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Penetration of didanosine in semen of HIV-1-infected men

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***Objectives*: The disposition of antiretroviral agents into genital tissue and fluids is one of the factors implicated in the control of viral replication within the male genital tract and should be an objective of highly active antiretroviral therapy. We have investigated didanosine penetration in seminal plasma of 16 HIV-infected patients.**

***Patients and methods*: A total of 16 patients on didanosine (200 mg every 12 h or 400 mg once daily) participated in the pharmacokinetic study. After the didanosine morning dose, peripheral blood plasma and semen plasma were collected within the intervals 0–4, 4–8 and 8–12 h in the twice-daily regimen and 0–4, 4–12 and 12–24 h in the once-daily regimen.**

***Results*: Within each sampling time interval didanosine concentrations in seminal plasma were higher than in blood. The interquartile range of concentrations in seminal plasma was 292–1217 ng/mL, compared with 50–150 ng/mL in blood plasma. Didanosine could be detected in 14 of the 16 semen samples analysed and in 8 of the 16 blood samples.**

***Conclusions*: We have demonstrated that didanosine penetrates into the seminal plasma in higher concentrations than in blood plasma.**

Keywords: antiretroviral therapy, pharmacokinetics, drug distribution, sanctuary site

Introduction

Sexual transmission is the principal mode of spread of human immunodeficiency virus type 1 (HIV-1) throughout the world. Since the sexual transmission of HIV-1 is influenced by the viral load in semen, control of viral replication within the male genital tract should be one of the objectives of highly active antiretroviral therapy (HAART).[1,2] Despite the availability of HAART, HIV-1 may persist in both cellular (e.g. resting CD4+ T lymphocytes) and anatomical reservoirs, which include the male genital tract.[3]

The penetration of antiretroviral agents into the male genital tract is likely to be an important factor that may affect HIV-1 viral replication in semen and the development of resistant strains.[2,4,5] Poor drug penetration in the male genital tract may lead to suboptimal viral suppression and predisposition to the emergence of drug resistance-associated viral mutations. Studies regarding the penetration of antiretroviral agents in the male genital tract are therefore timely and relevant. The penetration of antiretroviral agents in semen of HIV-1-infected patients has been reviewed by Taylor *et al.*[6] Data on the semen concentration are available for zidovudine, lamivudine, stavudine, abacavir and tenofovir among nucleoside/nucleotide analogues (NRTIs); for nevirapine and efavirenz among non-nucleoside reverse transcriptase inhibitors (NNRTIs); for indinavir, ritonavir, saquinavir, nelfinavir, amprenavir and lopinavir among protease inhibitors (PIs); and for the fusion inhibitor enfuvirtide.[6–9] Despite the large clinical experience with didanosine as a component of antiretroviral therapy, the concentration of this drug in semen has only been reported in two patients.[10] Here we present the data on the concentration of didanosine in blood and semen of 16 HIV-1-infected men.

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Table 1. Baseline characteristics of 16 patients enrolled in the pharmacokinetic study

Median age (range), years	38 (30–57)
Median weight (range), kg	73 (50–89)
CD4 count, no. cells/mm^3, median (range)	379 (158–1107)
Viral load, log copies/mL, median (range)	3.1 (1.7–5.1)
HAART regimens, no. patients	
All didanosine +	
stavudine + nelfinavir	3
stavudine + indinavir	2
stavudine + efavirenz	1
abacavir + efavirenz	3
abacavir + nelfinavir	2
abacavir + indinavir	1
abacavir + indinavir/ritonavir	1
abacavir + stavudine	1
zidovudine + indinavir	1
zidovudine + epivir	1

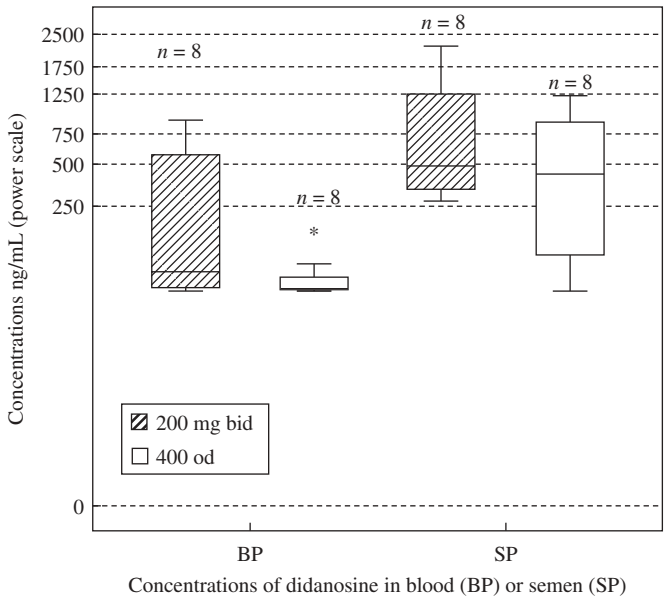

Figure 1. Box plots showing didanosine [200 mg twice daily (bid) or 400 mg once daily (od)] concentrations in blood and seminal plasma. The lower limit of each box represents the 25th percentile, the upper limit of each box represents the 75th percentile and the horizontal line within each box represents the median value. Error bars on vertical lines indicate the 5th and 95th percentiles. Outlying values are represented by *.

Patients and methods

Adult HIV-1-infected patients from the participating centres were enrolled in a prospective protocol aimed at evaluating the concentrations of didanosine in seminal plasma. Demographics and main baseline characteristics of enrolled patients are shown in Table 1. All the patients provided written informed consent, and the protocol was approved by the Institutional Review Board of the Infectious Diseases Department, San Martino Hospital, Genoa. Subjects included were required to be on stable antiretroviral treatment with didanosine as a component of the HAART regimen for at least 4 weeks. Didanosine was administered at a dosage of 200 mg every 12 h in eight patients and 400 mg once daily in the remaining eight patients. Peripheral blood and semen (after 3 days of sexual abstinence) were collected after a morning dose of didanosine within the following intervals: 0–12 h in the twice-daily regimen and 0–24 h in the once-daily regimen. Patients collected the seminal fluid at their homes and were advised to carefully record the time of didanosine intake and seminal fluid production. The samples of seminal fluid were kept refrigerated (+4°C) and taken to the hospital centre within 1 h from production, where matched blood samples for the determination of didanosine concentrations had to be collected. After 1:1 dilution with PBS, semen was centrifuged at 800 **g** for 10 min; the supernatant was then re-centrifuged at 1000 **g** for 15 min. Blood plasma and seminal plasma were stored at –20°C until assayed. Didanosine concentration in seminal plasma and blood plasma was determined using a validated HPLC assay, with a lower limit of sensitivity of 50 ng/mL. A descriptive statistical analysis was performed. Distributions of drug concentration in each compartment are summarized with interquartile ranges and box plots. Samples that fell below the lower limits of sensitivity were assigned a value equal to the limit of sensitivity.[7]

Results and discussion

Didanosine levels in blood and seminal plasma are shown in Figure 1 and Table 2. Semen samples were obtained from all the enrolled patients. Didanosine could be detected in 14 of the 16 semen samples analysed and in 8 of the 16 blood samples. Two patients in the once-daily regimen had semen concentrations below the limit of sensitivity 9.5 and 14.0 h after a single administration of a 400 mg didanosine dose. Didanosine blood concentrations were below the limits of sensitivity in 6 out of 8 patients after the once-daily regimen and in 2 out of 8 patients in the twice-daily regimen. Median didanosine concentrations were 455 ng/mL (range <50–2190 ng/mL) in seminal plasma and <50 (range <50–860 ng/mL) in blood. Large interindividual variability was noted (Table 2).

Didanosine, which was approved for the treatment of HIV infection in 1991, remains an attractive alternative to zidovudine as a part of dual nucleoside 'backbone' for initial combination therapy.[11] Data from the present study confirm the large interindividual variability associated with didanosine pharmacokinetics. It has been suggested that variations in bioavailability mostly resulting from changes in drug absorption may be a major source of the observed variability. Within each sampling time interval we have found that didanosine penetrates into the seminal plasma with concentrations which remain sustained for a longer period than the respective blood plasma concentrations.

The present study, as well as other studies on this subject, has some limitations. The timing of study-drug administration and semen collection was self-reported. Moreover, semen and plasma were not collected simultaneously, though in the large majority of patients the interval between specimen collections was ≤2 h. Therefore we decided to refrain from calculating the plasma/semen penetration ratio, which can vary with time and give a static observation of the dynamic process of drug accumulation and elimination.[5] Nonetheless, with the exception of two patients who had both semen and plasma concentrations below the limit of detection, didanosine concentrations within seminal plasma reached higher concentrations than in blood. In fact, the interquartile range of concentrations in semen was 292–1217 ng/mL, compared with 50–150 ng/mL in blood.

Table 2. Didanosine concentrations in blood plasma and seminal plasma at different time intervals after administration

Didanosine regimen	Time interval (h)	Blood plasma concentrations (ng/mL)	No. of observations	Seminal plasma concentrations (ng/mL)	No. of observations
200 mg twice daily	0–4	50–860	5	<50–2190	4
	4–8	<50–70	2	480–1250	2
	8–12	<50	1	350–1250	2
400 mg once daily	0–4	<50	1	200	1
	4–12	<50–90	2	430–1240	2
	12–24	<50–170	5	<50–1150	5

Drug penetration into seminal plasma is drug specific and depends on a variety of drug-specific physiochemical determinants, including partition coefficient, dissociation constant and protein binding. The low plasma-protein binding (<5%) and the drug pKa (=9) can play a part in the accumulation of didanosine in semen.

In a study aimed at developing a sensitive method for the simultaneous determination of didanosine and stavudine in plasma and other human matrices, Huang *et al.*[10] evaluated the concentration of didanosine in seminal plasma and in blood of two HIV-infected patients. Didanosine concentrations were higher in seminal plasma than in blood (205 and 884 ng/mL compared with 54 and 290 ng/mL, respectively). Our report confirms the favourable penetration of didanosine in the male genital tract. Data on the penetration of other NRTIs are available.[6,8,9,12,13] Zidovudine, lamivudine, abacavir and tenofovir demonstrated a good penetration in the male genital tract, with semen concentrations higher than blood concentrations. Both of the NNRTIs nevirapine and efavirenz have been found to achieve therapeutic concentrations in semen, though the semen/plasma concentration ratio was <1.[9] Concentrations in the semen were ~60% of those in the blood plasma for nevirapine and 10% for efavirenz. Data on PI penetration into the male genital tract are somewhat sparse. Results from several reports have shown that lopinavir, ritonavir, nelfinavir and saquinavir did not reach effective semen concentrations.[6,7] In contrast, indinavir semen concentrations were high and similar to concurrent plasma concentrations; the addition of ritonavir increased indinavir semen concentrations significantly.[14] Amprenavir semen concentrations as determined in the semen of 31 men were consistently lower than the blood plasma concentrations; drug concentrations in semen were above the protein-corrected IC_{95} in blood plasma in some but not all patients.[13]

The disposition of antiretroviral agents into genital tissue and fluids is one of the factors implicated in the control of viral replication within the male genital tract and should be an objective of HAART. We have demonstrated that didanosine penetrates into the seminal plasma in higher concentrations than in blood plasma. The impact of these findings in terms of virus suppression and evolution of resistance requires further study.

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Transparency declarations

None to declare.

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