Absence of HIV-1 shedding in male genital tract after 1 year of first-line lopinavir/ritonavir alone or in combination with zidovudine/lamivudine

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Background: New strategies such as boosted-protease inhibitor (PI) monotherapy are being investigated. However, a concern remains regarding the efficacy of this strategy in viral sanctuaries such as the male genital tract. More than 80% of untreated HIV-infected men have detectable HIV-RNA in semen and such a strategy could favour local selection of resistant variants, given the poor penetration of most PIs in semen.

Objectives: To evaluate the impact of a first-line lopinavir/ritonavir alone or standard triple combination on HIV-1 shedding in the genital tract.

Methods: HIV-1-infected men enrolled in the Monark randomized trial were eligible for the present study after 48 weeks of a first-line lopinavir/ritonavir alone or in combination with zidovudine and lamivudine. Single-paired samples of blood and semen were collected at week 48. Blood plasma HIV-RNA and seminal plasma HIV-RNA were measured at week 48. Lopinavir and ritonavir concentrations were measured in blood and in semen at week 48 by high-performance liquid chromatography.

Results: Ten patients were included: five of them received lopinavir/ritonavir monotherapy and five received a triple combination. At week 48, all patients had blood plasma HIV-RNA <1.7 log10 copies/mL. Median lopinavir and ritonavir concentrations were within the expected therapeutic target range in blood plasma (4896 and 130.5 ng/mL, respectively), whereas both lopinavir and ritonavir were undetectable in all seminal plasma samples (<30 ng/mL). All 10 patients had undetectable seminal plasma HIV-RNA at week 48 (<2.3 log10 copies/mL).

Conclusions: No local viral production was evident in semen, despite the local absence of therapeutic antiretroviral drug concentrations in the five patients receiving lopinavir/ritonavir alone.

Keywords: protease inhibitor, compartmentalization, semen, sanctuary site
Efficacy of LPV/r monotherapy in semen

Introduction

Long-term toxicities of prolonged use of combined antiretroviral drugs have led to evaluation of alternative treatment strategies for HIV-1-infected patients. Regarding this issue, monotherapy with ritonavir-boosted protease inhibitors (PIs) is being investigated. So far, ritonavir-boosted indinavir, atazanavir1,2 and lopinavir have been used as maintenance regimens in pre-treated patients with controlled HIV replication on a triple-drug regimen. In addition, lopinavir/ritonavir monotherapy has been investigated in antiretroviral-naive patients in the randomized Monark trial.3

One concern about boosted-PI monotherapy is its ability to control HIV-1 replication in sanctuary anatomical reservoirs such as the male genital tract. Indeed, drug disposition in semen is influenced by drug ionization, lipophilicity, molecular weight, the degree of protein-binding, affinity for membrane transporters and semen pH.4 The biochemical characteristics of most PIs suggest that they may not penetrate the blood–testis barrier well, being more lipophilic and extensively bound to blood plasma proteins. We and others have previously shown that the penetration of boosted amprenavir, saquinavir, lopinavir and atazanavir in semen was poor,5 contrasting with that of indinavir which achieved therapeutic concentrations in semen.5

Triple combination with two nucleoside reverse transcriptase inhibitors and one PI has been shown to efficiently reduce HIV-1 shedding in semen of most patients.6 However, little is known about the impact of PI monotherapy on HIV-1 shedding in semen, and the few available results are conflicting.1,2 Therefore, our objective was to evaluate, in HIV-1-infected men enrolling in the randomized Monark trial3 and starting a first-line therapy, the impact on HIV-1 shedding in the genital tract after 48 weeks of lopinavir/ritonavir alone or standard triple combination with lopinavir/ritonavir plus zidovudine and lamivudine.

Patients and methods

Participation in this study was proposed to all male patients enrolled in the Monark trial in four French clinical sites, after reaching 48 weeks on study treatment (lopinavir/ritonavir alone or lopinavir/ritonavir plus zidovudine and lamivudine). This study was approved by Bicêtre Hospital Ethics Committee. Ten men, with no clinical or biological signs of acute genital infection, were willing to participate and were included in this cross-sectional study after giving written informed consent. At inclusion in the Monark trial, their median blood plasma HIV-RNA was 4.36 log10 copies/mL (range 4.00–4.88) and median CD4 cell count was 221 cells/mm3 (range 132–289). All patients received 100 mg ritonavir and 400 mg lopinavir twice daily with the fixed co-formulation of lopinavir/ritonavir soft gel capsules (Kaletra®). Patients randomized in the triple combination arm also received 300 mg zidovudine and 150 mg lamivudine, both twice daily in a fixed combination (Combivir®).

Five patients were assigned to receive lopinavir/ritonavir alone, and the five remaining patients were randomized in the triple combination arm. At week 48, adherence to study drugs was 100% (self-questionnaire on missed doses during the previous 4 days). Steady-state conditions were ensured. Single-paired samples of blood and semen were collected the same day within 1 h at the week 48 visit (±2 weeks), always before the morning drug intake. Semen was obtained by masturbation after a recommended 2 day period of sexual abstinence. Blood and semen were processed for viral quantification as described elsewhere.7 The lower limit of quantification of HIV-RNA was 50 copies/mL in blood plasma, and for semen that requires dilution in RPMI to decrease viscosity, the lower limit of quantification was 200 copies/mL. Blood plasma and seminal plasma were assessed for pharmacological measurements of lopinavir and ritonavir using a validated specific reverse-phase high-performance liquid chromatography assay coupled with ultraviolet photodiode array detection as described previously.8 After the liquid–liquid phase extraction, no matrix effect between blood plasma and seminal plasma was detected. In both matrices, lower limit of quantification was 30 ng/mL for both lopinavir and ritonavir.

Results

The results of concentrations of lopinavir and ritonavir in blood plasma and in seminal plasma are summarized in Table 1.

All 10 patients had blood plasma HIV-RNA <50 copies/mL at week 48, and the median plasma lopinavir trough concentration (4896 ng/mL) was above the recommended cut-off target of 3000 ng/mL according to the French guidelines. Plasma lopinavir trough concentrations did not differ between patients receiving monotherapy or triple combination. At week 48 in seminal plasma, all 10 patients had HIV-RNA <200 copies/mL and undetectable lopinavir and ritonavir concentrations.

Discussion

This is the first study evaluating, in antiretroviral-naive patients starting a first-line single agent boosted PI, the impact of 1 year of lopinavir/ritonavir alone on HIV-1 shedding in the male genital tract. Moreover, this is the first study in the male genital tract with data on viral quantification and pharmacological measurements in the context of a first-line boosted-PI

Table 1. Lopinavir and ritonavir concentrations in blood plasma and in seminal plasma at week 48

<table>
<thead>
<tr>
<th>Patient</th>
<th>Concentrations (ng/mL)</th>
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<th>LPV</th>
<th>RTV</th>
<th>LPV</th>
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<tr>
<td></td>
<td>blood plasma</td>
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</table>

RTV, ritonavir; LPV, lopinavir; MED, median.
monotherapy. Lopinavir, like most other PIs, is a large hydrophobic molecule that is highly bound to plasma proteins and is a substrate for the multidrug resistance pump (P-glycoprotein). Hence, the pharmacological properties of lopinavir make some viral reservoirs (such as the central nervous system and the male genital tract) virtually inaccessible to this drug. This is of major interest, since sexual transmission is the main route of infection worldwide. In addition, assessing HIV-RNA and antiretroviral drug quantification in sanctuary sites through non-invasive procedures is more feasible by exploiting semen, the central nervous system requiring invasive collection of cerebrospinal fluid via lumbar puncture.

HIV in semen clearly represents some combination of compartmental production and diffusion. Interestingly, despite the lack of penetration of lopinavir in seminal plasma of the five men on lopinavir/ritonavir alone, we did not detect HIV-RNA in seminal plasma, suggesting that, in these selected patients with no acute genital infection, HIV-1 in semen originated from passive diffusion from blood plasma rather than compartmentalization with local viral production. Indeed, HIV-RNA free particles are detected in seminal plasma of >90% of untreated men or patients receiving suboptimal treatment. Although we did not measure HIV-RNA in seminal plasma prior to antiretroviral treatment initiation, we can assume that HIV-RNA was locally detectable. Thus, the optimal suppression of HIV-1 replication in blood plasma probably caused the absence of detection of HIV-RNA in semen. The five patients receiving conventional triple combination had undetectable levels of HIV-RNA both in blood and seminal plasma. Undetectable HIV-RNA in the semen of these five patients could be attributed to both the activity of zidovudine/lamivudine and the full suppression of viral replication in blood.

Our assay was able to detect lopinavir concentrations as low as 30 ng/mL. It could be argued that effective drug concentrations required in seminal plasma might be lower than those usually expected in blood plasma, given the relatively protein-free environment in seminal plasma. However, early data from the manufacturer suggest that the IC_{50} values in the presence and absence of 50% human serum are 63 and 14 ng/mL, respectively. Correcting the latter value for 98% protein binding gives an estimated protein-binding-corrected IC_{50} of 692 ng/mL (Abbott Laboratories, data on file: ABT-387/r in PI Experienced Patients, Study M97-765; Abbott Laboratories, Abbott Park, IL, USA). These data suggest a poor penetration of lopinavir in compartments and if the membrane is intact, direct antiviral efficacy of lopinavir in the sanctuary of the male genital tract is probably unlikely.

Two small studies describing seminal plasma antiretroviral activity of a boosted PI when used as sole agent are available but are limited by short duration and absence of determination of drug exposure in the genital tract. Moreover, these two studies involved patients who had an already suppressed HIV replication on a triple combination before switching to boosted-PI monotherapy. These two studies provided conflicting results, one showing no detection of HIV-RNA in seminal plasma of eight patients after 24 weeks on boosted atazanavir monotherapy, whereas in the other study, high levels of HIV-RNA were detected in seminal plasma of 2/15 patients tested at week 24, despite full viral suppression in blood. Thus, while our results with lopinavir/ritonavir are reassuring, they might not be extended to the whole PI class.

This study was a cross-sectional one and the results are based on a single determination of virus titre in semen after 48 weeks of therapy. Few studies have performed viral quantification on multiple semen samples from the same patient taken over a short-time interval, and the belief that HIV-1 shedding is constant remains controversial. However, in a recently published study assessing viral dynamics of HIV viral load in blood and semen of patients under highly active antiretroviral therapy, HIV-RNA titres in blood and semen were stable throughout therapy in patients with available longitudinal samples.

In conclusion, in this small study, no local viral production was detected in semen, despite the local absence of therapeutic antiretroviral drug concentrations in the five patients receiving lopinavir/ritonavir alone. However, no safety conclusions can be drawn given the small sample size, and the selection of patients free from sexually transmitted diseases. Further work to better characterize lopinavir disposition and its antiretroviral activity in semen is warranted to monitor the role of local drug-selective pressure on the risk of persisting local replication and emergence of viral resistance in the male genital tract.

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Transparency declarations

I. C.-C. is an employee of Abbott. All other authors have none to declare.

References


