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MAJOR ARTICLE

Antiretroviral Drug Concentrations and HIV RNA in the Genital Tract of HIV-Infected Women Receiving Long-Term Highly Active Antiretroviral Therapy

Awewura Kwara,[1,2] Allison DeLong,[3] Naser Rezk,[4] Joseph Hogan[3], Heather Burtwell,[1] Stacy Chapman,[1] Carla C. Moreira,[2] Jaclyn Kurpewski,[1] Jessica Ingersoll,[5] Angela M. Caliendo,[5] Angela Kashuba,[4] and Susan Cu-Uvin[1,2]

[1]Miriam Hospital, [2]Warren Alpert Medical School of Brown University, and [3]Center for Statistical Science, Brown University, Providence, Rhode Island; [4]Division of Pharmacology and Experimental Therapeutics, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill; and [5]Department of Pathology and Laboratory Medicine and Center for AIDS Research, Emory University School of Medicine, Atlanta, Georgia

Objective. Our objective was to determine antiretroviral drug concentrations and human immunodeficiency virus (HIV) RNA rebound in cervicovaginal fluid (CVF) in relation to blood plasma (BP) in women receiving suppressive highly active antiretroviral therapy (HAART).

Methods. Thirty-four HIV-infected women who had plasma HIV RNA levels ≤80 copies/mL for at least 6 months were enrolled. Sixty-eight paired CVF and BP drug concentrations and HIV RNA levels were determined before and 3–4 h after drug administration. For each woman and antiretroviral drug, the CVF:BP drug concentration ratios before and after drug administration were calculated. The nonparametric Wilcoxon rank sum test was used to determine if these ratios were different from 1.0.

Results. Lamivudine (administered to 20 patients) and tenofovir (administered to 16) had significantly higher concentrations in CVF than in BP before drug administration, with mean CVF:BP concentration ratios of 3.19 (95% confidence interval, 1.2–8.5) and 5.2 (95% confidence interval, 1.2–22.6), respectively. Efavirenz (administered to 13 patients) and lopinavir (administered to 6) had significantly lower concentrations in CVF, with mean CVF:BP concentration ratios of 0.01 (95% confidence interval, 0.00–0.03) and 0.03 (0.01–0.11), respectively. During the study visit (median time after enrollment, 6 months), BP and CVF detectable HIV RNA levels were observed 7 patients (20.6%) and 1 patient (2.9%), respectively.

Conclusion. Despite lower CVF concentrations of key HAART components, such as efavirenz and lopinavir, virologic rebound was rare. The high concentrations of tenofovir and lamivudine in CVF may have implications for the prevention of sexual transmission during HAART and for pre-exposure or postexposure prophylaxis.

Combination antiretroviral therapy and zidovudine monotherapy have been associated with a reduced risk of maternal transmission of HIV infection [1, 2]. Furthermore, the use of HAART within defined communities has been associated with reduced sexual transmission of HIV infection [3, 4]. This public health benefit of HAART is likely to be attributable, in part, to treatment-associated reductions in HIV loads in the genital tract, because transmission is believed to occur through direct contact with virus in the genital tract [5, 6]. Reductions in HIV RNA levels in cervicovaginal fluid (CVF) have been found to parallel those in plasma during HAART [7, 8]. However, over time, discordance in viral replication and the emergence of drug-resistant variants between the blood and the genital tract compartments has been observed [9, 10]. It is unclear whether this discordance in viral replication is a result of differences in antiretroviral drug concentrations and local antiviral selective pressure in the different compartments.

The standard initial HAART regimen is composed of 2 nucleoside reverse-transcriptase inhibitors (NRTIs) plus either a protease inhibitor (PI) or a nonnucleoside reverse-transcriptase inhibitor (NNRTI) [11]. A triple

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Reprints or correspondence: Dr. Awewura Kwara, The Miriam Hospital, 164 Summit Ave., Providence, RI 02906 (akwara@lifespan.org).

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combination of drugs from at least 2 classes is designed to achieve maximum suppression of viral replication. Cell-free virus in the genital tract is thought to arise from both blood plasma (BP) transudation and independent local viral replication and evolution [9, 10, 12, 13]. Therefore, one would expect that penetration of all components of the treatment regimen into compartments such as the female genital tract at concentrations sufficient to inhibit viral replication would be necessary to prevent the emergence of drug-resistant variants during HAART. Previous studies have found lower concentrations of some PIs and the NNRTIs in CVF, compared with levels in BP [14, 15], but there are limited published data on the concentrations of NRTIs in CVF. Also, there are scarce data on the relationship between CVF drug concentrations and suppression of viral replication in this compartment. The objective of this study was to assess paired CVF and BP antiretroviral drug concentrations and to correlate local drug concentrations with HIV RNA rebound in patients receiving long-term suppressive HAART.

PATIENTS, MATERIALS, AND METHODS

Study population. Thirty-four HIV-infected women who had achieved plasma HIV RNA levels ≤80 copies/mL after receiving HAART for ≥6 months were prospectively enrolled from 5 September 2003 through 15 November 2005. These women were part of a longitudinal study designed to understand the relative dynamics of viral failure and viral replication in the female genital tract, to assess drug exposure and patterns of drug resistance in the female genital tract, and to evaluate cellular reservoirs of HIV in the female genital tract. During the study period, we enrolled 47 women who had achieved full HIV RNA suppression for ≥6 months (present study) and 9 women who had experienced failure of therapy and needed to switch regimens. All enrolled patients received HAART under standard medical care at the Immunology Center of The Miriam Hospital (Providence, Rhode Island). This study was reviewed and approved by the Lifespan Institutional Review Board, and written informed consent was obtained prior to enrollment.

Sample collection and handling. Enrolled subjects had a baseline evaluation that examined their demographic characteristics and medical, sexual, and reproductive histories. At enrollment, each patient had urine specimens tested for *Neisseria gonorrhea* and *Chlamydia trachomatis.* Pelvic examination and tests for genital tract infections were performed, and paired BP and CVF samples were obtained at all study visits (by Snostrips [Akorn] or Tear Flo [Hub Pharmaceuticals]). The study visit during which samples were obtained for measurement of drug concentrations and quantitative HIV RNA could be scheduled at any time within 12 months after enrolment, with a mean time to sampling of 5.5 months (median time to sampling, 6 months; range, 1–11 months). On the day of study sampling, patients reported to the clinic before their morning dose of medications. Paired CVF and blood samples were obtained prior to and 3–4 h after antiretroviral drug administration. The blood sample was obtained first, and then the CVF was collected within 5 min after blood sampling. CVF from the posterior fonix of the vagina was collected with a volumetric vaginal aspirator and transferred to a 1.2-mL cryovial and frozen at −70°C until shipment for testing. Approximately 10 mL of blood was collected in vacutainers containing EDTA and centrifuged at 1500 *g* for 10 min, and the plasma was aliquoted and stored at −70°C. All frozen samples were shipped on dry ice for testing.

Drug concentration determination. Drug concentrations in BP were measured using validated high-performance liquid chromatography with UV detection methods [16–18]. The lower limit of quantitation for plasma specimens was 10 ng/mL for emtricitabine, tenofovir, lamivudine, zidovudine, didanosine, abacavir, and stavudine and 25 ng/mL for fosamprenavir, efavirenz, lopinavir, atazanavir sulfate, ritonavir, and nelfinavir.

Concentrations in CVF were quantified using a validated high-performance liquid chromatography, tandem mass spectrometry method [19]. In brief, CVF concentrations were measured using a simultaneous assay for 17 antiretroviral drugs. Samples underwent solid-phase extraction using Bond Elut C-18 columns (Varian), as described elsewhere [18]. Cimetidine (in acetate buffer; pH 5.0) was used as internal standard and was applied directly to the conditioned column prior to CVF introduction. An Agilent 1100 binary pump (Agilent) and an HTC Pal thermostatted (6°C) autosampler (LEAP Technologies) connected to an Applied Biosystems API4000 triple quadruple mass spectrometer and Turbospray ion source (Applied Biosystems) with an Aquasil C18 column (Thermo-Electron) was used for the analysis. Multiple reaction monitoring and positive-to-negative polarity switching were used: 3 analytes (stavudine, didanosine, and zidovudine) were monitored in negative mode, and the remaining analytes were monitored in positive mode. The lower limits of quantitation in CVF were 1 ng/mL for fosamprenavir, nevirapine, nelfinavir, and abacavir; 5 ng/mL for efavirenz, emtricitabine, and tenofovir; 10 ng/mL for lopinavir; 50 ng/mL for lamivudine, zidovudine, didanosine, and stavudine; and 75 ng/mL for ritonavir. Overall assay precision, expressed as coefficient of variation, was 2.0%–14.3%, and accuracy was 88%–113%. Recovery for the drugs studied ranged from 80% for ritonavir and lopinavir to 99% for lamivudine, didanosine, and abacavir. All analytical work was performed by the University of North Carolina Center for AIDS Research Clinical Pharmacology and Analytical Chemistry Core (Chapel Hill, North Carolina), which participates in quarterly national and international external proficiency testing [20, 21].

These results consistently demonstrate high levels of accuracy and precision for our antiretroviral assays.

HIV RNA determination. Nucleic acid sequence–based amplification (bioMerieux) was used to measure HIV RNA levels. All results are expressed as copies per mL, with a lower limit of detection of 80 copies/mL for BP and 3300 copies/mL for CVF collected by Sno-strips.

Statistical analysis. Antiretroviral drug concentrations in 2 pairs of genital tract and BP samples from each woman were examined. When the result was below the limit of detection, the concentration was set to 0 ng/mL. When the result was below the limit of quantitation but above the lower limit of detection, the concentration was set to 50% of the lower limit of quantitation.

For each compartment and time period, the mean and associated 95% CI of the concentrations of each drug were calculated. As expected, the concentrations showed a skewed distribution, attributable to some very high concentrations and a lower bound of zero. For each woman and antiretroviral drug, we calculated the ratio of drug concentration in the genital tract to drug concentration in the BP at each time period. Ratios >1 indicated that genital tract concentrations were greater than BP concentrations. The means and 95% CIs of the ratios were estimated using the Student's t test.

The nonparametric Wilcoxon rank-sum test was used to determine whether the ratios were different from 1.0 (i.e., whether drug concentrations were different in the 2 biological compartments at either time period or whether concentrations were different in the genital tract after drug administration, compared with before administration). Statistical significance was determined at the $\alpha = 0.05$ level.

Table 1. Participant demographic data and clinical characteristics at baseline.

Characteristic	Patients (n = 34)
Age, median years (range)	45 (32–62)
Race/ethnicity	
Black	15 (44)
White/non-Hispanic	12 (35)
Hispanic	5 (15)
Other	2 (6)
CD4 cell count	
<200 cells/mm^3	2 (6)
200–499 cells/mm^3	20 (59)
⩾500 cells/mm^3	12 (35)
Hysterectomy	8 (24)
Test results positive for sexually transmitted infection	
Chlamydia	0/24 (0)
Syphilis	0/23 (0)
Gonorrhea	0/23 (0)
Trichomonas	1 (3)
Candida	3 (9)
Bacterial vaginosis	8 (24)
Herpes simplex virus 2	
By IgG	32/33 (97)
By PCR	5/32 (16)
Antiretroviral therapy	
NRTI and PI	17 (50)
NRTI and NNRTI	13 (38)
3 NRTIs	3 (9)
NNRTI and PI	1 (3)

NOTE. Data are no. (%) of patients, unless otherwise indicated. NNRTIs, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.

RESULTS

Patient characteristics. During the study period, 47 women met the selection criteria and were enrolled. Of these women, 13 (28%) did not complete the study visit for sampling of drug concentrations and quantitative HIV RNA. Of these 13 patients, 3 withdrew from the study, 3 were lost to follow-up, 3 had scheduling conflicts, 2 moved out of state, 1 was incarcerated, and 1 died.

Of the 34 women who completed the study, the median age was 45 years (range, 32–62 years), 44% were black, 35% were non-Hispanic white, 15% were Hispanic, and 6% were of other ethnicities (table 1). Fifty-nine percent of the patients had CD4 cell counts of 200–500 cells/mm^3, and 35% had counts ⩾500 cells/mm^3. Thirty-two (97%) of 33 patients were seropositive for herpes simplex virus 2 IgG, 8 (24%) of 34 had test results that were positive for bacterial vaginosis, and 5 (16%) had PCR results that were positive for herpes simplex virus 2 in CVF (table 1). Thirty patients (88%) received at least 2 NRTIs plus a PI or an NNRTI, 3 (9%) were given 3 or 4 NRTIs, and only 1 patient (3%) received a regimen that did not contain an NRTI. Ritonavir was given to boost other PIs in all but 1 patient. All except 3 patients received either lamivudine (n = 15) or tenofovir (n = 11) or both (n = 5) in their treatment regimen; 2 of the other 3 women received emtricitabine, and 1 was given a non–NRTI-based regimen.

Antiretroviral drug concentrations in BP and CVF. A total of 4 specimens were collected from each of the 34 women, who were taking a total of 13 different antiretroviral combinations. The number of women taking each drug ranged from 2 for nevirapine to 20 for lamivudine. Test results and 95% CIs associated with antiretrovirals with <5 samples were not considered to be reliable; they are included in the tables as preliminary information. Furthermore, 8 of 9 concentrations of zidovudine in the CVF were below the limit of quantitation (<50 ng/mL) before drug administration; 3 of the BP concentrations were below the limit of detection, 2 were below the limit of quantitation (<10 ng/mL), and 4 were <25 ng/mL. As a result, we could not compare CVF and BP concentrations of

zidovudine prior to drug administration. Finally, in 2 instances, the 95% CIs for the ratios include the value 1, but the *P* value is stated to be <.05. Such inconsistencies are expected, because 2 different methods were used to construct the 95% CIs and to perform significance tests. The Wilcoxon rank-sum test is nonparametric, makes fewer distributional assumptions than the Student's *t* test, and is a conservative test of significance.

Table 2 shows the steady state concentrations at the end of the dosing interval prior to taking morning medications and the CVF:BP concentration ratios. Lamivudine (administered to 20 patients) and tenofovir (administered to 16) had significantly higher concentrations in CVF than in BP at the end of the dosing interval, with mean CVF:BP ratios of 3.19 (95% CI, 1.2–8.5) for lamivudine and 5.2 (95% CI, 1.2–22.6) for tenofovir. On the other hand, efavirenz (administered to 13 patients) and lopinavir (administered to 6) had significantly lower concentrations in CVF samples than in paired BP samples, with mean CVF:BP concentration ratios of 0.01 (95% CI, 0.0–0.03) and 0.03 (95% CI, 0.01–0.11), respectively. All of the other PIs and NNRTIs had lower concentrations in CVF, compared with concentrations in BP, with CVF:BP concentration ratios <1 but with statistically insignificant differences.

The mean concentrations of each drug in CVF and BP 3–4 h after administration are shown in table 3. Abacavir, efavirenz, ritonavir, and lopinavir had significantly lower concentrations in CVF than in BP 3–4 h after administration, with CVF:BP ratios significantly <1. The CVF:BP drug concentration ratios decreased for all of the drugs except efavirenz, lopinavir, nelfinavir, and nevirapine after administration, indicating that most drugs accumulated faster in BP than in CVF.

Virologic rebound in BP and CVF. There were 7 patients who had detectable HIV RNA in BP at the time of sampling, with viral loads of 100–1400 copies/mL. Of the 7 patients who had low plasma viremia at the study visit, 3 had fully suppressed plasma viral loads on subsequent visits, 2 subsequently experienced failure of therapy because of treatment nonadherence, and data was unavailable for 2 patients, because the study visit was their last follow-up visit. Only 1 (3%) of the 34 patients had a detectable HIV RNA level of 6000 copies/mL in the CVF sample at the time of paired sampling; this patient also had an HIV RNA level of 1400 copies/mL in the BP sample. At the time of evaluation, the patient was receiving didanosine, lamivudine, and efavirenz and was noted to be nonadherent to therapy. The didanosine concentration before administration was below the limit of detection in both BP and CVF samples, and efavirenz concentrations were low, compared with our observed mean concentration for all patients, in BP (913.9 ng/mL vs. 2087.8 ng/mL) and in CVF (6.9 ng/mL vs. 18.4 ng/mL). However, the concentration of lamivudine in the patient's BP sample was higher than the observed mean concentration for all of the patients (455.8 ng/mL vs. 123.7 ng/mL).

DISCUSSION

Maximum suppression of viral replication in the genital tract is believed to be essential to prevent evolution and transmission of drug-resistant virus during HAART. The ability of components of a triple-drug regimen to reach the genital tract in concentrations adequate to inhibit local viral replication may be important to prevent the evolution of drug-resistant variants of HIV in the genital tract during HAART, because HIV replication has been shown to be compartmentalized at this site [9, 10, 12, 13]. Also, it may be desirable that antiretroviral drugs given for pre– or post–sexual exposure prophylaxis penetrate and accumulate in high concentrations in the CVF, which is the likely site of exposure and initial infection. This study examined the concentrations of components of HAART in the CVF of women who had achieved excellent viral suppression in BP and sought to correlate local drug concentrations with subsequent virologic rebound. In general, the NRTIs demonstrated good penetration into the CVF, with lamivudine and tenofovir achieving concentrations in CVF of nearly 3–5 times their concentration in BP at the end of the dosing interval. The excellent accumulation of these agents in the CVF may be beneficial for the prevention of HIV transmission during HAART and for pre– or post–sexual exposure antiretroviral prophylaxis. On the other hand, selective accumulation of NRTIs but not the PIs or NNRTIs in the CVF may provide an environment for the selection of drug-resistant variants in this compartment. Transmission of antiretroviral drug–resistant HIV variants has been shown to occur through sexual contact as well as from mother to child [22–24]. However, it is not clear whether the drug-resistant strains arose from the genital tract or systemically. Also, the underlying mechanism for the protective effect of antiretroviral therapy against vertical transmission of HIV is not well understood, because therapy that maximally suppressed viral replication in BP demonstrated the best benefit in reducing mother-to-child transmission of HIV, but zidovudine monotherapy that did not have significant sustained effects on BP viral loads also reduced transmission [1, 2].

Our data also confirmed that the PIs and NNRTIs penetrate poorly into CVF, as has been reported elsewhere [12, 13]. At the end of the dosing interval, the concentrations of these classes of drugs in CVF was only 3%–33% of the concentrations in the paired BP. Efavirenz and lopinavir, which were prescribed to 19 (55.8%) of the 34 patients, achieved lower pre- and post-dose concentrations in CVF, compared with BP. Despite the lower concentrations of these agents in CVF, sustained suppression of HIV RNA levels was observed in the genital tract compartment. It is possible that there was a bias in our study, because we enrolled only women who had achieved maximum viral suppression in BP for at least 6 months, and the follow-

Table 2. Steady state antiretroviral drug concentrations at the end of an 8–12-h dosing interval.

Drug	Drug class	No. of patients	Concentration in CVF, mean ng/mL (95% CI)	Concentration in BP, mean ng/mL (95% CI)	CVF:BP, mean ratio (95% CI)
3TC[a]	NRTI	20	394.17 (134.7–1153.7)	123.74 (63.08–242.72)	3.19 (1.19–8.53)
TDF[a]	NRTI	16	84.03 (24.47–288.56)	16.3 (7.04–37.74)	5.15 (1.18–22.6)
ZDV[b]	NRTI	9	31.25 (18.68–52.25)	5.3 (1.85–15.19)	5.9 (2.11–16.52)
ABC	NRTI	8	28.17 (5.73–138.6)	48.26 (8.74–266.52)	0.58 (0.16–2.19)
ddI	NRTI	6	26.06 (23.42–29.01)	2.63 (0.22–31.45)	9.92 (0.81–121.63)
FTC	NRTI	4	250.51 (65.99–950.89)	167.14 (29.51–946.58)	1.5 (0.1–22.72)
EFV[c]	NNRTI	13	18.4 (6.95–48.73)	2087.81 (1483.43–2938.42)	0.01 (0–0.03)
NVP	NNRTI	2	272.02 (2.49–29737.16)	3499.78 (961.05–12744.92)	0.08 (0–2.33)
RTV	PI	11	58.73 (13.71–251.61)	72.71 (20.88–253.18)	0.81 (0.14–4.54)
LPV[c]	PI	6	74.64 (15.09–369.3)	2870.42 (1114.75–7391.19)	0.03 (0.01–0.11)
NFV	PI	4	113.7 (11.01–1173.87)	2363.78 (1523.0–3652.1)	0.05 (0.01–0.37)
ATV	PI	3	390.23 (81.94–1858.33)	1188.04 (585.2–2411.87)	0.33 (0.07–1.64)
FPV	PI	3	54.84 (2.21–1360.56)	1009.88 (125.76–8109.63)	0.05 (0–0.61)

NOTE. 3TC, lamivudine; ABC, abacavir; ATV, atazanavir sulfate; BP, blood plasma; CVF, cervicovaginal fluid; ddI, didanosine; EFV, efavirenz; FPV, fosamprenavir; FTC, emtricitabine; LPV, lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RTV, ritonavir; TDF, tenofovir; ZDV, zidovudine.

[a] Drugs that had significantly higher concentrations in CVF than in BP at the end of the dosing interval.

[b] Statistical significance cannot be determined, because many observations were below the limit of quantitation of the assay.

[c] Drugs that had higher concentrations in BP than in CVF at the end of the dosing interval.

up period was relatively short (median duration, 6 months). It was not possible for us to determine the relationship between CVF drug concentrations and viral rebound, because there was only 1 patient with HIV RNA rebound in the genital tract compartment. Other investigators have proposed that failure to fully suppress BP HIV RNA levels, rather than CVF drug concentrations, is the main determinant of genital tract viral shedding [25–29]. Alternatively, it is also possible that adequate drug concentration of the PIs and NNRTIs are achieved at the intracellular site of HIV replication, as demonstrated in studies that have examined intracellular pharmacokinetics [30–32].

We recognize that these data have limitations and should be interpreted with caution, especially for the drugs that were administered to <5 patients. Second, sampling for measurement of drug concentrations and viral loads was performed at only 1 time during the 12-month study period and at only 2 points

Table 3. Steady state antiretroviral drug concentrations 3–4 h after administration.

Drug	Drug class	No. of patients	Concentration in CVF, mean ng/mL (95% CI)	Concentration in BP, mean ng/mL (95% CI)	CVF:BP, mean ratio (95% CI)
3TC	NRTI	20	782.9 (279.6–2192.6)	805.2 (351.8–1843.2)	0.97 (0.41–2.3)
TDF	NRTI	16	67.4 (16.5–274.9)	90.0 (58.9–137.5)	0.75 (0.16–3.49)
ZDV	NRTI	9	62.2 (23.7–162.9)	151.1 (67.9–336.4)	0.41 (0.12–1.43)
ABC[a]	NRTI	8	168.4 (82.5–343.7)	1596.1 (916.8–2778.8)	0.11 (0.04–0.28)
ddI	NRTI	6	39.2 (3.3–472.7)	34.3 (1.6–741.4)	1.14 (0.02–53.08)
FTC	NRTI	4	374.8 (1.4–97493.4)	823.0 (268.3–2524.8)	0.46 (0–293.05)
EFV[a]	NNRTI	13	29.8 (6.3–142.1)	3195.8 (2559.1–3991.0)	0.01 (0–0.05)
NVP	NNRTI	2	565.4 (364.8–876.2)	4334.8 (547.2–34340.8)	0.13 (0.01–1.6)
RTV[a,b]	PI	11	146.6 (44.4–483.6)	752.1 (471.2–1200.6)	0.19 (0.04–0.89)
LPV[a]	PI	6	215.1 (62.8–737.5)	6625.9 (4715.4–9310.4)	0.03 (0.01–0.11)
NFV	PI	4	162.4 (69.4–379.9)	2965.8 (1564.1–5623.4)	0.05 (0.01–0.2)
ATV	PI	3	724.9 (425.3–1235.7)	5068.1 (3660.3–7017.5)	0.14 (0.06–0.34)
FPV	PI	3	41.3 (0.04–47578.5)	5739.8 (1411.4–23341.8)	0.01 (0–4.57)

NOTE. 3TC, lamivudine; ABC, abacavir; ATV, atazanavir sulfate; BP, blood plasma; CVF, cervicovaginal fluid; ddI, didanosine; EFV, efavirenz; FPV, fosamprenavir; FTC, emtricitabine; LPV, lopinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; NVP, nevirapine; PI, protease inhibitor; RTV, ritonavir; TDF, tenofovir; ZDV, zidovudine.

[a] Drugs that had significant lower CVF:BP drug concentrations 3–4 h after doing.

[b] Drugs that had significant increases in the genital tract concentrations after observed administration.

in the 8–24-h period of drug exposure during the dosing interval. Finally, we did not measure intracellular drug concentrations or the triphosphate form of the NRTIs, which are important for pharmacologic action. Notwithstanding these limitations, our data demonstrate that lamivudine and tenofovir accumulate well in the female genital tract and may have potential for use as part of combination therapy in reducing the sexual transmission of HIV. In addition, despite lower concentrations of the PIs and NNRTIs in the CVF, compared with concentrations in BP, we found no virologic rebound in the genital tracts of any of the patients who had undetectable viral loads in BP during a median follow-up period of ~6 months.

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References

1. Cooper ER, Charurat M, Mofenson L, et al. Combination antiretroviral strategies for the treatment of pregnant HIV-1–infected women and prevention of perinatal HIV-1 transmission. J Acquir Immune Defic Syndr **2002**; 29:484–94.
2. Garcia PM, Kalish LA, Pitt J, et al. Maternal levels of plasma human immunodeficiency virus type 1 RNA and the risk of perinatal transmission. N Engl J Med **1999**; 341:394–402.
3. Porco TC, Martin JN, Page-Shafer KA, et al. Decline in HIV infectivity following the introduction of highly active antiretroviral therapy. AIDS **2004**; 18:81–8.
4. Fang C-T, Hsu H-M, Twu S-J, et al. Decreased HIV transmission after a policy of providing free access to highly active antiretroviral therapy in Taiwan. J Infect Dis **2004**; 190:879–85.
5. Tuomala RE, O'Driscoll PT, Brewer JW, et al. Cell-associated genital tract virus and vertical transmission of human immunodeficiency virus type 1 in antiretroviral-experienced women. J Infect Dis **2003**; 187: 375–84.
6. Mostad SB, Kreiss JK. Shedding of HIV in the genital tract. AIDS **1996**; 10:1305–15.
7. Cu-Uvin S, Synder B, Harwell JI, et al. Association between paired plasma and cervicovaginal lavage fluid HIV-1 RNA levels during 36 months. J Acquir Immune Defic Syndr **2006**; 42:584–7.
8. Vettore MV, Schecter M, Melo MF, Boechat LJ, Barroso PF. Genital HIV-1 viral load is correlated with blood plasma HIV-1 viral load in Brazilian women and is reduced by antiretroviral therapy. J Infect **2006**; 52:290–3.
9. De Pasquale MP, Leigh Brown AJ, Cu Uvin S, et al. Differences in HIV-1 *pol* sequences from female genital tract and blood during antiretroviral therapy. J Acquir Immune Defic Syndr **2003**; 34:37–44.
10. Si-Mohamed A, Kazatchkine MD, Heard I, et al. Selection of drug-resistant variants in the female genital tract of human immunodeficiency virus type 1–infected women receiving antiretroviral therapy. J Infect Dis **2000**; 182:112–22.
11. Hammer SM, Saag MS, Schecter M, et al. Treatment for adult HIV infection: 2006 recommendations of the International AIDS Society-USA Panel. JAMA **2006**; 296:827–43.
12. Ellerbrock TV, Lennox JF, Clancy KA, et al. Cellular replication of human immunodeficiency virus type 1 occurs in vaginal secretions. J Infect Dis **2001**; 184:28–36.
13. Tirado G, Jove G, Kumar R, et al. Differential virus evolution in blood and genital tract of HI-infected females: evidence for the involvement of drug and non–drug resistance-associated mutations. Virology **2004**; 324:577–86.
14. Min SS, Corbett AH, Rezk N, et al. Protease inhibitors and nonnucleoside reverse transcriptase inhibitor concentration in the genital tract of HIV-1–infected women. J Acquir Immune Defic Syndr **2004**; 37: 1577–80.
15. Ghosn J, Chaix M-L, Peytavin G, et al. Penetration of enfurvitide, tenofovir, efavirenz and protease inhibitors in the genital tract of HIV-1–infected men. AIDS **2004**; 18:1958–61.
16. Rezk NL, Tidwell RR, Kashuba ADM. Simultaneous determination of six HIV nucleoside analogue reverse transcriptase inhibitors and nevirapine by liquid chromatography with ultraviolet detection. J Chromatogr B Analyt Technol Biomed Life Sci **2003**; 791:137–47.
17. Rezk NL, Crutchley RD, Yeh RF, et al. Full validation of an analytical method for the HIV-protease inhibitor atazanavir in combination with 8 other antiretroviral agents and its applicability to therapeutic drug monitoring. Ther Drug Monit **2006**; 28:517–25.
18. Rezk NL, Crutchley DR, Kashuba ADM. Simultaneous quantification of emtricitabine and tenofovir in human plasma using high-performance liquid chromatography after solid phase extraction. J Chromatogr B Analyt Technol Biomed Life Sci **2005**; 822:201–8.
19. Jung BW, Rezk NL, Bridges AS, Kashuba AD. Simultaneous determination of 17 antiretroviral drugs in human plasma for quantitative analysis with liquid chromatography-tandem mass spectrometry. Biomed Chromatogr **2007**; 21:1095–104.
20. Holland DT, DiFrancesco R, Connor JD, Morse GD. Quality Assurance Program for Pharmacokinetic Assay of Antiretrovirals: ACTG proficiency testing for pediatric and adult pharmacology support laboratories, 2003 to 2004. Ther Drug Monit **2006**; 28:367–74.
21. Droste JAH, Aarnoutse RE, Koopmans PP, Hekster YA, Burger DM. Evaluation of antiretroviral drug measurements by an interlaboratory quality control program. J Acquir Immune Defic Syndr **2003**; 32: 287–91.
22. Yerly S, Kaiser L, Race E, Bru J-P, Clavel F, Perrin L. Transmission of antiretroviral-drug resistant HIV-1 variants. Lancet **1999**; 354:729–33.
23. Colgrove RC, Pitt J, Chung PH, Welles SL, Japour AJ. Selective vertical transmission of HIV-1 antiretroviral resistance mutations. AIDS **1998**; 12:2281–8.
24. Neihues T, Walter H, Homeff G, Wahn V, Schmidt B. Selective vertical transmission on HIV: lamivudine-resistant maternal clone undetectable by conventional resistance testing. AIDS **1999**; 13:2482–4.
25. Debiaggi M, Zara F, Spinillo A, et al. Viral excretion in cervicovaginal secretions of HIV-1 infected women receiving antiretroviral therapy. Eur J Clin Microbiol Infect Dis **2001**; 20:91–6.
26. Lowe SH, Wensing AMJ, Droste JAH, et al. No virological failure in semen during properly suppressive antiretroviral therapy despite subtherapeutic local drug concentrations. HIV Clin Trials **2006**; 7:285–90.
27. Gupta P, Mellors J, Kingsley L, et al. High viral load in semen on human immunodeficiency virus type 1-infected men at all stages of disease and its reduction by therapy with protease and nonnucleoside reverse transcriptase inhibitors. J Virol **1997**; 71:6271–5.

28. Vernazza PL, Gilliam BL, Flepp M, et al. Effect of antiviral treatment on the shedding of HIV-1 in semen. AIDS **1997**; 11:1249–54.
29. Vernaza PL, Gilliam BL, Dyer J, et al. Quantification of HIV in semen: correlation with antiviral treatment and immune status. AIDS **1997**; 11:987–93.
30. Ford J, Boffito M, Wildfire A, et al. Intracellular and plasma pharmacokinetics of saquinavir-ritonavir, administered at 1,600/100 milligrams once daily in human immunodeficiency virus–infected patients. Antimicrob Agents Chemother **2004**; 48:2388–93.
31. Khoo SH, Hoggrad PG, Williams I, et al. Intracellular accumulation of human immunodeficiency virus protease inhibitors. Antimicrob Agents Chemother **2002**; 46:3228–35.
32. Ford J, Khoo SH, Back JD. Intracellular pharmacology of antiretroviral protease inhibitors. J Antimicrob Chemother **2004**; 54:982–90.