Efficacy of prolonged tenofovir therapy on hepatitis delta in HIV-infected patients

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Background: Hepatitis delta virus (HDV) produces the most severe form of chronic viral hepatitis. We explored whether prolonged tenofovir exposure might be beneficial on hepatitis delta in HIV-infected patients.

Methods: All HIV-infected patients with hepatitis delta followed at our institution since year 2000 were retrospectively examined. Serum HBV-DNA and HDV-RNA were quantified using commercial assays. Liver fibrosis was measured using elastometry.

Results: A total of 19 HIV/delta patients were identified. All were viremic for HDV and 11 for HBV. After a median tenofovir exposure of 58 months, all had undetectable HBV-DNA and 10 (53%) had undetectable HDV-RNA. The median drop in HDV-RNA in the remaining nine HDV viremic patients at the end of follow-up was 2.4 log copies/ml. A reduction above 30% in liver stiffness occurred in six out 10 (60%) patients who achieved undetectable HDV-RNA, whereas hepatic stiffness did not change in the remaining HDV viremic patients ($P = 0.03$). Serum HBsAg concentrations did not decline significantly, although HBsAg seroclearance occurred in three patients, all of whom became negative for HDV-RNA.

Conclusion: Long-term exposure to tenofovir significantly reduced serum HDV-RNA apart from completely suppressing HBV-DNA in HIV-infected patients with hepatitis delta. This virological benefit is accompanied by significant improvements in liver fibrosis.

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Introduction

Hepatitis D virus (HDV) is a small unique subviral particle that only infects individuals with hepatitis B virus (HBV) infection [1,2]. More than 15 million persons are estimated to be infected with HDV worldwide [3,4]. HDV transmission overlaps HBV mechanisms, contagion being mainly the result of sexual and parenteral exposures [5]. Large HBV vaccine campaigns in adults and universal HBV vaccination of children in Western countries have translated in dramatic declines of hepatitis delta, being immigration from endemic countries the most frequent source of current HDV cases [6–8].

Infection with HDV produces the most severe form of chronic viral hepatitis [9,10]. There is no specific antiviral treatment for hepatitis delta, although peginterferon alpha given for at least 1 year may improve liver enzymes and reduce viremia [11,12]. This benefit, however, is generally only transient as HDV replication resumes thereafter in most treated patients [13–16]. New hopes for hepatitis delta patients rely on the use of peginterferon

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lambda, which is better tolerated than alpha, and prenylated inhibitors [17–19].

As HDV replication requires HBsAg and tenofovir is amongst the most potent anti-HBV agents and is widely used as part of many HIV therapeutic combinations, we explored whether prolonged tenofovir exposure might be of benefit on hepatitis delta in HIV-coinfected patients.

Patients and methods

All HIV-infected patients who were HBsAg+ and reactive for HDV antibodies followed at a large reference HIV clinic in Madrid since year 2000 were retrospectively identified. The main demographics, laboratory parameters and clinical information were recorded in a case report form specially designed for this study. The variables recorded were age, sex, risk group category, HBV-DNA, HBV genotype, HDV-RNA, HDV genotype, HBsAg, anti-HCV antibodies and serum HCV-RNA. Given the retrospective design of the study, laboratory parameters were recovered testing stored samples when they had not being tested originally.

Serum HDV-RNA was quantified using a commercial real-time PCR method (DIA.PRO; Diagnostic Bioprobes Srl, Vienna, Austria), which has a lower limit of detection of 10 copies/ml. Serum HBsAg was measured using the ARCHITECT assay (Abbott, Madrid, Spain), which has a dynamic range between 0.05 and 250 IU/ml. Serum HBV-DNA was quantified using a real-time PCR assay (Abbott), which exhibits a lower limit of detection of 10 IU/ml. HBV genotyping was performed using Inno-LiPA (Innogenetics, Ghent, Belgium). HDV genotyping was made using an in-home assay, with conditions adapted from protocols reported elsewhere [20].

Transient elastography (FibroScan; EchoSens, Paris, France) was used to assess the extent of liver fibrosis. It was performed by experienced operators using a single machine. The right lobe of the liver was explored through intercostal spaces on patients lying on the back, with the right arm at maximal abduction. At least 10 valid measurements were required before producing a result. Examinations with a success rate (ratio between number of validated and total measurements) less than 0.7 were excluded. Final liver stiffness was reported as the median value of all valid measurements, expressed as kiloPascals (kPa). Values less than 7.5 kPa were considered as equivalent to histologic Metavir stages F0-F1, values 7.5–9.4 kPa reflected F2, values 9.5–14.4 kPa were considered as F3 and values more than 14.5 kPa were equivalent to F4 (cirrhosis), following the information derived from studies conducted in hepatitis C, including HIV-HCV coinfected patients [21,22]. Advanced liver fibrosis was considered for Metavir F3 or F4 estimates. All patients underwent liver fibrosis assessment periodically at around 12-month intervals.

Statistical analysis

Results are reported as absolute values, percentages or as median and interquartile ranges (IQRs). Baseline and end of follow-up characteristics were compared using the chi-square-test and Fisher exact test for categorical variables and the Wilcoxon nonparametric test for continuous variables. Univariate and multivariate analyses were performed using logistic regression. All variables with P values below 0.2 in the univariate analysis were included in the multivariate analysis. All statistical analyses were performed by the SPSS software version 15.0 (SPSS Inc., Chicago, Illinois, USA).

Results

A total of 19 HIV-infected patients with hepatitis delta were identified in our database updated up to December 2012. Their main characteristics were as follows: 84% were reactive for HCV antibodies (three of them also positive for HCV-RNA); 79% were former IDUs; 90% were men; and median age was 48 years. HDV genotype 1 was found in all cases. HBV genotypes D and A were the most common (53 and 26%, respectively).

All patients at different time points began tenofovir therapy (time 0 for our analysis). Prior lamivudine exposure (median 59 months) before beginning tenofovir was recognized in nine (47%), being the rest naive to any anti-HBV agent. All patients were on stable antiretroviral therapy for longer than 6 months and with undetectable plasma HIV-RNA before beginning tenofovir therapy. Of note, all patients received tenofovir coformulated with emtricitabine (Truvada), being the third antiretroviral agent raltegravir in 14, efavirenz in three and darunavir/ritonavir in two.

Median $CD4^+$ cell count at baseline was 289 cells/μl and only slightly increased during the study period to 309 cells/μl. Moreover, the mean CD4 : CD8 ratio did not change significantly during tenofovir exposure in the study population. At baseline, all patients were viremic for HDV and 11 for HBV before beginning tenofovir (300 mg/day) as part of their HIV therapy. Interferon was never used either before or during follow-up on tenofovir, neither in the three patients viremic for HCV.

After a median tenofovir exposure of 58 (34–93) months, all patients had undetectable HBV-DNA and 10 (53%) HDV-RNA less than 10 copies/ml. In the last group, the median time to reach undetectable HDV-RNA was 54 (33–72) months. In the remaining nine HDV viremic patients at the end of follow-up, the median HDV-RNA had dropped to 2.42 (1.27–3.09) log copies/ml (Fig. 1).

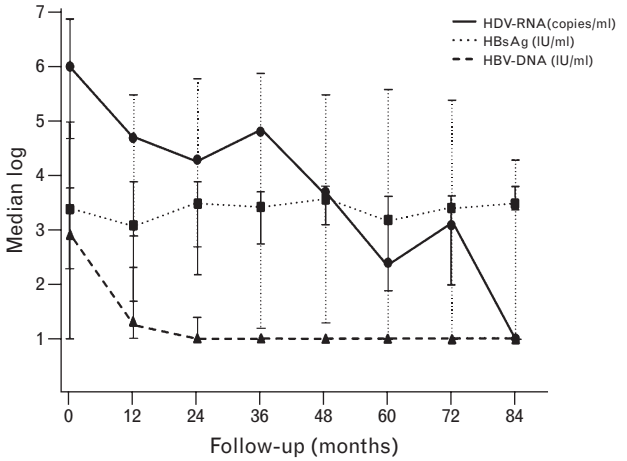

Fig. 1. Evolution of main virological markers over time.

Although there was an initial decline in serum HBsAg following initiation of tenofovir therapy, it was not sustained over time (Fig. 1). Overall, there were no significant changes in serum HBsAg concentrations during the whole study period, although HBsAg seroclearance occurred in three patients. Median baseline HDV-RNA was lower in these three individuals than in the rest [2 (1–5.1) vs. 5.8 (4.3–6.7) log copies/ml]. Interestingly, these three patients became all negative for serum HDV-RNA.

During tenofovir therapy, there was an overall reduction in liver stiffness from a median of 21.9 to 13.8 KPa ($P = 0.34$). More than 30% reduction in liver stiffness during the study period occurred in six out of 10 (60%) patients who achieved undetectable HDV-RNA, whereas liver fibrosis did not change much in the remaining nine HDV viremic patients ($P = 0.03$) (Fig. 2). Regression of cirrhosis was recognized in five patients, all of whom had achieved undetectable HDV-RNA.

Median liver enzymes slightly declined during the study period [from 78 to 59 IU/ml for aspartate aminotransferase (AST), and from 84 to 68 IU/ml for alanine aminotransferase (ALT)], but the difference did not reach statistical significance. Moreover, all but four patients remained with elevated ALT.

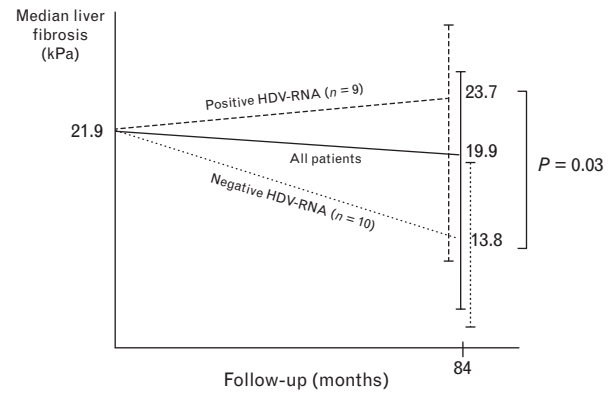

Fig. 2. Liver fibrosis progression according to serum HDV-RNA suppression.

Table 1 records the most important baseline characteristics of HIV/delta patients stratified by the achievement of undetectable serum HDV-RNA after long-term tenofovir exposure. Then, we investigated which variables could be associated with suppression of serum HDV-RNA in univariate and multivariate analysis (Table 2). Sex, age, $CD4^+$ cell counts, serum HDV-RNA, HBsAg concentrations, HBV genotype, presence of HCV antibodies, liver fibrosis staging and IL28B alleles were not significantly associated with the achievement of serum HDV-RNA suppression on tenofovir.

Discussion

Chronic hepatitis delta is a neglected disease [23]. Despite producing the most aggressive form of chronic viral hepatitis, exclusion of hepatitis delta is often forgotten in chronic hepatitis B carriers. The need for testing hepatitis delta markers in all individuals with chronic hepatitis B should be particularly reminded in HIV-HBV coinfected patients, in whom liver fibrosis progression may accelerated [5,24]. Nearly 10% of HIV-infected patients in Europe are serum HBsAg positive [25], of whom 15% are superinfected with HDV [26]. Given that tenofovir is one of the most active antivirals against HBV and is widely used as part of most antiretroviral regimens, we hypothesized that prolonged tenofovir exposure might indirectly be of benefit on hepatitis delta throughout its inhibitory effect on HBV replication. Ultimately, HBsAg is a necessary element for the HDV envelope.

Previous studies that checked the clinical effect of nucleos(t)ide analogues (i.e. lamivudine or adefovir) on hepatitis delta failed to demonstrate any significant benefit [27,28]. Tenofovir has been assessed testing small series of individuals experiencing either acute episodes of hepatitis delta [29], or in chronic hepatitis D patients treated for relatively short periods and/or in combination with interferon [30–32], always in the absence of histological assessment. More recently, the HIDIT-2 trial tested the potential benefit of adding tenofovir to peginterferon for 96 weeks and found only a slight benefit on HDV-RNA reduction in the combination group over placebo that did not reach statistical significance [33]. In our study, we report outcomes for viral markers and histological estimates in a relatively large series of chronic hepatitis delta patients treated with tenofovir and followed for a median of nearly 5 years. Complete HBV suppression was achieved in all patients along with significant reductions in HDV replication (with clearance of HDV-RNA in more than a half) along with improvements in liver

Table 1. Baseline characteristics of patients according to the achievement of HDV-RNA suppression on tenofovir.

Variable	Negative HDV-RNA $n=10$	Positive HDV-RNA $n=9$	P
Male sex, *n* (%)	10 (100)	8 (89)	0.28
Median age, years (IQR)	47 (46–53)	49 (44–49)	0.66
Median $CD4^+$ cell count (cells/μl, IQR)	284 (95–523)	294 (142–501)	0.72
Median HDV-RNA (log IU/ml, IQR)	5.85 (2.54–6.49)	6.17 (5.39–7.21)	0.32
Serum HBV-DNA <10 IU/ml, *n* (%)	4 (40)	4 (44)	0.67
Median HBsAg, log IU/ml (IQR)	2.90 (−0.06 to 3.89)	3.57 (2.71–3.68)	0.77
HBV genotype, *n* (%)			
A	2 (22)	3 (43)	0.37
D	7 (78)	3 (43)	0.15
Reactive HCV antibodies, *n* (%)	8 (80)	8 (89)	0.59
Advanced liver fibrosis (>9.5 kPa), *n* (%)	5 (50)	6 (67)	0.46
IL28B CC alleles, *n* (%)	4 (57)	4 (67)	0.72

HBV, hepatitis B virus; HDV, hepatitis delta virus; IQR, interquartile range.

fibrosis (with regression of cirrhosis in five out of 10). In our knowledge, this is the first study that demonstrates unquestionably a clinical benefit of oral antivirals on chronic hepatitis delta. On the basis of these data, we propose that tenofovir should be considered as a therapeutic option for HDV infection, being aware that its benefit would mainly be recognized in the long term and perhaps not in all patients.

Our results suggest that the benefit of tenofovir on delta hepatitis is most likely the result of an indirect effect that follows the potent antiviral activity of the drug on HBV. Reduction and/or suppression of HDV-RNA occurred over months and followed complete HBV-DNA suppression in all patients. However, concentrations of serum HBsAg did not follow the same trend and remained relatively stable during the whole length of the study. Similar unaffected kinetics for HBsAg in patients treated with tenofovir have been reported by others [34–39], which argues against a benefit of tenofovir on delta hepatitis throughout reducing the production of the HBV envelope protein. However, we did not check whether a dysbalance in the release of distinct HBsAg fractions under prolonged tenofovir therapy had occurred in our patients. Hypothetically, a reduction in the production of the large HBsAg isoform on long-term tenofovir treatment could impair HDV infectivity of new hepatocytes and in this way ultimately result in HDV-RNA suppression [40].

It is noteworthy that the improvement in liver fibrosis was significantly linked to the achievement of complete suppression of serum HDV-RNA. Although almost all our patients with delta hepatitis experienced declines in serum HDV-RNA on long-term tenofovir, liver fibrosis did not improve in those who did not completely suppress HDV-RNA, suggesting that hepatic damage in delta patients is mainly driven by HDV replication rather than by HBV infection.

We could not identify any predictor of the achievement of HDV-RNA suppression in our series. Whereas HBV-DNA became negative soon after introducing tenofovir in all patients, the median time to reach negative HDV-RNA in the 10 individuals who cleared HDV was 54 months. This observation suggests that any benefit of tenofovir on delta hepatitis would be manifest only after extended periods.

We should acknowledge several limitations of our study. The first is the lack of a control group of HIV-infected individuals with delta hepatitis followed for long periods

Table 2. Predictors of serum HDV-RNA suppression on long-term tenofovir therapy.

	Univariate analysis OR (95% CI) P	Multivariate analysis OR (95% CI) P
Age (per year)	1.08 (0.88–1.34) 0.45	–
Baseline HBV-DNA <10 IU/ml	0.67 (0.11–4.35) 0.67	–
HBsAg concentration (per log IU/ml)	**0.61 (0.20–1.84) 0.35**	–
Baseline $CD4^+$ cell count (per cells/μl)	1.01 (0.99–1.02) 0.63	–
Advanced liver fibrosis (>9.5 kPa)	0.62 (0.93–4.22) 0.63	–
Baseline HDV-RNA (per log IU/ml)	**0.63 (0.32–1.25) 0.16**	0.44 (0.08–2.56) 0.36
Reactive HCV antibodies	0.50 (0.04–6.68) 0.52	–
HBV genotype (A vs. D)	**0.38 (0.04–3.34) 0.38**	–
IL28B alleles (CC vs. CT/TT)	0.67 (0.07–6.41) 0.72	
Baseline ALT (per IU/l)	0.99 (0.97–1.02) 0.48	–

The variables in bold entered the multivariate analysis. ALT, alanine aminotransferase; CI, confidence interval; HBV, hepatitis B virus; HDV, hepatitis delta virus; OR, odds ratio.

not being treated with tenofovir. This population should have provided reliable information about liver fibrosis progression and changes in viremia for both HBV and HDV. However, most antiretroviral guidelines recommend tenofovir as part of any antiretroviral regimen in HIV-HBV coinfected individuals [41–43], which precluded us to find this subset of patients in our cohort. A second limitation of our study is that we used the thresholds in liver stiffness applied in chronic hepatitis C for distinguishing hepatic fibrosis stages, given the lack of studies validating paired liver biopsies and hepatic stiffness in patients with delta hepatitis. However, fibrosis deposition seems to be a phenomenon that follows chronic inflammation of the liver caused by any cause, and therefore, we are confident that our estimates of liver fibrosis severity would be reliable, mainly as a uniform trend was seen in almost all patients over time.

A third limitation was that all but one of our 19 patients received tenofovir along with emtricitabine or lamivudine, which are also active against HBV. Therefore, our results might have been influenced hypothetically by this dual antiviral pressure. It should be noted, however, that nine patients had been on lamivudine before beginning tenofovir despite which all of them were viremic for HDV. Moreover, other authors have already shown that lamivudine does not exhibit any recognizable antiviral effect on HDV [27]. Accordingly, we are confident that our results are mainly if not uniquely attributable to long-term tenofovir exposure. A fourth limitation of our study derives from its retrospective design. Patients with hepatitis delta who died for any reason or were lost to follow-up before the censuring date were not analysed. In this regard, we cannot exclude an overestimation of the benefit attributed to tenofovir examining the surviving population cohort. Finally, it should be noted that our results with tenofovir were recorded in a population infected with HIV and that outcomes in hepatitis delta monoinfected individuals might be different. However, $CD4^+$ cell counts were relatively high and HIV replication was suppressed in almost all our patients, which makes reasonable to accept that our results should confidently be applicable to both populations.

In summary, thanks to the unique opportunity represented by a population of HIV patients with delta hepatitis, we investigated the long-term effect of tenofovir, which exhibits dual antiviral effect against both HIV and HBV, and was marketed as an antiretroviral agent 10 years ago. Serum HDV-RNA steadily declined in all patients receiving tenofovir and became undetectable in more than a half of them by 5 years. This virological outcome resulted in significant improvements in hepatic fibrosis as measured by transient elastography, with regression of cirrhosis in five out of nine patients. In our knowledge, this study is the first to provide robust evidence in favour of a clinical benefit of oral antivirals on chronic hepatitis delta. In the absence of current, more well tolerated medications, we propose that tenofovir should be considered as a therapeutic option for HDV infection, acknowledging its benefit would mainly be recognized after prolonged exposure and perhaps not in all patients.

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R.S.E., E.V. and V.S. designed the study. J.V.F.-M., V.S., E.V., P.L. and P.B. were in charge of clinical data collection. E.V. and R.S.-E. filled the database and did the statistical analysis; R.S.-E. and Cd.M. did the laboratory analyses. All contributed to manuscript preparation.

Conflicts of interest

There are no conflicts of interest.

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