminated lymphoma. Most probably, elevated transaminases in these cases were a result of hepatic involvement with lymphoma or were secondary to hepatoxicity of drug or infection, but were not a result of HBV. Neither HBV reactivation nor hepatic failure was observed in this group. In some cases, treatment with lamivudine even led to loss of HBsAg, to seroconversion from HBsAg to anti-HBc, and to disappearance of HBV DNA.

It is important to note that 6 of 7 lymphoma patients received higher doses of steroids than the 4 patients with inflammatory diseases and that the other 3 patients (1 lymphoma patient and 2 colon cancer patients) did not receive steroids at all. Even though patients were treated with steroids and were more susceptible to HBV reactivation, lamivudine treatment prevented HBV reactivation and hepatic damage in all patient subgroups. Interestingly, both patients who lost their HBsAg had colon carcinoma and were not treated by steroids.

Our study has a few limitations. First because it is retrospective, the treatment schedules were not identical; however, most of the patients initiated and stopped the therapy within a narrow time frame, which made management decisions applicable to general treatment. It is also unlikely that large, randomized prospective studies of lamivudine prophylaxis will be conducted in the near future.

Although these preliminary data are not controlled, we believe that the results in the literature citing a high reactivation rate, together with the relatively high reactivation rate in 2 of 5 of the historical controls used in this study, show the merits of using lamivudine.

In conclusion, the data presented suggest that prophylaxis with daily administration of lamivudine to HBsAg carriers who are candidates for chemotherapy or immunosuppressive therapy is safe and may prevent immunosuppression-induced or associated reactivation. Therapy should be offered to all chemotherapy and immunosuppression candidate HBsAg-positive patients regardless of whether they have evidence of active viral replication. Administration of lamivudine should begin shortly before the first chemotherapy cycle, preferably within 2 to 4 weeks before, to allow optimal suppression of viral replication. On the basis of current available information, the duration of therapy with lamivudine should continue as long as there is immunosuppression and up to 6 months following the last immunosuppressive treatment.

References
21. Yeo W, Steinberg JL, Tam JS, et al. Lamivudine in
16. Deinstag JL, Perrillo RP, Schiff ER, Bartholomew
14. Thung SN, Gerber MA, Klion F, Gilbert H. Mas-
41. Brechot C, Knoop C, Delforge ML, et al. Reacti-
38. Allen MI, Deslauriers M, Andrews CW, et al. Iden-
37. Honkoop P, Niesters HG, De Man RA, Osterhaus
34. Lau GKK, Liang RHS, Wu PC, Lee CK, Lim WL, Au WY. Use of famciclovir to prevent HBV reactiv-
33.963-971.
29. Clark FL, Drummond MW, Chambers S, Chap-
28. Ahmed A, Keefe EB. Lamivudine therapy for che-
27. Maguire CM, Crawford DGH, Hourigan LF, Clous-
26. Farrell G. Immunosuppression and reactivation of hepatitis B in the lamivudine era: opportunities for preven-
23. Honkoop P, Gerber MA, Klion F, Gilbert H. Mas-
22. Nakamura Y, Motokura T, Fujita A. Severe hepatic-
21. Deinstag JL, Perrillo RP, Schiff ER, Bartholomew
20. Yeo W, Steinberg JL, Tam JS, et al. Lamivudine therapy for chronic hepatitis B: a six-month ran-
14. Thung SN, Gerber MA, Klion F, Gilbert H. Mas-
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