

Case report

Unusual selection of rtA181V HBV mutants cross-resistant to adefovir following prolonged lamivudine monotherapy: report of two cases

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Development of hepatitis B virus (HBV)-resistant strains following nucleos(t)ide analogue treatment is a major concern. The A181V mutation within the reverse transcriptase (RT) of HBV has been shown to be associated with HBV resistance to adefovir dipivoxil (ADV), and its level of sensitivity to other nucleos(t)ide analogues is an important issue. This article reports two cases of chronically HBV infected patients who developed rtA181V HBV mutants following lamivudine (LAM) monotherapy. This was subsequently associated with virological breakthrough under

LAM monotherapy or LAM/ADV bi-therapy, which were rescued by tenofovir disoproxil fumarate treatment. These observations suggest that rtA181V mutation may unusually emerge under LAM monotherapy, and may be associated with cross resistance to LAM and ADV, but remains sensitive to tenofovir disoproxil fumarate. Moreover, they highlight that HBV sequence analysis is an essential tool to optimize therapeutic management of HBV chronic infection in clinical practice in order to choose the appropriate nucleos(t)ide analogues.

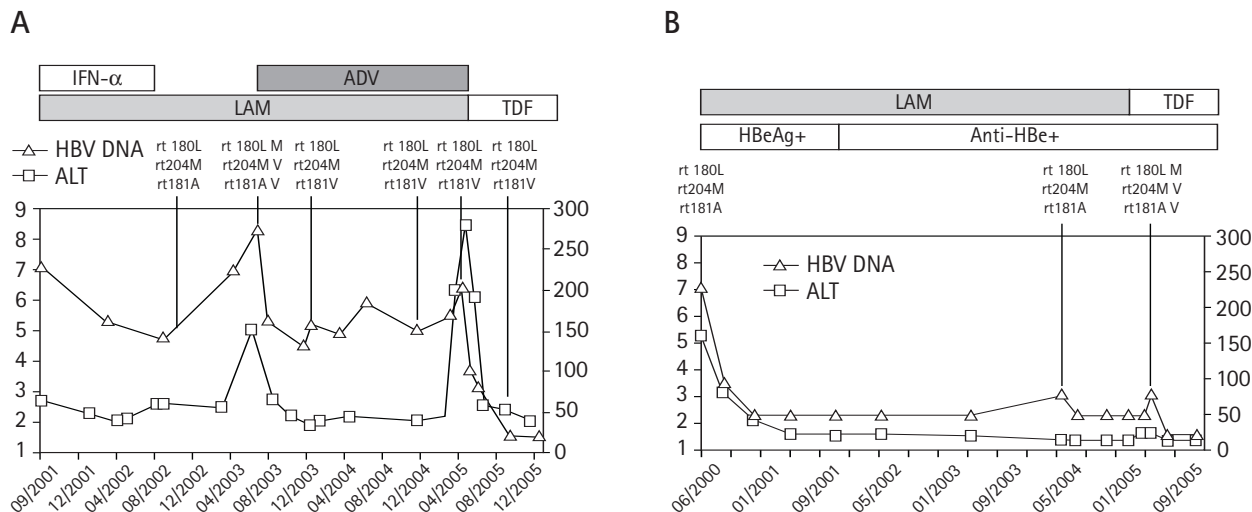
Introduction

The nucleoside/nucleotide analogues lamivudine (LAM) and adefovir dipivoxil (ADV) are widely used in the treatment of chronic hepatitis B virus (HBV) infection [1–6]. The emergence of HBV strains resistant to these antivirals that lead to disease exacerbation is a major concern [7]. Development of HBV mutants that are resistant to LAM has been observed in 70% of patients following 4 years of therapy [8–10]. By contrast, resistance to ADV has been observed in only 11 and 18% of patients after 3 and 4 years of therapy, respectively [11]. Three mutations associated with LAM resistance have been mostly described within the HBV polymerase (M204V/I in the C domain, V173L and L180M in the B domain). The nucleotide analogue ADV is the alternative of choice in these patients presenting LAM resistance. Indeed, its activity has been demonstrated against wild-type HBV, pre-core mutants and, most impor-

tantly, LAM-resistant HBV [12–14]. Two major mutations associated with ADV resistance have been described so far. Firstly, the N236T mutation located in the D domain of the HBV polymerase, and secondly, the A181V mutation within its B domain. Both mutations have been shown to confer *in vitro* and *in vivo* resistance to ADV [11,15,16]. However, it is uncertain whether those ADV escape mutants retain complete sensitivity to LAM *in vitro* and *in vivo* even though preliminary data suggest that rtN236T mutation may be more sensitive to LAM than rtA181V mutation [17,18].

We present herein the cases of two patients who developed rtA181V mutation in association with viral breakthrough on LAM monotherapy. One of them did not respond to combination therapy with LAM and ADV, and both were rescued by tenofovir disoproxil fumarate (TDF).

Figure 1. Evolution of HBV DNA (\log_{10} copies/ml), ALT (UI/l) levels, HBV genotypic patterns, and antiviral regimen for patient 1 (A) and patient 2 (B)



Amino acids in bold are different of wild-type. Alanine aminotransferase (ALT) normal values <40 UI/l. ADV, adefovir; HBV, hepatitis B virus; LAM, lamivudine; RT, reverse transcriptase; TDF, tenofovir disoproxil fumarate.

Patient 1

A 42-year-old man was known to be chronically infected with HBV since 1999. In September 2001, the patient was hepatitis B surface antigen (HBsAg) positive, hepatitis B e antigen (HBeAg) negative, anti-HBe positive (Axsym Abbott microparticles enzyme immunoassay, Wiesbaden, Germany) and HBV DNA positive (12,500,000 copies/ml, Cobas Amplicor HBV Monitor Assay, Roche Diagnostics, Meylan, France). Transaminases were increased (2×normal). LAM (100 mg/d) was started in association with interferon- α (3 million units 3 times a week). Interferon- α was stopped in October 2002 while LAM was continued. At that time, the HBV DNA level was 52,000 copies/ml and transaminases were within the normal range. Liver histology performed after the end of interferon therapy showed significant improvement compared with pre-treatment status (Knodell score = 5 vs 10). In July 2003, alanine aminotransferase (ALT) rose again (1.5×normal) and a viral breakthrough occurred, with HBV DNA increasing to more than 100 million copies per ml. ADV (10 mg/d) was added to LAM. During ADV/LAM bi-therapy, HBV DNA diminished by more than 3 \log_{10} , reaching 4.5 \log_{10} , but then progressively rose again and fluctuated between 4.9 and 5.9 \log_{10} during year 2004. ALT levels were persistently within the normal range in this period. In April 2005, viral and clinical breakthrough occurred since HBV DNA rebounded to more than

2,690,000 copies/ml in association with increasing ALT levels (2×normal). ADV/LAM bi-therapy was stopped and replaced by TDF. HBV DNA markedly decreased to less than 100 copies/ml (Cobas Ampliprep/Cobas TaqMan 48 assay, Roche Diagnostics) after 3 months of TDF treatment and was still undetectable after 8 months of follow-up.

HBV sequencing was retrospectively performed from serum samples collected during the different phases of treatment. HBV DNA sequences (core and pol genes) were obtained from serum using in-house amplification and sequencing protocols, as previously described [19]. Of note, HBV was of genotype D and harboured a precore G1896A mutation. The sequences obtained were aligned and compared with a reference HBV sequence (SeqScape software, Applied-Biosystems, Branchburg, NJ; HBV reference sequence: GenBank accession number X65259).

The evolution of viral load and HBV RT genotypic patterns is presented in Figure 1. No mutation was detected in HBV RT in October 2002 when interferon was stopped after 13 months on LAM. By contrast, rtL180M, rtM204V and rtA181V mutations were concurrently detected in July 2003. This was concomitant with a virological rebound. Five months after addition of ADV, rtL180M and rtM204V mutations were no more found and HBV strains bearing rtA181V were exclusively detected. No other mutation within HBV RT was detected at that time.

Patient 2

A 39-year-old man was chronically infected with HBV since October 1999. In April 2000, the patient was HBsAg positive, HBeAg positive, anti-HBe negative and HBV DNA was over 369,000,000 copies/ml. ALT were increased (5×normal) and liver biopsy demonstrated liver cirrhosis (Knodell score of 12, METAVIR A2F4). In June 2000, HBV DNA was 11,323,902 copies/ml and LAM (100 mg/d) was started. HBV DNA decreased under the lower limit of detection of 200 copies/ml within 6 months of treatment and ALT were within the normal range in 9 months. HBe seroconversion occurred after 2 years of therapy. In June 2002 liver biopsy demonstrated an improvement of liver histology with a Knodell score of 3 and METAVIR A1F2. LAM treatment was continued. In May 2004 HBV DNA transiently reappeared (1,194 copies/mL [Cobas TaqMan 48 assay, Roche Diagnostics]), but ALT remained normal and HBeAg remained negative. LAM therapy was continued. In June 2005, HBV DNA reappeared and HBV sequencing analysis was performed. RtM204V, rtL180M and rtA181V mutations were concurrently detected. At that time, patient was still on LAM monotherapy, viral breakthrough was obvious and due to the presence of rtA181V mutation we decided to add TDF to LAM by the end of June 2005. The viral response was rapid with disappearance of HBV DNA <34 copies/ml within 1 month and remained negative since that time. Retrospective analysis of sera available since 2000 demonstrated that rtL180M, rtA181V and rtM204V occurred on LAM monotherapy (Figure 2).

Discussion

We report the occurrence of rtA181V HBV mutants during LAM monotherapy in two patients, followed by a virological failure to subsequent ADV and LAM bi-therapy in one case. These observations clearly suggest that LAM may promote the emergence of ADV-resistant rtA181V mutants, in addition to the classical rtM204V and rtL180M mutations.

In our observations, the virological breakthrough occurring after 21 and 60 months of LAM monotherapy, respectively, was associated with the emergence of HBV strains harbouring rtA181V concurrently with rtL180M and rtM204V mutations classically associated with LAM resistance [7]. The presence of rtA181V mutation probably explains that in patient 1 the response to ADV was of relatively short duration. Indeed, the classical LAM resistance mutations (rtM204V, rtL180M, rtV173L) are not associated with resistance to ADV, and most patients presenting with LAM resistance are efficiently treated

by ADV [13,14]. Furthermore, once ADV was introduced in addition to LAM, rtA181V-mutated HBV strains were exclusively detected as pure mutants 5 months later. Therefore, in our observations, ADV-resistant HBV strains harbouring rtA181V mutation were selected during LAM monotherapy. In the same line, another mutation at codon 181 within the HBV polymerase, namely the rtA181T mutation, has been previously described following prolonged LAM monotherapy in LAM-resistant patients. Finally, in a recent report, the rtA181V/T mutations conferring resistance to ADV were found more common in LAM-resistant patients as compared with treatment-naïve patients [20,21]. Together with our observations, these data suggest that prolonged LAM monotherapy may promote the emergence of rtA181T/V-mutated HBV viral strains precluding the antiviral efficacy of ADV.

Furthermore, our first observation clearly suggests that the rtA181V mutation may confer resistance to LAM with virological and clinical breakthrough. If the role of mutation rtA181V in resistance to ADV is established, its impact on the sensitivity of HBV strains to LAM is not so clear. Thus, it has been reported that in patients in whom rtA181V HBV mutants emerged on ADV, the addition of LAM resulted in a virological response [17,18]. By contrast, in patient 1, the rtA181V mutation was associated with virological failure despite continuous LAM administration with good compliance to treatment, even though LAM-resistance mutations rtM204V and rtL180M were no more detected. It must be outlined that no other mutation within the HBV RT gene known to confer resistance to nucleo(t)side analogues (that is, I233V, N236T, etc) could be detected in patient number 1 and sequencing of the RT gene did not demonstrate significant difference, other than A181V mutation, as compared with a reference HBV sequence. This suggests that rtA181V mutation alone might be associated with resistance to LAM. Although recent *in vitro* data have reported that it could promote a 12- to 15-fold reduction in the antiviral efficacy of LAM [17,18], clinical evidence of rtA181V-associated LAM resistance, which is indicated by our observations, has not been reported so far.

The sensitivity of the rtA181V-mutated HBV strains to new antiviral drugs is not well known. A lower *in vitro* sensitivity to TDF or next available nucleoside analogues (clevudine, telbivudine) has been recently reported [17]. However, our observations suggest that the rtA181V-resistant mutant is sensitive to TDF in clinical practice.

Although the association between the rtA181V mutation and clinical resistance to ADV+LMV requires further *in vitro* and *in vivo* confirmation, our observations may have major consequences in the management of HBV-infected patients. Firstly, they suggest that the

emergence of rtA181V mutation within the HBV polymerase may be promoted by LAM monotherapy prior to any ADV therapy. Secondly, they suggest that this mutation may induce cross-resistance to LAM and ADV resulting in subsequent failure of alternative therapeutic strategies and worsening of hepatitis. Altogether, the present data stress the importance of HBV polymerase sequencing to detect drug-resistance mutations and to guide the choice of nucleos(t)ide analogues in patients who are chronically infected with HBV.

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