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A post-mortem analysis of tenofovir, lamivudine, efavirenz and fluconazole penetration in female genital tissues

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Background: Optimal penetration of anti-infectives in the female genital tract (FGT) is paramount in the treatment and prevention of infectious diseases. While exposure of anti-infectives in lower FGT tissues (e.g. cervix, vagina) has been described, little data exist on upper genital tissues (e.g. ovary, uterus).

Methods: Autopsies were performed and post-mortem tissues were collected within 24 h of death for female participants with advanced HIV in Uganda ($n = 27$). Tenofovir, lamivudine, efavirenz and fluconazole concentrations were measured using LC-MS/MS in plasma, ovarian, uterine, cervical and vaginal tissues. Tissue penetration was calculated as tissue-to-plasma concentration ratios (TPRs).

Results: TPRs of tenofovir, lamivudine and fluconazole were highest in vaginal tissue (medians 1.86, 1.83 and 0.94, respectively), while the TPR of efavirenz was highest in ovarian tissue (median 0.65). With cervix as a reference compartment, vaginal TPRs were significantly higher than cervical for all four drugs; TPRs of efavirenz in uterine and ovarian compartments were also significantly higher than cervical. Most of the post-mortem FGT samples had a TPR of greater than 1 for tenofovir and lamivudine, while less than 50% had a TPR of greater than 1 for both efavirenz and fluconazole.

Conclusions: Penetration of anti-infectives was not homogeneous among the FGT compartments. Approximately 70% of FGT tissues had a TPR of greater than 1 for tenofovir and lamivudine, favouring the prevention of local HIV replication and transmission in the FGT.

Introduction

The female genital tract (FGT) is a significant anatomical site of pathogen persistence and sexual transmission of infectious diseases. Optimal drug exposure in the FGT is paramount for prevention and treatment of FGT-involved infectious diseases, such as HIV and vaginal candidiasis. Although much of the focus on mucosal HIV transmission in women has been the lower FGT, HIV infection can occur throughout the entire FGT, as demonstrated in the rhesus macaque vaginal transmission model of simian immunodeficiency virus.[1] Candidiasis, while more often defined as vulvovaginal compared with other FGT sites, can, in rare cases, occur in the upper FGT as well.[2] Despite this, our knowledge of FGT tissue exposure to antiretrovirals and antifungals has predominantly been obtained through sampling of the lower FGT, mostly via cervicovaginal tissue and cervicovaginal fluid.[3–9] Studies related to FGT drug exposure in upper tissue tracts (i.e. ovarian and uterine tissue) in non-pregnant women are scarce due to lack of easiness of sampling.

In order to fill the knowledge gap surrounding exposure of anti-infectives in solid tissues of the upper and lower FGT, we explored tissue penetration of two commonly prescribed NRTIs, tenofovir and lamivudine, and an NNRTI, efavirenz, in addition to an antifungal, fluconazole, in post-mortem FGT tissues (i.e. ovarian, uterine, cervical and vaginal) from a population of Ugandan women living with HIV/AIDs prior to the time of death.

Methods

Study participants and sample collection

All study participants were hospitalized patients living with HIV/AIDs who passed away at Mulago National Referral Hospital in Kampala, Uganda from 2017 to 2020. Written informed consent was obtained from the next of kin. ART history, including recent medication adherence, was extracted from medical charts and from interviews with caretakers.

Autopsies were performed shortly after consent and time of death (typically within 24 h). During autopsies, approximately 1–2 g tissue sections from various organs and anatomical sites (including vagina, cervix, uterus and ovary) were collected and immediately snap-frozen in liquid nitrogen or dry ice/ethanol bath. Whole blood from the femoral vein was collected into EDTA vacutainers and spun at 2400–3000 rpm at 4°C for 10 min to separate plasma. Tissue and plasma specimens were transferred via liquid nitrogen to −80°C freezers prior to analysis. The study protocol was approved by the Research Ethics Review Committee at Mulago National Referral Hospital.

Sample processing and quantification of anti-infective concentrations

Plasma and tissue concentrations of tenofovir, lamivudine and fluconazole were quantified simultaneously, while efavirenz was quantified separately from the other three drugs, according to the methods previously described.[10] Briefly, 200 μL of plasma was spiked with 20 μL of a mixed internal standard of tenofovir-d6, lamivudine-$^{13}C_1$, d2, efavirenz-d4 and fluconazole-d4. Protein was then precipitated by adding 800 μL of ice-cold acetonitrile and removed after centrifugation at 15 000 **g** for 15 min. The supernatant was then condensed under a nitrogen flow, following reconstitution with mobile phase and injection for LC-MS/MS assay. The method for FGT tissue processing and LC-MS/MS quantification was adapted from the aforementioned plasma method, with some modification. Briefly, for each female genital specimen (i.e. ovarian, uterine, cervical and vaginal tissue), 0.3 g of tissue was weighed, cut into small pieces, and homogenized in 0.6 mL of PBS with a portable rotor stator (Omni TH). Two hundred microlitres of supernatant were collected after centrifuging the homogenate at 4696 **g** for 15 min, then spiked with 20 μL of the mixed internal standard followed by the same process as plasma.

The simultaneous detection and quantification of tenofovir, lamivudine and fluconazole in FGTs were performed using UPLC (Thermo Scientific) coupled with a TSQ Quantum triple-stage quadrupole mass spectrometer (Thermo Electron, San Jose, CA, USA) (LC-MS/MS). The chromatographic separation was performed with an ACQUITY UPLC HSS T3 (2.1×50 mm), reversed-phase column with a 1.8 μm particle size. The mobile phase used for the gradient elution consisted of (A) 0.1% formic acid in deionized water and (B) 0.1% formic acid in acetonitrile. The chromatographic conditions were isocratic from 0 to 1.50 min at 0% B, followed by a linear gradient at 1.5 to 2.75 min of 0%–45% B and then returning to the starting conditions (at 3.0 min) with a flow rate of 0.3 mL/min, for a total run time of 6 min. The column temperature was maintained at 30°C. The detector settings of the mass spectrometer were: ESI with the stainless-steel spray needle, positive polarity ionization and multiple reaction monitoring (MRM) mode. The ion transitions (*m/z*) were as follows: 288 to 176 for tenofovir, 230 to 112 for lamivudine, and 307 to 238 for fluconazole.

Chromatographic separation of efavirenz was performed with an ACQUITY UPLC BEH C18 (2.1×50 mm), reversed-phase column with a 1.7 μm particle size (Waters, Milford, MA, USA), on the same LC-MS/MS system mentioned above. The mobile phase used was a mixture of 10 mM ammonium acetate in water, pH 6.8 (30%), and acetonitrile (70%) with an isocratic elution flow rate of 0.25 mL/min and a total run time of 2 min. The column temperature was maintained at 30°C. The detector settings of the mass spectrometer were: ESI with the stainless-steel spray needle, negative polarity ionization and selective reaction monitoring (SRM) mode. The ion transition (*m/z*) was 314 to 244 for efavirenz.

Efficiency (or recovery) of extraction from tissue homogenate was estimated by repeating the step of homogenizing the sample with PBS three additional times for four randomly chosen samples. Supernatant from each homogenate was analysed by the above assay, and an average extraction efficiency factor of 0.7 was derived by fitting exponential regression curves for the remaining amount of tenofovir, lamivudine and fluconazole in the same samples.

Statistical analysis

The penetration metric was defined as the ratio between tissue and plasma concentration (tissue-to-plasma ratio; TPR). TPR was used rather than raw concentration as there was expected variability in concentrations due to the various times since the last dose and, in the case of fluconazole, variable dosages. Concentrations and TPRs of tenofovir, lamivudine, efavirenz and fluconazole in each tissue compartment were expressed as median (IQR). Friedman test (non-parametric analysis for repeated measures) was used for the global comparisons of difference among the four FGT compartments and the paired Wilcoxon test was used for comparisons between other FGT compartments and the cervical compartment, as it was the most common tissue compartment measured and has been used to broadly refer to FGT in previous studies.[3–5,7] Pearson correlation coefficients (r) and the corresponding *P* values were used to show the correlation between plasma concentration and tissue concentration. Linear regression was used to analyse the relationship between TPR and post-mortem interval.

Results

Participant demographics

Post-mortem tissues were sampled from 27 female Ugandan participants living with HIV/AIDs at the time of death. Demographic characteristics of deceased participants can be seen in Table 1. The majority of study participants were young to middle-aged. Twenty-two participants were receiving ART at the time of death and 13 were receiving fluconazole for the treatment of cryptococcal meningitis. Of the 21 participants with available dosing history, all had received ART for at least 7 days prior to the time of death and 67% were said to be adherent to their ART. The most common ART regimen prescribed was tenofovir disoproxil fumarate/lamivudine/efavirenz (44.4%). The most common causes of death were cryptococcal meningitis and TB. Other causes included various types of cancer, anaemia, respiratory failure, heart failure, etc. Tissue concentrations were only reported for those with detectable corresponding plasma concentrations.

NRTIs

The median concentration of tenofovir was highest in vaginal tissue across the four FGT compartments (i.e. vaginal, cervical, uterine and ovarian) and median vaginal concentrations were 64% higher than cervical concentrations ($P=0.03$) (Figure 1a). When normalizing for plasma, the TPR of tenofovir in vaginal tissue was greater than ovarian, uterine and cervical tissues as well (Table 2). More than 50% of participants attained TPR > 1 in all four FGT compartments, with the highest proportion of TPR > 1 being in vaginal tissue (Table 3).

Similarly, vaginal concentrations of lamivudine were higher than those of other FGT compartments and median vaginal concentrations were 50% higher than the cervical concentrations ($P=0.0003$) (Figure 1b). TPRs of lamivudine were greater in vaginal tissue when compared with the other three tissue compartments (Table 2). More than 50% of participants attained TPR > 1 in all four FGT compartments, with vaginal tissue again having the highest proportion of participants with TPR > 1 (Table 3).

Table 1. Demographic characteristics and medication use

Characteristic	Median (IQR) unless otherwise noted
Age (years)	34 (26–45)
Height (cm)	161 (156–163)
Most recent CD4+ T cell count (cells/mm^3)[a]	23 (5–112)
eGFR (mL/min/1.73 m^2)	14 (9–32)
Time on current ART regimen (days)	200 (42–558)
Time on fluconazole regimen (days)	7.5 (3–15)
Most recent viral load (copies/mL)[b]	Undetectable–1 100 000
Time since last antiretroviral dose prior death (h)[c]	20.2 (13.6–42.4)
Time since last fluconazole dose prior to death (h)	18.9 (10.9–24.8)
Post-mortem interval (h)	7.5 (5.2–12.2)
Characteristic	*N* = 27
Cryptococcal meningitis, *n* (%)	12 (44.4)
TB, *n* (%)	12 (44.4)
Antiretrovirals, *n* (%)	
Tenofovir disoproxil fumarate	17 (63.0)
Lamivudine	21 (77.8)
Efavirenz	11 (40.7)
Dolutegravir	7 (25.9)
Nevirapine	3 (11.1)
Abacavir	2 (7.4)
Zidovudine	2 (7.4)
Fluconazole	15 (55.6)
200 mg, *n*	3
400 mg, *n*	1
800 mg, *n*	2
1200 mg, *n*	5
Unknown, *n*	4

[a]Time interval between death and the most recent CD4+ T cell counts ranged from 2 days to 6.9 years.
[b]Most recent viral load is shown as range for seven participants. Time interval between death and the most recent viral load ranged from 11 days to 783 days.
[c]Time of last ART dose was provided by next of kin or taken from medical records.

NNRTI

Median concentration of efavirenz was highest in ovarian tissue, and concentrations in ovarian, vaginal and uterine tissues were all significantly higher than in cervical tissue by 82% ($P=0.01$), 37% ($P<0.01$) and 33% ($P=0.03$), respectively (Figure 1c). TPRs of efavirenz demonstrated the same relationships among the four FGTs as concentration (Table 2). All four FGT compartments had less than 50% of participant samples attaining TPR > 1. Ovarian tissue had the highest TPR > 1 proportion compared with the other FGT tissues (Table 3).

Antifungal

While the median concentration of fluconazole was highest in the ovarian tissue, only vaginal concentrations were statistically higher (22%) than cervical concentrations ($P=0.02$) (Figure 1d). TPRs of fluconazole in the four FGT compartments were comparable, with the largest median in vaginal tissue (Table 2). Vaginal TPRs were significantly larger than cervical TPRs ($P=0.02$). The proportion of participants attaining TPR > 1 was less than 50% in all of the four FGT compartments, with vaginal tissue having the highest proportion (Table 3).

Correlation between plasma and tissue concentration

Figure S1, available as Supplementary data at *JAC* Online, shows the correlation between plasma and tissue concentration. Tenofovir concentrations in plasma were not correlated with any FGT compartment. Plasma concentrations of lamivudine and efavirenz were only significantly correlated with uterine concentration ($r=0.68$, $P<0.001$ and $r=0.6$, $P=0.4$, respectively). Fluconazole plasma concentrations were significantly correlated with fluconazole in all tissue compartments (ovarian: $r=0.9$, $P<0.001$; uterine: $r=0.93$, $P<0.001$; cervical: $r=0.91$, $P=0.03$; vaginal: $r=0.91$, $P<0.001$).

Impact of post-mortem redistribution

To examine the impact of post-mortem redistribution, we explored the relationship between TPR and post-mortem intervals (Figure S2). Post-mortem interval was not significant as a predictor for TPR in any of the models for tenofovir, lamivudine, efavirenz or fluconazole in any of the FGT tissue compartments ($P>0.05$), apart from lamivudine in uterine tissue, where longer time to post-mortem was associated with increased TPR ($P=0.036$, slope coefficient = 0.066, suggesting an 0.066 increase in TPR every hour post-mortem).

Discussion

In this study, we simultaneously measured the concentration of two commonly prescribed NRTIs, tenofovir and lamivudine, and an NNRTI, efavirenz, in addition to the antifungal fluconazole, in post-mortem tissues of the reproductive tracts of Ugandan women living with HIV/AIDs prior to death. We normalized these data to plasma to understand the relative penetration into the FGT. Our major findings were: (1) penetration ratios of the four drugs were different among the ovarian, uterine, cervical and vaginal tissues, thus, cervical tissue may not be an adequate representative for the entire FGT; and (2) penetration ratios of tenofovir and lamivudine in female genital tissues were >1 for most participants, but not for efavirenz and fluconazole.

Several studies have measured tenofovir exposure in cervical or vaginal biopsies from healthy, non-pregnant women without HIV infection, and results are very variable given different study designs.[5,11–13] In a 2021 study conducted by Thurman and colleagues,[11] at 24 h after a 14 day multiple dose period, tenofovir concentrations (medians 56 ng/mL for blood, 52.8 ng/g for cervical tissue and 63.1 ng/g for vaginal tissue) were more than 50-fold less than what we measured, but both were notably similar to plasma, as we reported. Hendrix *et al.*[13] measured tenofovir concentration in vaginal biopsy of HIV-uninfected women who took oral tenofovir disoproxil fumarate daily for 6 weeks. However, tenofovir concentrations of half of the vaginal biopsy samples were lower than the lower limit of quantification of

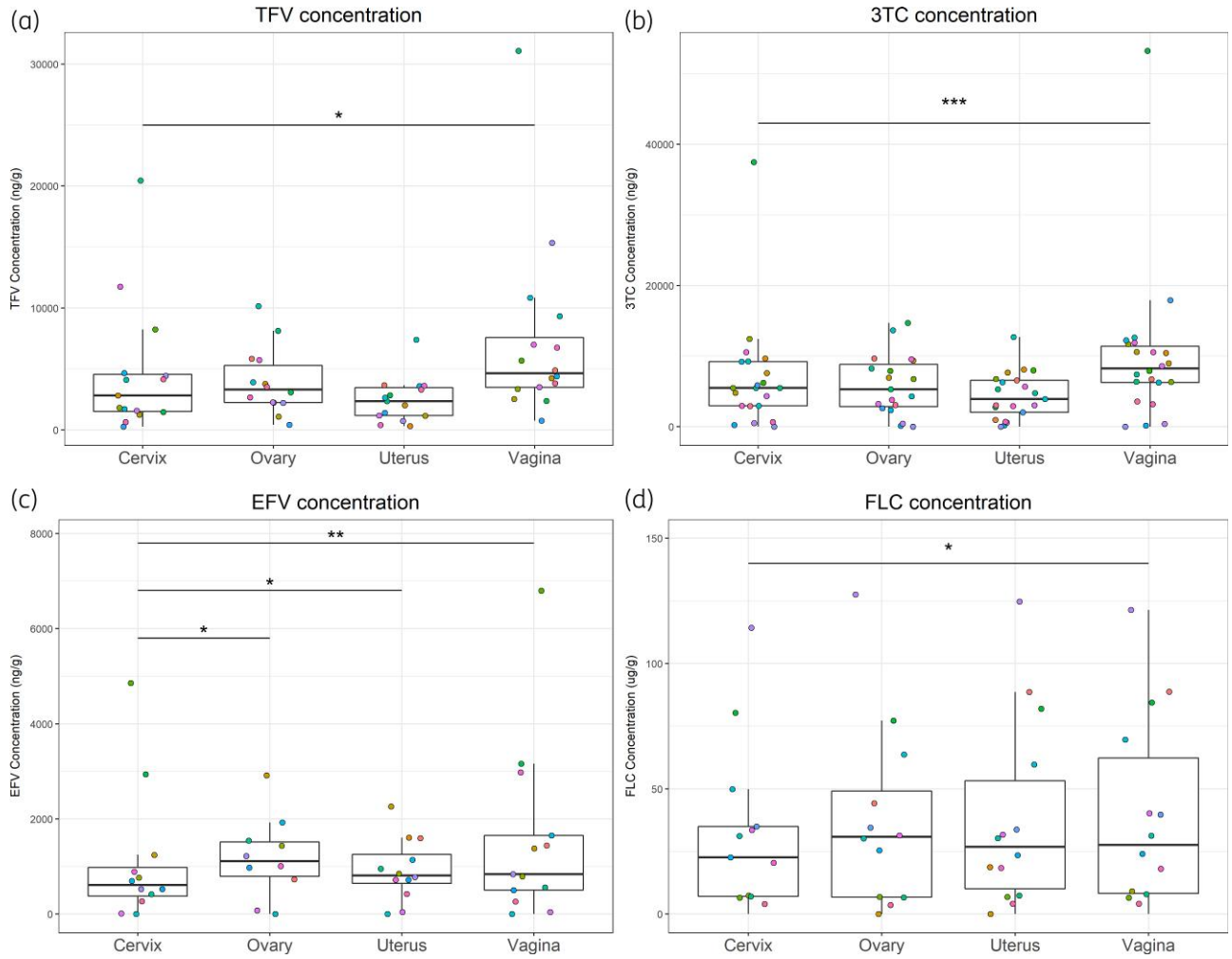

Figure 1. Concentrations of tenofovir (a), lamivudine (b), efavirenz (c) and fluconazole (d) in ovarian, uterine, cervical and vaginal tissue. In each plot, points with the same colour represent the same individual. *$P < 0.05$; **$P < 0.01$; ***$P < 0.005$. TFV, tenofovir; 3TC, lamivudine; EFV, efavirenz; FLC, fluconazole. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 2. TPRs in ovarian, uterine, cervical and vaginal tissue

	Cervix TPR			Ovary TPR			Uterus TPR			Vagina TPR		
Drug	Median (IQR)	*n*	*P*	Median (IQR)	*n*	*P*	Median (IQR)	*n*	*P*	Median (IQR)	*n*	*P*
Tenofovir	1.22 (0.77–1.96)	15	ref	1.21 (0.88–1.89)	14	0.95	1.14 (0.65–2.91)	15	0.41	1.86 (1.31–5.54)	16	0.03
Lamivudine	1.24 (0.98–1.67)	21	ref	1.34 (0.97–1.72)	19	0.81	1.05 (0.94–1.63)	21	0.36	1.83 (1.11–2.92)	22	0.001
Efavirenz	0.51 (0.22–0.82)	12	ref	0.65 (0.24–1.23)	10	0.01	0.51 (0.35–0.88)	12	0.04	0.59 (0.28–0.7)	13	0.02
Fluconazole	0.92 (0.90–0.96)	13	ref	0.85 (0.64–0.94)	12	1	0.89 (0.86–0.94)	14	0.69	0.94 (0.87–1.10)	14	0.017

Values of *n* are the sample size and *P* values are from the Wilcoxon test between the other FGT tissues and the cervical tissue (indicated as 'ref').

that particular assay, such that a measure of tissue penetration compared with blood was not accessible.[13] There are other studies, including that conducted by Thurman *et al.*,[11] which measured tenofovir in cervical/vaginal biopsies after a single oral dose of tenofovir disoproxil fumarate, but they are not discussed here or comparable with our results as our participants were considered to be at steady state.[5,11,12] Data on lamivudine exposure in the FGT at steady state are even more scarce. Herrera and colleagues[14] measured exposure of lamivudine in vaginal tissues of 18 healthy, non-HIV participants during and after they took the

Table 3. The proportion of TPR greater than 1 by tissue and drug

	Tenofovir		Lamivudine		Fluconazole		Efavirenz	
Tissue	*n*	TPR > 1 (%)	*n*	TPR > 1 (%)	*n*	TPR > 1 (%)	*n*	TPR > 1 (%)
Ovary	14	71	19	68	12	17	10	40
Uterus	15	60	21	57	14	14	12	8
Cervix	15	60	21	71	13	15	12	25
Vagina	16	88	22	86	14	43	13	23
% of TPR > 1 in all tested tissue samples	70		71		23		23	
% of participants with at least 1 tissue with TPR > 1	100		91		43		29	

combination of lamivudine and raltegravir for 7 days, and the average lamivudine TPR was 8, which is higher than what we found (median 1.86).

So far, only two studies have measured tenofovir or lamivudine in upper reproductive tissues. Rahangdale *et al.*[3] measured tenofovir in endometrial tissues of non-pregnant women living with HIV and reported values ~1% (median 26 ng/g) of what we measured in the uterine tissues (median 2356.6 ng/g). In addition, Yeh *et al.*[15] measured ART concentrations in amniotic fluid of women living with HIV during delivery; however, the sample size was small ($n = 1$ for tenofovir; $n = 6$ for lamivudine). The concentrations of tenofovir and lamivudine in amniotic fluid were one-third and one-quarter of the median tenofovir and lamivudine concentrations of uterine tissues in our study but comparisons are challenging given the small sample size in this group, and the different tissue compartment/matrix sampled.[15]

Before our current study, efavirenz concentration had only been measured in cervicovaginal fluid as representative of its exposure in the FGT by Kwara *et al.*[7] and Dumond *et al.*[4] Both studies measured efavirenz concentration in paired cervicovaginal fluid and blood plasma samples at steady state of women who were living with HIV.[4,7] In the study by Kwara *et al.*,[7] the mean cervicovaginal fluid-to-plasma ratio was 0.01 for both samples taken before and 3–4 h after administration. Dumond *et al.*[4] had more intensive sampling and derived the penetration (i.e. cervicovaginal fluid-to-plasma ratio) with AUCs of efavirenz concentration in cervicovaginal fluid and blood plasma, and the median ratio was 0.004. Since a different biomatrix was used in our study for FGT, the results are not directly comparable, but the relationship of FGT penetration of efavirenz compared with the NRTIs is the same (i.e. penetration of efavirenz at FGT was less than that of tenofovir and lamivudine).[4,7]

Exposure of fluconazole in the lower FGT from two previous studies by Mikamo *et al.*[8] and Houang *et al.*[9] are lower than what we reported; however, these were both single-dose studies using a dose lower than any used in our cohort. The two studies investigated the pharmacokinetics of fluconazole after a single oral dose of 150 mg for treatment of vaginal candidiasis, where the peak concentrations in vaginal tissue were estimated as 3.8[8] and 2.4 μg/g,[9] respectively. Meanwhile, Mikamo *et al.*[8] is the only study published to date that has measured fluconazole exposure in the upper FGT (i.e. the uterus, endometrium, oviduct and ovary), with estimated C_{max} of 4, 4.1, 4.5 and 3.9 μg/g, respectively, following a 150 mg dose. The exposure of fluconazole in the FGT was reported by Mikamo *et al.*[8] to be similar to that in serum, which implies a TPR of about 1, similar to our findings. Most importantly, these exposures are considered optimal as MICs of fluconazole for *Candida albicans* are within 0.4–0.8 mg/L (or μg/mL).[9]

Aside from different biological sample matrices and varying study design, there are other possible explanations for the high drug concentrations in plasma and FGT tissues in our study. As all our participants were hospitalized and critically ill at the time of death, multi-organ dysfunction would not be unexpected. As a consequence, the pharmacokinetics of drugs may have been affected as a collective result of altered absorption, distribution, elimination and excretion under a critically ill status. For instance, change of gastrointestinal function and hypoperfusion of vital organs reduces absorption, and albumin escape increases the free drug portion that is subject to greater distribution and clearance. However, it is worth noting that tenofovir, lamivudine and fluconazole have relatively low protein binding, which implies that decreased albumin should only minimally affect their free drug portion available for elimination. Hepatic and renal failure usually result in reduced metabolic capacity and excretion.[16] The majority of participants in our study had an estimated glomerular filtration rate (eGFR) less than 60 mL/min/1.73 m^2, which indicates a degree of renal impairment and is presumably a contributing factor of the high concentrations of tenofovir, lamivudine and fluconazole, as all three are primarily renal excreted, whereas efavirenz concentrations in our study were more similar to those observed clinically. Moreover, tissue concentration after death may change due to post-mortem redistribution, under mechanisms including cell death, ongoing blood movement, passive diffusion along a concentration gradient and putrefaction,[17] but the exact 'necrokinetics' are still unclear. Commonly proposed properties that make a drug subject to post-mortem redistribution include having a volume of distribution (V_d) greater than 3 L/kg and being lipophilic and basic.[17,18] Tenofovir, lamivudine and fluconazole have a V_d of less than 3 L/kg and relatively small logP; however, lamivudine and fluconazole are weak bases, which may lead to post-mortem redistribution. On the contrary, efavirenz is lipophilic and has a rather high V_d. Nonetheless, we conducted most of our autopsy within 24 h post-death (median 7.5 h), and all but one of the TPRs were not significantly associated with post-mortem interval, thus, we believe post-mortem redistribution is not particularly worrisome in this case. Finally, there were some outliers in our cohort who had extremely high

FGT concentrations. This could be a reflection of the nature of post-mortem sampling and unaccounted-for variables such as the high variability in patient deterioration in their hospital course before death, variable dosing before death, and variable post-mortem storage conditions.

Optimal penetration of antiretrovirals is important when used as prevention to protect women from new infection and viral replication, but it is also critical in women living with HIV to minimize viral shedding and help protect their sexual partners. Female genital shedding of HIV has been shown in previous studies to be correlated with plasma viral load and is reduced by ART.[19–21] Furthermore, Kourtis *et al*.[22] found that higher efavirenz concentration in plasma was associated with lower risk of HIV genital shedding (however, the association was not significant for efavirenz concentration in cervicovaginal fluid and genital shedding). Though the relationship between systemic or local exposure of other antiretrovirals and genital viral shedding remains unexplored, we would reasonably assume that an optimal systemic/local exposure will suppress viral shedding and viral reservoir at the transmission site. We would also generally assume that when antiretrovirals are used as pre-exposure prophylaxis (PrEP), an optimal exposure in female genital tissues is critical in prevention of HIV acquisition from sexual transmission, which is supported, for example, by the CAPRISA study, which suggested higher tenofovir concentration in the cervicovaginal fluid correlated with higher protection against HIV infection.[23] Generally, for the NRTIs tenofovir and lamivudine, most of our study participants had tissue concentrations in the FGT close to or larger than the plasma concentrations, implying an optimal penetration for the antiviral effect; the highest penetration in vaginal tissue implies even better protection at the common inter-sex transmission site. In addition, the low penetration of efavirenz emphasizes the importance of combined therapy for HIV. However, efficacy of oral tenofovir-based PrEP for women reported by clinical trials is controversial, which is likely confounded by non-adherence.[24] Therefore, further studies of the exposure–efficacy relationship are needed and so are more alternative PrEP options for women.

There are several limitations to our study. First, we only measured total drug concentrations in tissues, which are inevitably higher than the free drug concentrations. For the NRTIs tenofovir and lamivudine, attainment of expected therapeutic effect depends on the free drug portion that can diffuse into infected CD4+ T cells, transforming into the active metabolites tenofovir diphosphate and lamivudine triphosphate before suppressing the intracellular viral replication. As there are multiple binding species in tissues, such as phospholipids, interstitial albumin and lipoproteins,[25] the free drug concentrations are lower than the total concentration we measured. Second, limited by the nature of post-mortem analysis, we were not able to sample over a period of time for calculation of AUC and derive penetration ratios based on AUCs, which may be a more relevant measure of relative penetration. Third, target concentrations or a TPR cut-off for these antiretrovirals have not been established to relate to efficacy of HIV suppression or prevention. Thus, the cut-off of 1 was chosen as it could be intuitively interpreted as the 'penetrating ability' relative to the concentration in the circulating system.

In conclusion, the current study measured concentrations and determined penetration ratios of tenofovir, lamivudine, efavirenz and fluconazole in post-mortem ovarian, uterine, cervical and vaginal tissues from a population of Ugandan women previously living with HIV/AIDs. The post-mortem analysis provides a unique opportunity to evaluate antiretroviral exposure in submucosal tissues. At steady state, tenofovir and lamivudine had better penetration at the solid tissue compartments of the FGT, while penetration of efavirenz was relatively poorer. Fluconazole exposure in FGT was consistently less than that in plasma but still expected to reach FGT tissues in concentrations adequate to treat target pathogens. In addition, although it is often used as an FGT surrogate, we found that cervical tissue may not be able to represent the entire FGT when measuring penetration of anti-infective drugs.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online.

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Transparency declarations

All authors: none to declare.

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