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Tenofovir monotherapy is effective in hepatitis B patients with antiviral treatment failure to adefovir in the absence of adefovir-resistant mutations☆

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See Editorial, pages 383–386

***Background/Aims*: We sought to identify mutations associated with treatment failure to adefovir (ADV) and to determine virologic response to tenofovir (TDF) alone and in combination with emtricitabine (FTC) in these patients.**

***Methods*: Serum samples prior to and after the change in treatment to TDF/TDF + FTC from 13 patients were analyzed by direct sequencing and clonal analysis.**

***Results*: ADV-resistant mutations, rtA181V and rtN236T, were detected on direct sequencing in 3 of 8 patients who had virologic breakthrough. Among patients with suboptimal virologic response, rtA181T, rtI233V, and rtN236T were present on clonal analysis in 3 patients. Ten patients received TDF, 8 achieved virologic response. One had ADV-resistance at baseline and persistence of ADV-resistant mutations during TDF treatment, addition of FTC resulted in a further decrease in HBV DNA. Another patient had no ADV-resistance at baseline, and selection of ADV-resistant mutations during TDF treatment. All 3 patients who received TDF + FTC had undetectable HBV DNA within 3–12 months including 2 who had ADV-resistance at baseline.**

***Conclusions*: TDF monotherapy is effective for patients with virologic breakthrough or suboptimal response to ADV, but combination therapy with a nucleoside analogue should be considered in patients with ADV-resistance. No novel mutations were detected.**

***Keywords*: Adefovir; Tenofovir; Emtricitabine; Antiviral resistance; Hepatitis B treatment**

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Abbreviations: ADV, adefovir; FTC, emtricitabine; HBV, hepatitis B virus; LAM, lamivudine; TDF, tenofovir.

1. Introduction

Adefovir dipivoxil (ADV), an acyclic phosphonate, has antiviral activity against both wild type and lamivudine (LAM)-resistant hepatitis B virus (HBV). Initial studies of nucleoside-naïve patients reported no evidence of drug resistance after 48 weeks of ADV treatment [1]. However, ADV-resistant mutations with substitutions of valine for alanine at position 181 (rtA181V) and threonine for asparagine at position 236 (rtN236T) in the reverse transcriptase region of the HBV polymerase have been reported [2,3]. These mutations have subsequently been observed in 29% of

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nucleoside-naïve hepatitis B e antigen (HBeAg) negative patients after 5 years of ADV treatment [4]. A higher rate of resistance has been reported in patients with LAM-resistance, who were switched to adefovir monotherapy [5,6]. *In vitro* studies showed that rtA181V and rtN236T mutations decrease susceptibility to ADV by 4.3- to 23-fold [2,3,7]. Several other mutations have been proposed to be associated with ADV-resistance but the significance of these mutations is unclear [8–12]. Clinical studies have observed that as many as 50% of patients have suboptimal virologic response to ADV [5,13]. Some possible causes for this include the low approved dose of ADV to avoid nephrotoxicity, LAM-resistance, and high HBV DNA at the start of ADV treatment. A recent report of 3 patients suggested that a novel mutation with substitution of valine for isoleucine at position 233 (rtI233V) is associated with primary non-response to ADV [14], but this finding was not confirmed in another study [15].

Tenofovir disoproxil fumarate (TDF), a nucleotide analogue closely related to ADV, has similar antiviral activity against wild type and LAM-resistant HBV as ADV in *in vitro* studies [16]. TDF is approved for the treatment of human immunodeficiency virus (HIV) infection; it is available on its own and as a co-formulation with emtricitabine (FTC), which has comparable antiviral activity and resistance profile as LAM. Clinical studies have observed that TDF is more effective in suppressing HBV replication than ADV [17,18], possibly due to the higher approved dose: 300 vs. 10 mg. Thus, patients with suboptimal virologic response to ADV have been reported to experience further viral suppression when treatment was switched to TDF [19]. Nevertheless, *in vitro* studies showed that susceptibility of HBV isolates with rtN236T and rtA181V to TDF is decreased by 4- and 3.2-fold, respectively, indicating that tenofovir may be less effective for ADV-resistant HBV [7,20].

The aims of this study were to identify mutations associated with suboptimal virologic response to ADV and to determine virologic response to TDF, alone and in combination with FTC, in patients with suboptimal response or breakthrough during ADV treatment.

2. Patients and methods

2.1. Patients

Adult patients with compensated chronic hepatitis B referred to the University of Michigan Liver Clinic between August 1999 and January 2007 who had suboptimal virologic response to ADV or virologic breakthrough during ADV treatment and subsequently received rescue therapy with TDF or TDF + FTC for at least 6 months were included. Clinical and laboratory data were reviewed.

Quantitative HBV DNA and liver panel were tested every 3 months and patients were assessed at 6- to 12-month intervals. Serial serum samples were collected before, and every 6–12 months after change in therapy. All samples were stored at −80°C. Written informed consent for the collection of blood samples was obtained from all patients and the study was approved by the Institutional Review Board at the University of Michigan.

2.2. HBV quantification

HBV DNA was quantified using COBAS Amplicor HBV Monitor Assay (Roche, Branchburg, NJ), which has a lower detection limit of 200 copies/mL. Samples with values >100,000 copies/mL were retested after 1:100,000 dilutions.

2.3. Nested polymerase chain reaction and direct sequencing

DNA extraction was carried out with QIAamp® DNA Mini Kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. Nested polymerase chain reaction (PCR) was performed as described previously [21]. The amplicons spanned domains A through F of the reverse transcriptase region of the HBV polymerase gene (rt1-rt280). PCR products were purified by QIAquick PCR Purification Kit (Qiagen) and directly sequenced at the DNA sequencing core facility, University of Michigan Medical Center, using the standard protocol for the ABI 3730xl DNA Analyzer (Applied Biosystems Co., Foster City, CA). The DNA sequences were aligned using Seqman™ II and EditSeq™ software (DNASTAR Inc., Madison, WI).

2.4. Cloning

Cloning was carried out as previously described [22]. PCR-amplified HBV DNA was cloned into pGEM T Easy Vector (Promega Co., Madison, WI), and 20–31 colonies with HBV insert were selected for each sample. Recombinant plasmid DNA was purified, electrophoresed after digestion with restriction enzymes XbaI and PstI (Roche Diagnostics Co., Indianapolis, IN), and sequenced using primers SP6 or T7. The sequences of all the clones from each sample were compared using MegAlign™ software (DNASTAR Inc., Madison, WI).

2.5. Definitions

Suboptimal virologic response was arbitrarily defined as HBV DNA $>4\ \log_{10}$ copies/mL after $\leqslant 6$ months of antiviral treatment [21]. Virologic breakthrough was defined as $\leqslant 1\ \log_{10}$ copies/mL increase in HBV DNA from nadir.

Sequences of samples collected prior to TDF or TDF + FTC treatment were compared to consensus sequences of the same HBV genotype derived from GenBank database. Sequences of follow-up samples were compared to those at the start of TDF or TDF + FTC treatment. Changes in amino acid residues were classified as one of the following categories: [1] previously observed polymorphisms, [2] novel residues at polymorphic sites, and [3] conserved site mutations.

3. Results

3.1. Baseline characteristics of patients before TDF or TDF + FTC

Baseline characteristics of all 13 patients who met inclusion criteria are listed in Table 1. The median age was 51 years (range 35–72). Eleven patients were men and 8 were Caucasians. Six patients had genotype A infection. All patients had compensated liver disease and five had cirrhosis. Nine patients had received prior LAM; of these, 8 were switched to ADV monotherapy due to LAM-resistance, the remaining patient was