

Hepatitis B virus mutations potentially conferring adefovir/tenofovir resistance in treatment-naïve patients

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INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health problem, with approximately 350 million individuals chronically infected worldwide. Chronic HBV carriers are exposed to the risk of complications, including chronic hepatitis, cirrhosis and hepatocellular carcinoma. Up to one million people die every year from complications of HBV infection^[1].

The discovery and clinical use of antiviral agents, targeting in particular the viral reverse transcriptase, have revolutionized therapy for patients chronically infected with HBV^[2]. While each nucleotide or nucleoside produces efficient viral suppression, they induce only modest rates of HBV surface antigen (HBsAg) seroconversion and require long-term administration to control disease in patients. The need for long-term therapy necessitates drug safety and the ability to delay or manage the emergence of resistant HBV strains^[3-5]. The clinical benefit of these therapies has been compromised by the emergence of resistant viral strains that carry specific mutations in the polymerase gene^[6,7]. Elsewhere, mutations can be observed in treatment-naïve patients, but very little is known about the frequency and impact

Abstract

Anti-hepatitis B virus (HBV) therapy leads to the emergence of mutant viral strains during the treatment of chronic hepatitis B with nucleos(t)ides analogues. The existence of HBV variants with primary antiviral resistance may be important for treatment choice. We studied two patients with chronic HBV infection by sequencing the HBV polymerase gene. They had adefovir- and tenofovir-related mutations in the viral polymerase, although they had never been treated. These mutations were rtV214A/rtN238T in one patient and rtA194T in the other. Thus, mutations in untreated patients deserve cautious surveillance. These data indicate that mutations that can theoretically confer adefovir or tenofovir resistance may emerge in treatment-naïve patients.

of these HBV variants^[8-11].

In this context, different treatment options for the optimal management of chronic hepatitis B deserve to be mentioned. According to the European Association for the Study of the Liver, interferon alpha or nucleos(t)ides analogues can be used^[12]. The indications for treatment, for patients with HBe antigen (HBeAg)-positive or -negative chronic HBV infection, are based on three criteria: serum HBV DNA > 2000 IU/mL, serum alanine aminotransferase (ALT) above the upper limit of normal, and liver biopsy showing at least grade A2 or stage F2 by METAVIR scoring. As a summary of recommendations, interferon can be proposed in patients with high baseline ALT (> 3 times upper limit of normal) and HBV DNA < 2 × 10⁶ IU/mL at baseline. Interferon gives higher rates of HBeAg and HBsAg seroconversion but has frequent side effects. The second option is treatment with nucleos(t)ides analogues with potent antiviral efficacy, good tolerance but lower rates of HBe and HBs seroconversion, possibly indefinite duration of treatment, and risk of HBV resistance.

In the context of the introduction of HBV sequencing in the Virology Laboratory of Strasbourg University Hospital, with the aim to explore genotypic viral resistance, 14 untreated patients with chronic HBV infection were investigated for the HBV polymerase gene. Two of 14 patients showed, in the absence of treatment pressure, mutations theoretically linked to adefovir and tenofovir pressure.

CASE REPORT

In December 2005, a 50-year-old Vietnamese man was diagnosed with chronic HBV infection, without co-infection by hepatitis delta virus, hepatitis C virus (HCV) or human immunodeficiency virus (HIV). This diagnosis was established by exploring asthenia associated with arthralgia. As mentioned during the medical investigation when the patient was admitted to the University Hospital of Strasbourg, the infection was acquired by vertical transmission, which is common in Asia^[13,5]. At the time of diagnosis, HBsAg and HBeAg were positive and anti-HBc antibodies were detected. The patient's ALT and aspartate aminotransferase (AST) values were seven times the upper limit of normal. The viral load was 1.08 × 10⁸ IU/mL (COBAS TaqMan HBV test; Roche Diagnostics). In March 2006, a liver biopsy confirmed the diagnosis of chronic hepatitis B with moderate activity and extensive fibrosis (Metavir score A2F3). Sequencing of the HBV polymerase (TRUGENE® HBV genotyping kit; Siemens Medical Solutions Diagnostics, France) revealed two mutations potentially linked to adefovir resistance: rtV214A and rtN238T (confirmed by a second sequencing).

The second patient, a 35-year-old Moroccan man, was diagnosed with HBV infection in May 2003, without co-infection by hepatitis delta virus, HCV or HIV. He presented no past history of hospitalization, transfusion or drug addiction. HBsAg was positive, HBeAg was negative, and anti-HBc antibodies were detected in

the absence of anti-HBs antibodies. The viral load was 43200 copies/mL (Amplicor HBV test; Roche Diagnostics). ALT and AST values were normal. A liver biopsy performed in July 2003 confirmed the diagnosis of chronic hepatitis B (Metavir score A1F0). Therefore, a simple follow-up was proposed to the patient. In December 2005, sequencing of the viral genome revealed an rtA194T mutation of the polymerase (confirmed by a second sequencing), possibly linked to tenofovir resistance.

DISCUSSION

In these two cases, viral variants with mutations that confer potential adefovir or tenofovir resistance were discovered in patients who had never been treated.

The rate of selection of adefovir resistance is around 30% after 5 years of treatment. Adefovir resistance is associated with a primary mutation in the D domain at rtN236T. In addition, a number of other mutations have been detected that cluster into three distinct regions of the polymerase: the D and A domains (rtP237H, rtN238T/D, rtV84M and rtS85A); the B domain at rtA181T/V; and the C-D interdomain (rtV214A, rtQ215S). These mutations may be regarded as secondary resistance mutations, as they are associated only with very low-level resistance *in vitro*. These secondary mutations have also been detected in the absence of rtN236T (both alone and in combination) in patients who have either not responded or have had a virological breakthrough during adefovir treatment^[6]. The rtN238T mutation may be involved in disruption of triphosphate binding in viral polymerase. However, other authors have suggested that background polymorphisms including rtV214A and rtN238T could exist without any impact on antiviral treatment failure^[8].

Tenofovir resistance conferred by rtA194T in association with the changes that cause lamivudine resistance, i.e. rtL180M and rtM204V, has been observed in individuals who are co-infected with HBV and HIV-1^[14,15]. However, the analysis reported by other authors has not provided a clear association between rtA194T and viral load rebound^[4]. Thus, the potential impact of this mutation on tenofovir susceptibility deserves further study.

Two hypotheses come to mind. The first one is that the mutations appeared in the course of the chronic infection. The probability of selecting antiviral resistance is usually proportional to the intensity of selection pressure and the diversity of HBV quasispecies^[6]. In our cases, treatment was not the means of selection pressure that allowed the mutated clones to take over. In our study, the pressure may correspond to the patients' immune system, or the mutant clones that bear the rtV214A, rtN238T or rtA194T mutations may confer a replication advantage on the wild-type virus. This hypothesis suggests that there is a natural polymorphism in the population with chronic hepatitis B, which might predispose to resistance to certain antiviral agents. The second hypothesis is that the patients may have been

infected with strains from other patients who had been treated with the corresponding nucleotide analogues.

Although the fact that two treatment-naïve patients were infected by HBV strains with mutations in viral polymerase is interesting, two limitations have to be considered. First, since only 14 patients were studied, the prevalence of these changes in treatment-naïve patients cannot be safely established. Large-scale investigations in HBV-infected patients, before any anti-HBV treatment, should be conducted in order to determine this prevalence. Second, the changes observed in positions 194, 214 and 238 of HBV polymerase do not represent well-established HBV resistance mutations^[4,8]. Based on *in vitro* results, with controversial data reported for the rtA194T change by Delaney *et al.*^[4] and for rtV214A and rtN238T by Borroto-Esoda *et al.*^[8], the clinical significance of these mutants remains questionable.

In conclusion, our results concerning HBV mutations in treatment-naïve patients that potentially confer resistance suggest the need for studies on large cohorts. By analyzing HBV sequences before antiviral therapy with analogues in treatment-naïve patients, the clinical impact of pre-treatment mutations on the efficacy of antiviral therapy may be better characterized^[16].

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