Differential Diffusions of Indinavir and Lopinavir in Genital Secretions of Human Immunodeficiency Virus-Infected Women

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Plasma and cervicovaginal secretion (CVS) samples were collected from 19 human immunodeficiency virus type 1-infected women on lopinavir- or indinavir-containing regimens. Lopinavir and indinavir were detectable in 29 and 93% of CVS samples, respectively, a finding that may be ascribed to these drugs’ differences in protein binding and pKa. The relationship between lopinavir and indinavir pharmacodynamics and viral evolution in the female genital tract should be assessed over time.

Differential viral load suppression and rate of emergence of drug resistance-associated viral mutations have been observed between blood and genital compartments, a fact which has possibly been related to inadequate penetration of antiretroviral drugs into sanctuaries (3, 4, 9, 11, 12, 14, 15). Numerous studies have evaluated the penetration of antiretrovirals into the male genital tract (7, 8, 16, 17). In contrast, data on the diffusion of antiretrovirals in the female genital tract are scarce, but preliminary results evidenced major differences between drugs in terms of detection in cervicovaginal secretions (CVS) (8, 14). Protease inhibitors (PIs) exhibit variable protein binding capacities and physicochemical properties, which might influence PI diffusion into the female genital tract. We thus decided to compare the pharmacokinetics of indinavir alone or combined with ritonavir and that of lopinavir-ritonavir in the plasma and genital secretions of human immunodeficiency virus type 1 (HIV-1)-infected women.

HIV-1-infected women who were on a lopinavir- or indinavir-containing regimen for a minimum of 4 weeks were recruited between October 2001 and July 2002. They had no symptoms of genital infection and no genital bleeding during the 5 days preceding the visit and were asked to avoid sexual intercourse and intravaginal medications within 2 days before sample processing. To assess whether the patients were adherent to their treatment, they were asked to fill out a simple self-administered questionnaire regarding their drug intake just before and 3 to 4 h after intake of the last drug for 15 min after collection of the genital secretion, a blood sample was taken for the measurement of plasma lopinavir or indinavir concentration. None of the women had taken drugs susceptible to interaction with the metabolism of either indinavir or lopinavir. For each woman, samples were precisely collected just before and 3 to 4 h after intake of the last drug for measurements of 12-h trough and peak plasma concentrations simultaneously with that of drug levels in genital secretions. Both peak and trough concentrations were measured at two different visits separated by 1 to 3 months. The study protocol was approved by the Ethics Committee of the Pitie-Salpêtrière Hospital, Paris, France, and signed informed consent was obtained from all women. Measurements of antiretroviral drug concentrations in paired samples of plasma and acellular fractions of cervicovaginal lavage samples were performed by high-performance liquid chromatography (HPLC) coupled with ultraviolet detection (2). For indinavir, in the concentration range of 0.02 to 4 µg/ml, the between-day coefficient of variation (CV) decreased from 12 to 7%, while the within-day CV decreased from 12 to 3%. For lopinavir, in the concentration range of 0.025 to 10 µg/ml, the between-day CV decreased from 10 to 5% and the within-day CV decreased from 9 to 3%. The method used to assess the dilution of genital secretions has been previously described (1) and was taken into account for the measurement of PI concentration in CVS. The method consists of adding lithium chloride in the washing buffer used to carry out the vaginal washing for collection of CVS as a marker of dilution. Measurements of its concentration before and after the CVS specimen is sampled permit the calculation of the dilution factor introduced by the sample collection. The limits of quantification (LOQ) were 20 ng/ml for indinavir and 25 ng/ml for lopinavir in plasma samples and approximately 200 ng/ml for indinavir and 250 ng/ml for lopinavir in genital secretions, depending on the exact dilution. In case of a high CVS-to-plasma drug concentration ratio, indinavir concentrations were also further measured by HPLC with a diode-array detector and the absorbance spectrum of the peak occurring at the indinavir retention time was compared with that of pure indinavir treated in the same way.
Nineteen HIV-1-infected women were included in the study: five were treated with indinavir (800 mg administered three times a day), four were treated with the combination of indinavir (400 mg administered twice a day [b.i.d.]) and ritonavir (100 mg b.i.d.), and 10 were treated with ritonavir-lopinavir (100 mg of ritonavir and 400 mg of lopinavir b.i.d.). Demographic and major biological characteristics of these women are summarized in Table 1.

A total of 66 pairs of time-matched plasma and genital secretion samples were obtained for pharmacokinetic analysis of indinavir (28 pairs) and lopinavir (38 pairs).

The median plasma lopinavir concentrations at peak (3 to 4 h after lopinavir intake) and trough (11 to 16 h after the last lopinavir intake) were 7,670 ng/ml (range, 140 to 27,300 ng/ml) and 5,100 ng/ml (range, 110 to 22,300 ng/ml), respectively (Fig. 1). Lopinavir was detected at peak concentrations in 7 of 19 (37%) CVS samples and at trough concentrations in 4 of 19 (21%) CVS samples. Among women having detectable lopinavir concentrations, the median CVS drug concentrations at peak and trough were 480 ng/ml (range, 300 to 600 ng/ml) and 480 ng/ml (range, 360 to 720 ng/ml), respectively. Median CVS-to-plasma drug concentration ratios for lopinavir at peak and trough were 0.076 (range, 0.013 to 0.429) and 0.070 (range, 0.026 to 0.091), respectively.

The median plasma indinavir concentrations at peak (3 to 4 h) and trough (11 to 16 h) were 2,000 ng/ml (range, 410 to 6,500 ng/ml) and 450 ng/ml (range, 100 to 1,570 ng/ml), respectively (Fig. 2). Indinavir was detected in 14 of 15 (93%) CVS samples at peak (median, 2,220 ng/ml; range, 200 to 28,100 ng/ml) and 12 of 13 (92%) samples at trough (median, 2,135 ng/ml; range, <200 to 5,410 ng/ml). Among women having detectable indinavir concentrations, the median CVS-to-plasma drug concentration ratios for indinavir at peak and trough were 1.32 (range, 0.08 to 5.62) and 3.8 (range, 0.99 to 10), respectively.

An interference in the determination of indinavir concentration in the vaginal fluid was suspected in the case of two samples, which were further analyzed by HPLC with a diode-array detector. The spectra were clearly different (data not shown), proving the occurrence of an interference with an endogenous compound present in vaginal fluid.

We were able to demonstrate an increased diffusion of indinavir compared with that of lopinavir at steady state in the

![FIG. 1. Lopinavir concentrations in plasma and CVS samples from 10 HIV-1-infected women. Results are expressed in log_{10} nanograms per milliliter. Values under the LOQ were plotted at the LOQ divided by 2. n, number of samples under the LOQ.](http://aac.asm.org/)

![FIG. 2. Indinavir concentrations in plasma and CVS samples from 9 HIV-1-infected women (five received indinavir alone and four received it in association with ritonavir). Results are expressed in log_{10} nanograms per milliliter. Values under the LOQ were plotted at the LOQ divided by 2. n, number of samples under the LOQ.](http://aac.asm.org/)
genital compartments of HIV-infected women. These results were not related to the reduced plasma concentrations of lopinavir obtained, as they were comparable to those previously obtained and described (6). The plasma indinavir concentrations obtained here were also comparable to those previously published (5). The differential diffusion of indinavir and lopinavir in the CVS samples observed here might be explained by differences in protein binding (60% for indinavir versus >98% for lopinavir) and differences in ionization in plasma (pH of 2.8, is ionized in both fluids and does not concentrate in CVS; indinavir, a weak base with pKₐ of 6.2 and 3.8, is much more ionized in CVS than in plasma, resulting in a higher total concentration in CVS than in plasma. However, binding of indinavir to components of CVS or active transport from plasma to CVS cannot be excluded. In a preliminary study examining the diffusion of antiretrovirals in the genital tracts of 58 HIV-infected women (14), indinavir was detected in 8 of 9 CVS samples of women receiving this drug. In contrast, nelfinavir, ritonavir, and saquinavir were not detected in genital samples.

The differences observed here between indinavir and lopinavir concentrations in CVS were also recently found by others who used similar methodologies in examining the male genital tract (13, 15, 16) and cerebrospinal fluid (10, 15, 18) of HIV-infected patients. Whether or not such differences in the PI concentrations observed in various sanctuaries may be associated with differences in local virological outcome should now be assessed prospectively. Indeed, if such pharmacokinetic differences observed in the female genital tract closely influence virological dynamics, our results may be relevant not only for the prevention of local HIV infection in cases of sexual intercourse with an HIV-infected partner but also for the better prevention of materno-fetal transmission of HIV (19).

Regarding the interpretation of indinavir concentrations in the cervicovaginal fluid, we were intrigued by the HPLC profiles obtained from samples collected from two different women which exhibited a very high peak in the region of indinavir but with a small difference in retention time compared to authentic indinavir. We thus decided to analyze these latter samples using a more specific HPLC method that allowed the absorbance spectrum of the eluted substances to be obtained. This method clearly revealed that these high peaks had a spectrum different from that of indinavir and resulted from an endogenous compound, which appeared at high concentration in only some CVS samples. It should be noted that results of previous studies evaluating indinavir concentrations in male genital secretions also disclosed such plasma-to-fluid indinavir concentration ratios above 1 (15, 16). These results were obtained using HPLC methods similar to ours and were not further controlled for potential interferences with endogenous components by using another more specific technique. Such pharmacokinetic discrepancies might attenuate the differential diffusions of indinavir and lopinavir that we and others obtained.

The present study has some limitations. It has no clinical relevance since HIV RNA level in the genital fluid was not determined. We also did not address the mechanism by which indinavir or lopinavir got into the cervical fluid. Overall, the present results obtained in the female genital tract and those obtained in semen by others warrant a large prospective study questioning the relationship between the pharmacodynamics of lopinavir and indinavir and virological course in sanctuaries of HIV-infected patients.

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