Concentrations of Tenofovir and Emtricitabine in Saliva: Implications for Preexposure Prophylaxis of Oral HIV Acquisition

Victoire de Lastours,1* Julien Fonsart,2 Ruxandra Burlacu,1 Bernard Gourmel,2 and Jean-Michel Molina1

Infectious Diseases Department, Saint-Louis Hospital, Paris, France,1 and Biochemistry Department, Saint-Louis Hospital, Paris, France2

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To prevent acquisition of HIV through oral sex, drugs used for preexposure prophylaxis (Prep) need to diffuse in saliva. We measured tenofovir (TFV) and emtricitabine (FTC) concentrations simultaneously in the plasma and saliva of 41 HIV-infected patients under stable antiretroviral treatment. Mean ratios of saliva/plasma concentration were 3% (±4%) and 86.9% (±124%) for TFV and FTC, respectively. Tenofovir disoproxil fumarate (TDF) should be used in combination with FTC to prevent oral acquisition of HIV.

Thirty years into the HIV pandemic, a high incidence of new HIV infections persists, particularly in the subgroup of men having sex with men (MSM), where HIV transmission is sustained (16). Innovative approaches to prevent HIV transmission include the use of antiretrovirals as preventive tools, known as preexposure prophylaxis (Prep), either as oral pills or topical microbicides containing an active antiviral agent (13). Recent studies have shown a partial reduction in HIV acquisition in women using a vaginal tenofovir gel (1) and in MSM taking a daily oral tenofovir disoproxil fumarate (TDF)-emtricitabine (FTC) combination pill (11), the favored combination for Prep.

While it is agreed that oral sex carries a much lower HIV infection risk than vaginal and anal sex, studies have convincingly demonstrated that both insertive and receptive oral sex are independent risk factors for transmission of HIV (6, 19, 20). The risk increases with frequency of activity, the presence of oral ulcers, oropharyngeal inflammation, or sexually transmitted infections in the oropharynx. This route of transmission should not be underestimated, in particular in MSM, where oral sex is rarely protected (8). In order for Prep to prevent HIV transmission through oral sex also, antiviral drugs should be taken orally, and their saliva concentrations should be sufficient to inhibit viral replication. Yet, no data are available on the penetration of tenofovir (TFV) and FTC in saliva. The aim of this work is to measure TFV and FTC saliva concentrations and saliva-to-plasma ratios in HIV-infected patients receiving either a TDF- or a TDF-FTC-based treatment.

HIV-infected patients regularly followed in our HIV clinic were included if they were receiving a stable antiretroviral regimen, including 245 mg TDF daily and/or 200 mg FTC daily for at least 3 months, and gave informed consent. The study was approved by the Paris Saint-Louis Institutional Review Board. Blood and saliva samples were collected simultaneously during a routine follow-up evaluation of their HIV infection. Saliva samples were obtained by having participants spit 10 ml saliva in a sterile pot. To confirm that saliva had been sampled, samples were tested by validated colorimetric assays for total proteins (Biuret test) and saliva α-amylase (S type, enzymatic method) levels, using a Roche Modular analyzer (Roche Diagnostics France, Meylan, France). Both plasma and saliva TFV and FTC levels were measured by a validated high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS-MS), adapted from Le Saxe et al. (15). The plasma assay was linear over the range of 4 (lower limit of quantification [LLOQ]) to 400 ng/ml for TFV and 15 (LLOQ) to 1,500 ng/ml for FTC. The saliva assay was linear over the range of 0.25 (LLOQ) to 50 ng/ml for TFV and 2 (LLOQ) to 2,000 ng/ml for FTC. A Student t test was used to compare saliva and plasma concentrations for each drug and to compare saliva-to-plasma ratios between TFV and FTC. A P value of 0.05 was considered significant.

Forty-one HIV-infected patients accepted to participate. Clinical characteristics are reported in Table 1. Plasma and saliva samples were obtained between 2 and 26 h following drug intake. The time since the last treatment intake was a median of 11.5 h. Amylase and protein concentrations in saliva samples were in the expected ranges for all patients (36,206 to 40,5230 IU/liter and 0.38 to 2 g/liter, respectively). Results of TFV and FTC concentrations in saliva and plasma and the saliva-to-plasma ratios are reported in Fig. 1 and Table 2. Plasma drug concentrations were above the estimated median inhibitory concentration (IC50) for wild-type HIV in all patients for both TFV and FTC (10 ng/ml for TFV and 2 ng/ml for FTC) (12). In saliva, only one patient had TFV levels above the IC50, whereas FTC levels were above the IC50 for all patients. Saliva-to-plasma ratios were significantly different between TFV and FTC (P < 0.001), with a very poor diffusion of TFV in saliva, while a high proportion of FTC plasma concentration diffused in saliva.

This work is the first to address the question of saliva penetration of TFV and FTC, the favored candidate drugs for Prep. TFV saliva penetration is insufficient to inhibit viral replication, whereas concentrations of FTC in saliva, although

* Corresponding author. Mailing address: Infectious Diseases Department, Hôpital Saint-Louis, Assistance-Publique Hôpitaux de Paris, 1 Avenue Claude Vellefaux, 75010 Paris, France. Phone: 33 1 42 49 45 72, Fax: 33 1 42 49 48 20. E-mail: vdelastours@hotmail.com.

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beneath the IC\textsubscript{50} for wild-type HIV, whereas FTC penetration of TFV is also poor, with concentrations consistently lower than those of TFV. A recent work has shown that TFV diffused relatively well, reaching concentrations above the IC\textsubscript{50} of TFV in saliva (23). FTC seems to be more suitable to prevent HIV acquisition because it is phosphorylated intracellularly and saliva is a mostly nonionized medium.

It may be surprising that TFV penetrates so little in the saliva. Indeed, TFV has shown high rates of penetration in semen, male and female genital tracts, rectal tissue, and amniotic fluid in pregnant women (22). A recent work has shown, however, very poor diffusion of TFV in breast milk, whereas FTC diffusion was relatively well, reaching concentrations above the virus IC\textsubscript{50} (2). Additionally, cerebrospinal fluid (CSF) penetration of TFV is also poor, with concentrations consistently lower than those of FTC in blood (23).

Many factors are important in the salivary distribution of drugs, such as protein binding, hydrophilicity, ionization, and molecular mass, which may interfere with saliva concentrations of TFV and FTC (17). TFV and FTC poorly bind to human plasma proteins (<0.7% and <4%, respectively). Therefore, low protein concentrations in saliva may not explain such low levels of TFV concentrations. Additionally, both drugs have similar log half-lives (3, 12). In contrast, because it is already phosphorylated, TFV is more hydrophilic (partition coefficient log P of -1.6) than FTC (log P of -0.43), which is not yet phosphorylated. Because ionization increases hydrophilicity, only nonionized drugs diffuse through biological membranes. Based on the molecules’ known pK\textsubscript{a} and the usual pH of saliva, TFV will have a much lower ionization rate in saliva than FTC (3, 12). We hypothesize that the higher hydrophilicity and lower ionization of TFV may be among the factors influencing its low diffusion in saliva by increasing its reabsorption compared to that of FTC.

Protease inhibitors (PI) lopinavir and ritonavir also penetrate poorly in saliva (9) and semen (10), probably because they are extensively bound to plasma proteins. Maraviroc is another drug favored for Prep because of its activity on C5 viruses. Recently, it was reported that saliva-to-plasma concentration ratios of maraviroc in 12 HIV-negative individuals was only 30% (4). Conversely, more than 90% of plasma concentrations of nevirapine can be found in saliva, with a high level of correlation with plasma levels, allowing the development of simple dosing methods in developing countries (18).

Although only one saliva sample and one plasma sample were available for each patient in this study and the number of patients tested was limited, the saliva-to-plasma ratios remained stable for both TFV and FTC, as we had ensured that all patients had steady-state conditions by selecting patients on stable antiretroviral regimens for at least 3 months.

In conclusion, our study demonstrates that FTC diffuses relatively well in saliva, whereas diffusion of TFV is poor. In a Prep perspective, the use of a combination pill of TFV and FTC seems to be more suitable to prevent HIV acquisition...
through oral sex than the use of TFV alone. Further studies of TFV and FTC concentrations in the saliva of volunteers are warranted to confirm these findings.

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### REFERENCES


### TABLE 2. Plasma and saliva concentrations of tenofovir (TFV) and emtricitabine (FTC) following oral drug administration

<table>
<thead>
<tr>
<th>Drug</th>
<th>Plasma concn (ng/ml)</th>
<th>Saliva concn (ng/ml)</th>
<th>Saliva/plasma ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>TFV (n = 41 patients)</td>
<td>98.62</td>
<td>51.5</td>
<td>7.7–279</td>
</tr>
<tr>
<td>FTC (n = 37 patients)</td>
<td>365.9</td>
<td>408</td>
<td>50–1,606</td>
</tr>
</tbody>
</table>

* Coefficient of variation.