Role of plasmatic and urinary concentration of tenofovir disoproxil fumarate in a cohort of patients affected by chronic hepatitis B

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Abstract

The aim of this study was to evaluate plasmatic and urinary therapeutic drug monitoring (TDM) of tenofovir disoproxil fumarate (TDF) in a cohort of patients with chronic hepatitis B (CHB). In 68 enrolled patients, the estimated glomerular filtration rate (eGFR) was 68 mL/min in naive subjects, while in adefovir dipivoxil (ADV)-pretreated patients, it was 55.5 mL/min (p < 0.001). The HBV E genotype was associated with lower TDF levels (β = -0.698, p < 0.001). The urinary TDF concentration was associated with ADV pretreatment (β = 0.829, p < 0.001). Determination of urinary concentrations of TDF may be useful in the clinical management of older CHB patients and those with previous treatment with ADV.

Introduction

Hepatitis B infection affects millions of people worldwide, causing a large number of deaths due to cirrhosis and hepatocellular carcinoma every year [1]. Two different strategies are currently available as a first-line therapy: a limited course of treatment with pegylated interferon alpha (PEG-IFN) or long-term treatment with nucleos(t)ide analogues (NAs). Several factors, such as age, comorbidity, cirrhosis, and family history of hepatocellular carcinoma, extrahepatic manifestations, virological and serological response predictors, and coinfection with hepatitis D virus (HDV) should be considered before initiation of treatment [2].

Since IFN is not always indicated and the response rate is low, NAs are widely used in the majority of patients who have previously experienced failure of PEG-IFN treatment. Undetectable hepatitis B virus (HBV) DNA after 24–48 weeks and HBsAg reduction or loss during treatment are valuable response predictors in both HBeAg-positive and HBeAg-negative patients [3].

Among NAs, we distinguish drugs with a low genetic barrier of resistance (lamivudine, telbivudine, and adefovir) and those with a high genetic barrier (entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide) [2].

Despite its high genetic barrier and high effectiveness, tenofovir disoproxil fumarate (TDF) has been reported to cause kidney failure, hypophosphatemia, osteoporosis, and bone fractures, especially in older patients and when taken for a long period of time [4, 5]. Maggi et al. [6] quantified TDF toxicity based on estimated glomerular filtration rate (eGFR), proteinuria (>200 mg/24 h), hypophosphatemia (phosphate <2.5 mg/dL), increased parathyroid hormone (>65 pg/mL), plasmatic levels of vitamin D, and DEXA scanning. The authors recommended routine monitoring of eGFR and phosphatemia in TDF-treated patients and indicated that more information on kidney biomarkers is needed to identify early impairment.

Recently, tenofovir alafenamide (TAF) was proposed as a safer alternative to TDF for treatment of HBV infection in older patients (age > 60 years) and in patients with bone or kidney disease [7]. TAF is a prodrug of TDF with higher plasmatic stability and a longer plasmatic half-life, thus allowing the use of lower doses, resulting in less kidney excretion and bone accumulation compared to TDF [8]. A novel approach using therapeutic drug monitoring (TDM) of TDF was applied in the clinical management of patients with human immunodeficiency virus (HIV); the role of plasmatic
and urinary levels of TDF were found to be related to the risk of renal damage and treatment failure [9]. However, no data are available about using this approach in patients with chronic hepatitis B (CHB) who are being treated with TDF.

The aim of this study was to evaluate the role of plasmatic and urinary levels of TDF in a cohort of chronic HBV patients according to clinical outcome (virological, serological, biochemical) and toxicity (renal failure, treatment interruption).

**Materials and methods**

We performed a retrospective cohort study at the Infectious Disease Unit of Amedeo di Savoia Hospital, Torino, Italy. We included all HBV HBeAg-positive and negative naïve or experienced patients treated with TDF from March 2019 and June 2019. The patients were tested for HDV antibodies (HDV IgG) and HDV RNA. All HIV-coinfected patients were excluded from the study.

The study was approved by our local ethics committee (number 002360, January 15th, 2015) and was conducted in agreement with the Helsinki Declaration, with all patients providing written informed consent.

Serum HBV DNA levels were quantified by real-time PCR (COBAS AmpliPrep/COBAS TaqMan HBV Test 2.0, Roche Molecular Systems, NJ, USA). HBV genotyping was performed using INNO-LiPA (Innogenetics, Belgium). HBsAg, HBeAg, and anti-HBe were detected using an Elecsys assay (Roche Diagnostics, Italy), but quantification of the S antigen was instead carried out using an ARCHITECT analyzer (Abbott Diagnostics, Ireland) with a range of 0.05-250.0 IU/mL; values of qHBsAg > 250.0 IU/mL were subsequently diluted and retested. Pharmacokinetic analysis was performed on samples collected 24 hours before drug administration (Cthrough) at the last visit, according to the TDF plasmatic dosage method published by De Nicolò et al. [10]. The ratio of urinary TDF concentration to TDF plasmatic concentration was also calculated. Therapeutic drug monitoring on TDF plasma and urinary Cthrough was performed.

We collected the following baseline data: age, sex, weight, height, BMI, geographic origin, HBV genotype (A, B, C, D, E), level of education, current job, probable route of transmission according to medical history (unknown, sexual, IDU, vertical, familial, iatrogenic). Information about other medications and comorbidities were also collected.

We divided our population in two groups: naïve and experienced patients. For experienced patients we collected data on previous treatments, including PEG-IFN, lamivudine, adefovir, entecavir, tenofovir, and telbivudine and on resistance-associated mutations, as appropriate.

We collected data on alanine aminotransferase (ALT) and aspartate aminotransferase (AST), platelets, qHBsAg, HBV DNA, HBeAg, and HDV coinfection at baseline (treatment initiation) and at their last four visits of follow-up. Tolerability was evaluated at the same time points by measuring plasmatic creatinine and eGFR, calculated by the MDRD (Modification of Diet in Renal Disease) formula.

Hepatic staging data were collected based on liver stiffness at transient elastography (Fibroscan) or by liver biopsy, if available. In transient elastography, 9 kPa was the cutoff to identify patients with cirrhosis. For liver biopsy samples, the Ishak score was determined. Indirect signs of cirrhosis observed in ultrasound scans and the most recent alpha-fetoprotein (AFP) values were also recorded.

Continuous variables for descriptive analysis are reported as the median with the interquartile range [IQR] from the 25th to the 75th percentile. Categorical variables are described as frequencies and percentages. All variables were compared using the Shapiro-Wilk test. Categorical variables were compared using the Mann-Whitney and Kruskal-Wallis tests. Continuous variables were evaluated using Spearman’s correlation. Associations were assessed using the χ² test. Univariate and multivariate analyses for plasmatic and urinary TDF levels were performed using a linear regression model. Multivariate analysis was adjusted for the following variables: age, gender, BMI, baseline qHBsAg, HBV DNA baseline, HBV genotype, eGFR, liver stiffness, presence of HBV resistance, and treatment experience.

**Results**

We examined 68 patients treated with TDF. Plasmatic and urinary TDF data were available for 29 patients out of 68, whereas 28 patients were not included in the follow-up. The baseline characteristics of the study population are shown in Table 1: 32 (47.1%) of the patients were Italians, 14 (20.6%) were Africans, 12 (17.6%) were Chinese, nine (13.3%) were from other European countries, and one (1.5%) was from South America.

The median age was 50 years (IQR, 34-62), and 51 (75%) of the subjects were males. The distribution of genotypes was as follows: 8 (11.8%) A, 3 (4.4 %) B, 9 (13.2%) C, 37 (54.4%) D, 11 (16.2%) E. Forty-five (66.2%) of the subjects were employed, and 23 (33.8%) were unemployed. The median BMI was 24 kg/m² (IQR, 22.65-26.38). The route of transmission of the infection was unknown in 33 cases (48.5%), sexual exposure in four (5.9%), due to intravenous drug use (IDU) in six (8.8%), vertical in one (1.5%), familial in 17 (25%), and iatrogenic in seven (10.3%). At baseline, 32 (47.1%) subjects were naïve for antiviral treatment, and 36 (52.9%) had experienced antiviral treatment previously. Previous treatment included PEG-IFN (11, 30.5%), lamivudine (17, 47.2%), adefovir dipivoxil (ADV) (11, 30.5%), entecavir (ETV)
The reason for switching regimens was the onset of resistance, which occurred in 18 (27%) patients. The median duration of therapy was 8.7 years (IQR, 7-11). All patients were screened for HDV coinfection at baseline, and positive serology for HDV IgG was observed in four (5.9%) patients who were negative for HDV RNA. At baseline, HBeAg was positive in 24 (35.3%) of the 68 patients and negative in 44 (64.7%).

In this population, the median qHBsAg baseline was 1850 IU/mL (IQR, 404.75-9850), 3.27 log IU/mL (IQR, 2.61-3.99). The median HBV DNA baseline was 375 IU/mL (IQR, 20-260800.5), 2.72 log IU/mL (IQR, 1.3-5.44). Median stiffness was 7 kPa (IQR, 6-10.43): 35 patients (51.5%) showed stiffness less than 7 kPa; 14 (20.6%), 7-9.5 kPa; five (7.4%), 9.6-12.5 kPa; 14 (20.6%), more than 12.5 kPa.

The median ALT and AST values were 43.5 IU/mL (IQR, 22-81.75) and 33 IU/mL (IQR, 22.5-81.75), respectively. The median eGFR was 80.15 mL/min (IQR, 69.25-88.86). The median plasma concentration of TDF (Cthrough) was 45 ng/mL (IQR, 34-57.5), whereas the median urinary concentration of TDF (Cthrough) was 17490 ng/mL (IQR, 12307.5-24858). The median ratio of the urinary TDF concentration to the plasma TDF concentration was 393.66 (IQR, 254.44-622.99).

The median eGFR in naïve patients was 68 mL/min (IQR, 59.5-76), while in ADV-pretreated patients, it was significantly lower: 55.5 mL/min (IQR, 51.75-60) ($p < 0.001$).

The median eGFR decline observed was 4 mL/min (IQR, 1-6) in naïve patients and 15.5 mL/min (IQR, 13.25-21) in subjects with previous treatment with ADV ($p < 0.001$).

The median TDF urinary concentration in ADV-pretreated patients was 26,590 (IQR, 18,360-33,871.75), which was statistically higher than in naïve subjects: 6650 ng/mL (IQR, 5552.25-8700) ($p < 0.001$) (Fig. 1).

Linear regression showed a direct correlation between the urinary concentration of TDF (ng/mL) and the decrease in eGFR compared to baseline (Z-coefficient, -6.434; $y = 1666.6x + 3.894$; $r^2 = 0.510$; $p < 0.001$) (Fig. 2).
Fig. 1  Urinary concentration of TDF in the study population according to previous ADV treatment

Fig. 2  Relationship between urinary concentrations of TDF and eGFR reduction from baseline in the study population
The median liver stiffness (kPa) value was higher in ADV-pretreated patients: 18.5 kPa (IQR, 12-30) than in naïve patients: 7.05 kPa (IQR, 6-8.43) (p < 0.001).

The median treatment duration was 12 years (8-14) for naïve patients and 8 years (6-12) for experienced patients, but the difference was not statistically significant (p = 0.017).

Considering patients’ genotypes, the median plasmatic concentration of TDF (ng/mL) in patients with genotype E was 26 (IQR, 22-31.5), and this was statistically lower than in patients with a different genotype; 54.5 (IQR, 45-66; p < 0.001).

In multivariate analysis, the liver stiffness results were predictive of a higher plasmatic TDF concentration (β = 0.894, DS = 0.319, p = 0.009), while HBV genotype E was associated with lower TDF levels (β = -0.698, DS = 0.050, p < 0.001). Pretreatment with ADV was not associated with plasmatic TDF concentration (β = -0.124, DS = 0.390, p = 0.122).

In multivariate analysis, a higher urinary TDF concentration was associated with pretreatment with ADV (β = 0.829, DS = 0.202, p < 0.001).

Discussion

In this real-life study, we have, for the first time, investigated the role of plasmatic and urinary concentrations of TDF in patients with CHB. TDF is widely used in the treatment of HIV and HBV infections, with a high degree of effectiveness in viral suppression and a low rate of drug resistance. Although TDF is generally well tolerated, several studies have found potential kidney and bone toxicity during a long course of therapy [5]. Mild or moderate renal failure is frequently associated with TDF treatment, while severe kidney injury is rare and is more often related to other comorbidities or previous kidney diseases. Major risk factors related to renal failure include older age, HIV infection, elevated baseline creatinine levels, and long-term exposure to TDF [11]. In more detail, TDF leads to tubular toxicity with an involvement of mitochondrial alterations due to oxidative stress. Tubular proximal cells are responsible for the secretion of intracellular TDF, but mitochondrial failure due to exposure to high levels of TDF leads to cell damage and nephrotoxicity [12].

The observation of TDF-related toxicity is more frequent in patients with HIV infection, prolonged TDF exposure, other potential drug-drug interactions with higher risk of reduced tubular secretion, proteinuria, bone mineral density decline, and proximal tubulopathy [13]. Conversely, in the treatment of CHB, TDF is less frequently related to moderate/severe nephrotoxicity, and only moderate eGFR reduction has been observed [6]. This aspect may be explained by differences in the immune responses to CHB and HIV and the lower chance of drug interaction with higher risk of toxicity. Despite the identification of possible biomarkers, the TDM of TDF is not yet used in current clinical practice in CHB, although the TDF plasma concentration was found to be predictive of TDF discontinuation due to renal toxicity in a cohort of HIV-treated patients [9]. Based on these data, we investigated the plasmatic/urinary levels of TDF in patients with CHB. Interestingly, the urinary concentrations of TDF are related to eGFR reduction from baseline, with a greater impact in older patients who had undergone previous treatment with ADV. For this reason, ADV-pretreated patients have a higher risk of developing chronic kidney failure, unlike naïve patients undergoing TDF treatment. The role of ADV in nephrotoxicity was examined previously, with some studies showing evidence of interstitium fibrosis due to accumulation of the drug outside the cells when tubular uptake was inhibited [14, 15]. For this reason, the interstitial damage due to long-term ADV treatment can become chronic and irreversible, with a progressive reduction of eGFR in a large proportion of patients and frequent evolution to interstitial nephritis and Fanconi syndrome [16].

Our data suggest that older ADV-pretreated patients may experience a greater benefit by switching to TAF. This aspect could be useful in clinical practice because, although the use of ADV has been discontinued, a large proportion of patients who take TDF were previously treated with ADV and were at high risk of rapidly worsening renal function.

Finally, the association of the HBV E genotype with plasmatic TDF concentration is an interesting finding, and its clinical significance should be assessed further in future studies.

In conclusion, our study provides evidence of the predictive role of urinary TDF concentrations for assessing the risk of renal injury in patients with CHB and focuses attention mainly on individuals who have previously undergone treatment with ADV as being at major risk of nephrotoxicity and in immediate need of switching to TAF therapy.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Ethical approval The present study was approved by our local ethics committee as protocol “HBV-analogues” on 15 May 2015. This study was approved by the local ethics committee as “HBV-Analogue Study” (Prot. N°002360; 26/1/2015), and all included subjects provided written informed consent.

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