The predictive value of liver fibrosis in determining the effectiveness of interferon and lamivudine therapies for chronic hepatitis B

Michiko Shindo1, Kazushige Hamada2, Kenichi Nishioji1, Akira Muramatsu1, Yoko Oda1, and Tadao Okuno1

1 Division of Liver Diseases, Department of Internal Medicine, Akashi Municipal Hospital, 1-33 Takashoumachi, Akashi 673-8501, Japan
2 Department of Biotechnology, Kyoto Institutes of Technology, Kyoto, Japan

Introduction

Chronic hepatitis B is a leading cause of cirrhosis and hepatocellular carcinoma worldwide.1,2 The objective of treating chronic hepatitis B is to halt progression of liver injury by suppressing or completely eliminating viral replication. Sustained loss of the markers of active viral replication, such as hepatitis B e antigen (HBeAg) and hepatitis B virus (HBV) DNA, has been shown to result in biochemical, clinical, and histologic remission. Treatment of chronic hepatitis B is a rapidly evolving field. Interferon (IFN) had been the only approved treatment of choice for chronic hepatitis B during the past decade. A 4-month course of IFN alpha leads to loss of HBeAg and persistent loss of hepatitis HBV DNA in approximately 33% of patients.3,4 This outcome is associated with long-term improvement in hepatic histology and lack of progression of the disease.5,6 In addition to the low response rate, IFN is expensive and poorly tolerated. Recently, lamivudine (LAM), (-)-B-L-2',3'-dideoxy-3'-thiacytidine, an oral cytosine nucleoside analogue, has been shown to induce a rapid decrease in HBV DNA and improvement in transaminase levels and liver histology, and to enhance the rate of loss of HBeAg.7–10 The greatest drawback with LAM treatment is the selection of drug-resistant hepatitis B virus mutants.11–16 These mutations have been detected in 14% to 32% of patients after 1 year of treatment, increasing to 38%, 49%, and 66% after 2, 3, and 4 years of treatment, respectively.12–15 The most common mutation affects the highly conserved YMDD motif in the catalytic domain of the HBV reverse transcriptase/DNA polymerase (P gene).12–16 In addition, the M552V mutation was frequently accompanied by a leucine 528-to-methionine (L528M) substitution.17,18 Recently it has been reported that viral control after liver transplantation was more difficult in patients with LAM resistance.19 If patients already harbor LAM-resistant viruses, treatment would be very difficult and

Background. To determine the best indicator of the effective use of interferon and lamivudine for the treatment of hepatitis B e antigen-positive chronic hepatitis, we retrospectively analyzed histologic and virologic status in 200 patients who were treated with interferon and 45 patients who were treated with lamivudine. Methods. Histological grading and staging scores were determined by international criteria and the METAVIR scoring system. The YMDD motif associated with lamivudine resistance was analyzed by the sequencing of hepatitis B virus (HBV) DNA. Results. Of 200 interferon-treated patients, 62 (31%) seroconverted to anti-hepatitis B e (anti-HBe). Multivariate analysis showed that the significantly important predictors of response were a higher grading score (P = 0.0056) and lower staging score (P = 0.0010). Twenty (44%) of the 45 lamivudine-treated patients seroconverted to anti-HBe, and multivariate analysis showed that the significantly important predictors of response were a higher alanine aminotransferase (ALT) level (P = 0.0034) and lower hepatitis B e antigen levels (P = 0.0128). YMDD mutations occurred during therapy in 12 patients (27%). The significantly important predictor of the development of mutation was a higher staging score (P = 0.0226). Conclusions. Both interferon and lamivudine were effective for patients with high ALT levels, but interferon’s efficacy appeared to be limited by the degree of fibrosis. Lamivudine appeared to be effective irrespective of the degree of fibrosis, but YMDD mutations seemed to develop sooner in patients with advanced liver fibrosis.

Key words: chronic hepatitis B, interferon, lamivudine, liver fibrosis

Received: April 14, 2003 / Accepted: August 15, 2003
Reprint requests to: M. Shindo
complicated, as reported in the above study. Moreover, as LAM-resistant viruses spread in the population, this mode of treatment would become increasingly less useful. Thus, the best use of LAM is to select patients who are most likely to respond to this agent, with a low probability of developing the mutations. Recently, predictors of LAM responsiveness, such as high alanine aminotransferase (ALT) levels and a high histologic activity index (HAI) score have been reported.20 Interestingly, the LAM predictors were similar to those for IFN.21–25 Thus, these predictors may not be so helpful in selecting either IFN or LAM for the first choice of treatment of HBeAg-positive chronic hepatitis patients. Moreover, these parameters were not useful for predicting the development of mutations. To make the best use of these two antiviral agents for this disease we need to understand which patients are less likely to respond to which agent, and also to know which patients are less likely to develop LAM resistance mutations. In order to determine such indicators we have retrospectively analyzed the histologic and virologic status of 200 patients who were treated with IFN between 1990 and Oct 2000, and 45 patients who were treated with LAM after November 2000.

**Patients, materials, and methods**

**Patients**

A total of 245 biopsy-proven hepatitis B surface antigen (HBsAg)- and HBeAg-positive chronic hepatitis patients were studied. Because IFN was the only approved antiviral agent in Japan until October 2000, 200 patients who were seen between 1990 and October 2000 were treated with IFN, and 45 patients who were seen after November 2000 were treated with LAM. All 245 patients had had elevated ALT levels for at least 6 months before treatment, and were negative for anti-hepatitis C virus (HCV). Pretreatment liver biopsy revealed chronic hepatitis in 223 and cirrhosis in 22 patients. No patients had clinically decompensated cirrhosis.

**IFN and LAM therapy**

All patients were treated at Akashi Municipal Hospital and all of the protocols were approved by the ethics committee of the hospital. After initial evaluation, 200 patients were started on IFN α (Sumiferon; Sumitomo Pharmaceuticals, Osaka, Japan; or Intron A; Schering-Plough, Tokyo, Japan) 6 to 10 million units, three times per week (TIW), for 4 months, and 45 patients were started on LAM, 100mg/day, for 1 to 1.5 years. All patients were followed in the outpatient clinic, questioned about symptoms and possible side-effects, and had blood drawn every 2 weeks during treatment, and monthly to bimonthly for more than 1 to 12 years (mean, 5 years) after the cessation of therapy.

Responders to IFN were defined as patients who lost HBeAg and seroconverted to anti-HBe (loss of detectable levels of HBeAg and HBV DNA in serum and the appearance of antibody to HBeAg) during IFN therapy or within 6 months after the cessation of the therapy. Responders to LAM were defined as those who lost HBeAg and seroconverted to anti-HBe during LAM therapy or within 6 months after the cessation of the therapy.

**Laboratory and virologic testing**

Routine laboratory tests taken at monthly to bimonthly clinic visits included ALT and aspartate aminotransferase (AST), serum direct and total bilirubin, albumin, and complete blood counts. Blood was also tested for HBeAg, anti-HBe by commercial immunoassays (Dainabott, Tokyo, Japan) and for HBV DNA by a branched DNA (bDNA) probe assay, which has a lower limit of detection of 0.7 × 10^6 genome equivalents (10^6 genome equivalents = 1Meq) per ml. Samples with HBV DNA below 0.7Meq/ml were tested by quantitative polymerase chain reaction (Amplisor PCR; Roche, Tokyo, Japan), which has a lower limit of detection of 4 × 10^2 copies/ml. All samples were tested for genotyping of HBV using commercially available HBV genotyping enzyme immunoassay (EIA) kits according to the manufacturer’s procedure.26

Sera were also tested for the existence or emergence of LAM-resistant mutations in LAM-treated patients, monthly or bimonthly, using sequencing of the HBV DNA. Methionine at codon 552 replaced by an isoleucine (M552I) or a valine (M552V), and a leucine 528-to-methionine (L528M) substitution were examined. The region including L528M and the YMDD motif was amplified by nested reverse transcription (RT)-PCR with two sets of primers, as follows: external sense DNA. Methionine at codon 552 replaced by an isoleucine (M552I) or a valine (M552V), and a leucine 528-to-methionine (L528M) substitution were examined. The region including L528M and the YMDD motif was amplified by nested reverse transcription (RT)-PCR with two sets of primers, as follows: external sense (nucleotide nos. 580-600), 5'-TTCGGACGGAAAC TGCACTTG-3'; external antisense (nucleotide nos. 865-895); 5'-TCCCTTACATTCTGGAATGATGA ATTTGAA-3', internal sense, (nucleotide nos. 613-633), 5'-ATCATCCTGGGCTTTGCAGAAG-3'; internal antisense (nucleotide nos. 851-870), 5'-AACGTGGGGCTACTCCCTT-3'. After electrophoresis, amplified DNA was extracted and purified from the low-melting-point agarose gel slice with phenol/chloroform, and then sequenced directly, using the internal sense oligonucleotide described above as the sequencing primer, with Dye Terminator Cycle Sequencing kits (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and a 373A sequencer (Applied Biosystems, Foster City, CA, USA).