Sustained HBs seroconversion during lamivudine and adefovir dipivoxil combination therapy for lamivudine failure

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The efficacy of chronic hepatitis B therapy with lamivudine is limited by the emergence of drug resistant mutants (1). Mutations confering resistance to lamivudine are mainly located in the C domain of the reverse transcriptase within the YMDD motif, ie M204V or M204I, and may be associated with compensatory mutations in the C domain, ie V173L or L180M (2). Adefovir Dipivoxil (ADV) has recently been licensed for the treatment of chronic hepatitis B in the United States and in Europe. ADV has demonstrated activity against wild type, pre-core mutant as well as lamivudine resistant strains of HBV (3-5). ADV has a proven clinical benefit in the treatment of lamivudine resistance with a significant inhibition of viral replication and improvement in liver function after 1 year of therapy. Despite the fact that ADV and lamivudine are not cross-resistant (6, 7), it is still debated whether the best treatment regimen for lamivudine resistance should rely on the switch to adefovir dipivoxil or on the addition of this drug to ongoing lamivudine (3-5).

In the Lyon Liver Department, we followed a cohort of 60 patients that received adefovir dipivoxil in a compassionate use program from the AFSSAPS (French Drug Agency) for lamivudine resistance. All patients received ADV in addition to lamivudine. The patients were prospectively followed up for a mean period of 10.3 months (range 3 months to 21 months). One of these patients lost serum HBsAg (Figure 1). This 41-year-old man with chronic hepatitis B failed entecavir therapy in a clinical trial, then received lamivudine and became resistant to lamivudine. He was treated succesfully with ADV in addition to lamivudine. Lamivudine resistance was associated with the selection of a L180M+M204V polymerase mutant. After the addition of ADV, serum HBV DNA levels droped by more than 4 Log_{10} which was followed by HBsAg clearance after 22 months of combination therapy. This occurred simultaneously with HBeAg clearance and was not accompanied by an hepatitis flare. Moreover, anti-HBs antibody titers rose to 1000 mIU/mL after 32 months of the new treatment regimen. In parallel, HBV DNA declined below 100 copies / mL by a quantitative real time PCR assay. Analysis of intrahepatic viral DNA showed a significant decline of total HBV DNA and cccDNA (figure 1) which was accompanied by a decrease of the number of infected cells expressing viral antigens below the detection limit of immunostaining (data not shown). Evolution of serum HBsAg levels was parallel to that of HBV DNA in serum and liver. In parallel, liver histology analysis showed an improvement in both the activity index and fibrosis score. No virological or biochemical relapse was observed until the last visit three months after cessation of therapy.

It is very unlikely that this favorable outcome is due to spontaneous HBs seroconversion. Indeed, in the integrated analysis of phase III trials for lamivudine therapy, Lai et al observed HBeAg seroconversion in 10% up to 33% patients with lamivudine resistance who continued lamivudine and were followed for one up to 4 years respectively. However, no case of HBsAg clearance was noted (1). Furthermore, in two studies of ADV therapy for

lamivudine resistant chronic hepatitis B, no case of HBs seroconversion was observed in the 67 patients who received lamivudine plus placebo for 48 weeks (3, 4). Three patients lost HBsAg in these two clinical trials. All three received a combination of lamivudine plus adefovir dipivoxil. One patient out of 20 receiving this combination lost HBsAg after 44 weeks of therapy in the setting of non decompensated chronic hepatitis B (4). In the other study, 2 patients out of 84 lost HBsAg during combination therapy, but both had undergone liver transplantation and received HBIg (3); it is therefore unknown if the addition of ADV was the only cause of HBsAg clearance.

In our case, viral load declined progressively for the first 19 months of the add-on therapy and then a sharp decline of viremia occurred and was followed by HBsAg clearance and subsequently by the rise in anti-HBsAb titers. None of these events was associated with ALT flares which could have suggested a cytotoxic T cell attack of infected hepatocytes. Indeed, ALT levels became rapidly normal or at the level of the ULN after the addition of ADV. However, we cannot exclude killing by T cells of a minor proportion of the liver since ALT levels were slightly elevated but close to the ULN before HBsAg elimination. Furthermore, HBeAg clearance was simultaneous to that of HBsAg, and did not precede it. Interestingly, the longitudinal analysis of viral genome sequence, confirmed that lamivudine resistance was due to a known mutation in the viral polymerase, and neither a selection of basic core promoter or pre-core stop codon mutation nor a selection of an adefovir resistant mutant was observed throughout the evolution. Analysis of intrahepatic viral DNA showed a sharp decline of total intrahepatic viral DNA consistent with the inhibition of viral DNA synthesis. Furthermore, a significant decline of viral cccDNA by 2 log10 copies/cell was observed in agreement with the results of another study in patients receiving adefovir dipivoxil (8). It was associated with a significant decline of HBV antigen – expressing cells below the detection limit of immunostaining assays. Therefore, one may hypothesize that the decline of intrahepatic viral load may have been associated with the sudden restoration of non cytolytic TH1 response followed by HBs seroconversion (9).

Furthermore, the combination of the virological and biochemical response was associated with an improvement of liver histology including a significant regression of liver fibrosis score.

In conclusion, we have reported the case of a patient with chronic hepatitis B failing lamivudine therapy who received a combination of adefovir dipivoxil and lamivudine and showed a dramatic drop in viral load both in the serum and the liver that was accompanied by HBsAg seroconversion and significant improvement in liver histology. In patients who previously failed lamivudine therapy, proactive antiviral treatment may lead to a beneficial virological and clinical effect.

Acknowledgments

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Figure 1 Legend

Panel A. Evolution of biochemical markers and HBV DNA in a patient who failed entecavir and lamivudine therapy, and showed HBsAg seroconversion during lamivudine – adefovir dipivoxil combination therapy. Viral load was analyzed by versant HBV DNA 3.0 assay and expressed in Log_{10} IU/mL. Results of HBV serology are indicated below the graph. The results of viral polymerase gene sequence analysis are also indicated (WT, wild type sequence; L180M+M204V, lamivudine resistant mutant). The time points of liver biopsy (LB) are indicated by an arrow. **Panel B.** Results of quantification of serum HBsAg and anti-HBs during the course of the disease. **Panel C.** Results of intrahepatic HBV DNA quantification on sequential liver biopsies. Total viral DNA and cccDNA were detected using a real time PCR assay. Total viral DNA and cccDNA are expressed in copy number/cell.