

Concentrations of nevirapine, lamivudine and stavudine in semen of HIV-1-infected men

Stephen Taylor^{ab}, Rolf P.G. van Heeswijk^c, Richard M.W. Hoetelmans^c, Judith Workman^a, Susan M. Drake^b, David J. White^b and Deenan Pillay^a

Objective: To determine the concentrations of nevirapine (NVP), lamivudine (3TC) and stavudine (D4T) in seminal and blood plasma in HIV-1-infected men.

Methods: Twelve HIV-1-infected men on NVP-containing regimens including 3TC (n = 8) or D4T (n = 11) provided 23 blood plasma and 22 seminal plasma samples for drug concentration and viral load quantitation. Concentrations of all drugs were assessed by sensitive validated high performance liquid chromatography (HPLC) assays. Blood plasma and seminal plasma viral loads were measured using nucleic acid sequence-based amplification (NASBA). Samples were grouped according to time after drug ingestion, 0–2, 2–4, 4–8 and 8–12 h. For matched seminal and blood plasma samples, obtained within 1 h of each other, a seminal : blood plasma ratio was calculated.

Results: The concentration of NVP in seminal plasma appeared to mirror the concentrations in blood plasma. Absolute median seminal plasma NVP concentrations at 0–2, 2–4, 4–8 and 8–12 h were 3.1 µg/ml (range 1.7–4.89), 2.68 µg/ml (2.5–3.9), 2.5 µg/ml (2.3–2.7) and 3.09 µg/ml (1.3–9.1). The median seminal : blood plasma ratios for the four time periods were 0.54 (range 0.34–0.85), 0.83 (range 0.43–1.08), 0.53 (0.48–0.59), and 0.61 (0.59–0.78). 3TC and D4T appeared to reach concentrations in seminal plasma of a similar magnitude or higher than concentrations in blood plasma. The median seminal plasma viral load for all patients was less than 800 copies/ml (range < 800–11 000). The median blood plasma viral load was less than 400 copies/ml (< 400–1100).

Conclusion: NVP reaches concentrations in the semen approximately 60% of those in the blood plasma throughout the 12 h dosing period. In a smaller dataset, 3TC and D4T concentrations in blood plasma and seminal plasma were similar. These data may well have implications for the evolution of drug-resistant virus within the genital tract.

© 2000 Lippincott Williams & Wilkins

AIDS 2000, **14**:1979–1984

Keywords: HIV-1, lamivudine, nevirapine, pharmacokinetics, semen, stavudine

From the ^aPHLS Antiviral Susceptibility Reference Unit, Division of Immunity and Infection, University of Birmingham, Birmingham, UK; ^bDepartment of Sexual Medicine, Birmingham Heartlands Hospital, Birmingham, UK; and ^cDepartment of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, the Netherlands.

Sponsorship: S.T. is supported by a West Midlands NHSE Sheldon Clinical Research Fellowship. Support is also acknowledged from Boehringer Ingelheim for an unrestricted educational grant.

Correspondence to: Dr Stephen Taylor, PHLS Antiviral Susceptibility Reference Unit, Division of Immunity and Infection, University of Birmingham Medical School, B15 2TT, UK.

Fax: +44 (0)1926 741115; e-mail: s.taylor.1@bham.ac.uk

Received: 25 October 1999; revised: 3 March 2000; accepted: 24 May 2000.

Introduction

Treatment of HIV-1-infected men with highly active antiretroviral therapy (HAART) generally leads to a reduction in seminal plasma viral load, which parallels that seen in the blood plasma [1,2]. However, some patients demonstrate discordance between viral load suppression in blood plasma and that in semen [3,4], especially in the presence of concurrent urethritis [5,6].

HIV genotypic differences between semen and blood viral populations have been documented [7], including the recent finding of differences in drug resistance-associated mutations in antiretroviral-experienced patients [8–10]. The male genital tract may therefore represent a distinct ‘compartment’ in which viral replication and evolution are subject to different selective pressures compared with blood.

One determinant of virus evolution may be the concentration of antiviral drugs achieved in this compartment [11]. Poor drug penetration may lead to sub-optimal viral suppression and predispose to the more rapid emergence of drug-resistant variants within the genital tract.

In this study we determined the concentrations of nevirapine (NVP), lamivudine (3TC) and stavudine (D4T) in the blood plasma and seminal plasma of 12 HIV-1-infected men. NVP is a commonly prescribed first-line agent both within our unit and the UK. In addition there is no published data on non-nucleoside analogue drug concentrations in semen. It was our primary aim to measure NVP concentrations, as it is known that there is a dose–response relationship with plasma NVP levels and antiviral activity [12,13]. In turn low levels within semen may reduce antiviral activity in this compartment. Little data is available for nucleoside analogue penetration into semen and we also undertook this analysis within this cohort of patients.

Methods

Twelve antiretroviral drug-experienced HIV-1-infected patients attending Birmingham Heartlands Hospital were enrolled prospectively. They were all established on NVP-containing triple therapy regimens (200 mg twice a day) for over 12 weeks. Eight patients concurrently received 3TC (150 mg twice a day) and 11 patients D4T (40 mg twice a day). Written informed consent and local ethics committee approval were obtained for the study.

Semen samples were produced by masturbation and ejaculation into a sterile wide-necked container. Times of drug ingestion and sample production were carefully

documented. Blood samples were obtained by venepuncture.

A single patient was enrolled to undertake a detailed pharmacokinetic study of NVP in semen and blood plasma. This was performed to guide appropriate sample collection times for the remaining 11 patients. At the time of the study it was unknown whether there was a delay in the uptake and elimination of NVP into semen.

Briefly, a single patient took NVP 200 mg, 3TC 150 mg and D4T 40 mg at 08.00 h on study day 1. He had been instructed to ejaculate several times on the days preceding study day 1. One hour after drug ingestion he produced a semen sample and within 30 min provided a matched blood sample. On day 2 of the study he produced his semen sample at 2 h after drug ingestion followed by a blood sample. This procedure continued on a daily basis with close attention paid to the timing of drug ingestion and sample production. This continued until he had produced daily samples at 1, 2, 3, 4, 6 and 10 h after drug ingestion (Fig. 1). No two samples were produced on the same day. On the basis of the results of this study we prospectively enrolled a further 11 patients. We asked patients to provide three matched samples on separate days, one pair within the 0–4 h time period, one within the 4–8 h time period and one within the 8–12 h time period. (in the analysis samples produced in the 0–4 h time period were further divided into 0–2 and 2–4 for analysis). The duration of the study was planned to finish when all three samples had been

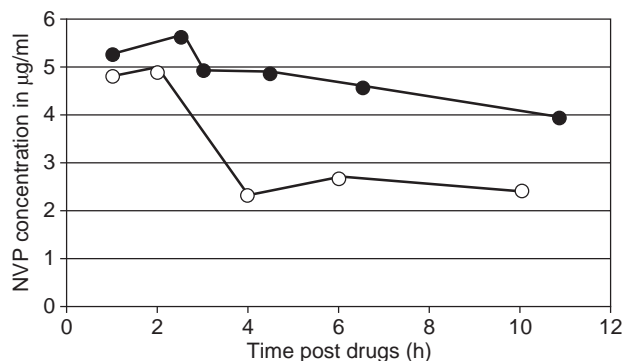


Fig. 1. Nevirapine drug concentration versus time plots for blood plasma and seminal plasma in a single patient pharmacokinetic study. Patient 10 produced multiple time-matched semen and blood samples to establish the pattern of nevirapine (NVP) concentration in seminal plasma and blood plasma in relation to the amount of time elapsed after drug insertion. Semen samples were produced at 1, 2, 3, 4, 6 and 10 h after drug insertion on sequential days. Blood was drawn within 1 h of semen sample production (mean time between samples 27 min). The semen sample at time 3 h was of insufficient volume for drug concentration determination.

collected from each patient or until they were unable or unwilling to produce further samples.

Semen and blood samples were centrifuged without delay at 3000g for 10 min. Plasma and cell fractions were separated, aliquoted and stored at -70°C until analysis. For each patient, a urethral swab was taken for microscopy and culture, a two-glass urine test performed, and a urine sample obtained for *Chlamydia trachomatis* nucleic acid detection (Ligase chain reaction, Abbott Diagnostics, Abbot Park, Illinois, USA).

Drug level and viral load analysis

Concentrations of NVP in plasma and semen were quantified using a modified version of a validated, sensitive high-performance liquid chromatography (HPLC) assay [14]. To separate NVP from interfering endogenous compounds in semen, the pH of the mobile phase was adjusted to 3.5. Semen samples were diluted with human plasma (1:4 v/v) before analysis. Briefly, sample pre-treatment consisted of protein precipitation with acetonitrile. The analyte was separated from endogenous compounds by isocratic reversed-phase HPLC with ultraviolet detection at 282 nm. The method was validated for analysis of NVP in plasma over the range of 50–10 000 ng/ml using 250 μl plasma. Concentrations of D4T and 3TC in plasma and semen were measured simultaneously using an HPLC assay as previously described [15]. D4T and 3TC were extracted from semen and plasma samples using silica extraction columns before reversed-phase HPLC with ultraviolet detection at 270 nm. The method was validated over the range of 10–5000 ng/ml using a 0.5 ml sample volume.

Determination of viral RNA titres was performed using the nucleic acid sequence-based amplification (NASBA)/Nuclisens assay system (Organon Teknica, Dur-

ham, NC, USA). This gave a lower limit of detection for 100 μl of seminal plasma of 800 copies/ml and 400 copies/ml for an input of 200 μl blood plasma.

Blood plasma and seminal plasma results were grouped according to time as 0–2, 2–4, 4–8 or 8–12 h after drug ingestion. Median values were calculated and data graphically displayed as box plots (Fig. 2(a–c)). A comparison of median blood plasma and seminal plasma drug levels at any one period was undertaken using the Mann–Whitney U test (SPSS version 9 for Windows). Seminal to blood plasma ratios were only calculated if blood plasma and seminal plasma samples were obtained within 1 h of each other and no drugs were ingested between the production of these matched samples.

Results

The 12 patients had a median age of 40.5 years and a median CD4 cell count of 347 cells/mm³. All except two patients had undetectable blood plasma and seminal plasma viral loads at the time of drug level analysis. No patients tested had evidence of urethritis at the time of sample production. All drug regimens and viral load results are outlined in Table 1.

Nevirapine concentrations in blood plasma and seminal plasma

Twenty-three blood plasma and 22 seminal plasma samples from 12 patients were available for NVP determination. Median NVP levels in blood plasma and seminal plasma at 0–2, 2–4, 4–8 and 8–12 h are displayed in Fig. 2(a). Seminal plasma levels reached approximately 60% of blood plasma levels at all time-points. This difference only achieved statistical signifi-

Table 1. Patient demographics and status at time of blood and semen collection.

Patient	Age (years)	Risk	Drugs	CD4 (cells/ μl)	Nuclisens NASBA Blood plasma (copies/ml)	NASBA Seminal plasma (copies/ml)
1	29	Heterosexual	NVP, 3TC, D4T	406	< 400 ^a	< 800
2	32	Homosexual	NVP, 3TC, D4T	485	< 400	1119
3	53	Heterosexual	NVP, 3TC, D4T	347	< 400 ^a	< 800
4	33	Homosexual	NVP, 3TC, D4T	499	< 400	< 800
5	42	Homosexual	NVP, 3TC, D4T, HU	126	< 400	< 800
6	27	Homosexual	NVP, 3TC, ZDV	302	< 400	< 800
7	43	Heterosexual	NVP, 3TC, D4T	281	1100	< 800
8	50	Homosexual	NVP, 3TC, D4T	347	< 400	< 800
9	43	Intravenous drug user	NVP, DDI, D4T	403	580	11 000
10	36	Homosexual	NVP, DDI, D4T	336	< 400	< 800
11	40	Homosexual	NVP, DDI, D4T	425	< 400	< 800
12	41	Homosexual	NVP, DDI, D4T, HU	162	< 400	< 800
Median	40.5			347	< 400	< 800

DDI, Didanosine; D4T, stavudine; HU, hydroxyurea; NASBA, nucleic acid sequence-based amplification; NVP, nevirapine; 3TC, lamivudine, ZDV, zidovudine.

^aIndicates samples analysed by Roche Amplicor Monitor assay (Roche Diagnostics, Branchburg, NJ, USA).

cance at the 0–2 and 2–4 h time period ($P < 0.04$) (Fig. 2(a)).

Fourteen matched blood plasma/seminal plasma pairs were available for calculation of a seminal : blood plasma ratio. The median 0–2, 2–4, 4–8 and 8–12 h seminal : blood plasma ratios for NVP were 0.54 (range 0.34–0.85), 0.83 (0.43–1.08), 0.53 (0.5–0.6) and 0.61 (0.59–0.78), respectively. Seminal : blood plasma ratios were plotted against time after dose in Fig. 3. The median time between matched seminal and blood plasma production was 30 min (range 5 min to 1 h).

Lamivudine concentrations in blood plasma and seminal plasma

Fifteen blood plasma and 14 seminal plasma samples from eight patients were available for 3TC concentration determination. Median 3TC concentrations in blood plasma and seminal plasma at 0–2, 2–4, 4–8 and 8–12 h after drug ingestion are displayed in Fig. 2(b). The median 3TC seminal plasma concentrations were consistently higher than the corresponding blood plas-

ma 3TC concentrations at all four time periods after drug ingestion. This difference reached statistical significance at the 2–4 and 8–12 time periods ($P = 0.004$ and $P = 0.04$, respectively). Ten matched blood plasma/seminal plasma pairs were available for the calculation of a seminal : blood plasma ratio. The median 0–2, 2–4, 4–8 and 8–12 h seminal : blood plasma ratios for 3TC were 4.2 (range 2.04–6.4), 4.6 (2.5–6.4), 22.0 (9.05–35) and 8.7 (4–16.3), respectively. For individual patients absolute seminal plasma 3TC concentrations were always higher than corresponding blood plasma concentrations.

Stavudine concentrations in blood plasma and seminal plasma

Seventeen blood plasma and 18 seminal plasma samples from 10 patients were available for D4T concentration determination (insufficient sample was available for one patient). D4T concentrations in both blood plasma and seminal plasma were variable in this study. In approximately one third of the blood plasma and seminal plasma samples analysed, D4T levels were below the

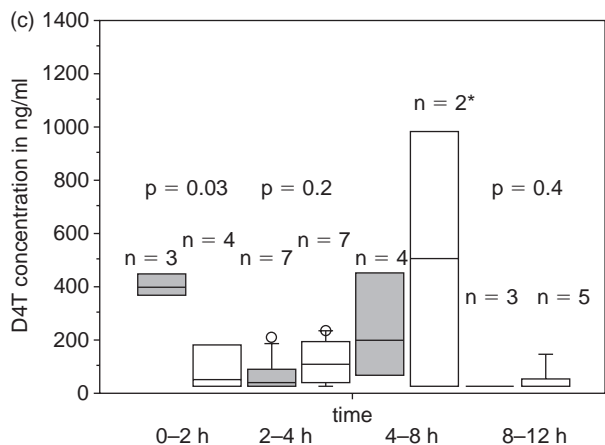
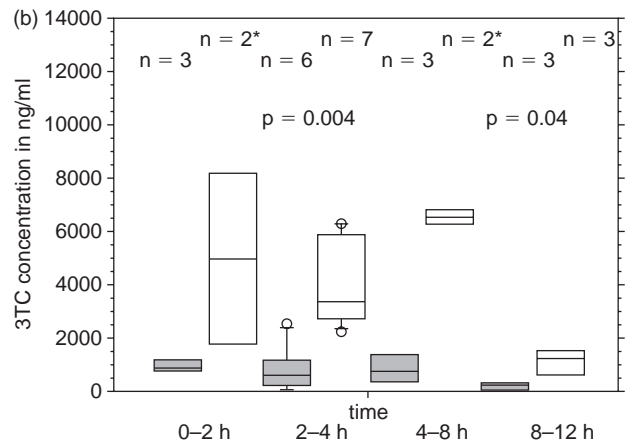
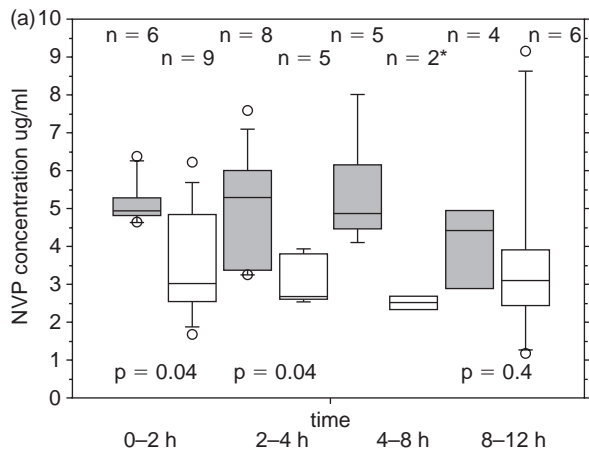


Fig. 2. (a) Nevirapine (NVP) concentrations in blood plasma and seminal plasma at different times after drug ingestion in 12 patients. (b) Lamivudine (3TC) concentrations in blood plasma and seminal plasma at different times after drug ingestion in eight patients. (c) Stavudine (D4T) concentrations in blood plasma and seminal plasma at different times after drug ingestion in 10 patients.

Boxplots illustrate drug concentrations at different time intervals after drug ingestion. Grey boxes represent blood plasma drug concentrations. White boxes represent seminal plasma drug concentrations. Horizontal lines within boxes represent median values. The lower limits of boxes represent 25th percentiles. The upper limits of boxes represent 75th percentiles. Vertical lines indicate 5th and 95th percentiles. White circles represent outlying values. Differences between blood plasma and seminal plasma drug concentrations have been compared using the Mann–Whitney U test. *Indicates only two values available in this time period. Mean values given with range provided. No statistical comparison with blood concentrations (provided as medians) undertaken.

limit of detection of the assay (25 ng/ml). Median D4T levels in blood plasma and seminal plasma at 0–2, 2–4, 4–8, and 8–12 h after drug ingestion are displayed in Fig. 2(c). Seminal plasma D4T concentrations were significantly lower than blood plasma levels only at the 0–2 h after dose period ($P = 0.03$). Twelve time-matched blood plasma/seminal plasma pairs were available for the calculation of a seminal : blood plasma ratio. The median 0–2, 2–4, 4–8 and 8–12 h seminal : blood plasma ratios for D4T were 0.46 (range 0.19–0.73), 2.7 (0.47–4.8) 5.9 (0.08–11.9) and 3.5 (1–6).

Discussion

We previously studied the penetration of the HIV-1 protease inhibitors ritonavir and saquinavir into semen, demonstrating that drugs were present in semen at concentrations of less than 5% of those concurrently measured in the blood plasma [16]. By contrast, we now show that the non-nucleoside reverse transcriptase inhibitor NVP shows good penetration into the semen with seminal plasma concentrations reaching approximately 60% of those simultaneously found in the blood.

Absolute seminal plasma NVP concentrations were approximately 100-fold greater than the NVP IC_{90} for wild-type virus (0.016 $\mu\text{g/ml}$), in all patients [12,13]. It is currently unknown to what degree free NVP will bind to protein once within the semen. On the assumption that the degree of NVP protein binding in seminal plasma is similar to that in blood plasma (i.e. 60% bound), then the free drug available for antiviral activity (~40%) should still exceed the IC_{90} by more than 40-fold.

When describing drug concentrations within body compartments, it is always important to describe absolute drug concentrations achieved in addition to ratios. This is because these ratios can be highly dependent on the time after drug ingestion at which they are calculated. This is particularly true for drugs that have a delayed uptake and or elimination from different body compartments. The cerebrospinal fluid : blood plasma ratio for 3TC and D4T thus appears to increase over time after drug ingestion [17]. Therefore, in the present study, the seminal : blood plasma ratios were plotted against time after dose (Fig. 3). This analysis demonstrated that the seminal : blood plasma ratio for NVP remains reasonably constant throughout the dosing period

Seminal plasma 3TC concentrations were always in excess of blood plasma concentrations. This apparent accumulation or delay in the removal of 3TC within

semen raises the possibility that there may be active transport of 3TC both into or out of the male genital tract. Examples of this type of drug modulation include recent data on nucleoside analogue efflux transporters [18] and the multidrug transporter P glycoprotein [19].

We did not discern a clear pattern of D4T penetration into the genital tract, unlike the case of NVP or 3TC. Furthermore, in several patients, D4T levels in both the seminal and blood plasma were below the limit of detection of the assay. It is not clear whether the co-administration of 3TC or NVP may affect the uptake and elimination of D4T from the semen.

There are several limitations to this study, many of which arise because of the inherent practical difficulties in working with HIV-1 in semen. These include patient recruitment, close matching of blood and semen samples and limited and non-consistent sample volumes. Taking this into consideration, this study provides some of the first data on semen drug concentrations of drugs widely used in clinical practice. The interpretation of nucleoside analogue plasma concentrations is problematical for several reasons. First, it has been shown that blood plasma levels do not consistently reflect the active intracellular triphosphate concentrations within peripheral blood mononuclear cells [20]. In addition, plasma concentrations can peak and fall very rapidly, whereas concentrations in other

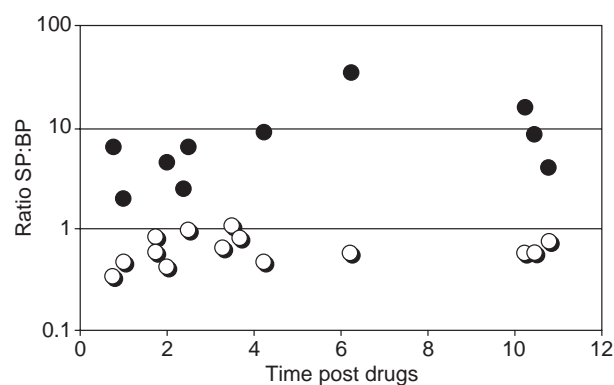


Fig. 3. Seminal plasma : blood plasma ratios for nevirapine and lamivudine plotted against time after the ingestion of drugs.

Black circles represent seminal plasma : blood plasma ratios for lamivudine at different times after drug ingestion. White circles represent seminal plasma : blood plasma ratios for nevirapine at different times after drug ingestion. Only truly matched seminal plasma and blood plasma drug levels obtained within 1 h of each other were used for the calculation of the seminal plasma : blood plasma ratio. The median time between the collection of blood plasma and seminal plasma samples was 30 min (range 5 min to 1 h). The time assigned to each ratio is the midpoint between the time the blood plasma and the seminal plasma specimens were obtained.

compartments may rise and fall more slowly. Ratio calculations between different anatomical sites thus have to be interpreted with care. Therefore we have restricted calculating seminal : blood plasma ratios only for those samples produced within a median of 30 min of each other (range 5 min to 1 h). Despite this it should be noted that the 0–2 h time interval may still contain both the blood plasma C_{\min} (considering absorption lag) and the C_{\max} for the nucleoside analogues.

Taking these important caveats into consideration, our finding that 3TC appears to accumulate within seminal plasma is interesting and corroborates the findings of Pereira *et al.* [21], who also found that seminal plasma 3TC concentrations were generally higher than corresponding blood plasma concentrations. Because the peripheral blood mononuclear cell half-life of 3TC-triphosphate appears prolonged compared with blood plasma 3TC levels [22], it is possible that our results may manifest in high levels of intracellular active drug within the genital tract. Unfortunately, the limited cell numbers in semen make such an investigation difficult.

Finally, it has been suggested that dual protease inhibitors alone may be insufficient to suppress the cerebrospinal fluid viral load fully [23] without the addition of nucleoside analogues. The same may also be true of viral suppression within the genital tract. Clearly, further information is required on the nature of virus replication within these sanctuary sites before data of the type presented here are used directly to determine antiretroviral use in clinical practice.

Acknowledgements

The authors would like to thank the patients and all staff involved with the semen studies, especially Pat Cane, Maxine Owen, Kevin Baker and Brian Coup-land.

References

- Gupta P, Mellors J, Kingsley L, *et al.* High viral load in semen of human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. *J Virol* 1997, **71**:6271–6275.
- Vernazza PL, Gilliam BL, Flepp M, *et al.* Effect of antiviral treatment on the shedding of HIV-1 in semen. *AIDS* 1997, **11**:1249–1254.
- Ball JK, Rowe T, Curran R, *et al.* Poor reduction of HIV-1 RNA titres in nucleoside reverse transcriptase inhibitor experienced patients treated with indinavir combination therapy. *Sex Transm Infect* 1999, **75**:337–339.
- Tachet A, Dulioust E, Salmon D, *et al.* Detection and quantification of HIV-1 in semen. identification of a subpopulation of men

- at high potential risk of viral sexual transmission. *AIDS* 1999, **13**:823–831.
- Winter AJ, Taylor S, Workman J, *et al.* Asymptomatic urethritis and detection of HIV-1 RNA in seminal plasma. *Sex Transm Infect* 1999, **75**:261–263.
- Taylor S, Cane P, Drake S, Pillay D. Multidrug resistant HIV-1 in semen: reduction by treatment of concurrent gonorrhoea [Abstract 40]. *Antivir Ther* 2000, **5** (Suppl. 2), in press.
- Kroodsma KL, Kozal MJ, Hamed KA, Winters MA, Merigan TC. Detection of drug resistance mutations in the human immunodeficiency virus type 1 (HIV-1) *pol* gene: differences in semen and blood HIV-1 RNA and proviral DNA. *J Infect Dis* 1994, **170**:1292–1295.
- Byrn RA, Kiessling AA. Analysis of human immunodeficiency virus in semen: indications of a genetically distinct virus reservoir. *J Reprod Immunol* 1998, **41**:161–176.
- Eron JJ, Vernazza PL, Johnston DM, *et al.* Resistance of HIV-1 to antiretroviral agents in blood and seminal plasma: implications for transmission. *AIDS* 1998, **12**:F181–F189.
- Youle M, Stuyver L, Ball J, *et al.* Genotypic differences between plasma and seminal fluid HIV RNA in reverse transcriptase inhibitor experienced patients [Abstract 128]. *Antiviral Ther* 1998, **3** (Suppl. 1):88.
- Kashuba AD, Dyer JR, Kramer LM, Raasch RH, Eron JJ, Cohen MS. Antiretroviral-drug concentrations in semen. implications for sexual transmission of human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 1999, **43**:1817–1826.
- Havlir D, Cheesman SH, McLaughlin MM, *et al.* High dose nevirapine. safety, pharmacokinetics, and antiviral effect in patients with human immunodeficiency virus infection. *J Infect Dis* 1995, **171**:537–545.
- Veldkamp AI, Hoetelmans RMW, Beijnen JH, *et al.* High exposure to nevirapine is associated with a higher initial HIV-1 clearance rate, a higher likelihood to reach undetectability and prolonged suppression of HIV-1 replication. *Program and Abstracts from the Seventh European Conference on Clinical Aspects of HIV Infection*. Lisbon, Portugal. 23–27 October 1999 [Abstract 239].
- Van Heeswijk RPG, Hoetelmans RMW, Meenhorst PL, Mulder JW, Beijnen JH. Rapid determination of nevirapine in human plasma by ion-pair reversed phase high-performance liquid chromatography with ultra-violet detection. *J Chromatogr* 1998, **713**:395–399.
- Hoetelmans RMW, Profit M, Meenhorst PL, Mulder FW, Beijnen JH. Quantitative determination of (–)-2'-deoxy-3'-thiacytidine (lamivudine) in human plasma, saliva and cerebrospinal fluid by high-performance liquid chromatography with ultraviolet detection. *J Chromatogr* 1999, **713**:387–394.
- Taylor S, Back DJ, Workman J, *et al.* Poor penetration of the male genital tract by HIV-1 protease inhibitors. *AIDS* 1999, **13**:859–860.
- Foudraïne NA, Hoetelmans RM, Lange JM, *et al.* Cerebrospinal-fluid HIV-1 RNA and drug concentrations after treatment with lamivudine plus zidovudine or stavudine. *Lancet* 1998, **351**:1547–1551.
- Schuetz JD, Connelly MC, Sun D, *et al.* MRP4. A previously unidentified factor in resistance to nucleoside-based antiviral drugs. *Nat Med* 1999, **5**:1048–1051.
- Kim RB, Fromm MF, Wandel C, *et al.* The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV protease inhibitors. *J Clin Invest* 1998, **101**:289–294.
- Barry MG, Khoo SH, Veal GJ, *et al.* The effect of zidovudine dose on the formation of intracellular phosphorylated metabolites. *AIDS* 1996, **10**:1361–1367.
- Pereira AS, Kashuba AD, Fiscus SA, *et al.* Nucleoside analogues achieve high concentrations in seminal plasma: relationship between drug concentration and virus burden. *J Infect Dis* 1999, **180**:2039–2043.
- Moore KHP, Barrett JE, Shaw S, *et al.* The pharmacokinetics of lamivudine phosphorylation in peripheral blood mononuclear cells from patients infected with HIV-1. *AIDS* 1999, **13**:2239–2250.
- Gisolf EH, Jurriaans S, Hoetelmans R, *et al.* Ritonavir (RTV)/saquinavir (SQV) versus RTV/SQV/D4T in cerebrospinal fluid (CSF): drug concentrations and the effect of these regimens on CSF HIV-RNA levels. *AIDS* 1998, **12** (Suppl.):S8.