Etravirine concentrations in seminal plasma in HIV-infected patients


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Objectives: To determine etravirine concentrations and the HIV-1 viral load (VL) in blood plasma (BP) and seminal plasma (SP) of HIV-infected patients.

Methods: Ten adult antiretroviral-experienced HIV-1 patients receiving an etravirine-containing regimen for at least 1 month were enrolled. Semen and blood samples were collected ≏12 or 24 h after the last etravirine dose, depending on twice-daily or once-daily dosing, respectively. Liquid chromatography tandem mass spectrometry was used to determine etravirine concentrations and HIV-1 VL was determined by real-time PCR (detection limit 40 copies/mL). Results are presented as the median (range) unless otherwise indicated.

Results: Ten blood and 20 semen samples were collected. The CD4 count was 502 (252–817) cells/mm³ and the BP VL was 40 (40–362) copies/mL. The time on etravirine was 52 (12–124) weeks. The BP etravirine concentration was 452.5 (258–751) ng/mL. The SP etravirine concentration was 62.9 (31.2–166.0) ng/mL and values were above the IC50 range (0.39–2.4 ng/mL) in all cases. The median etravirine SP:BP ratio was 0.16 (0.07–0.26). The SP VL was 40 copies/mL in all patients, whereas the BP VL was detectable in one patient with poor adherence to treatment.

Conclusions: Etravirine concentrations in male genital secretions are modest, reaching only 16% of the BP concentration. Nevertheless, they are more than 10 times greater than the wild-type IC50 range (not adjusted for protein binding).

Keywords: antiretrovirals, reservoirs, genital secretions

Introduction

Penetration of antiretroviral (ARV) drugs in anatomical compartments is important for maintaining long-term viral suppression. Good penetration of ARV drugs into the seminal plasma (SP), a suggested surrogate marker of viral activity in the male genital tract, may be associated with a decrease in viral replication and play an important role in the prevention of sexual HIV transmission. It may be postulated that the male genital tract can act as a separate compartment in which different viral resistance patterns may develop, mainly in patients receiving drugs with suboptimal penetration.

Etravirine is the first of a ‘second generation’ of non-nucleoside reverse transcriptase inhibitors and it is approved for the treatment of ARV-experienced patients. To date, there is little published information regarding etravirine concentrations in compartments. We recently reported results on etravirine penetration in CSF. To our knowledge, data on etravirine in the semen of HIV-infected patients have only been presented at an international conference, and there is a single, recent article on etravirine in the semen of non-HIV-infected men.

We present the data from a series of HIV-infected ARV-experienced patients receiving etravirine-containing regimens in whom etravirine concentrations and viral loads (VLS) were determined in blood plasma (BP) and SP.

Patients and methods

Ten asymptomatic adult, HIV-infected, ARV pre-treated patients with no clinical evidence of sexually transmitted disease were enrolled in our HIV Outpatient Unit. All had been taking etravirine as part of an ARV regimen for at least 1 month. In 50% of patients, etravirine dosage was set at twice daily according to the recommendations stated in the European Medicines Agency (EMA) scientific report (200 mg twice daily) and in the remaining patients the drug was taken in a once-a-day regimen (400 mg once daily). Adherence to ARV drugs was assessed using the simplified medication adherence questionnaire (SMAQ). The study was approved by the hospital Ethics Committee and the Spanish Drug Agency, and patients gave written informed consent to participate.

From each patient, one blood sample and two semen samples were collected, 1 day apart. On the first day, a blood sample was obtained by peripheral venous puncture and patients brought a first sample...
of semen, obtained by self-masturbation, to determine etravirine concentrations. On the next day, patients brought a second semen sample to determine HIV VL. All samples were processed within 2 h after acquisition and frozen at −70°C until analysis. In order to determine the lowest BP etravirine concentrations, blood and the first semen sample were taken on the same day, ~12 h after the last etravirine dose in patients taking a twice-daily regimen and 24 h after the etravirine dose in those taking the drug once a day. HIV-1 VL was analysed in the same blood sample and in the second semen sample. Therefore there was no more than a 1 h difference between collection of the plasma sample and the first semen sample for etravirine analysis and about a 24 h difference between the plasma sample and the second semen sample for VL determination.

The total etravirine concentration in BP and SP samples was determined by a validated liquid chromatography tandem mass spectrometry assay.7 Samples were analysed against a standard curve prepared from be set of plasma quality control (QC) samples and a set of semen QC samples. During the extraction process, the matrix was balanced to be 1:1 plasma:semen. The internal standard for the assay was a stable labelled compound (etravirine-D8) and all QC samples showed a percentage deviation from the expected values within acceptable limits. The internal standard showed consistent results throughout the run (Tandem Labs, West Trenton, NJ, USA). Results are presented as the median (range) unless otherwise indicated.

The HIV-1 VL was quantified with a real-time PCR technique (Abbot Molecular Inc., Des Plaines, IL, USA; limit of detection 40 copies/mL).

**Results**

The median (range) age of the patients was 45.5 (33–64) years. The BP HIV-1 VL was <40 (<40–362) copies/mL and the CD4 count was 502 (252–817) cells/mm³. Patients had received etravirine for 52 (12–124) weeks. The ARV regimen of each patient, along with individual pharmacokinetic and virological data, are presented in Table 1. Sixty percent (6/10) of participants were on two nucleos(t)ide analogues plus etravirine alone, while 40% received a protease inhibitor with or without nucleos(t)ide analogues, in addition to etravirine (three darunavir/ritonavir; one lopinavir/ritonavir).

Paired BP and SP etravirine concentrations were determined in all 10 patients. All BP etravirine concentrations were greater than the minimal therapeutic concentration required to inhibit 50% of a wild-type HIV-1 strain (IC_{50} range 0.39–2.4 ng/mL), the median (range) value was 452.5 (258–751) ng/mL. In all SP samples, etravirine concentrations exceeded several times the upper limit of the IC_{50} range (0.39–2.4 ng/mL)1 (Figure 1). The median etravirine SP concentration was 62.9 (31.2–166.0) ng/mL and the median etravirine SP:BP ratio was 0.16 (0.07–0.26). There were no differences in median BP etravirine concentrations between patients taking etravirine twice daily (491 (258–751) ng/mL) or once daily (414 (307–592) ng/mL) or in median SP etravirine concentrations, at 68.1 (31.2–166.0) ng/mL versus 60.6 (36.9–104.0) ng/mL, respectively.

The BP VL was detectable in one patient (patient 7) at 362 copies/mL, while the patient was concomitantly taking darunavir/ritonavir/tenofovir/emtricitabine in addition to etravirine, but with poor adherence to treatment. Of note, the SP VL was <40 copies/mL in the same patient.

**Discussion**

In this study etravirine BP concentrations in all patients were similar to the plasma etravirine C_{min} in HIV-positive patients reported in the FDA product information chart (296.74 ± 377.52 ng/mL),1 and no differences in concentrations between patients taking etravirine once or twice daily were observed. Although etravirine is 99.9% bound to proteins in plasma (primarily to albumin at 99.6% and to α1-acid glycoprotein at 97.66% and 99.02% in vitro),5 recent data suggest that less etravirine is bound to proteins in semen (96.7%). Thus protein-free etravirine concentrations (active drug) would be more than three times higher in semen than in BP.6

In our study, total etravirine SP concentrations were higher than the upper end of the protein-free IC_{50} range (0.39–2.4 ng/mL)1 in all patients and the median etravirine SP concentration was 16% of the BP concentration, suggesting that unbound etravirine diffuses passively from blood to the seminal compartment through the blood–testis barrier because of its relatively low molecular weight and very high lipophilicity.5 Our results are in agreement with those recently published by Brown et al.,8 who found unbound etravirine trough concentrations of 1.2 ng/mL. Using the reported percentage of etravirine protein binding in semen (96.7%), the median estimated free etravirine concentration in our study was 2.07 ng/mL, which is within the wild-type IC_{50} range (not adjusted for protein binding) and above the protein-free IC_{50} (1.3 ng/mL).8

Etravirine exposure in semen was also in keeping with findings from the two previous studies, which reported an SP:BP ratio of 0.12–0.15 and 0.18, respectively.1,5

An extensive review of data regarding different patterns of ARV drug penetration into male genital secretions has been published recently.3 The pathogenesis of HIV and ARV pharmacodynamics in the male genital tract are complex. Moreover, it is difficult to compare drug concentrations in semen between different studies due to important design variabilities, such as...
sampling time, number of samples, potential drug interactions, techniques used and the pharmacokinetic and pharmacodynamic characteristics of the drug.

Several physicochemical characteristics influence drug penetration into genital tissue and concentrations in genital secretions, such as lipophilicity, molecular weight, protein-binding percentage, P-glycoprotein activity, semen pH and timing of the last ejaculation. Interestingly, etravirine is not a substrate for P-glycoprotein, therefore diffused drug cannot be transported out of the genital tract by this transporter. In comparison with other drugs, etravirine seems to be in the lower range of penetrability in semen, but it shows concentrations higher than the IC50 for wild-type virus and higher levels than those observed for other highly protein-bound drugs, such as efavirenz, or the protease inhibitors lopinavir, ritonavir or saquinavir, when the protein-corrected IC50 is considered.

Etravirine concentrations in female genital secretions seem to be clearly higher (130% of plasma concentrations) than those achieved in male genital fluids. This notion is further supported by the results of a recent publication by Clavel et al., in which the cervicovaginal fluid:BP ratio was 1.2. A similar situation is seen with other drugs, such as maraviroc (89% in semen and ~200% in cervicovaginal fluid).

A small sample size, etravirine determinations in only a single sample and a cross-sectional design are limitations of this study that could potentially bias the results. However, it is difficult to obtain several semen samples from the same patient and this is the first publication of etravirine semen concentrations in HIV-infected patients. Our findings coincide with those observed in HIV-negative men and with the limited data from HIV-infected patients. Thus we believe the results represent a potentially useful piece of information for physicians and patients.

In conclusion, this study shows that in male genital secretions etravirine reaches modest concentrations that are several times greater than the IC50. These findings suggest that etravirine in combination with other ARV drugs may contribute to suppress viral replication in semen.

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Transparency declarations
None to declare.

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