Effect of antiviral treatment on the shedding of HIV-1 in semen

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Objective: The potential role of antiretroviral treatment on the infectiousness of HIV-1-infected men was examined by studying the effect of antiviral treatment on the shedding of HIV-1 in semen.

Methods: Forty-four patients enrolled in various treatment protocols were asked to donate a semen sample before they began a new antiviral treatment and at a follow-up visit after 6 to 15 weeks of treatment. Since most patients were on blinded protocols, patients were stratified by response of blood viral load. The effect of each patient’s treatment was classified as good (n = 24), fair (n = 8) and marginal (n = 13) by measurement of the HIV RNA reduction in blood plasma (> 1.0 log10, 0.5–1.0 log10 and <0.5 log10 HIV RNA copies/ml reduction, respectively). The effect of treatment on shedding of HIV-1 in semen was documented by the reduction of HIV RNA concentration in seminal plasma and by quantitative HIV-1 seminal cell culture.

Results: Overall, antiviral treatment resulted in a significant fall in the viral load in semen (RNA and culture) that paralleled the reduction of viral load in blood. More pronounced reductions of HIV RNA in semen were observed as the effectiveness of treatment on blood HIV RNA levels increased (median drop from baseline 0, 0.3 log10 and 0.8 log10 RNA copies/ml in patients with marginal, fair and good treatment effect, respectively). Thirteen patients lost detectable HIV RNA in blood on treatment and all of these had undetectable levels of HIV-1 in semen by culture and RNA analysis at follow-up. In 19 of the 31 patients (62%) who still had HIV RNA in their blood during treatment, semen HIV levels were below detection in semen at follow-up.

Conclusions: Treatment-induced changes of HIV RNA concentration in blood are generally associated with a corresponding change in seminal HIV RNA. If confirmed in larger studies, potent antiretroviral therapy might reduce the spread of HIV-1.

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Keywords: Semen, sexual transmission, viral load, antiviral treatment, HIV RNA

Introduction

Recent advances in the development of antiviral treatment for HIV infection have resulted in therapeutic regimens that produce a marked suppression of viral replication and a significant extension of survival of infected individuals [1]. Suppression of HIV RNA in blood plasma to below the limit of detection has...
become a major goal of antiretroviral treatment strategies. Whether the benefit for the individual patient is matched by reduced spread of HIV-1 in the population is not known. Indeed, if transmission probability is not reduced by the treatment, the extension of the infectious period of infected individuals could lead to an increase of the AIDS epidemic [2,3]. Conversely, a treatment-induced reduction of the transmission probability might have a positive impact on public health. Preliminary evidence suggests that antiretroviral therapy might decrease transmission [4,5]. Because semen is an important vehicle for transmission of HIV-1 [3], we examined the effect of antiretroviral therapy on HIV RNA in blood and compared it with the shedding of HIV-1 in semen.

**Methods**

**Patients and antiretroviral therapy**

The study was conducted at the three participating HIV clinics (St Gallen, n = 16; Chapel Hill, n = 14; Zürich, n = 14). Antiretroviral-naive patients (n = 19) starting a new antiretroviral treatment or drug-experienced patients who were changing treatment regimens (n = 25) were asked to participate in the study. Blood and semen samples were obtained prior to the start of the new antiviral treatment and at regular intervals thereafter. Only patients who gave semen and blood samples at baseline and between 6 and 15 weeks thereafter were included in this analysis. When patients provided multiple post-treatment samples, the sample that was given closest to 10 weeks after baseline was selected as the follow-up sample. Patients were stratified into good (n = 24), fair (n = 7) and marginal (n = 13) treatment effect by measurement of the HIV RNA reduction in blood plasma (> 1.0 log_{10}, 0.5–1.0 log_{10} and <0.5 log_{10} copies/ml reduction, respectively). A more detailed presentation of the results from nine of the subjects was recently reported [5].

**Quantification of HIV RNA in blood**

Blood samples were drawn within 24 h of the semen collection. Plasma was stored at –75°C until used for HIV RNA detection. HIV RNA concentration in blood was determined using reverse transcriptase (RT)–polymerase chain reaction (PCR) assays [Roche, Basel, Switzerland (n = 35), Pharmacia Upjohn, Kalamazoo, Michigan, USA (n = 9)] [6]. The lower limit of detection of the RT-PCR assay was equivalent to 2.5 log_{10} copies/ml in the Roche assay.

**Processing of semen samples and semen microculture assay**

Semen samples were obtained by masturbation into a sterile container. A virus transport medium (penicillin plus streptomycin) was added to each sample. Semen samples were processed in the laboratory within 2–4 h of ejaculation. Semen samples were centrifuged at 1000 g for 10 min and seminal plasma was removed and frozen in portions at –75°C. Seminal cells were washed twice in phosphate-buffered saline (PBS) and half of the seminal cells were used for quantitative HIV culture. Culture of seminal cells were performed as previously described [7,8] and results were expressed as infectious units per ejaculate.

**Quantitative detection of HIV RNA in seminal plasma**

HIV-1 RNA in cell-free seminal plasma was quantified using NASBA (Organon Technika, Durham, North Carolina, USA), as previously described [8,9]. The lower limit of detection of this assay was 1000 copies/ml. The dilution factor of the semen samples with known amounts of transport medium was taken into account for the final calculation of the HIV RNA concentration in seminal plasma.

**Statistical methods**

The respective changes of HIV RNA concentration in semen and blood were compared in the baseline and follow-up samples. Samples with HIV RNA concentrations below the limit of detection were assigned the value of the detection limit. Fisher’s exact test or χ² test were used to compare distribution ratios. The Mann–Whitney U test was used to compare median values of RNA and the Mc-Nemar test to compare fractions of patients with undetectable RNA pre- and post-treatment. Wilcoxon’s signed rank test was used to compare pre- and post-treatment values from individual patients.

**Table 1. Baseline characteristics and antiviral treatment of study participants.**

<table>
<thead>
<tr>
<th>Treatment group (n)*</th>
<th>Drug-naive [(n (%)]</th>
<th>Asymptomatic stage (CDC AI n (%)]</th>
<th>Mean CD4 count (cells × 10^6/l)</th>
<th>Median HIV RNA (blood) (log_{10} copies/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (2)</td>
<td>0 (0)</td>
<td>1 (50)</td>
<td>100</td>
<td>3.6</td>
</tr>
<tr>
<td>B (5)</td>
<td>4 (80)</td>
<td>3 (60)</td>
<td>180</td>
<td>5.1</td>
</tr>
<tr>
<td>C (16)</td>
<td>3 (19)</td>
<td>3 (19)</td>
<td>110</td>
<td>4.6</td>
</tr>
<tr>
<td>D (21)</td>
<td>12 (57)</td>
<td>17 (81)</td>
<td>220</td>
<td>5.2</td>
</tr>
<tr>
<td>All patients (44)</td>
<td>19 (43)</td>
<td>23 (56)</td>
<td>170</td>
<td>4.9</td>
</tr>
</tbody>
</table>

*Treatment groups: A: monotherapy [zidovudine (1), indinavir (1)]; B: two reverse transcriptase inhibitors (RTI) (all zidovudine plus lamivudine); C: two RTI plus a protease inhibitor [ritonavir (14), indinavir (2)]; D: still blinded [zidovudine versus zidovudine plus zalcitabine versus zidovudine plus saquinavir versus zidovudine plus zalcitabine plus saquinavir (12); zidovudine versus zidovudine plus delavirdine (3); didanosine versus didanosine plus delavirdine (6)]. CDC, Centers for Disease Control and Prevention criteria.
Results

Forty-four patients were included in this study. Patients received either a triple-drug combination including a protease inhibitor (n = 14) or were assigned to one of four blinded protocols evaluating a combination of RT inhibitors with or without a protease inhibitor (n = 30). At the time of submission of the manuscript, the authors were still blinded for the treatment of 21 of the patients included in the study. Antiviral treatments used in this study are summarized in Table 1.

At baseline, HIV RNA was detectable in the blood of all patients, with a median concentration of 4.9 log_{10} copies/ml (range, 2.8–6.7). HIV-1 RNA was detected in seminal plasma in 30 patients (68%) and HIV-1 was recovered in 16 (37%) by HIV-1 coculture of seminal cells (Table 2). All 14 patients with undetectable HIV RNA in seminal plasma also had negative HIV-1 seminal cell cultures at baseline. These findings are consistent with our previous results of a cross-sectional analysis of 101 patients [7].

Antiviral treatment resulted in a significant reduction of the median HIV-1 RNA level in blood and semen. The proportion of subjects with detectable virus in semen (either by HIV RNA assay or by culture) was significantly lower at follow-up (Table 2). When the treatment effect in blood was stratified, the reduction of HIV RNA concentration in semen was most apparent in patients with good treatment effect in blood. The median change of HIV RNA in semen was 0.8 log_{10}, 0.3 log_{10} and 0 copies/ml in patients with good, fair and marginal treatment effect, respectively (Fig. 1). Thirty patients had detectable HIV RNA in semen at baseline. Among those 30 subjects, the median HIV RNA level in semen at baseline was slightly higher in the 17 patients with good treatment effect compared with the 13 patients for whom treatment was less effective (4.82 versus 4.56 log_{10} copies/ml). Despite this, individuals with good treatment effect were more likely to become HIV RNA-negative in the follow-up sample compared with subjects who received less effective therapy [Fig. 1; 14 out of 17 versus 6 out of 13; odds ratio (OR) 5.44, 95% confidence interval (CI): 1.04–28.5, \( P = 0.04 \)].

Under treatment, 13 patients had a decrease in HIV RNA levels in blood to below the limit of detection. In this group of patients, HIV-1 was detectable in the baseline semen samples in eight and four patients by RNA and culture analysis, respectively. None of these

### Table 2. Detection of HIV-1 in blood and semen pretreatment and on treatment.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Follow-up</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV RNA in blood plasma (n = 44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number detectable (%)</td>
<td>44 (100)</td>
<td>32 (73)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Median (log_{10} copies/ml)</td>
<td>4.96</td>
<td>3.51</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>Range (log_{10} copies/ml)</td>
<td>2.8–6.7</td>
<td>2.5–5.8</td>
<td></td>
</tr>
<tr>
<td>HIV RNA in seminal plasma (n = 44)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number detectable (%)</td>
<td>30 (68)</td>
<td>12 (27)</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Median (log_{10} copies/ml)</td>
<td>3.91</td>
<td>3.0</td>
<td>&lt; 0.001†</td>
</tr>
<tr>
<td>Range (log_{10} copies/ml)</td>
<td>&lt; 3.0–6.9</td>
<td>&lt; 3.0–6.7</td>
<td></td>
</tr>
<tr>
<td>Seminal cell culture (n = 43)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number detectable (%)</td>
<td>16 (37)</td>
<td>5 (12)</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Range (IUPE)</td>
<td>0–500</td>
<td>0–12</td>
<td></td>
</tr>
</tbody>
</table>

*Mc-Nemar test. †Wilcoxon rank signed test. IUPE, Infectious units per ejaculate.

Fig. 1. HIV RNA concentration in semen for patients were grouped by the effectiveness of treatment on blood HIV RNA levels, as described in the text. Each line represents one patient’s log_{10}-transformed HIV RNA levels (copies/ml) in semen at baseline (left) and during follow-up (right). Whisker’s plots at left and right represent the range of HIV RNA values at baseline and follow-up, respectively. Comparisons of semen HIV RNA values at baseline and post-treatment were made using Wilcoxon’s signed rank test.
13 patients had detectable HIV-1 in semen at follow-up either by HIV RNA measurement or by culture (Fig. 2). In contrast, among the remaining 31 subjects who did not achieve that degree of HIV RNA suppression in blood, clearance of HIV from semen was only observed in 12 out of 22 and 10 out of 12 patients by RNA or culture analysis, respectively ($P = 0.02$, in a comparison of RNA detection by Fisher’s exact test).

The treatment-induced reduction of HIV RNA in semen was compared with the corresponding reduction in blood in all patients where pre- and post-treatment measurements were above the detection limit of the method ($n = 10$). The change in HIV-1 RNA load associated with the antiviral treatment was similar in semen and blood (within 0.5 log$_{10}$ variation of the method) only one patient had a marked decrease (1.7 log$_{10}$ copies/ml) in HIV viral load in semen despite an insignificant (0.4 log$_{10}$ copies/ml) change in blood HIV-1 viral load. Although, in general, the drop of HIV RNA in semen parallels the drop in blood, the limited sample size of this study precludes any conclusions on the correlation between the two compartments.

**Discussion**

Measurement of HIV RNA reduction in blood is a direct reflection of the degree of therapeutic effect and is an excellent prognostic marker for the effect of treatment on clinical outcome [10,11]. Since most of our patients were on blinded protocols, we analysed the effect of therapy on HIV shedding in semen using the degree of viral load suppression in blood as a marker. We chose the value of $< 0.5$ log$_{10}$ copies/ml for marginal treatment effect since this is the biological variability of the assay and chose 1.0 log$_{10}$ for the cutoff between fair and good treatment effect. Treatment resulted in a significant reduction in the proportion of men with detectable HIV-1 in both blood and semen as well as in a significant drop of median HIV RNA concentrations in both compartments. Patients receiving effective treatment (as defined by a drop of blood HIV RNA of more than 1 log$_{10}$ copy/ml) were significantly more likely to have seminal HIV RNA levels below detection after treatment compared with patients who had a less pronounced treatment effect in blood. This longitudinal study supports previous results from several cross-sectional studies that have documented an association of antiviral treatment with lower detection rates of HIV in semen [7,12,15] and extends our preliminary longitudinal results on nine patients [5].

The decrease in HIV-1 in semen to levels below the limit of detection in the majority of men treated with effective antiretroviral therapy raises the possibility that effective antiviral treatment may result in a biologically relevant reduction of an individual’s sexual infectiousness for HIV. The significance of this finding, however, needs further evaluation. First, the concentration of HIV-1 in semen does not necessarily correlate with infectiousness. Second, the role of cell-free versus cell-associated virus in the transmission of HIV-1 has yet to be determined. Third, infectious HIV-1 may be present in genital secretions at levels below the detection limit of the methods used in this study. However, even though relative reduction in infectiousness of the donor may not reduce the chance of infection among discordant partners to zero, an overall reduction in infectiousness of HIV-positive persons in a population should result in a decrease in the spread (or reproductive rate) of the epidemic in that population[16].

Recent work from our group and others indicates that detectable amounts of HIV-1 in semen correlate with what is known about sexual transmission of HIV: the detection of HIV-1 both by seminal cell culture and HIV RNA measurement is enhanced in patients with low CD4 counts [7,12,17,18]. These observations correlate with epidemiological data showing increased transmission rates in partner studies from patients with lower CD4 counts and more advanced stages of disease [19–22]. Similarly, reduction of HIV RNA load in semen after treatment of sexually transmitted diseases [23] supports the findings of decreased transmission in populations who are treated for these diseases [24]. Longitudinal studies are under way to define further the role of HIV-1 in genital secretions for the transmission of HIV.

![Fig. 2. HIV RNA in semen (log$_{10}$ copies/ml) in 13 patients who had no detectable HIV RNA in blood during treatment. See Fig. 1 for details.](image-url)
In vitro studies indicate an important role of seminal lymphocytes for the transmission of HIV-1 to the cervical mucosa [25], though the precise contribution of cell-free and cell-associated virus in transmission of HIV-1 is unknown. The quantification of cell-free HIV RNA is more precise and sensitive than quantitative HIV culture from seminal cells. Even if future studies demonstrate that cell-free virus is not important for transmission, the correlation of HIV RNA detection with the cell culture method [7,18] supports the use of quantitative HIV RNA detection as a surrogate marker for the quantitative determination of cell-associated HIV-1 in semen. HIV-1 RNA levels in seminal plasma may also be a surrogate marker for infectiousness, just as HIV-1 RNA levels in blood have correlated with parental and vertical transmission [26–28]. The fact that RNA levels in blood correlate with RNA levels in seminal plasma, and blood RNA levels correlate with the likelihood of sexual transmission [29], also lends support to the hypothesis that HIV RNA levels in seminal plasma may correlate with infectiousness.

The inoculum needed for efficient transmission of HIV-1 is not known. One drawback of our study is the high level of HIV RNA required for detection in semen. However, the similarity in the degree of HIV RNA reduction in semen and blood, in the 10 patients where this comparison was possible, suggests that a potent suppression of blood viral load might be paralleled by a similar effect in semen.

One observation in our study that raises concern is the presence of detectable RNA in the semen of 10 men on therapy. The transmission of zidovudine-resistant virus has been documented [30]. The transmission of virus resistant to other agents, including protease inhibitors, could result in decreasing efficacy of antiretroviral treatment in the HIV-infected population over time.

This study provides evidence that effective antiviral treatment can be expected to have a beneficial impact on the spread of the epidemic. Long-term studies of the effect of antiretroviral therapy on HIV-1 levels in semen need to be conducted to confirm these findings and to evaluate whether suppression of HIV in semen is persistent over time and whether HIV-1 resistance emerges in semen.

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References


