Combination of HBIG and Lamivudine-Resistant Mutations: A Formula for Trouble?

Selection of hepatitis B virus polymerase mutants with enhanced replication by lamivudine treatment after liver transplantation. *Bock CT, Tillmann HL, Torresi J, Klempnauer J, Locarnini S, Manns MP, Trautwein C.* Gastroenterol 2002;122:264-273.(Reprinted with permission from the American Gastroenterological Association.)

Abstract

Background & Aims: **Lamivudine has become a main therapeutic option for treating hepatitis B virus (HBV) infection. Although drug resistance develops, the clinical course after selection of antiviral-resistant HBV mutants seems to be benign. However, we observed a severe clinical course of hepatitis B infection in several liver transplant recipients after the emergence of lamivudine resistance. This was associated with high viral load in the blood.** *Methods:* **In this report, we characterize the molecular mechanisms underlying drug-dependent enhanced replication of particular lamivudine-resistant HBV mutants selected in these patients, which were associated with sudden onset of liver failure.** *Results:* **The clinical course was characterized by a sudden rise in serum bilirubin, prothrombin time, and transaminase. HBV sequence analysis of these patients revealed both mutations in the "a-determinant" of the envelope and the YMDD (tyrosine, methionine, aspartate, aspartate) motif (domain C) of the polymerase protein. Transfection experiments with replication competent vectors indicated that the "a-determinant" changes were not associated with resistance, whereas mutations in the YMDD motif conferred resistance to lamivudine. More importantly, combinations of mutations in the "a-determinant" and the YMDD motif in patients with a severe hepatitis were not only resistant to lamivudine treatment, but showed enhanced replication in vitro in the presence of lamivudine. This observation was confirmed in separate laboratories.** *Conclusions:* **Severe and fatal hepatitis B infection can occur during lamivudine therapy and may be associated with certain HBV mutants selected during sequential nucleoside and HBIg treatment. The lamivudine-enhanced replication shown by these mutants suggests that continuation of therapy with lamivudine could be deleterious in some patients. (Gastroenterology 2002;122:264-273.)**

Comments

The use of hepatitis B immune globulin (HBIG) and lamivudine has resulted in excellent patient and graft survival rates for patients who have undergone liver transplantation for hepatitis B virus (HBV)–related liver failure; however, HBIG is expensive. In addition, 20% to 40% of patients who received HBIG mono-

therapy develop recurrent HBV infection.[1-3] Inadequate HBIG in patients with high viral load is the most important cause of early HBV recurrence, whereas selection of HBV S gene mutants is the predominant cause of late HBV recurrence.[4] Lamivudine monotherapy has been shown to decrease recurrent HBV infection after liver transplantation, but the efficacy diminishes with time because of the selection of resistant mutations in the YMDD motif of the HBV polymerase (P) gene.[5,6] The most important mutation involves substitution of the methionine at position 204 (550 or 552 depending on the numbering system) for valine or isoleucine (M204V/I).[7] An additional mutation involving substitution of leucine at position 180 (526 or 528) for methionine is present in most patients. In vitro studies showed that the M204V/I mutants have decreased replication fitness compared with wild-type HBV.[8] Clinical studies found that most patients with these mutants have low HBV DNA and aminotransferase levels. Nevertheless, some patients harboring lamivudine resistant mutants may develop progressive liver disease.[9] Furthermore, additional mutations in the HBV genome have been reported in some patients during prolonged lamivudine therapy.[10,11] It has been suggested that these changes may represent compensatory mutations that restore the replication fitness of HBV.

In the article by Bock et al,[12] the molecular mechanisms that may be responsible for the poor clinical outcomes observed in three patients who developed lamivudine resistance posttransplantation were presented. Of note, HBIG immunoprophylaxis and subsequent therapy with famciclovir had previously failed in all three patients. At 13, 24, and 64 weeks after the introduction of lamivudine, progressive graft failure developed that resulted in death or retransplantation. Viral sequencing showed that all three patients had M550V and L526M mutations and additional mutations in the HBV P gene that corresponded to the a determinant region of the surface (S) gene. Two patients had a P120T mutation in the envelope region.

Using site-directed mutagenesis, they created constructs with individual and combined HBV P and S gene mutations identified in these three patients. These constructs were then transfected into human hepatoma cells and analyzed for HBV replication fitness in the presence or absence of lamivudine. They confirmed that the M550V mutant was resistant to lamivudine. In addition, they showed that constructs with combined mutations in the P (L526M+M550V) and S (P120T or G145R) genes also were resistant to lamivudine. Furthermore, constructs with combined P and S gene

mutations had increased replication fitness in the presence of lamivudine compared with wild-type HBV. The authors concluded that the enhanced replication of the combined a-determinant and P gene mutants observed in vitro accounted for the poor clinical course observed in the three patients studied.

Do these study results indicate that lamivudine should not be used in patients who developed recurrent hepatitis B secondary to failure of HBIG prophylaxis? Many studies have shown that lamivudine treatment of patients who developed recurrent hepatitis B secondary to failure of HBIG prophylaxis results in viral suppression as well as clinical improvement.[13,14] Although progressive liver disease has been reported in some patients who developed lamivudine resistance, short-term follow-up (1 to 4 years) showed that most patients continue to benefit from lamivudine treatment. Bock et al[15] studied three patients with adverse outcome but control patients who developed lamivudine resistance during treatment for recurrent hepatitis B after liver transplantation and who had stable clinical course were not examined. Thus, the causal relationship between their findings and clinical outcome remain to be established. Previous studies found that in most patients, mutations in the a-determinant revert back to wild-type S sequence after discontinuation of HBIG. It is surprising that the a-determinant mutants persisted in these three patients. Unfortunately, the pretransplant viral sequences were not reported and the exact timing of sequence analysis in relation to discontinuation of HBIG was not provided. The rapidity with which breakthrough infection occurred after initiation of lamivudine therapy was also unusual and may be related to prior treatment failure with famciclovir. L526M mutation has been reported in patients with famciclovir resistance.[16] Although the investigators were unable to detect this mutation at the onset of lamivudine therapy, it is possible that preselection of the L526M mutation has occurred, albeit at low levels. This may account for the rapid selection of the M550V mutation on introduction of lamivudine. Results of this study argue against sequential therapy of weak antiviral agents.

Despite the shortcomings of this study, Bock et al[12] showed that additional or compensatory mutations restore and even enhance replication fitness of lamivudine-resistant HBV mutants. Similar findings have been reported in the HIV literature.[17] Thus, patients who are maintained on lamivudine therapy after the emergence of lamivudine resistance must be closely monitored. Further studies are needed to confirm the findings of Bock et al and to identify the factors that are associated with adverse clinical outcome in patients

with lamivudine resistance. As new antiviral agents with efficacy against lamivudine resistant HBV such as adefovir, tenofovir, and entecavir become available, the management of patients with lamivudine resistance will need to be reassessed.[18,19] Addition or substitution of these new agents for lamivudine will likely be recommended. This is particularly important for patients who have developed lamivudine resistance prior to liver transplantation.

This study raised other intriguing questions. Will patients with vaccine escape a-determinants in the HBV S gene have decreased response to lamivudine therapy or worse outcome when they develop lamivudine resistance? Two decades after the introduction of HBV vaccination, there is no proof that the efficacy of current HBV vaccines is declining and no evidence that the prevalence of a-determinant mutants in the community is increasing. Thus, the question raised may be irrelevant. Of more concern to the transplant community is the efficacy of combination prophylaxis with lamivudine and HBIG in the prevention of HBV recurrence in patients who had been on lamivudine monotherapy for extended duration before transplantation. To date, most studies using combination prophylaxis have reported recurrence rates of less than 10%.[20-22] However, several investigators found that despite the use of combination prophylaxis, HBV recurrence occurred in most patients who had lamivudine resistance before transplantation.[23,24] Whether the treatment failures were attributable to suboptimal dose of HBIG, enhanced HBV replication secondary to combination of HBV P and S gene mutations, or diminished efficacy of HBIG in patients with lamivudine resistant mutations remain to be determined. It has been suggested that lamivudine-resistant P gene mutations may lead to changes in the overlapping S gene, thereby decreasing the affinity of the HBV surface protein for HBIG. This concern has not been proven, but merit studying as combination prophylaxis with HBIG and lamivudine is the standard in most transplant centers.

Robert J. Fontana, MD
Anna S.F. Lok, MD
Division of Gastroenterology
University of Michigan Medical School
Ann Arbor, MI

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