Antiretroviral drug concentrations in semen of HIV-infected men: differential penetration of indinavir, ritonavir and saquinavir

Stephen Taylor\textsuperscript{a,b,*}, David J. Back\textsuperscript{c}, Susan M. Drake\textsuperscript{b}, Judith Workman\textsuperscript{a}, Helen Reynolds\textsuperscript{c}, Sara E. Gibbons\textsuperscript{c}, David J. White\textsuperscript{b} and Deenan Pillay\textsuperscript{a}

\textsuperscript{a}PHLS Antiviral Susceptibility Reference Unit, Division of Immunity and Infection, University of Birmingham; \textsuperscript{b}Department of Sexual Medicine, Birmingham Heartlands Hospital, Birmingham; \textsuperscript{c}Department of Pharmacology \& Therapeutics, University of Liverpool, UK

Variable drug penetration of antiretroviral drugs into the genital tract may contribute to the differential evolution of HIV and the emergence of drug resistance. This, in turn, may have an impact on the sexual transmission of resistant HIV in patients treated with antiretroviral drugs. We have measured concentrations of the HIV-1 protease inhibitors indinavir, ritonavir and saquinavir in the blood plasma (BP) and seminal plasma (SP) of 23 HIV-1-positive men. Forty-five time-matched blood and semen samples were obtained. SP concentrations of indinavir exceeded the EC\textsubscript{95} of indinavir, corrected for protein binding, of 42 ng/mL at all time intervals. In contrast, the median ritonavir and saquinavir SP concentrations were below the relevant EC\textsubscript{95} at all times post drug ingestion. The median SP:BP concentration ratios for indinavir were 0.6, 0.8 and 1.4, respectively, at 0–2, 2–6 and 6–8 h post-drug ingestion. In contrast, the median SP:BP concentration ratios at 0–3, 3–9 and 9–12 h post-drug ingestion were <0.02, <0.04 and <0.04, respectively, for both ritonavir and saquinavir. These differences justify further study of HIV-1 evolution and development of resistance in the genital tract of men taking these anti-HIV drugs.

Introduction

Highly active antiretroviral therapy (HAART) has revolutionized the treatment of people infected with HIV. However, up to 50% of patients may suffer virological relapse within 1 year of starting HAART treatment.\textsuperscript{1} Reasons for antiviral failure are numerous and include difficulties in adhering to medication, sub-therapeutic plasma drug concentrations and the development of drug resistance. A further potential cause of failure is the existence of anatomical sanctuary sites, in which viral replication and evolution may persist despite apparent virological control in the blood.

The central nervous system and genital tract represent two such sanctuary sites. Although the antiviral responses are usually concordant in the blood and genital tract, discordant viral load suppression in these compartments and differences in the rate of emergence of drug resistance-associated viral mutations have been documented.\textsuperscript{2} Differential penetration of antiviral drugs into semen may be one factor influencing viral suppression and evolution in the male genital tract. In this study we have compared the concentrations of three protease inhibitors in the semen and blood of HIV-1-infected men.

Materials and methods

Patients

HIV-1-infected men taking indinavir or a combination of ritonavir plus saquinavir, in combination with one or two nucleoside or non-nucleoside reverse transcriptase inhibitors, were studied. All had been taking their medication for at least 4 weeks before samples were obtained. All
provided written informed consent and the study was approved by the local ethics committee. All patients were tested for evidence of other sexually transmitted infections at the time of semen production.

**Viral load determination**

Viral loads (VL) in blood plasma (BP) and semen plasma (SP) were determined by nucleic acid sequence-based amplification (NASBA; Nuclisens, Organon Teknika, Durham, NC, USA) as described previously, with a lower limit of detection of 400 copies/mL for BP and 800 copies/mL for SP.

Sample collection times were carefully documented in relation to the time of drug ingestion. When samples of semen and blood were obtained within 1 h of each other and with no intervening drug administration, a SP:BP concentration ratio was calculated. The sample pairs were divided into groups according to their time of collection (0–2, 2–6 or 6–8 h post-drug ingestion for ritonavir analysis and 0–3, 3–9 or 9–12 h for ritonavir and saquinavir calculations). These intervals enabled the initial groups to include peak BP concentrations during the absorption phase and the final groups to capture the lowest BP concentrations.

**Drug concentration analysis**

Drug concentrations were analysed by sensitive high-performance liquid chromatography assays as described previously. The lower limits of quantification (LLQ) of the saquinavir, ritonavir and indinavir assays were 20, 25 and 20 ng/mL, respectively. Samples that fell below the LLQ were assigned a value equal to the LLQ for statistical analysis.

Drug concentrations were compared with EC95 values for the respective drugs (EC95 = concentration of drug required to inhibit viral replication by 95% in an in vitro cell culture assay). In this study the reported EC95 values were adjusted for the plasma protein binding of the drugs as described by Molla et al. This produced EC95 values of 42.9 ng/mL (68 nM) for indinavir, 515 ng/mL (2100 nM) for ritonavir and 278 ng/mL (416 nM) for saquinavir.

Differences in BP and SP drug concentrations in sample pairs and differences in SP:BP ratios generated at different time points within the same individuals were compared using the Wilcoxon signed rank test.

**Results**

Twenty-three HIV-1-positive men participated in this study. Sixteen were taking ritonavir 400 mg bd plus saquinavir 400 mg bd; five indinavir 800 mg tds; and two indinavir 600 mg tds. Twenty-one patients were also taking stavudine 40 mg bd; four lamivudine 150 mg bd; three didanosine 400 mg bd; two delavirdine 400 mg bd; and one zidovudine 250 mg bd. The median age of men in the study was 37 years (range 28–54 years). The median time on antiviral medication was 28 weeks (range 4–114 weeks) and the median CD4 cell count was 205 cells/mm³ (range 81–763 cells/mm³). Nineteen patients had undetectable viral loads in the SP and BP. The other four patients were all taking ritonavir/ saquinavir-containing regimens, and had BP (SP) VLs of 1100 (42 000), 8100 (25 000), 7200 (4400) and 620 (2700) copies/mL, respectively. A total of 45 pairs of closely time-matched BP and SP samples were obtained for pharmacokinetic analysis of indinavir (21 pairs), ritonavir (24 pairs) and saquinavir (18 pairs) concentrations.

**Indinavir**

Median BP indinavir concentrations at 0–2, 2–6 and 6–8 h were 4473 ng/mL (range 1380–6020 ng/mL, n = 7), 937 ng/mL (range 360–3070 ng/mL, n = 7) and 360 ng/mL (range 201–2090 ng/mL, n = 7), respectively (Figure 1a). The paired SP indinavir concentrations at 0–2, 2–6 and 6–8 h were 2527 ng/mL (range 319–3775 ng/mL), 1259 ng/mL (range 216–3567 ng/mL) and 558 ng/mL (range 272–3178 ng/mL), respectively. Only during the 0–2 h interval were SP and BP concentrations significantly different from each other (P < 0.01). All BP and SP indinavir concentrations exceeded the EC95 for indinavir of 42 ng/mL.

Median SP:BP indinavir ratios at 0–2, 2–6 and 6–8 h were 0.59 (range 0.23–0.75), 0.79 (range 0.55–3.96) and 1.44 (range 0.67–5.79), respectively. The 0–2 and 6–8 h values (Figure 2) were significantly different from each other (P < 0.01).

**Ritonavir**

Median BP ritonavir concentrations at 0–3, 3–9 and 9–12 h were 16 310 ng/mL (range 3493–33 610 ng/mL, n = 11), 9815 ng/mL (range 2391–14 000 ng/mL, n = 4) and 6020 ng/mL (range 894–12 510 ng/mL, n = 9), respectively (Figure 1b). The paired SP ritonavir concentrations were 319 ng/mL (range 60–790 ng/mL), 406 ng/mL (range <25–790 ng/mL) and 295 ng/mL (range <25–870 ng/mL), respectively. SP concentrations were lower than BP concentrations in all time intervals post-drug ingestion (P < 0.003, P = 0.06, P < 0.007, respectively). All but one patient had detectable SP ritonavir concentrations at all time periods; however, the median SP ritonavir concentrations were below the EC95 of ritonavir (515 ng/mL) throughout the dosing interval. In contrast, BP ritonavir concentrations were above the EC95 at all time points.

Median SP:BP ritonavir ratios at 0–3, 3–9 and 9–12 h were 0.02 (range 0.01–0.05), <0.04 (range <0.01–0.06) and <0.04 (range <0.02–0.11) (data not shown).

**Saquinavir**

Median BP saquinavir concentrations at 0–3, 3–9 and 9–12 h were 1904 ng/mL (range 742–6260 ng/mL, n = 7),
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1547 ng/mL (range 1081–3236 ng/mL, *n* = 4) and 661 ng/mL (range 502–1376 ng/mL, *n* = 7), respectively (Figure 1c). The paired SP saquinavir concentrations were 40 ng/mL (range <20–84 ng/mL), <20 ng/mL (<20–83 ng/mL) and <20 ng/mL (range <20–34 ng/mL), respectively. SP saquinavir concentrations were below BP saquinavir concentrations at all time intervals (*P* < 0.01, *P* = 0.07, *P* < 0.02, respectively). All BP saquinavir concentrations were above the EC95 for saquinavir (278 ng/mL) at all time points post-drug ingestion. In contrast, 13/18 SP saquinavir concentration were below the limit of detection of the assay and, of the seven detectable concentrations, all were below the EC95 of saquinavir.

SP:BP saquinavir ratios at 0–3, 3–9 and 9–12 h were 0.02 (range 0.003–0.11), 0.04 (range 0.01–0.07) and <0.02 (<0.02–<0.06), respectively (data not shown).

**Discussion**

We have shown that the penetration of the HIV-1 protease inhibitors indinavir, ritonavir and saquinavir into the semen differs. Indinavir concentrations within SP reached similar concentrations to those in blood, whereas ritonavir and saquinavir were present in SP at concentrations <5% of concurrent BP concentrations throughout the interval between doses. However, we have also demonstrated that the SP:BP ratios for indinavir, ritonavir and saquinavir are dependent on the time after drug administration, with higher SP:BP ratios observed at trough blood concentrations and lowest ratios achieved when blood concentrations are highest. These relative changes in ratio over time reflect differences in disposition and elimination of drug between these two compartments and indicate that the drugs enter the semen relatively slowly. Multiple time point sampling from single patients confirmed that the absorption and elimination phases are much more dynamic within the BP than SP (data not shown).

When trying to establish reasons for these differences, the physicochemical properties of this class of drugs must be considered. All three drugs are of similar size, are lipid soluble and are weak bases, so that ion trapping within the prostate gland (pH 6.6) may contribute to lower rates of elimination, although the degree of active drug transport into and out of the genital tract is unknown. The most plausible reason for the differences in semen penetration is the different protein binding of the drugs. Indinavir is 60% bound to plasma proteins but ritonavir and saquinavir are each >98% protein bound. This hypothesis is supported by the results with amprenavir, which is c. 90% protein bound and achieves semen concentrations of c. 20% of plasma concentrations.

Since all the patients enrolled in this study were receiving nucleoside analogues in addition to protease inhibitors (PIs), it is difficult to draw conclusions regarding the efficacy of different PI combinations on suppression of

![Figure 1. Box and whisker plots showing (a) indinavir (IDV), (b) ritonavir (RTV) and (c) saquinavir (SQV) concentrations in BP (□) and SP (○) at different times post-drug ingestion. 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 open circles. Differences between matched BP and SP drug concentrations have been compared using the Wilcoxon signed rank test. Upper dashed lines represent protein corrected EC95 values. Lower dashed lines represent the lower limit of detection for the assay.](image)
Our observations support previous findings that indinavir concentrations in semen exceed protein-corrected EC\textsubscript{95} values\textsuperscript{9} and thus would be expected to be effective. The SP concentrations of saquinavir and ritonavir were, however, usually low. It is interesting that addition of low-dose ritonavir significantly increased seminal plasma indinavir concentrations in another study; the proposed mechanism suggested to be via inhibition of P glycoprotein.\textsuperscript{9} However, in our study, despite all patients taking saquinavir in combination with ritonavir, saquinavir concentrations remained low and, because no patients were taking saquinavir alone, we were unable to determine whether ritonavir increased seminal saquinavir concentrations to any significant extent. The semen concentrations were generally below the calculated EC\textsubscript{95}. This needs to be interpreted with some caution, however, since it is not known to what degree these drugs become protein bound within the semen and what effect this will have on the free drug available for either antiviral activity or active drug transport. Furthermore, although four patients receiving ritonavir and saquinavir had elevated SP VL, they all had detectable virus in the BP (where concentrations were above the EC\textsubscript{95}, and three had been receiving therapy for \textlesss than 4 weeks, which may be insufficient time to fully suppress the VL). The fourth patient has been studied in more detail, and virus in both compartments demonstrated the presence of PI resistance mutations.\textsuperscript{10}

In summary, we have demonstrated major differences between PIs in their capacity to penetrate the male genital tract. The impact of such differential penetration into this compartment on virus suppression and evolution of resistant virus requires further study.

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**References**


