Potent antiretroviral treatment of HIV-infection results in suppression of the seminal shedding of HIV

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Objective: The amount of HIV in semen likely influences infectiousness. Antiretroviral therapy decreases HIV-RNA in semen, but data on HIV concentrations in semen in a large cohort of men with suppressed HIV-RNA in blood is unavailable.

Methods: Male patients with a treatment-induced reduction of HIV-RNA load in plasma below 400 copies/ml were asked to donate a semen and blood sample. Blood and seminal plasma were tested for the presence of HIV-RNA by the NucliSens method (detection limit 400 copies/ml). Seminal cell samples from 67 patients were further analysed for the presence of HIV-DNA using a nested DNA-polymerase chain reaction. Results of RNA and DNA testing in semen were compared with 55 HIV-positive antiretroviral therapy-naive men.

Results: A total of 114 patients participated in the study. Seminal plasma HIV-RNA was detectable in only two patients [1.8%, 95% confidence ratio (CI), 0–4.2%] compared with a detection frequency of 67% in untreated controls [Odds ratio (OR), 0.01; 95% CI, 0–0.03]. Detection of cell-associated HIV-DNA in semen was significantly less frequent (16 versus 38%) in patients receiving suppressive therapy compared with untreated controls (OR, 0.32; 95% CI, 0.12–0.80).

Conclusion: In patients with treatment-induced suppression of blood viral load the likelihood of having detectable HIV in semen is very low (< 4%). In addition, seminal shedding of cell-free and cell-associated HIV is significantly lower than in an untreated population of HIV-infected asymptomatic men. On a population basis, this effect of therapy may help to reduce sexual transmission of HIV. However, individual patients may still be infected as evidenced by continued shedding of cells harbouring the HIV provirus.

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Introduction

Potent antiretroviral therapy of HIV infection results in a marked suppression of HIV-RNA concentration in the blood of infected individuals. This effect of therapy is also associated with a significant increase in CD4 count, CD4 cell function and a reduction in mortality [1]. In a substantial fraction of patients receiving triple

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drug combination, HIV-RNA can no longer be detected in blood, even with the use of highly sensitive polymerase chain reaction (PCR) technology. Recent work indicates that treatment results in a comparably potent suppression of HIV-RNA in the lymphoid tissue although the effect of highly suppressive therapy in other biological compartments is also important [2–4].

The amount of shedding of HIV-RNA in the seminal plasma is likely to correlate with the degree of infectiousness of an individual [5]. However, whether potent antiretroviral therapy is associated with a reduction of the infectiousness of treated individuals is not known. We and others have demonstrated a significant reduction of the HIV-RNA concentration in the seminal plasma of patients treated with antiretrovirals [6–9]. However, these studies involved small numbers of patients, and some of the patients were not treated with highly active drug combinations. Recent reports comparing sequences in viral isolates obtained from blood and semen indicate that HIV may be compartmentalized in the genital tract, and semen may serve as a sanctuary site for residual HIV infection, even with use of potent antiretroviral treatment [10–13]. Thus, it is conceivable that continuous HIV-1 replication in the genital tract might occur even in the face of HIV suppression in the systemic compartment. Previous studies have been too small to exclude with any certainty that a substantial minority of men who have HIV RNA below detectable limits in the blood will have persistent HIV shedding in semen. In the context of transmission, future studies will also have to focus more on HIV-infected cells in the genital tract. The aim of this study was to investigate the frequency of residual HIV-replication in the genital tract in a large number of men whose HIV-RNA levels are below detectable limits in blood.

Methods

Study design and patient selection

This study was conducted in three HIV clinics at the University of Zürich and at the Kantonsspital St. Gallen, Switzerland and at the University of North Carolina at Chapel Hill, North Carolina, USA. All HIV-infected men who donated a semen sample at one of the three institutions while receiving antiretroviral treatment were selected as cases if their blood viral load was suppressed below 400 copies/ml (NucliSens, Organon Teknika, Boxtel, The Netherlands). In patients with detectable HIV-RNA in semen, blood plasma was further tested with a more sensitive PCR assay (lower limit = 20 copies/ml [14]. A group of drug-naive, HIV-positive men from one centre (St. Gallen) was also studied to provide a comparison. This population was an unselected, unmatched, consecutive group of HIV-positive men with a median CD4 count of 280×10^6 cells/l who were asked to give a semen and blood specimen. None of the subjects had symptoms of urethritis. All samples were frozen until tested by PCR in the same series of experiments.

HIV detection in semen

Detection of HIV-RNA in seminal plasma was quantified by NucliSens technology. Seminal plasma was separated from seminal cells by centrifugation (10 min at 1000 *g*) and frozen within 4 h of ejaculation. Two hundred microlitres of seminal plasma was used per assay (detection limit: 400 copies/ml). In the samples from the two Swiss centres seminal cells were washed twice after separation of seminal plasma, and an equivalent of one-sixth of the ejaculate was used to detect HIV-DNA by nested PCR. The DNA-extraction was performed using the QIAamp spin column procedure (Qiagen, Basel, Switzerland) and one-sixth of the extracted DNA was tested with nested HIV PCR using *gag*-based primer pairs.

Statistical methods

Results of RNA and DNA testing in semen from treated men were compared with the results in the historical control by χ^2 (Yates corrected). The Kruskal–Wallis test was used to compare the median duration of HIV-RNA suppression in blood in the patients with and without detectable HIV-DNA in semen.

Results

One hundred and fourteen patients on therapy with an HIV-RNA concentration below 400 copies/ml of blood were enrolled (Zürich, n = 28; St. Gallen: n = 45, Chapel Hill: n = 41). Treatment consisted of a triple combination of two nucleoside RT-inhibitors with a protease inhibitor or nevirapine in 97 patients (14 nelfinavir; 55 indinavir; 23 ritonavir +/− saquinavir; four saquinavir; one nevirapine). One patient was receiving didanosine + hydroxyurea, one patient was on zidovudine + lamivudine + abacavir and 15 patients had a combination therapy with two reverse transcriptase (RT)-inhibitors. The median time of suppressed HIV-RNA in blood below 400 copies/ml was 7.2 months (range, 0–19.3). For comparison, HIV was measured in the semen of 55 drug-naïve HIV-positive men. The median concentration of HIV-RNA in blood of these untreated men was 4.8 $\log_{10}$ copies/ml.

HIV-RNA in seminal plasma was detected in only two of the 114 men [1.8%; 95% confidence interval (CI), 0–4.2%]. The detection frequency was 67% (36 of 55) in the group of untreated men. [odds ratio (OR), 0.01; 95% CI, 0–0.03; $P < 0.0001$, Fig. 1). The treatment

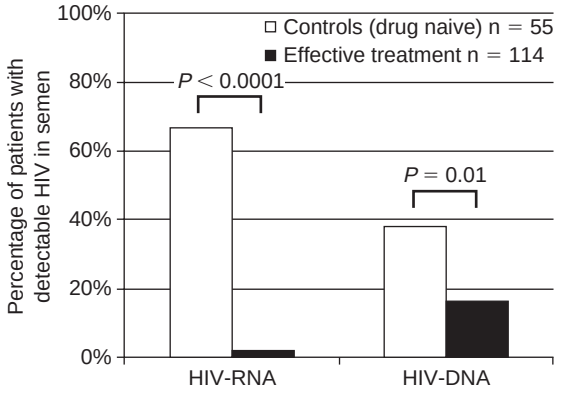

Fig. 1. Detection rate of HIV in semen. Detection rates of cell-free and cell-associated HIV in drug naïve historical controls (white) and treated individuals (black) in seminal plasma (HIV-RNA) and seminal cells (HIV-DNA). Seminal cells were only analysed in a subgroup of men from one centre.

regimen, duration of therapy and HIV-RNA levels in blood and semen of the two men who had detectable virus in semen despite blood level < 400 copies/ml are shown in Table 1. When the blood of these two patients was re-tested by ultrasensitive PCR, the HIV-RNA concentrations were 2.5 and 2.2 $\log_{10}$ copies/ml. Baseline samples (at the start of antiretroviral therapy) were available for these two patients. One man had a very high seminal viral load at the start of treatment, 2 $\log_{10}$ copies/ml above the value in blood. In the 55 treatment-naïve men, the HIV-RNA concentration was generally lower in semen than in blood with a median difference of 0.9 $\log_{10}$ copies/ml.

Seminal cells were available from a subgroup of 67 patients for HIV-DNA testing. Detection of cell-associated HIV-DNA in semen was significantly less frequent in patients receiving potent therapy compared with untreated individuals (11 of $67 = 16\%$ versus 21 of $55 = 38\%$; OR, 0.32; 95% CI, 0.12–0.80; $P = 0.01$). Cases with detectable HIV-DNA in semen had a shorter duration of therapy (median 5.3 months below detection level) in comparison with the cases with negative HIV-DNA detection in semen (median 9.2 months, $P = 0.09$).

Discussion

The purpose of this study was to determine the proportion of men with treatment-induced suppression of HIV-RNA < 400 copies/ml of blood who continued to have detectable HIV-RNA in seminal plasma. Such men, even if present in a substantial minority, may not have been detected in earlier small studies. A cut-off of 400 copies/ml was selected because lower detection levels have not been consistently achieved due to inhibitors present in semen [15].

In this large study, only two of 114 treated subjects with HIV-RNA < 400 copies/ml of blood had detectable HIV-RNA in the seminal plasma. In fact, all 97 men who were receiving at least three-drug therapy, which included a potent protease inhibitor or nevirapine, were below 400 copies/ml of semen. Given the size of our study, we can say with 95% confidence that in treated patients with a blood viral load below 400 copies/ml the proportion of men with detectable HIV-RNA in semen would not be greater than 4.2% in similar populations. As a point of reference, a group of previously studied drug-naïve asymptomatic, HIV-positive men were also studied. Seminal shedding in this group was by far more frequent (67 versus 1.8%) than in the study group but similar to previously reported studies of asymptomatic drug-naïve men [16,17].

The seminal HIV-RNA levels in the two subjects above the 400 copies/ml cut-off value were only slightly above the limit of detection of the assay and only marginally above the concentration found in the blood. In fact, the drug combinations used in these two patients are currently not recommended as highly potent antiretroviral drug combinations [18]. One of these men had substantially higher HIV RNA in semen than in blood prior to therapy. In men who demonstrate this phenomenon, suppression of HIV in the genital tract may be more difficult [19] and they may be at greater risk of shedding resistant virus [12].

Even under optimal conditions, recovery rates of replicating virus from seminal plasma are low [20].

Table 1. Summary of two patients with detectable HIV-RNA in semen under suppressive antiretroviral therapy.

	Week of therapy	HIV-RNA[a]		Treatment
		Blood	Semen	
Patient 1	0	3.4 (2500)	5.4 (2.5×10^5)	DDI + HU
	8	2.5[b] (300)	3.0 (1000)	
Patient 2	0	4.5 (30000)	3.0 (1000)	ZDV + DDC + SQV
	7	2.2[b] (160)	2.8 (630)	

[a] Log_{10} (copies/ml).
[b] Measured by ultrasensitive RNA-PCR, was negative by NucliSens (detection limit 2.6).
DDI, didanosine; HU, hydroxyurea; ZDV, zidovudine; DDC, zalzitabine; SQV, saquinavir.

Therefore, we and others have focused primarily on detection of HIV RNA in seminal plasma, as a reflection of viral burden [15–17]. Although vaginal transmission of simian immune-deficiency virus (SIV) is much easier by cell-free than cell-associated virus [21], it still remains uncertain, whether cell-associated HIV is an important vehicle for the sexual transmission of HIV. HIV can be isolated *in vitro* from seminal cells [22–24] and viral growth directly correlates with HIV-DNA detection in seminal cells [25].

In this study, we chose to assay for the presence of HIV-DNA in seminal cells. Detection of HIV DNA was significantly lower in treated patients when compared with the untreated men. However, HIV DNA was still detectable in a substantial proportion of men with HIV RNA < 400 copies/ml in blood. Although the significant difference of DNA detection in treated and untreated individuals and the decreasing likelihood of DNA detection with time on effective therapy are reassuring; the DNA assay used in this study was not quantitative and has limited sensitivity. Zhang *et al.* demonstrated the preservation of replication-competent HIV in seminal cells from three of seven men on highly active antiretroviral therapy (HAART) [9]. Intuitively, one would expect that treatment-associated reduction of HIV-DNA in semen results from suppression of HIV replication and that the number of infected cells would decrease over time. However, the effect of HAART on HIV-DNA shedding and the presence of infected cells in semen must be further evaluated in large longitudinal studies. It remains to be determined whether replication-competent HIV can be completely and permanently eradicated from the semen of patients on prolonged therapy, so as to eliminate this as a viral reservoir. Furthermore, the kinetics of the infected cell turnover and the presence of latently infected lymphocytes in the genital tract are unknown.

Sexual transmission of HIV almost certainly relates to the concentration of HIV in semen, as documented for transmission by all other routes [5]. HAART is now recommended for most patients with HIV infection, and the majority of patients have an excellent therapeutic response, with blood viral burden suppressed below detection with our most sensitive tests [14]. In this study we demonstrated with confidence that less than 4.2% of men on HAART who have HIV RNA below detectable levels in blood are likely to have detectable virus in semen. Absence of detectable HIV-RNA in semen certainly does not prove that such patients cannot transmit HIV. Indeed, we do not know the relative importance of cell-free and cell-associated virus for the sexual transmission of HIV, and we have emphasized the recovery of HIV DNA from seminal cells in some patients in whom no HIV-RNA is detected in blood and seminal plasma. Even if HIV-RNA is completely absent in semen, patients can still sexually transmit cells that contain the provirus. However, suppression of HIV in semen seems highly desirable, and ongoing biological and epidemiological studies should help to determine whether HAART reduces transmission of HIV within a population.

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Appendix

The members of the Swiss HIV Cohort Study are: M. Battegay (Co-Chairman of the Scientific Board), E. Bernasconi, Ph. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (Chairman of the Group Clinics), P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011- Lausanne), H.J. Furrer, P. Grob, B. Hirschel (Chairman of the Scientific Board), B. Ledergerber, R. Malinverni, L. Matter (Chairman of the Group Laboratories), A. Meynard, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, W. Pichler, J-C. Piffaretti, M. Rickenbach (Head of Data Center), P. Sudre, J. Schupbach, A. Telenti, P. Vernazza, R. Weber.