Male reproduction and HIV-1 infection

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Semen quality and drug concentrations in seminal plasma of patients using a didanosine or didanosine plus tenofovir containing antiretroviral regimen

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Abstract

Data on the concentrations of didanosine (ddI) and tenofovir (TFV) in seminal plasma are sparse. Subtherapeutic drug concentrations within the lumen of the male genital tract may have implications for selection and transmission of drug-resistant HIV strains. On the other hand, sufficient penetration of these drugs into the male genital tract has potential toxic effects on the spermatozoa and their precursors. In the current study, the authors obtained paired semen and blood samples at variable time points after drug intake from 30 HIV-1-infected patients using a ddI (n = 15) or ddI + TFV (n = 15) containing antiretroviral regimen. Didanosine and TFV concentrations were measured in seminal and blood plasma and semen quality was assessed. Both ddI and TFV penetrated well into seminal plasma. Whereas blood plasma ddI concentrations dropped to near or below the lower limit of quantification of 0.017 μg/mL 9 hours after drug intake, the ddI concentration in seminal plasma remained detectable during the whole dosing interval with a median of 0.20 and 0.21 μg/mL in the ddI and ddI + TFV groups, respectively. Tenofovir was detectable during the whole dosing interval in both blood and seminal plasma with a median concentration of 0.12 and 0.25 μg/mL, respectively, and a median seminal-to-blood-plasma ratio of 3.3. Semen quality was within the normal range according to the criteria of the World Health Organization, except for the percentage of progressively motile sperm, which was low in both groups of patients. The authors conclude that ddI and TFV penetrate well into seminal plasma and that the reduced sperm motility deserves further study.

Introduction

Not all antiretroviral drugs penetrate sufficiently into the lumen of the male genital tract. Subtherapeutic drug concentrations may allow local selection of drug-resistant HIV [1,2]. On the other hand, sufficient penetration into seminal plasma exposes the spermatozoa and their precursors to potentially toxic effects of antiretroviral drugs. In this context, it is of importance to note that several antiretroviral drugs, including didanosine (ddI), are associated with mitochondrial toxicity [3]. Because mitochondria are abundant in spermatozoa, and necessary for their progressive motility, these drugs may thus affect sperm motility.

Available data concerning the concentration of ddI and tenofovir (TFV) in seminal plasma are sparse. There is only one study that suggests that ddI accumulates in seminal plasma [4]. Similarly, TFV concentrations have only been studied in four patients using tenofovir disoproxil fumarate (TDF); this study suggested accumulation of TFV in seminal plasma [5]. Didanosine and TDF have a pharmacokinetic interaction that makes it necessary to reduce the dose of ddI when it is used in combination with TDF; in patients with a bodyweight of 60 kg or more, the recommended dose of ddI is 250 mg instead of 400 mg once daily [6,7]. No studies exist on the effect on seminal plasma drug concentrations when these two drugs are used simultaneously.

The purposes of the current cross-sectional study were to assess the penetration of ddI and TFV into seminal plasma in patients using a ddI or ddI plus TDF-containing antiretroviral regimen and to evaluate semen quality in these patients.

Materials and Methods

Patients were eligible for this study if they used ddI (enteric-coated) or ddI (enteric-coated) plus TDF as part of their current potent combination antiretroviral regimen (also called highly active antiretroviral therapy) for at least 6 weeks and were adherent to their therapy. Exclusion criteria were vasectomy, a genitourinary tract infection (assessed by urinalysis and *Chlamydia trachomatis* ligase chain reaction assay in urine within 8 weeks before semen collection, and a negative medical history on the day of semen collection), an intercurrent medical condition, and renal insufficiency. The study was approved by the Institutional Review Board and all patients gave written informed consent.

Semen was produced by masturbation after at least 2 days of sexual abstinence. The ejaculate was collected in a sterile container and analyzed within 1 hour. All semen analyses were performed according to the World Health Organization guidelines for routine semen analysis [8]. After liquefaction at 37°C, semen volume and pH were measured. Subsequently, concentration and motility of spermatozoa were assessed using a counting chamber (Léjà products B.V., Nieuw Vennep, The Netherlands) and the percentage of spermatozoa with a normal morphology was determined by counting 100 Quick diff-stained spermatozoa.

The whole semen sample was then centrifuged at 1200 *g* for 10 minutes and the supernatant, consisting of seminal plasma, was stored at −20°C until analysis of drug concentrations.

Within 2 hours before or after semen collection, a venous blood sample was collected in heparinized tubes for measurement of the blood plasma ddI and TFV concentrations. Heparinized blood was centrifuged at 1200 *g* for 10 minutes and the plasma was stored at −20°C.

Time of last intake of the drugs, production of the semen sample, and blood collection were recorded. As time point of the seminal-to-blood-plasma-drug ratio, the mean of the interval between drug intake and semen or blood collection was taken.

Concentrations of ddI in blood plasma were measured using a previously described validated high-performance liquid chromatography method with ultraviolet light detection [9]. The lower limit of quantification (LLOQ) was 0.017 μg/mL. Concentrations of ddI in seminal plasma were determined using the same method with an adapted sample preparation, i.e., solid phase extraction was used as described for urine [10]. The seminal plasma LLOQ with this method was also 0.017 μg/mL and the calibration curve was linear over a range of 0.017 to 5.19 μg/mL. Recovery after extraction from seminal plasma was 108% and accuracy ranged from 101% to 109% whereas intraday and interday precision were 4.3% and 10.1%, respectively (data not shown).

Tenofovir blood and seminal plasma concentrations were measured by a previously described high-performance liquid chromatography method with fluorimetric detection [11]. The LLOQ for tenofovir was 0.015 μg/mL for blood plasma and 0.048 μg/mL for seminal plasma. Both ddI and TFV assays were externally validated by the Quality Assurance Program for Clinical Measurement of Antiretrovirals of the AIDS Clinical Trials Group [12].

Descriptive statistics were performed using SPSS statistical programs version 11.5.1. (SPSS UK, Woking, Surrey, UK).

Results

From January until November 2004, 30 patients were included, 15 using ddI and 15 using ddI plus TDF-containing highly active antiretroviral therapy. Baseline characteristics of the patients are presented in Table 1. All patients weighed more than 60 kg and their daily dose of ddI was 400 mg (ddI-only group) and 250 mg (ddI + TDF group) as recommended. Of the patients using ddI only, 14 had a blood plasma HIV-1 RNA concentration (pVL) of less than 50 copies/mL [Versant HIV-1-RNA (branched-DNA) assay; Bayer Corp., Tarrytown, NY] in the 8 weeks before semen collection. One patient had a virologic blip of 78 copies/mL 6 weeks before semen collection. In the patients using ddI plus TDF, two had a detectable pVL around the time of semen collection (1042 and 472 copies/mL, respectively).

Data on drug intake and drug concentrations are shown in Figures 1 through 3. In most patients in both groups, the ddI seminal plasma concentrations were higher than the blood plasma concentrations (median concentration 0.20 µg/mL versus 0.09 µg/mL in the ddI-only group and 0.21 µg/mL versus 0.02 µg/mL in the ddI plus TDF group) (Fig. 1). In both groups, the ddI blood plasma concentrations peaked at approximately 2 to 7 hours after drug intake, whereas after 14 to 16 hours, the ddI blood plasma concentrations were all below the LLOQ.

Table 1. Characteristics of patients using didanosine (ddI) or ddI plus tenofovir DF-(TDF) containing antiretroviral regimen

	All patients	ddI	ddI + TDF
No.	30	15	15
Age (years)	43 (38-52)	43 (39-53)	42 (37-48)
Body weight (kg)	77 (70-86)	73 (66-84)	78 (75-87)
Present CD4 count (cells/µL)	515 (398-663)	520 (410-620)	510 (360-670)
Number with pVL less than 50 RNA copies/mL	26	14	12
Time on current HAART (months)	18 (11-30)	28 (17-37)	12 (7-18)
Time on HAART (months)	64 (37-95)	53 (34-83)	90 (42-96)
Time on ART (months)	76 (37-122)	61 (34-113)	104 (42-122)

Data are expressed as median and interquartile range where applicable.
HAART, highly active antiretroviral therapy. ART, Antiretroviral therapy (includes non-HAART and HAART regimens).

For seminal plasma, in both groups, the ddI concentration peaked at approximately 12 to 13 hours after drug intake, whereas after 15 to 18 hours, the ddI concentrations were all in the lower range (Fig. 1).

In the patients from the ddI-only group and the ddI plus TDF group in whom a seminal-to-blood-plasma-ddI ratio could be calculated, this ratio clearly increased during the course of
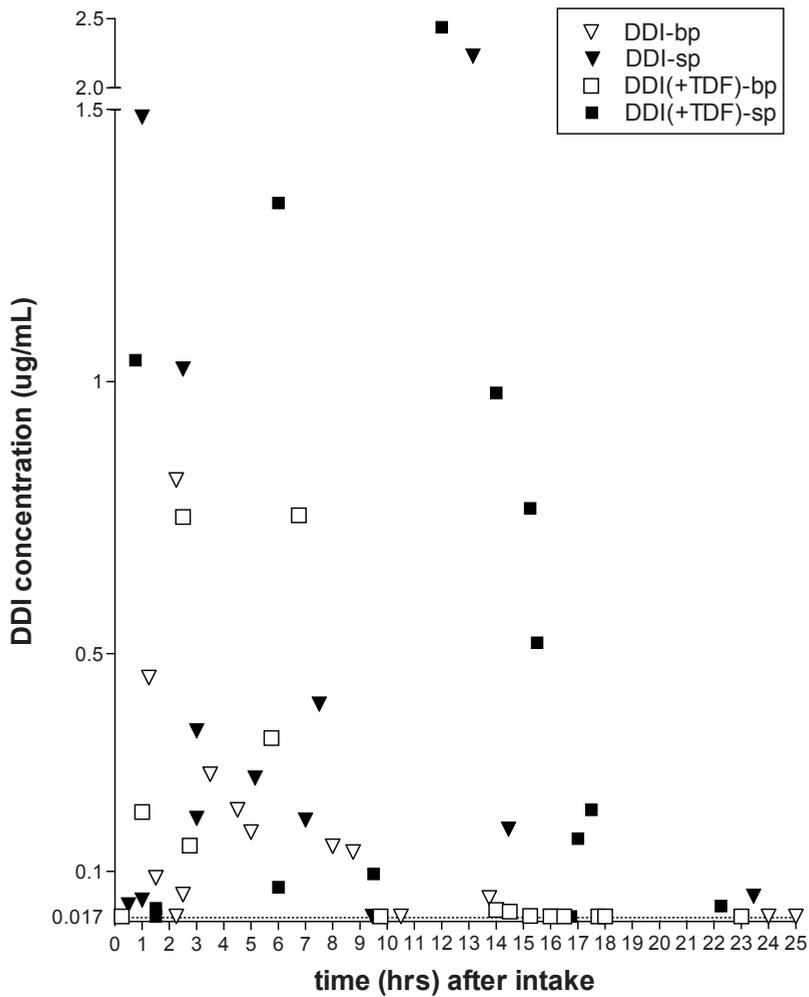

Figure 1. Didanosine (ddI) concentrations in blood (bp) and seminal plasma (sp) with or without tenofovir-DF (TDF) coadministration. Dotted line indicates lower limit of quantification of didanosine in blood and seminal plasma.

the dosing interval. Because of plasma concentrations less than LLOQ, from 16 hours onward, a seminal-to-blood-plasma-ddI ratio could not be calculated (Fig. 3).

Tenofovir was detectable in all blood and seminal plasma samples of the patients using ddI plus TDF with a median concentration of 0.12 µg/mL and 0.25 µg/mL, respectively. There was
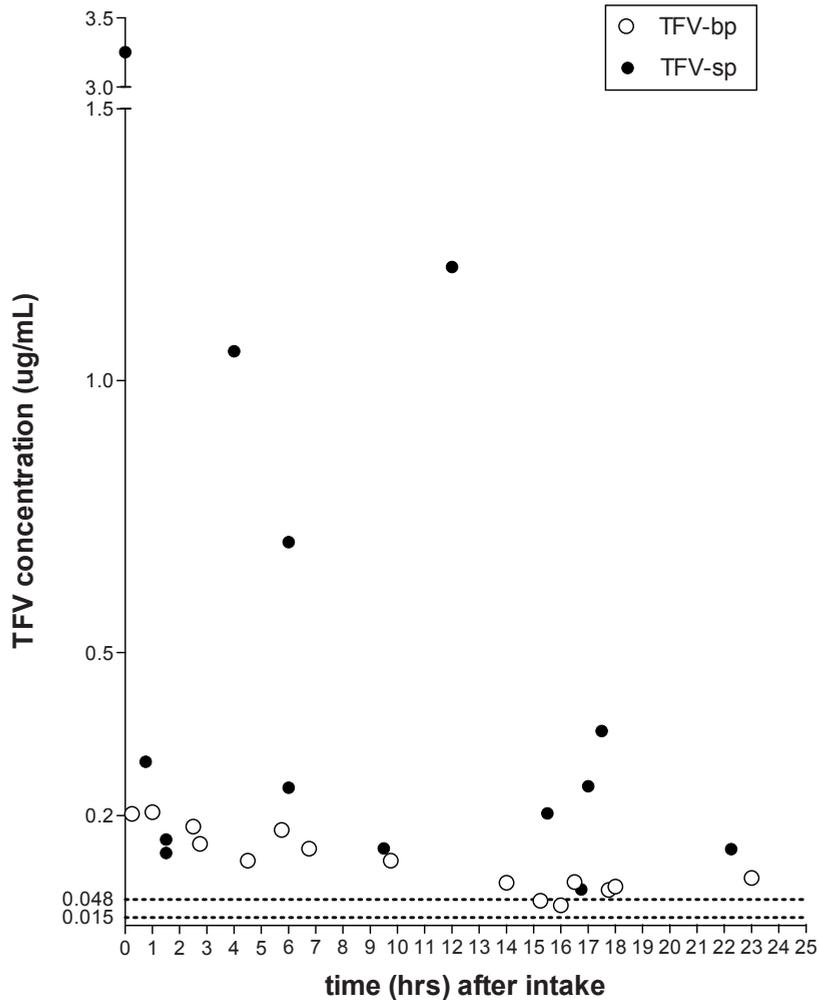

Figure 2. Tenovofir (TFV) concentrations in blood (bp) and seminal plasma (sp). Dotted lines indicate lower limit of quantification of tenofovir in blood plasma (0.015) and seminal plasma (0.048).
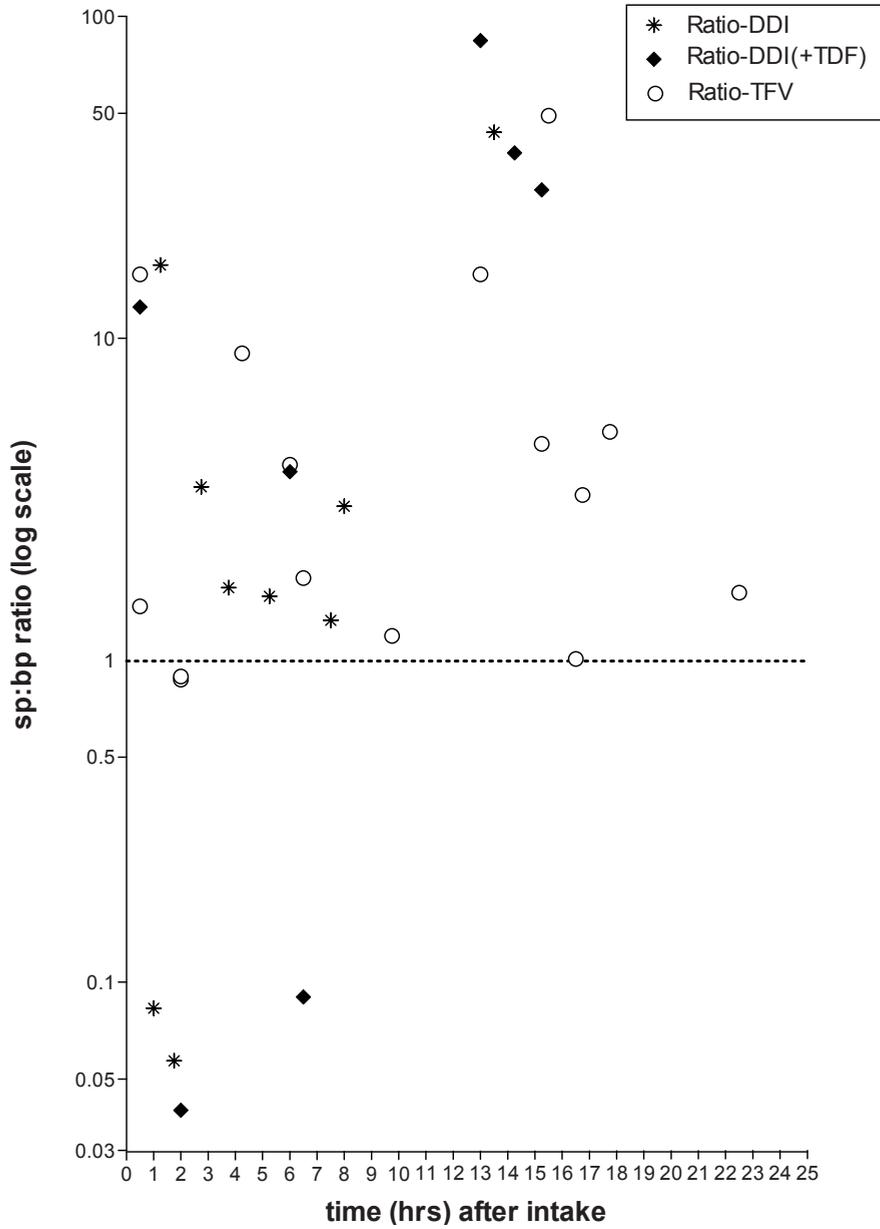
Figure 3. Seminal plasma (sp) to blood plasma (bp) ratio of didanosine (ddI) and tenofovir (TFV). Dotted line indicates ratio = 1.

a clear relationship between the interval after TDF intake and the TFV concentration in blood plasma (correlation coefficient -0.8, $P = 0.01$) (Fig. 2).

There was no clear relationship between the interval after TDF intake and the TFV concentration in seminal plasma (Fig. 2). In most patients, concentrations of TFV in seminal plasma were higher than in blood plasma with a median seminal/blood plasma ratio of 3.3 (range, 0.9–49.2) (Fig. 3).

Data on semen quality are given in Table 2. The semen volume was on the lower side of normal values and the percentage of progressively motile sperm was below World Health Organization criteria in both groups [8].

Discussion

This study is the largest on ddI and tenofovir concentrations in seminal plasma and, with respect to the combination of ddI and tenofovir, the first published study. In this study, we found that both ddI and TFV penetrate well into seminal plasma. In contrast to blood plasma ddI concentrations, the ddI concentration in seminal plasma remained detectable during a larger part of the dosing interval and compared with blood plasma, the concentration peak in seminal plasma was approximately 8 hours later. During the last part of the dosing

Table 2. Semen quality parameters

	ddI	ddI + TDF	normal values*
No.	15	15	
Abstinence (days)	2 (2.0–3.0)	2 (2.0–4.0)	
Volume (mL)	2.5 (1.40–3.00)	2.2 (1.70–4.00)	>2
PH	7.5 (7.2–7.5)	7.5 (7.5–7.5)	>7.2
Spermatozoa concentration (x 10^6/mL)	50 (40.0–73.0)	65 (50.0–108.0)	>20
Spermatozoa motility (%)			
Progressive (grade a) (%)	22 (15.0–46.0)	26 (16.0–47.0)	>50
Slow (grade b + c) (%)	15 (11.0–25.0)	9 (7.0–15.0)	
Immotile (grade d) (%)	53 (42.0–72.0)	59 (45.0–70.0)	
Spermatozoa with normal morphology (%)	34 (26.0–41.0)	41 (35.0–64.5)	>30

Data are expressed as median and interquartile ranges.
*Reference 8.
ddI, didanosine; TDF, tenofovir disoproxil fumarate.

interval also, the ddI concentrations in seminal plasma drop to low levels. So, with some delay, the ddI concentrations in seminal plasma parallel those of blood plasma, explaining the increasing seminal-to-blood-plasma-ddI ratios during the course of the dosing interval (Figs. 1 and 3). For indinavir not boosted with ritonavir and stavudine, both also antiretroviral drugs with a short blood plasma half-life (approximately 1.5 hours), the same delay between the blood and seminal plasma concentrations has been found [13-15]. Our data also suggest that concurrent use of tenofovir-DF, with a dose reduction of ddI as recommended, has no apparent effect on the blood and seminal plasma ddI concentrations.

Our results are in line with previous small studies on ddI and TFV in seminal plasma [4,5]. Furthermore, our results confirm previous findings that antiretroviral drugs with a protein binding of less than 90% penetrate well into the seminal plasma; ddI and TFV both have a protein binding of less than 10% [2].

The potential downside of good penetration is that spermatozoa and their precursors are exposed to more of the antiretroviral drugs. Most studies suggest that semen parameters are normal during asymptomatic HIV-1 infection [16-18]. However, a direct effect of HIV-infection on semen volume and sperm motility has been noted before [19]. Our study suggests that there was a tendency toward a lower semen volume and a decreased percentage of progressively motile sperm in antiviral therapy exposed HIV-1-infected patients. The patients were, on average, already 6 years on antiretroviral treatment, so the influence of previous regimens and that of other drugs in the current regimen, or the HIV infection itself, cannot be excluded.

Data describing the effects of antiretroviral therapy on semen quality are limited. Only one longitudinal study has been performed on semen parameters before and during highly active antiretroviral therapy, but the 4 to 12 weeks follow-up of this study may have been too short to evaluate possible adverse effects on semen quality [20].

Thus, larger and more extended prospective longitudinal studies are needed to elucidate any potential detrimental effects of antiretroviral combination therapy on semen quality.

Acknowledgements

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