Penetration and antiviral efficacy of total and unbound maraviroc, raltegravir and rilpivirine in both female and male genital fluids from HIV-positive patients receiving regimens containing these antiretrovirals

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Background: Sub-optimal penetration of antiretroviral drugs in genital compartments might promote local HIV persistence and increase the risk of HIV transmission.

Objectives: To describe the penetration of maraviroc, raltegravir, raltegravir glucuronide and rilpivirine in seminal plasma and cervico-vaginal secretions (CVS) and to assess local antiretroviral efficacy in HIV-1-positive patients.

Methods: This was a prospective, multicentre study. Inclusion criteria were HIV-1 positive, age >18 years, receiving regimens containing maraviroc and/or raltegravir and/or rilpivirine for >1 month, and good self-reported adherence. Paired blood and genital samples were collected 12 h (raltegravir and maraviroc) or 24 h (rilpivirine) post-dose. These concentrations were determined (UPLC–MS/MS) in blood and seminal plasma (total and unbound) and CVS (total, dried spots) and HIV-RNA was quantified in paired blood and genital samples.

Results: Among the 54 enrolled patients, 15 received maraviroc (6 men), 27 received raltegravir (14 men) and 20 received rilpivirine (10 men), corresponding to 54 total and 52 unbound plasma concentrations, 29 total CVS samples and 23 total and 18 unbound seminal plasma samples. Maraviroc and raltegravir displayed a ratio of genital fluids/plasma concentrations >0.5 in both male and female genital tracts. Conversely, rilpivirine displayed a low ratio. Antiretroviral free fractions were consistent with historical data. Nine patients had blood plasma HIV-RNA >50 copies/mL (2/9 had sub-optimal antiretroviral blood plasma exposure) and two other patients had detectable HIV-RNA in genital fluids.

Conclusions: Maraviroc and raltegravir demonstrated good penetration in genital compartments, yielding good local virological response in genital compartments, whereas rilpivirine presented a low penetration profile but good local response.

Introduction
The advent of highly active combined ART (cART) regimens raises the issue of anatomical sanctuary sites (including male and female genital tracts) where the different antiretroviral compounds might not be as bioavailable and effective as in the systemic compartment. In the past, discordant patterns of virological response or
genotypic drug susceptibility profiles were reported between plasma and sanctuary sites. In deep compartments, HIV replication might be autonomous because of the specific immune pressure or antiretroviral drug availability. As HIV infection is mostly acquired through sexual transmission, genital fluids might be one of the main vectors of transmission. Also, optimal genital exposure might decrease the risk of mother-to-child transmission in the case of vaginal delivery. Maraviroc (CCR5 antagonist), raltegravir (inhibitor integrase) and rilpivirine (NNRTI) are largely prescribed in association with other antiretrovirals for the treatment of HIV-1 infection. Data on their penetration in genital fluids are still scarce and wide differences in distribution between drugs with regard to their physicochemical and pharmacokinetic characteristics have been reported.

The active form of most of the antiretrovirals is the unbound fraction, which might theoretically penetrate through physiological barriers into the sanctuary sites. The objectives of this study were to describe, in HIV-1-infected patients, the penetration of maraviroc, raltegravir and rilpivirine in semen and cervico-vaginal secretions (CVS) and to assess the efficacy of regimens containing these drugs on HIV-1 viral load measured in both genital fluids.

Materials and methods

In this prospective, multicentre study, HIV-1-infected patients were eligible if they met the following criteria: age >18 years; receiving stable regimens containing maraviroc (150 mg twice daily with PI/ritonavir or 300 mg twice daily without PI/ritonavir) and/or raltegravir (400 mg twice daily) and/or rilpivirine (25 mg once daily) for >1 month; and good self-reported adherence. In addition, women had to have (i) a negative pregnancy test, (ii) absence of metrorrhagia and (iii) absence of sexual intercourse or intra-vaginal wash/treatment within 48 h before sampling. Patients with symptoms suggestive of sexually transmitted infections were excluded.

Paired blood and genital samples (semen and CVS) were collected 12 h (maraviroc and raltegravir) or 24 h (rilpivirine) post-dose. Semen was obtained by auto-masturbation (after a recommended 48 h abstinence period) in sterile containers and processed within 1 h according to WHO recommendations. Seminal plasma was recovered after centrifugation (300 g, 20 min) on a two-layer Percoll discontinuous gradient (Sigma–Aldrich Chimie, France) then stored at −80 °C. CVS were obtained through cervico-vaginal lavage and cytobrush.

Minimal [24 or 12 h post-dose (C_{24/C_{12}})] antiretroviral concentrations (maraviroc, raltegravir, rilpivirine glucuronide, darunavir, ritonavir, rilpivirine and the associated NRTIs abacavir, emtricitabine, lamivudine and tenofovir) in blood and seminal plasma (total and unbound) and CVS (total) were determined by UPLC–MS/MS (Waters Acquity UPLC-TQD) with some modifications. Plasma protein binding analysis involved an ultrafiltration assay (Centrifree, Millipore) (coefficient of variation 15%). Ratios of C_{min} (genital fluids)/C_{min} (blood plasma) and free fractions in blood and seminal plasma were calculated. Interpretation of antiretroviral C_{24}/C_{12} was performed according to efficacy thresholds: maraviroc, raltegravir, rilpivirine glucuronide and rilpivirine C_{24}/C_{12} thresholds were set as 50 ng/mL [membrane CCR5 on all CD4 cells should be saturated by a maraviroc threshold of 10 ng/mL, a raltegravir threshold of 15 ng/mL (the IC_{50} in 50% human serum) and a rilpivirine threshold of 12 ng/mL (the IC_{50} in 50% human serum)].

HIV-RNA was quantified in seminal plasma, CVS and blood plasma. Quantification of HIV-1 RNA in blood plasma, seminal plasma and CVS was performed using COBAS AmpliPrep/TaqMan HIV-1 Test v2.0 (Roche Diagnostics, France). For blood plasma and CVS, the threshold was <50 copies/mL. To avoid PCR inhibition in the seminal plasma, a dilution (1/3 or 1/5 or 1/10) was performed. The threshold was <60, <100 or <200 copies/mL depending on the dilution.

Descriptive statistics and non-parametric Mann–Whitney tests were performed. Results are presented as median (IQR).

Ethics

All participants provided written informed consent and the study was approved by Cochin ethics committee.

Results

Fifty-four patients (29 women) were enrolled. Their median age was 45 years (40–49) [men 48 years (44–53), women 45 years (40–46)]. Among the 54 enrolled patients, 15 received maraviroc (6 men), 27 received raltegravir (14 men) and 20 received rilpivirine (10 men), corresponding to 54 total and 52 unbound plasma concentrations, 29 total CVS samples and 23 total and 18 unbound seminal plasma samples.

All total plasma maraviroc C_{12}, raltegravir C_{12} and rilpivirine C_{24} values post-dose were above efficacy thresholds except for raltegravir, with two patients presenting a raltegravir C_{12} <14 ng/mL. Median concentrations were 52 ng/mL (33–83; n = 15) for maraviroc C_{12}, 63 ng/mL (34–98; n = 27) for raltegravir C_{12}, 133 ng/mL (59–330; n = 27) for raltegravir glucuronide C_{12} and 111 ng/mL (66–163; n = 20) for rilpivirine C_{24}. However, 4 samples out of the 13 (31%) available presented unbound maraviroc C_{12} <10 ng/mL. CVS/blood plasma and seminal plasma/blood plasma ratios are summarized in Figure 1. From the dried spots of CVS, all antiretroviral total concentration ratios (maraviroc, raltegravir, rilpivirine glucuronide, rilpivirine, abacavir, lamivudine, emtricitabine and tenofovir) were >1.0. In seminal plasma, all ratios except for maraviroc and rilpivirine were >1.0. In blood plasma, free fractions were as follows: maraviroc 0.32 (0.30–0.39; n = 11); raltegravir 0.19 (0.16–0.22; n = 27); raltegravir glucuronide 1.19 (0.94–1.58; n = 27); and rilpivirine 0.01 (0.01–0.02; n = 20). In seminal plasma, free fractions were as follows: maraviroc 0.89 (0.79–0.92; n = 3); raltegravir 1.06 (0.94–1.18; n = 8); raltegravir glucuronide 0.24 (0.16–0.34; n = 8); and rilpivirine 0.05 (n = 1).

Cases with detectable HIV-RNA in blood and seminal plasma are described with antiretroviral C_{12}/C_{24} in Table 1. Nine patients (six men) presented detectable blood plasma HIV-RNA: 86 copies/mL (71–161). Among them, four had a regimen containing rilpivirine, three had a regimen containing raltegravir, one had a regimen containing maraviroc and one had a regimen containing both maraviroc and raltegravir. All HIV-1 RNAs tested in CVS were <50 copies/mL. Despite a blood plasma HIV-RNA <50 copies/mL, 3/48 samples corresponding to two patients (Patients 31 and 32; details of ART, plasma HIV RNA and antiretroviral concentrations are in Table 1) demonstrated a detectable seminal plasma HIV-RNA (amplification failed), yielding a prevalence of 6.25%.

Discussion

In our study, total blood plasma maraviroc, raltegravir and rilpivirine concentrations were considered as adequate and other antiretroviral concentrations were consistent with previous
studies. Minimal concentrations in both blood plasma and genital fluids were used for the assessment of the tissue penetration ratio. Interestingly, we showed a high penetration of maraviroc and raltegravir in both CVS and seminal plasma. However, concentrations of rilpivirine in genital fluids were low. Our results for seminal/blood plasma concentration ratios were consistent with previous studies. An increase in the protein free ratio was observed for maraviroc, raltegravir, raltegravir glucuronide and rilpivirine in seminal plasma according to the different proteins' composition in comparison with blood plasma. Indeed, seminal concentration of protein ranges from 20 to 60 g/L, which is significantly lower than in blood plasma. Furthermore, the main discrepancy between raltegravir and maraviroc ratios on the one hand and rilpivirine's on the other, might be explained by their respective binding to plasma proteins (83%, 76% and >99%, respectively) and their respective plasma elimination half-lives (9, 14 and 50 h, respectively; from Isentress®, Selzentry® and Edurant® FDA label information). Since the sampling method for CVS was different from most of the published techniques (i.e. direct aspiration etc.), our ratios could not be directly compared with previous studies. Nevertheless, the rank order of the different compounds was consistent with previous reports (Figure 1).

In total, 87% of our patients had a blood plasma HIV-RNA <50 copies/mL; three women and six men presented with a blood plasma HIV-RNA >50 copies/mL. Among these patients, two had sub-optimal antiretroviral C<sub>24</sub>/C<sub>12</sub> in blood plasma. Among the 54 patients, only 2 men presented detectable levels of HIV-1 RNA in seminal plasma. In the first patient, plasma antiretroviral C<sub>24</sub>/C<sub>12</sub> was interpreted as suboptimal; in the second patient, plasma antiretroviral exposure was interpreted as optimal, but the antiretroviral regimen based on the dual combination of maraviroc and raltegravir might not be adequate to suppress local HIV variants in the genital reservoir. In this study of HIV-infected men on cART with sustained control of HIV-1 replication in blood plasma, the prevalence of detected intermittent HIV-1 RNA in seminal plasma was 6.5%. It was very close to the results obtained in semen of HIV-1 treated MSM. We demonstrated again that HIV-1 RNA shedding in semen was intermittent.

In conclusion, maraviroc and raltegravir penetrate well in the genital tract, with ratios suggesting a potential efficacy of these compounds in genital fluids. Despite the fact that rilpivirine is highly bound to blood plasma protein, its long plasma elimination half-life might allow it to be present and probably also effective in these anatomical sanctuary sites, suggesting its potential use in pre-exposure prophylaxis strategies. We reported a high proportion of patients with undetectable viral load in genital fluids,

**Figure 1.** Distribution of total antiretroviral tissue penetration ratios from female and male genital tracts, total and unbound seminal plasma/unbound blood plasma ratios.
explained by a controlled viraemia consecutive to optimized and effective cART. However, the risk of a sequestrated resistant HIV strain archived during previous treatment lines should be considered, in particular in patients receiving a dual- or single-agent maintenance strategy.

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Table 1. Description of patients with detectable HIV-1 RNA in plasma or genital secretions and respective ART, plasma concentrations and genital tracts/blood plasma concentrations ratios

<table>
<thead>
<tr>
<th>Patient</th>
<th>Plasma HIV-1 RNA (copies/mL)</th>
<th>ART daily doses</th>
<th>Total antiretroviral plasma C12/C24 (ng/mL)</th>
<th>Genital secretion HIV-1 RNA (copies/mL)</th>
<th>Total genital secretion/plasma C12/C24 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>DRV/RTV 600/100 mg bid + RAL 400 mg bid</td>
<td>DRV C12 4758 RTV C12 717 RAL/G-RAL C12 81/465</td>
<td>&lt;50</td>
<td>DRV 0.8 RTV 0.7 RAL/G-RAL 50.2/20.7</td>
</tr>
<tr>
<td>13</td>
<td>58</td>
<td>FTC/TDF 200/300 mg bid + MVC 300 mg bid + RAL 400 mg bid</td>
<td>FTC C24 5 TFV C24 5 MVC C12 35 RAL/G-RAL C12 125/251</td>
<td>&lt;50</td>
<td>FTC 7.4 TFV 29 MVC 10.6 RAL/G-RAL 2.3/3.6</td>
</tr>
<tr>
<td>45</td>
<td>70</td>
<td>FTC/TDF/RPV 200/300/25 mg bid</td>
<td>FTC C24 153 TFV C24 39 RAL/G-RAL C24 154</td>
<td>&lt;50</td>
<td>FTC 4.9 TFV 12.2 RAL/G-RAL 0.10</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>168</td>
<td>ABC/3TC 600/300 mg bid + RAL 400 mg bid</td>
<td>ABC C24 5 3TC C24 5 RAL/G-RAL C12 84</td>
<td>&lt;100</td>
<td>ABC 1.8 3TC 13.7 RAL/G-RAL 4.3/53.5</td>
</tr>
<tr>
<td>28</td>
<td>74</td>
<td>MVC 300 mg bid + RAL 400 mg bid</td>
<td>MVC C12 84 RAL/G-RAL C12 96/648</td>
<td>&lt;100</td>
<td>MVC 4.2 RAL/G-RAL 31.0/5.1</td>
</tr>
<tr>
<td>31</td>
<td>&lt;50</td>
<td>FTC/TDF 200/300 mg bid + MVC 300 mg bid + RAL 400 mg bid</td>
<td>FTC C24 32 TFV C24 35 MVC C12 25 RAL/G-RAL C12 57-54</td>
<td>60</td>
<td>FTC 16.1 TFV 4.4 MVC 2.2 RAL/G-RAL 8.8/36.1</td>
</tr>
<tr>
<td>32</td>
<td>&lt;50</td>
<td>MVC 300 mg bid + RAL 400 mg bid</td>
<td>MVC C12 78 RAL/G-RAL C12 48/116</td>
<td>320</td>
<td>MVC 0.3 RAL/G-RAL 0.2/0.4</td>
</tr>
<tr>
<td>35</td>
<td>2685</td>
<td>FTC/TDF/RPV 200/300/25 mg bid + DRV/RTV 600/100 mg bid + MVC 150 mg bid</td>
<td>FTC C24 185 TFV C24 108 RAL/G-RAL C12 1325</td>
<td>&lt;100</td>
<td>FTC 3.5 TFV 0.1 MVC 0.4 RAL/G-RAL 0.1/0.07</td>
</tr>
<tr>
<td>53</td>
<td>86</td>
<td>FTC/TDF/RPV (200/300/25 mg)</td>
<td>FTC C24 146 TFV C24 63 RAL/G-RAL C24 127</td>
<td>&lt;60</td>
<td>FTC 3.8 TFV 0.3 RAL/G-RAL 0.01</td>
</tr>
<tr>
<td>59</td>
<td>71</td>
<td>FTC/TDF/RPV (200/300/25 mg)</td>
<td>FTC C24 65 TFV C24 36 RAL/G-RAL C24 67</td>
<td>&lt;100</td>
<td>FTC 8.3 TFV 13.3 RAL/G-RAL 0.01</td>
</tr>
<tr>
<td>60</td>
<td>153</td>
<td>FTC/TDF/RPV (200/300/25 mg)</td>
<td>FTC C24 187 TFV C24 80 RAL/G-RAL C24 268</td>
<td>&lt;60</td>
<td>FTC 8.3 TFV 0.1 RAL/G-RAL 0.08</td>
</tr>
</tbody>
</table>

ABC, abacavir; DRV, darunavir; FTC, emtricitabine; MVC, maraviroc; RAL, raltegravir; G-RAL, raltegravir glucuronide; RTV, ritonavir; RPV, rilpivirine; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; 3TC, lamivudine; qd, once a day; bid, twice a day.

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