Case Report

Lamivudine as an Alternative Therapy for Interferon-Resistant Chronic Hepatitis B and the Characteristics of Hepatitis B Virus: A Case Report

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TERUI, Y., SAITO, T., WATANABE, H., AOKI, M., HAGA, H., MIYANO, S., TAKEDA, T., SAITO, K., TOGASHI, H., SHINZAWA, H. and TAKAHASHI, T. Lamivudine as an Alternative Therapy for Interferon-Resistant Chronic Hepatitis B and the Characteristics of Hepatitis B Virus: A Case Report. Tohoku J. Exp. Med., 2000, 191 (4), 247–253 — A 27-year-old man who had been diagnosed as having chronic hepatitis B suffered disease exacerbation with marked reactivation of hepatitis B virus (HBV). Treatment with interferon (IFN) did not improve his condition, and his serum HBV DNA level increased to over 10,000 pg/ml during IFN administration. Following replacement with lamivudine, there was a substantial reduction in HBV DNA to an undetectable level, and liver function parameters subsequently improved to within the normal range. Quantitative analysis of the precore mutant HBV DNA, which is a variant that cannot express hepatitis B e antigen due to a G-to-A point mutation in the precore region of the viral genome, revealed that the amount present was greater than for the precore wild-type HBV DNA in the serum taken before IFN treatment. This case suggests that lamivudine would be an appropriate alternative to IFN, particularly in patients infected with HBV containing an excess of precore mutants resistant to IFN therapy. ———— lamivudine; interferon; hepatitis B; precore mutant; YMDD mutant © 2000 Tohoku University Medical Press

Chronic hepatitis B takes a variety of clinical courses, one of which, disease exacerbation with a high level of viral reactivation, occasionally causes life-threatening liver injury (Borg et al. 1998). An appropriate anti-viral therapy is necessary for inhibition of viral replication to prevent the progression of liver cell necrosis induced by an enhanced immunological response. At present, the

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approved anti-viral therapies for chronic hepatitis B virus (HBV) infection are interferon (IFN) or the nucleoside analog, lamivudine (Nevens et al. 1997; Lai et al. 1998). Although both therapeutic approaches characteristically result in a reduction of viral load, it is uncertain which agent is more appropriate for individual cases of chronic hepatitis B. The anti-viral effect derived from both agents may be limited in association with specific HBV genome mutation. The efficacy of IFN may be limited by the emergence of a variant HBV that cannot express hepatitis B e antigen (HBeAg) due to a G-to-A point mutation at nucleotide 1896 in the precore gene (precore mutant) (Naoumov et al. 1992; Brunetto et al. 1993; Alberti and Fattovich 1994; Nakahori et al. 1995; Ichikawa et al. 1998). The efficacy of lamivudine may also be limited by the emergence of mutations in the tyrosine (Y), methionine (M), aspartate (D), aspartate (D) motif (YMDD motif) in the RNA-dependent DNA polymerase (P) gene of the HBV genome (Chayama et al. 1998; Ono-Nita et al. 1999). We report a case of severe exacerbation of chronic hepatitis B positive for HBeAg, in which IFN therapy failed. Therapy replacement with lamivudine in this case resulted in a marked improvement in virological and biochemical findings. The patient’s HBV molecular characteristics showed an excess of precore mutant without mutation of the YMDD motif. Specific HBV genome mutation appeared to be related to the response of these agents in this case, yielding important information for making the appropriate choice of initial anti-viral agent.

Case Report

A 26-year-old man whose mother was an HBV carrier had been followed up for chronic active hepatitis B. He was admitted to Yamagata University Hospi-
tal complaining of general fatigue with marked jaundice. Laboratory findings on admission revealed exacerbation of chronic hepatitis B: his serum alanine aminotransferase (ALT) level was 1995 IU/liter (normal <30 IU/liter), total bilirubin 7.9 mg/100 ml (normal <1.3 mg/100 ml), direct bilirubin 6.8 mg/100 ml (normal <0.4 mg/100 ml) and prothrombin time 52% of normal. He was positive for hepatitis B surface antigen, HBeAg and antibodies against hepatitis B core antigen, and negative for anti-HBs and anti-HBe. The serum HBV DNA showed a high level of 2500 pg/ml. Tests for anti-HBc IgM, anti-hepatitis delta virus (HDV), anti-hepatitis C virus (HCV), anti-hepatitis A virus (HAV) IgM and anti-nuclear antibody all gave negative results. As the patient’s enhanced im-
munological response to HBV may have caused severe liver necrosis resulting in fulminant hepatitis, he was first treated with an immunosuppressive agent, prednisolone, using 40 mg daily for one week and subsequently reducing the dose by 10 mg each week for a further three weeks. One week after starting pred-
nisolone he was given IFN-α 6 megaunits daily for two weeks and was then maintained on the same dose three times a week as an anti-viral therapy to counteract the increased viral load. As shown in Fig. 1, the HBV DNA level
Fig. 1. The clinical course of the present patient with exacerbation of chronic hepatitis B. During initial anti-viral therapy with interferon, the hepatitis B virus DNA (HBV DNA; open circle) level decreased with a partial improvement in the alanine aminotransferase (ALT; closed circle) level, but neither marker returned to a normal level. Thereafter, the HBV DNA level gradually increased and eventually reached over 10,000 pg/ml with a subsequent increase in the ALT level. Replacement with lamivudine resulted in a dramatic response of the HBV DNA, decreasing the viral load to an undetectable level and normalizing the ALT level.

decreased first with a partial improvement in the ALT level, but neither marker returned to within the normal range. Liver biopsy ten weeks after IFN treatment showed severe liver necro-inflammation with bridging fibrosis. Thereafter, the HBV DNA level gradually increased, and eventually reached over 10,000 pg/ml. The ALT level subsequently increased. IFN was not effective, and was stopped at week 16 after the start of treatment. The patient was then given lamivudine 150 mg daily instead of IFN. Informed consent for the lamivudine therapy was obtained from the patient. Two weeks after starting the treatment, the HBV DNA decreased to an undetectable level with subsequent seronegativity of HBeAg. Ten weeks later, the ALT level normalized. Specific HBV genome mutations in both the precore and P genes of the HBV DNA genome, which may influence the anti-viral effect of these agents, were investigated. In the serum sample taken on admission in which the HBV DNA level was 2500 pg/ml, the precore mutant HBV DNA occupied a higher percentage over 76% than for the precore wild-type HBV DNA in total viremia (precore mutant HBV vs. precore wild-type HBV: 2 × 10^8 vs. 6 × 10^7 copies/ml). In the serum sample taken at the termination of IFN treatment in which the HBV DNA level was over 10,000 pg/ml, the precore mutant HBV DNA still occupied approximately 41% of the total
(precore mutant HBV vs. precore wild-type HBV: $7 \times 10^8$ vs. $1 \times 10^9$ copies/ml). Mutation of the YMDD motif in the P gene was not detected in either of the samples by direct sequencing.

**Methods**

Serum HBsAg, anti-HBs and anti-HBc were measured by enzyme-linked immunosorbent assay (ELISA; Behring Inc., Marburg, Germany). HBeAg, anti-HBe, anti-HDV, anti-HBc IgM and anti-HAV IgM were determined by radioimmunoassay (Dainabott Co., Ltd., Tokyo). Anti-HCV was assayed by the third-generation ELISA (Ortho Clinical Diagnostic Inc., Raritan, NJ, USA). The serum HBV DNA level was measured by a soluble hybridization method (Suzuki et al. 1997) at SRL, Inc. (Tokyo). The quantitation of precore mutant HBV DNA in the serum was performed by a competitive mutation-site-specific-assay (MSSA) according to the procedure previously described (Kinoshita et al. 1994) at Otsuka Laboratories Co., Ltd. (Tokyo). Mutation of the YMDD motif in the P gene of HBV was investigated in the serum sample. Direct sequencing method after a nested polymerase chain reaction (PCR) amplification (Watanabe et al. 1999) was applied. The primers used for a nested PCR amplification of the P gene were 5′ GTT GCT GTA CAA AAC CTT CGG AC 3′ and 5′ CAG GCA GCT TCC GAA AAC ATT GC 3′ for the first round reaction and 5′ CAT CCC ATC ATC CTG GGC TT 3′ and 5′ CCT GTG GTA AAG TAC CCC AA 3′ for the second round reaction.

**Discussion**

This patient was suffering from exacerbation of hepatitis B which threatened to progress to hepatic failure. When such an exacerbation is associated with a high level of HBV reactivation, a therapy which inhibits HBV replication using an anti-viral agent, would seem to be most appropriate, either alone or in combination with prednisolone, to suppress the enhanced immunological response to the virus, thus preventing massive liver cell necrosis due to immune-mediated lysis of hepatocytes expressing viral antigen (Honkoop et al. 1995). In this case, IFN was not effective, but therapy replacement with lamivudine resulted in a marked improvement of both virological and biochemical findings.

Which is the better choice for the initial treatment of hepatitis B, IFN or lamivudine? A preliminary report found no benefit of administering the two drugs simultaneously (Mutimer et al. 1998). Therefore, it was important to investigate the viral characteristics in this case, in which HBV was resistant to IFN but responded to lamivudine, marked by a reduction in replication.

The precore mutant plays an important role in the process of seroconversion from HBeAg-positive to anti-HBe-positive (Naoumov et al. 1992; Brunetto et al. 1993; Nakahori et al. 1995; Ichikawa et al. 1998). The emergence of this mutant is observed in a proportion of the viral clones in the HBeAg positive-phase, and
the amount is thought to increase as serocoversion develops (Nakahori et al. 1995; Ichikawa et al. 1998). The recently developed MSSA assay enables us to quantify the precore mutant in the HBeAg-positive phase (Kinoshita et al. 1994; Ichikawa et al. 1998). This HBV variant is thought to be more resistant to immunoclearence than the precore wild-type HBV (Naoumov et al. 1992; Alberti and Fattovich 1994). Although both precore wild-type and mutant HBVs are responsive to the direct antiviral activity of IFN (Aikawa et al. 1995), the mutant virus escapes immunoelimination more efficiently than wild-type HBV (Brunetto et al. 1993; Ichikawa et al. 1998). Therefore, the efficacy of IFN treatment for chronic hepatitis B may be partly associated with the emergence of the precore mutant. Brunetto et al. (1993) showed that when the precore mutant reaches greater than 20% of the total viremia in the serum, it causes a failure in responsiveness to IFN. In this case, the precore mutant accounted for a higher percentage (approximately 70%) of the total viremia than the precore wild-type HBV in the serum before IFN treatment, which may have produced an unexpected result. Little is known about the efficacy of lamivudine therapy in cases of HBV infection where the HBV consists of an excess of precore mutants. The efficacy of lamivudine may be unrelated to the emergence of the precore mutant, since it is effective in cases of chronic active hepatitis B with anti-HBe positivity, in which most of the viral clones are the precore mutant (Tassopoulois et al. 1999).

HBV with mutation in the YMDD motif of the P gene (YMDD mutant) causes resistance to the anti-viral effect of lamivudine (Chayama et al. 1998; Ono-Nita et al. 1999). We did not calculate the exact amount of the YMDD mutant in this case, as a quantitative assay is not available. Direct sequencing of the genome showed no mutation, which means that most of the viral clones did not have this mutation in the serum before lamivudine treatment. However, the long-term benefit of lamivudine therapy is uncertain because withdrawal of the treatment is occasionally associated with viral recrudescence together with the emergence of the YMDD mutant. Lamivudine have a direct anti-viral effect, but few cases showing seroconversion from HBeAg to anti-HBe, which is considered a critical step to ensure sustained HBV suppression, have been reported (Chien et al. 1999). Complete remission resulting from the administration of lamivudine may be due to immunoclearence of the infected cells as well as anti-viral effect (Chien et al. 1999). Currently, the use of this drug is not the first line of therapeutic option in the treatment of hepatitis B. Further studies are needed to improve the long-term prognosis.

In summary, this case suggests that lamivudine administration as an alternative to IFN is effective in reducing HBV containing an excess of the precore mutant resistant to IFN, and in bringing about a prompt improvement of virological and biochemical findings, thus preventing progression to liver failure in cases of severe hepatitis.
References


