A Compartmental Pharmacokinetic Evaluation of Long-Acting Rilpivirine in HIV-Negative Volunteers for Pre-Exposure Prophylaxis

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Rilpivirine long-acting (RPV-LA) is a parenteral formulation enabling prolonged plasma exposure. We explored its multiple-compartment pharmacokinetics (PK) after a single dose, for pre-exposure prophylaxis. Sixty-six HIV-negative volunteers were enrolled: women received an intramuscular dose of 300, 600, or 1,200 mg, with plasma and genital levels measured to 84 days postdose; men receiving 600 mg had similar PK determined in plasma and rectum. *Ex vivo* antiviral activity of cervicovaginal lavage (CVL) was also assessed. After a single dose, RPV concentrations peaked at days 6–8 and were present in plasma and genital-tract fluid to day 84. Vaginal and male rectal tissue levels matched those in plasma. At the 1,200 mg dose, CVL showed greater antiviral activity, above baseline, at days 28 and 56. All doses were well tolerated. All doses gave prolonged plasma and genital-tract rilpivirine exposure. PK and viral inhibition of repeated doses will be important in further dose selection.

Human immunodeficiency virus (HIV) pre-exposure prophylaxis (PrEP) refers to a strategy involving the use of antiretroviral (ARV) drugs to decrease the risk of HIV infection in uninfected individuals whose behavior would combine with local HIV prevalence to place them at high risk of infection. In 2012, the use of tenofovir disoproxil fumarate and emtricitabine (TDF/FTC) in combination was approved by the US Food and Drug Administration for use as PrEP, based on the results of the iPrEx[1–3] study and Partners PrEP,[4] with the former showing a 44% reduction in the incidence of HIV transmission in men who have sex with men as compared with placebo treatment, when combined with a comprehensive package of prevention. Partners PrEP showed a 67–75% relative reduction in the incidence of HIV infection using TDF/FTC among heterosexual couples in sexual partnerships containing one seronegative partner.

Although there is conceptual proof of PrEP in these specific contexts, recent negative results of two studies in women, FEM-PrEP[5] and VOICE,[6] showed no evidence of benefit of daily oral TDF/FTC. These negative outcomes were later ascribed to suboptimal adherence to the dosing regimen, thus indicating the need for high motivation in order to attain prevention success. Therefore, durable adherence is critical for a successful long-term prevention strategy.[2,4,6] In addition, the potential for side effects and toxicities associated with the use of TDF/FTC[2,7] remains a concern due to its widespread administration as HIV PrEP.

An optimal PrEP therapy should be safe to administer and be readily distributed to the relevant target tissues in concentrations that are sufficient to provide protection against HIV infection. Ideally, PrEP agents should be characterized by convenient dosing and by routes of administration that do not depend on the recipient maintaining daily adherence to dosing.

The nonnucleoside reverse-transcriptase inhibitor RPV is a diarylpyrimidine derivative that was approved by the Food and Drug Administration in 2011 for oral administration for the treatment of HIV infection in combination with other ARV drugs.[1,3] A parenteral formulation of rilpivirine (RPV-LA) with prolonged pharmacokinetic (PK) exposure is being developed, enabling improved adherence to ARV treatment over prolonged periods and having potential as an agent for HIV PrEP.[2,8,9]

The potential advantages of a long-acting formulation include infrequent parenteral administration and a low potential for gastrointestinal side effects associated with lifelong oral ARV intake.

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For HIV prevention, it is important that the traditional sequence of the drug development phases be followed as closely as possible to address whether a PrEP agent is safe and effective for use in humans. However, dose optimization studies are challenging because protective concentration targets in both plasma and the genital/rectal compartments are unknown. Therefore, they must be inferred from treatment efficacy studies, animal models, and/or *ex vivo* pharmacodynamic (PD) experiments.

We performed an adaptive design study to determine the plasma PK of RPV-LA and to measure, for the first time, RPV concentrations in cervicovaginal fluid (CVF), rectal fluid (RF), and tissue from the female genital tract and male rectum, after i.m. administration of a range of doses to HIV-negative volunteers. The study also aimed to assess the safety and tolerability of i.m. injections at 300, 600, and 1,200 mg of RPV-LA and to determine the effect of RPV in genital fluid on HIV replication *ex vivo*.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

✓ The concept of HIV PrEP has been proven with oral antiretroviral medication. Long-acting rilpivirine may be a useful PrEP medication, with parenteral i.m. injection affording prolonged plasma exposure and enabling monthly or longer dose intervals. However, its distribution to the common tissue sites of HIV infection is unknown.

WHAT QUESTION DID THIS STUDY ADDRESS?

✓ This study determined the relationship between a range of rilpivirine doses and the kinetics of exposure in plasma and in female vaginal and male RTs simultaneously, informing the feasibility of its use for PrEP.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

✓ This study demonstrates that this formulation provides prolonged exposure of rilpivirine in plasma and vaginal and RTs, consistent with levels that should provide some degree of protection. This is supported by enhanced *ex vivo* inhibition of virus in genital fluid obtained from participants.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY AND THERAPEUTICS

✓ The availability of a long-acting prophylactic medication may offer significant advantages over oral agents for reduction of rates of HIV acquisition by bypassing the poor adherence that limits efficacy of the latter.

RESULTS

Participant demographics, disposition, and safety

Of the 89 women screened for this study, 60 were enrolled; in three phases of 20, they received a single dose of RPV-LA and provided blood, fluid, and tissue samples for analysis over the ensuing 84 days in each phase of the adaptive study design (Table 1). All female participants who received the single dose completed the study with no withdrawals due to adverse events. Six men were screened and found eligible to enroll, completing the study after receiving the 600 mg i.m. dose.

Demographic information is presented in Table 2; the three groups of women receiving 300, 600, and 1,200 mg doses had similar demographic characteristics.

Participants tolerated the medication well. The majority of the adverse events experienced were mild in severity, and of those defined by the investigator as definitely or probably related to treatment, the most common were transient, self-limiting discomfort at the injection site and temporary presence of a palpable nontender nodule. This was considered to be the deposit of the administered product; no cases of this were complicated by any local signs of infection, and all cases resolved completely over the course of study involvement.

In the third phase of the study, one female participant receiving the 300 mg dose experienced a significant medical event of HIV infection after nonadherence to the use of barrier contraception with a new male sexual partner (subsequently found to be newly HIV seropositive) at approximately 6 weeks after receiving study medication.

RPV plasma PK

At the lowest dose of 300 mg, 19 of the 20 women had detectable plasma RPV above the lower limit of quantification (LLQ) at 4 h postadministration.

Table 1 Study phases and sample schedule according to adaptive design modifications

				Pharmacokinetic			Viral inhibition
				Plasma samples	Cervical fluid, rectal fluid (male)	Tissue: cervical, rectal (male)	Cervicovaginal fluid lavage
Study phase	Gender	Dose received (mg)	Number recruited	Sample schedule (study day)			
Phase I	Male	600	6	Day 0 (predose, 4- and 8 h postdose) days 1 to 84[a]	Day 0 (8 h postdose) days 1 to 84[a]	7 and 14	—
	Female	300	10			7 and 14 or 14 and 28	—
		600	10				—
Phase II	Female	600	10			14 and 28	—
		1,200	10				—
Phase III	Female	300	10			28 and 56	0 (predose), 28 and 56
		1,200	10				

[a]Days 1, 1, 3, 7, 11 (plasma only), 14, 21, 28, 42, 56, and 84.

In women, the geometric mean (GM) and 90% confidence intervals (CIs) for RPV concentrations in blood plasma for all participants over 84 days after dosing are depicted in **Figure 1**, and pharmacokinetic parameters are presented in **Table 3**. Mean peak concentration (C_{max}) in plasma of 33.7, 81.9, and 160.2 ng/ml was attained at 7.9, 6.0, and 6.2 days (mean time to C_{max} (T_{max})) after a single dose of 300, 600, and 1,200 mg, respectively. After this peak, the concentration–time curves describe a prolonged persistence of drug in plasma, with concentrations at 28 days (C_{28}) of 19.3, 44.2, and 82.9 ng/ml; at 56 days (C_{56}) of 9.1, 22.6, and 45.4 ng/ml; and at 84 days (C_{84}) of 6.4, 16.2, and 30.2 ng/ml after the 300, 600, and 1,200 mg dose, respectively. Pairwise comparisons of plasma log area under the concentration–time curve (AUC) and C_{max} (dose normalized) did not result in any significant differences in plasma exposures among the three dosing groups ($P \geq 0.1$). In addition, the 90%

Table 2 Study population demographics and dose distribution

		Female			Male
		300 mg	600 mg	1,200 mg	600 mg
Age (year)	Mean (±SD)	34 (±9)	35 (±8)	36 (±9)	36 (±15)
Height (m)	—	1.67 (1.51–1.79)	1.68 (1.58–1.76)	1.64 (1.52–1.76)	1.76 (1.69–1.81)
Weight (kg)	Median (range)	74.3 (51.2–96.7)	74.5 (55.6–100.4)	66.5 (47.8–90.6)	74.5 (61.0–100.4)
Body mass index (kg.m^{-2})	—	26.6 (19.5–34.7)	26.6 (20.1–34.7)	24.7 (17.1–34.1)	24.5 (20.4–30.0)
Ethnicity	—	—	—	—	—
Black	—	9 (45%)	14 (70%)	9 (45%)	1 (17%)
White	—	9 (45%)	5 (25%)	11 (55%)	5 (83%)
Asian	—	2 (10%)	1 (5%)	0	0

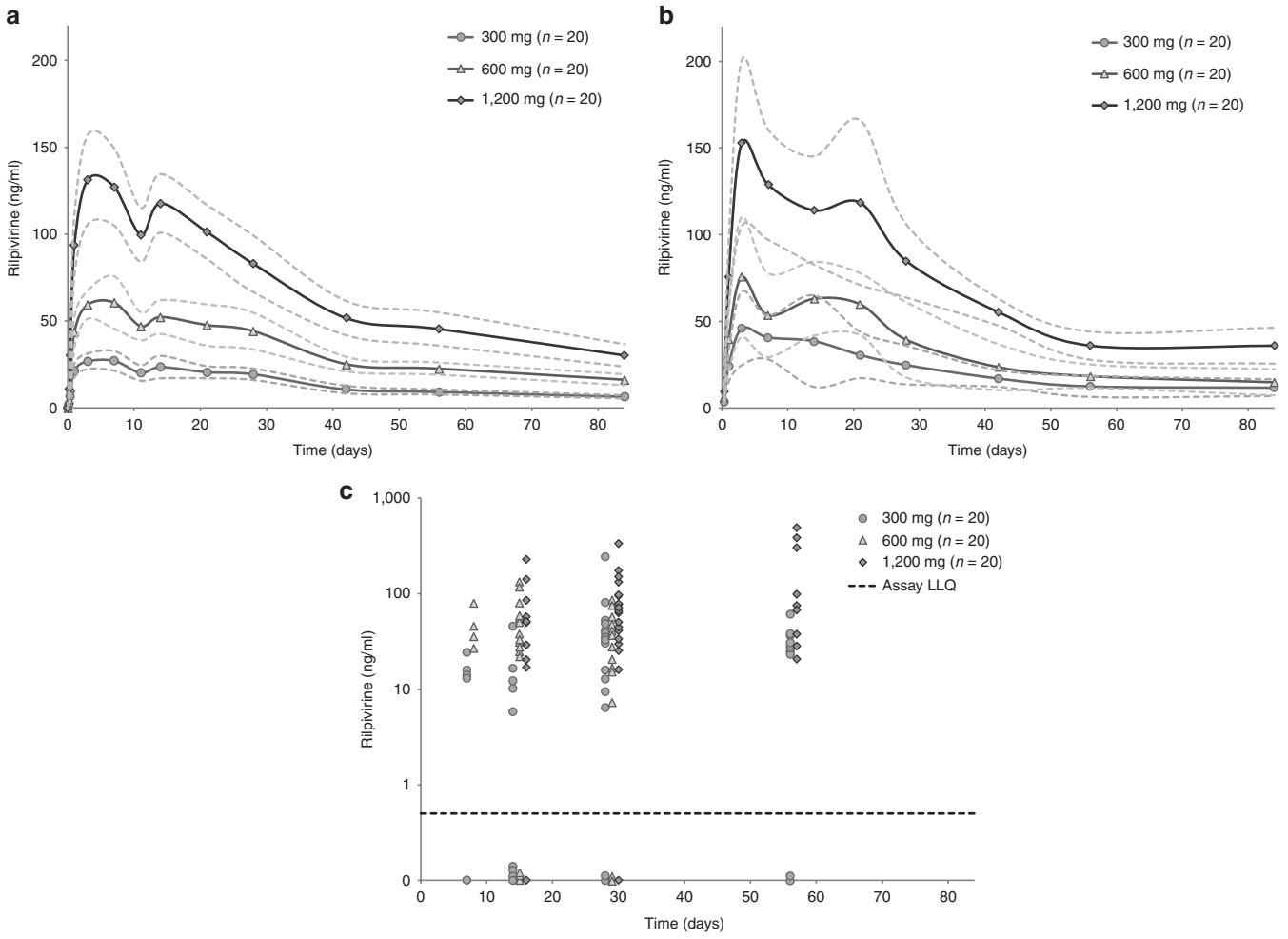

Figure 1 Rilpivirine (RPV) concentrations in women in (**a**) plasma, (**b**) cervicovaginal fluid, and (**c**) vaginal tissue, over 84 days postdose, when receiving long-acting RPV intramuscularly at 300, 600, or 1,200 mg.

CI of the regression slopes included unity. Dose proportionality was also demonstrated for rilpivirine CVF AUC and C_{max} (with the exception of C_{max} comparisons between the 1,200 and 300 mg doses; $P < 0.1$) using the same approach. These data suggest that both systemic and compartmental RPV concentrations are proportional to the administered i.m. dose.

In comparison, the six men receiving the 600 mg dose exhibited approximately 39% higher mean C_{max} as compared with women at the same dose (114 vs. 81.9 ng/ml), with subsequent higher concentrations at the sampling points C_{28} (33% higher) and C_{56} (21% higher) but with equivalent concentrations by day 84. This resulted in an increased overall exposure (AUC_{84d}) that was 32% above that measured in women at the same dose (**Table 4**).

CVF PK

RPV was detectable above the assay LLQ in the first CVF sample, 8-h postdose, in all female participants in whom sample collection was successful (57/60; 95%), the exceptions being three volunteers receiving the 300 mg dose.

RPV CVF mean T_{max} mirrored that in plasma of between 5 and 8 days, attaining higher mean peak concentrations of 67.4, 99.3, and 199.9 ng/ml at 300, 600, and 1,200 mg, respectively. Thereafter, RPV concentrations in CVF approximated those seen in plasma, with the GM $[RPV]_{CVF}/[RPV]_{PLASMA}$ ratio in paired samples maintained persistently at or above 0.8 at each dose level (**Table 3**). On day 84, RPV concentrations in CVF were still measurable, with a GM of 11.7, 14.9, and 36.0 ng/ml, respectively. Dose proportionality was also apparent in CVF,

Table 3 RPV PK in female plasma and genital tract (CVF); (geometric mean, 90% confidence intervals)

	Plasma			CVF			$[RPV]_{CVF}/[RPV]_{PLASMA}$		
Dose	**300 mg**	**600 mg**	**1,200 mg**	**300 mg**	**600 mg**	**1,200 mg**	**300 mg**	**600 mg**	**1,200 mg**
N	20	20	20	20	20	20	20	20	20
PK parameter	Geometric mean (90% confidence interval)								
C_{max} (ng/ml)	34 (28, 40)	82 (69, 95)	160 (137, 183)	67 (42, 93)	99 (67, 132)	200 (155, 245)	2.0 (1.6, 2.5)	1.2 (0.8, 1.6)	1.3 (1.0, 1.5)
T_{max} (days)	7.9 (4.3, 11.5)	6.0 (3.4, 8.6)	6.2 (4.3, 8.1)	5.3 (2.5, 8.2)	7.2 (3.1, 11.3)	8.5 (5.1, 11.9)	0.7 (0.1, 1.5)	1.2 (0.3, 2.1)	1.4 (0.1, 2.6)
$t_{½}$ (days)	43 (28, 58)	39 (33, 45)	38 (30, 47)	34 (23, 45)	31 (25, 37)	43 (31, 56)	0.8 (0.6, 1.0)	0.8 (0.7, 1.0)	1.2 (0.6, 1.8)
C_{28} (ng/ml)	19 (16, 23)	44 (34, 55)	83 (67, 99)	25 (14, 36)	39 (18, 61)	85 (64, 106)	1.2 (0.8, 1.6)	0.9 (0.3, 1.5)	1.0 (0.8, 1.3)
C_{56} (ng/ml)	9 (8, 11)	23 (19, 26)	45 (36, 55)	12 (7, 17)	15 (7, 22)	36 (26, 46)	1.3 (1.0, 1.7)	0.8 (0.5, 1.2)	0.8 (0.5, 1.1)
C_{84} (ng/ml)	6 (6, 7)	16 (13, 19)	30 (24, 37)	10 (6, 15)	12 (5, 20)	36 (26, 46)	1.7 (1.1, 2.2)	0.9 (0.6, 1.3)	1.2 (0.7, 1.7)
AUC_{84d} (ng/day/ml)	1,231 (1,054, 1,408)	2,934 (2,569 3,300)	5,982 (5,156, 6,807)	2,027 (1,409, 2,645)	3,207 (2,262, 4,152)	6,500 (5,264, 7735)	1.7 (1.3, 2.0)	1.1 (0.8, 1.4)	1.1 (0.9, 1.3)

AUC_{84d}, area under the curve from 0 to 84 days; CVF, cervicovaginal fluid; C_{max}, maximum concentration; C_{28}, drug concentration 28 days postdose; C_{56}, drug concentration 56 days postdose; C_{84}, drug concentration 84 days postdose; PK, pharmacokinetics; RPV, rilpivirine; T_{max}, time to reach C_{max}; $t_{½}$, half-life.

Table 4 RPV PK in male plasma and rectal compartment (RF); (geometric mean, 90% confidence interval)

	Plasma	RF	$[RPV]_{RF}/[RPV]_{PLASMA}$
Dose	**600 mg**	**600 mg**	**600 mg**
N	6	6	6
PK parameter	Geometric mean (90% confidence interval)		
C_{max} (ng/ml)	114.1 (88.8, 139.4)	35.7 (17.7, 53.6)	0.31 (0.20, 0.43)
T_{max} (days)	5.0 (0, 11.7)	6.2 (0, 13.6)	1.24 (0, 3.94)
$t_{½}$ (days)	30.5 (26.1, 35.0)	17.8 (9.3, 26.3)	0.61 (0.24, 0.97)
C_{28} (ng/ml)	58.9 (42.3, 75.5)	11.9 (0, 35.0)	0.20 (0, 0.49)
C_{56} (ng/ml)	27.4 (20.9, 33.8)	5.9 (3.4, 8.5)	0.22 (0.03, 0.41)
C_{84} (ng/ml)	15.9 (13.9, 17.9)	1.6 (0, 3.4)	0.10 (0, 0.22)
AUC_{84d} (ng/day/ml)	3,873.4 (3,283.7, 4,463.2)	935.0 (350.2, 1,519.8)	0.24 (0.10, 0.38)

AUC_{84d}, area under the curve from 0 to 84 days; C_{max}, maximum concentration; C_{28}, drug concentration 28 days postdose; C_{56}, drug concentration 56 days postdose; C_{84}, drug concentration 84 days postdose; PK, pharmacokinetics; RF, rectal fluid; RPV, rilpivirine; T_{max}, time to reach C_{max}; $t_{½}$, half-life.

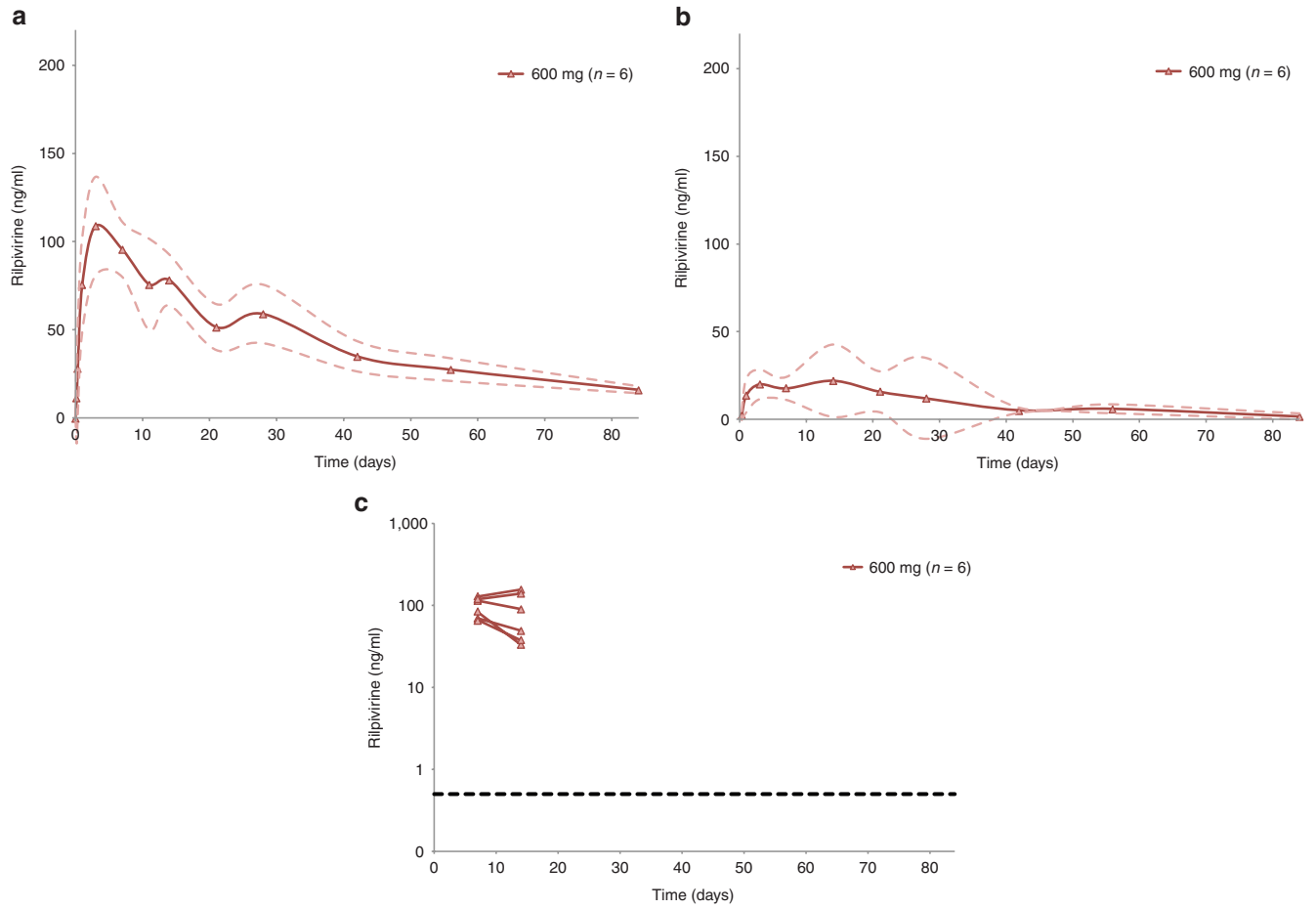

Figure 2 Rilpivirine (RPV) concentrations in men in (**a**) plasma, (**b**) rectal fluid, and (**c**) rectal tissue, over 84 days postdose, when receiving long-acting RPV intramuscularly at 300 mg.

although intersubject variation was higher than in the plasma compartment.

Rectal fluid and tissue

Rilpivirine was detectable in the first RF sample taken at 8 h postdose, in five of the six male participants, and in all six on day 1 (24 h postdose). The highest concentration in RF was observed at 6.2 days postdose and ranged from 15 to 92 ng/ml (**Table 4**; **Figure 2**), with the remaining measurable up to day 84 (from 0.4 to 7.4 ng/ml). RPV concentrations in RF were substantially lower than those in plasma or CVF. GM ratios of concentrations between RF and plasma ranged between 0.09 and 0.33 at different time points over the course of 84 days, with a ratio for the overall exposure, AUC_{0-84d} RF/plasma of 0.24.

Rectal tissue RPV concentrations in men ranged from 67 to 128 ng/ml of tissue on day 7 and from 33 to 156 ng/ml of tissue on day 14 (**Table 5**; **Figure 2**), with tissue/plasma ratios from 0.7 to 1.2 on day 7, and from 0.5 to 1.3 on day 14.

Vaginal tissue

Individual vaginal tissue (VT) RPV concentrations (GM, 90% CI) on days 7, 14, 28, and 56 are presented in **Figure 1** and **Supplementary Figure S4** online, and concentrations with associated $[RPV]_{VT}/[RPV]_{PLASMA}$ ratios are shown in **Table 5**.

In women in whom sampling was successful, RPV VT (ng/ml) concentrations (GM 90% CI presented in **Table 5**; **Figure 1**) in women receiving 300 mg ranged from 13 to 24 on day 7 (1 < LLQ), from 6 to 45 on day 14 (4 < LLQ), from 6 to 243 on day 28, (2 < LLQ), and from 23 to 61 ng/ml on day 56; with the GM of ratios in concentration—$[RPV]_{VT}/[RPV]_{PLASMA}$—on these days being 0.7, 0.6, 1.7, and 3.8, respectively.

In those receiving the 600 mg dose, concentrations ranged from 27 to 81, from 22 to 135 (3 < LLQ), and from 7 to 132 (3 < LLQ) in women who were on 600 mg on days 7 ($n = 5$), 14 ($n = 20$), and 28 ($n = 15$). $RPV]_{VT}/[RPV]_{PLASMA}$ GM ratios were 0.7, 0.8, and 0.8 on days 7, 14, and 28, respectively.

At the highest dose (1,200 mg), concentrations ranged from 17 to 228 (1 < LLQ), from 16 to 335 (1 < LLQ), and from 21 to 487 in women on days 14 ($n = 10$), 28 ($n = 19$), and 56 ($n = 10$). $RPV]_{VT}/[RPV]_{PLASMA}$ GM ratios were 0.5, 0.8, and 2.3 on days 14, 28, and 56, respectively.

Covariate analysis

A total of 520 paired plasma and CVF samples were available from the 60 female participants in the study. Plasma and CVF

Table 5 RPV concentrations in VT and RT and their relation to plasma concentrations; (geometric mean, 90% confidence intervals)

Females	Plasma ($n = 20$)			VT (n)			$[RPV]_{VT}/[RPV]_{PLASMA}$		
Dose	**300 mg**	**600 mg**	**1,200 mg**	**300 mg**	**600 mg**	**1,200 mg**	**300 mg**	**600 mg**	**1,200 mg**
PK parameter	Geometric mean (90% confidence interval)								
C_7 (ng/ml)	27.1 (21.4, 32.7)	60.6 (45.5, 75.8)	127.2 (104.9, 149.4)	16.4 (14.5, 18.2) 1 < LLQ/(5)	39.4 (31.2, 47.6) (5)	—	0.65 (0.51, 0.80)	0.72 (0.40, 1.05)	—
C_{14} (ng/ml)	23.4 (17.0, 29.9)	52.3 (42.6, 62.0)	117.7 (100.8, 134.4)	13.9 (8.1, 19.7) 4 < LLQ/(9)	41.4 (29.1, 53.8) 3 < LLQ/(20)	53.9 (28.6, 79.4) 1 < LLQ/(10)	0.59 (0.30, 0.87)	0.78 (0.55, 1.01)	0.45 (0.22, 0.67)
C_{28} (ng/ml)	19.3 (16.0, 22.6)	44.2 (33.6, 54.7)	82.9 (66.6, 99.2)	31.8 (9.1, 54.5) 2 < LLQ/(15)	33.8 (20.3, 47.3) 3 < LLQ/(15)	66.6 (38.8, 94.4) 1 < LLQ (19)	1.70 (0.58, 2.82)	0.80 (0.41, 1.19)	0.83 (0.48, 1.18)
C_{56} (ng/ml)	9.1 (7.7, 10.6)	22.6 (19.1, 26.1)	45.4 (35.8, 54.9)	31.9 (27.4, 36.4) 2 < LLQ/(10)	—	94.9 (33.3, 156.6) (10)	3.79 (2.35, 5.23)	—	2.31 (0.29, 4.90)

Males	Plasma	RT	$[RPV]_{RT}/[RPV]_{PLASMA}$
Dose	**600 mg**	**600 mg**	**600 mg**
PK parameter	Geometric mean (90% confidence interval) (n)		
C_7 (ng/ml)	95.5 (79.8, 111.1) (6)	93.7 (75.6, 111.8) (6)	0.98 (0.85, 1.11) (6)
C_{14} (ng/ml)	78.2 (63.7, 92.6) (6)	70.3 (34.2, 106.3) (6)	0.90 (0.59, 1.20) (6)

C_7, drug concentration 7 days postdose; C_{14}, drug concentration 14 days postdose; C_{28}, drug concentration 28 days postdose; C_{56}, drug concentration 56 days postdose; LLQ, lower limit of quantification; PK, pharmacokinetics; RPV, rilpivirine; RT, rectal tissue; VT, vaginal tissue.

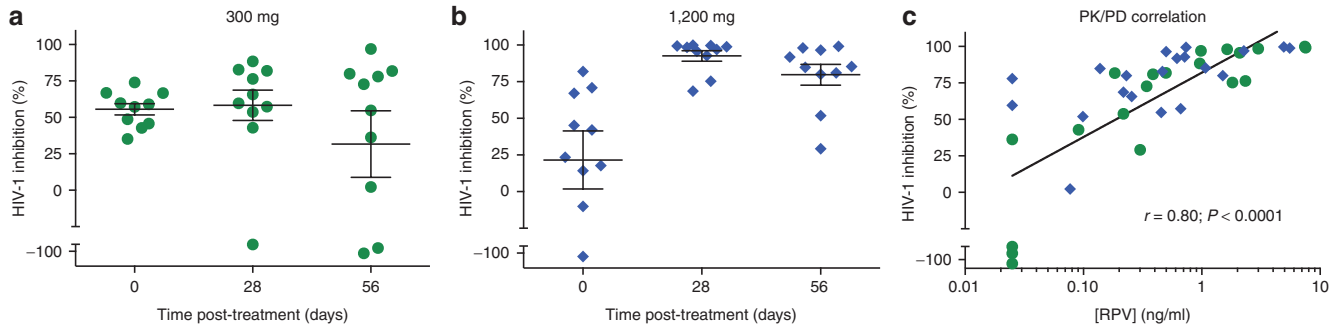

Figure 3 HIV viral inhibition percentage measured *ex vivo* in cervicovaginal lavage in female participants at baseline and at 28 and 56 days postdose: (**a**) 300 mg, $n = 10$; (**b**) 1,200 mg, $n = 10$. (**c**) Nonparametric Spearman's correlation between rilpivirine concentrations and viral inhibition at days 28 and 56.

concentrations were significantly correlated ($r^2 = 0.518$; $P < 0.01$). Furthermore, the association remained significant when stratifying by the dose administered (300 mg: $r^2 = 0.559$; $P < 0.01$; $n = 173$ pairs; 600 mg: $r^2 = 0.363$; $P < 0.01$; $n = 173$ pairs; 1,200 mg: $r^2 = 0.449$; $P < 0.01$; $n = 174$ pairs). Plasma and VT RPV concentrations from 100 paired samples were also significantly correlated ($r^2 = 0.139$; $P < 0.01$; $n = 100$), but the association was somewhat weaker, and the significance was lost when stratifying by dose.

When investigating the effects of predictors on RPV plasma concentrations, in a multivariate model, female gender and body mass index (BMI) were found to be independently associated with RPV C_{max}. Female gender was associated with an approximately 30% decrease in RPV C_{max} ($P = 0.013$), and a one-unit (kg/m^2) increase in BMI was associated with a 2.3% decrease in C_{max} ($P = 0.028$). In terms of the overall RPV exposure (AUC_{84d}) in plasma, only gender was a significant univariate predictor, in which women were associated with a ~28% decrease in RPV AUC_{84d}. Interestingly, there was no effect of either gender or BMI on RPV plasma concentrations beyond 28 days postdose. No effect of age, bodyweight, or ethnicity was observed for any of the RPV PK parameters in the plasma compartment.

In terms of predictors of RPV concentrations in the female genital tract (CVF and VT), in a multivariate analysis, age <40 years ($P < 0.031$), BMI > 25 kg/m^2 ($P = 0.005$), and RPV plasma concentrations ($P = 0.005$) were significant predictors of RPV concentrations in the female genital tract (CVF and VT), whereby age <40 years and BMI > 25 kg/m^2 were associated with a 35–40% reduction in RPV AUC and C_{max} in CVF, after adjusting for RPV plasma concentrations. There was no evidence of any colinearity between age and BMI, and the effects of age and BMI were lost beyond 28 days postdose. In a univariate analysis, RPV CVF concentrations and BMI (>25 kg/m^2) were predictors of RPV concentrations in VT at day 14. However, in a multivariate model, only BMI was a significant predictor, with an ~57% reduction in RPV tissue concentration ($P < 0.001$). No associations between BMI (or indeed other covariates) and RPV VT concentrations at days 28 or 56 were observed.

Viral inhibition in cervicovaginal lavage

RPV concentrations measured in cervicovaginal lavage (CVL) (GM; 90% CI) at days 28 and 56, respectively, were 0.45 ng/ml (0.08–0.83; 2 < LLQ) and 0.28 ng/ml (0.11–0.45; 4 < LLQ) for women who were on the 300 mg dose, and 1.90 ng/ml (0.66–3.13) and 0.63 ng/ml (0–1.79) for those who were on the 1,200 mg dose. However, it should be recognized that the procedure for CVL collection resulted in a considerable dilution of the naturally occurring fluid. Despite this, RPV concentrations in CVL and paired "undiluted" tear-test strips, both of which were taken on days 28 and 56 in phase III participants, were significantly correlated ($P < 0.00001$; Spearman's $r = 0.7$). CVL collected from women receiving a single dose of RPV-LA 1,200 mg i.m. during the third study phase ($n = 9$) had significantly greater antiviral activity on both days 28 (93 ± 12%; mean ± SD) and 56 (78 ± 23%), as compared with the baseline activity (28 ± 64%) (**Figure 3a**). By contrast, CVL obtained from women who were on the lower i.m. dose of 300 mg did not result in a significant increase in antiviral activity at either time point, as compared with baseline (**Figure 3b** and **Supplementary Figure S3** online). The activity correlated significantly ($P < 0.0001$; Spearman's $r = 0.8$) with RPV concentrations (**Figure 3c**). Because the endogenous antiviral activity of CVL is highly variable,[10–12] the data were also analyzed by subtracting the baseline inhibition from that observed at 28 and 56 days postdose, for each subject. The RPV-LA-driven inhibition after the 1,200 mg i.m. dose was higher at day 28 (71 ± 67%; mean ± SD) and persisted until day 56 (58 ± 74%; mean ± SD). By contrast, there was little to no increment after the 3,000 mg dose (**Supplementary Figure S5** online).

The volunteer who tested positive for HIV antibodies on study day 84 had detectable plasma HIV RNA of 370 and 175,060 copies/ml on study days 56 and 84, respectively. This volunteer received the lowest studied dose (300 mg i.m.) and the plasma and CVF RPV concentrations were 24.3 and 32.9 ng/ml, respectively, on day 28, 10.5 and 18.3 ng/ml, respectively, on day 42 (when presumed exposure to HIV occurred), 6.8 and 11.2 ng/ml, respectively, on day 56, and 7.5 and 14.0 ng/ml, respectively, on day 84. There was minimal change in viral inhibition in CVL obtained from this subject on day 28 (66%) or on day 56 (55%) from that at baseline (49%). These findings indicate that the 300 mg dose is not sufficient to protect against HIV infection.

DISCUSSION

This study was conducted to determine the concentrations of RPV in plasma, CVF, and VT in women after a single i.m. RPV-LA dose (300, 600, or 1,200 mg) and in the plasma, rectal compartment, and RT in men after 600 mg of RPV-LA i.m., over 84 days postdose.

Furthermore, the exploratory objective of this study was to investigate the effect of female genital-tract fluid (CVL) drug concentrations on HIV replication *ex vivo*, providing information on differential inhibitory effects of achieved RPV concentrations in biological fluids.

The rationale for the study, study design, and dose selection and sampling intervals was based on the need to investigate the potential role of RPV-LA as an HIV PrEP agent and on the results from a previously conducted healthy volunteer study by the drug manufacturer (unpublished data).

The study demonstrated that, after the administration of RPV-LA i.m. to both female and male healthy volunteers, measurable RPV plasma concentrations are achieved promptly (within 4 h postadministration) and persist for more than 84 days, in particular at higher doses. A secondary peak in the GM plasma concentration/time curves of the groups, observed at day 11, may reflect changes in the release of the drug from the injection site as well as complex RPV distribution kinetics (including immune cell trafficking of endocytosed nanoparticles from injection site to regional lymph nodes). It has been shown for long-acting preparations that the release profile can be influenced by changes in particle size distribution as more dissolution occurs over time. However, multiple peaks were observed in the individual curves (as shown in **Supplementary Figure S2** online); thus, the secondary peak appearing in the GM profiles may simply be due to increased relative sampling frequency during the first 14 days, as compared with thereafter.

RPV concentrations in the female genital tract (CVF and VT) are also achieved quickly and approximate those measured in plasma. Vaginal tissue concentrations were slightly lower than genital-tract fluid, possibly reflecting cell-to-fluid flux in response to concentration gradients.[5,13,14] RPV is highly (~99%) bound to plasma proteins, primarily to albumin. Therefore, high accumulation of RPV in CV fluid cannot be explained solely by protein binding. Instead, physicochemical properties, including the molecule's dissociation constant, partition constant, and low molecular weight, may favor active transport and low clearance from the CV compartment. One explanation is that the more acidic environment of CVF (pH 4–5) as compared with either plasma (pH 7.4) or RF (neutral to slightly basic) means that a greater percentage of RPV exists in a protonated form (pKa (logarithmic acid dissociation constant) 5.16), which may, in turn, result in mucosal "ion trapping" of the drug.

The RPV concentrations measured in the RT were equal to or higher than those in plasma, although RPV exposure in RF was approximately 75% lower than that in the plasma compartment. RF transudate may be diluted by luminal fluid derived from the

proximal gut, potentially indicating that this sample may not be a representative surrogate for RT concentrations with systemically delivered drug. Altogether, these data suggest a potential role of RPV-LA as a PrEP agent because of the exposures measured at the sites of HIV transmission.

Notably, target protective RPV concentrations in plasma or tissues are unknown and difficult to determine because of the lack of validated surrogate "efficacy" markers.

Therefore, potential efficacy markers are inferred from (i) animal models, (ii) human *in vitro* PD experiments, or (iii) phase III clinical trials of combination ARV treatment, including oral RPV. In the treatment trials, the mean RPV C_{trough} was 80 ng/ml, and the upper limit of the lowest quartile of exposures, in which group the virologic response was the lowest, was 50 ng/ml (unpublished data). However, a minimum effective RPV concentration *in vivo* for prevention has not been defined to date. It should be noted that the protein-corrected IC_{90} value (90% inhibitory concentration) for treatment is 12.1 ng/ml.[1,6]

In the third stage of the study, CVL samples were collected to determine whether RPV present in the vaginal lumen would inhibit HIV infection of susceptible cells *ex vivo*. This well-established assay was previously used to assess the antiviral activity of CVL samples collected from women exposed to a tenofovir gel formulation.[2,4,6,10] A limitation in quantifying drug in CVL is the unknown volume of vaginal fluid suspended in the saline wash; thus, it is not possible to derive a suitable correction factor for either drug or protein concentration. Nevertheless, experimental analysis correlating concentrations achieved with such inhibitory effects suggests that a single dose of 1,200 mg should deliver sufficient drug to the genital tract to provide protection against sexual exposure to HIV; however, more data, including direct challenge of cervical biopsies as another marker of pharmacodynamic effect, are needed to confirm this.

We have also shown that BMI and gender may influence the absorption (C_{max}) of parenteral RPV-LA. In particular, women with a BMI of >25 kg/m^2 may be susceptible to having lower RPV concentrations around the peak in the first 28 days after dosing, both in the systemic circulation and at the site of HIV transmission. For lipophilic agents such as RPV, the volume of distribution may be increased in subjects with high BMI, resulting in lower drug concentrations in both the central and peripheral compartments. However, these early findings are limited by an underrepresentation of men in the study cohort, and because BMI does not discriminate adipose tissue mass from muscular tissue, and men with high muscle mass are not well described. Indeed, differences between men and women may be related to a combination of factors, including different rates of release of drug from the i.m. depot and the higher body fat percentage and smaller water content in women, apart from regional differences in adipose tissue distribution.

A further consideration is that in the female genital tract, changes in pH, stage of the menstrual cycle and level of mucus production, and permeability of the vaginal epithelium may also impact RPV-LA distribution in CVF and vaginal tissue, explaining the high intraindividual variability observed in RPV concentrations in this compartment.

On the basis of these data, a phase I multiple-dosing study of RPV-LA for indication of HIV prevention is currently under way investigating steady-state PK with repeated administration of 600 and 1,200 mg doses using viral inhibition in tissue explants (both genital and rectal) as a surrogate marker for efficacy. The consideration of these aggregated data, analyzed by a population PK/PD model approach, will determine choice of dose magnitude and frequency in the design of a planned phase II study (assessing prophylactic efficacy in higher-risk populations) and, pending a favorable outcome, any subsequent global phase III studies. It is only during the latter two phases that a true determination of the efficacy and thus feasibility of parenteral HIV PrEP can be made.

ARV treatment depends on combination therapy, whereas current PrEP injectable agents are investigated as single drug prevention strategies because it is hypothesized that these would be sufficient to prevent HIV infection. However, delayed or missed injections could lead to prolonged periods of suboptimal drug exposure and increased risk of HIV acquisition; selective drug pressure in the presence of a replicating virus could enable expansion of drug resistance and onward transmission of resistant HIV strains. This may be especially true for nonnucleoside reverse-transcriptase inhibitors, in which single viral genotype mutations remarkably compromise the efficacy of this drug class,[15] whose constituents are the most commonly used third agents in first-line combination antiretroviral therapies.[16]

In conclusion, our study is the first to investigate RPV concentrations in the female genital tract and male rectal compartment after the i.m. administration of different RPV-LA doses to humans, and the first to assess the correlation between compartmental drug exposure and HIV growth *ex vivo*. This study encourages further development of RPV-LA as a potential PrEP intervention.

METHODS

Protocol development. This study was a phase I, prospective, open-label, exploratory dose-ranging study conducted at a single center (St. Stephen's Centre clinical trial unit, Chelsea and Westminster Hospital, London) with development of the protocol under the regulatory sponsorship of the St. Stephen's AIDS Trust. Funding for the study was provided by a grant from the Bill & Melinda Gates Foundation with the engagement of Tibotec (now Janssen R&D Infectious Diseases), which provided the investigational agent, provided protocol oversight, and formed part of the protocol steering committee. The protocol was approved by the Medicines Healthcare Regulatory Agency, UK, and ethical approval was obtained, before commencement of the study and after each protocol amendment, from the National Research Ethics Service, UK. The protocol concept was developed as an adaptive exploratory design with the aim of investigating RPV exposure in plasma, fluids, and tissues from the female genital tract and male rectum at up to four different doses of RPV-LA administered i.m.: 300 or 600 mg, with the option to explore either a 1,200 mg or a 150 mg dose, dependent on pharmacokinetic drug exposure attained with the former doses relative to historic data with the licensed oral therapeutic dose. For full details of the design, see **Supplementary Figure S1** online. The study was conducted in three phases, with a review of data by the protocol steering committee after each of the first two phases to determine the subsequent protocol amendments. In the first phase, 6 male participants were recruited to receive a single dose of 600 mg and 10 female participants

in each cohort received a single dose of 300 or 600 mg. After the first protocol steering committee review, the second phase enrolled two cohorts of 10 women to receive either a 600 or a 1,200 mg single dose, and the third phase completed the study with a further two cohorts of 10 women receiving either a 300 or a 1,200 mg single dose. In addition, on the basis of the protocol steering committee meetings, changes were made to the timing of vaginal tissue sampling for PK analysis (between phases) and to the timing and method of collection of CVF samples taken for exploratory PD assessments.

Study population and randomization. Male and nonpregnant, nonlactating female participants were eligible for enrollment if they provided written informed consent and met the following criteria: age between 18 and 50 years, BMI between 16 and 35 kg/m^2, and HIV-seronegative status at screening visit, with a low behavioral risk for acquisition of HIV in the preceding 6 months. Participants agreed to undergo regular HIV testing within the study and to abstain from sexual intercourse for the first 28 days of the trial and for 48 h before each subsequent study visit, before collection of genital or rectal samples.

Six male participants were recruited, and all received a single 600 mg dose. Female participants were randomly assigned (within each study phase) to receive either of the two doses being studied. A statistician created randomization lists, stratified by phase, with blinded allocation maintained by means of sealed envelopes kept in a restricted-access pharmacy. Once allocation was performed, doses were administered on an open-label basis.

Study design. Eligible participants attended on the morning of day 0, when they received a single i.m. dose of RPV-LA between 8 and 10 am.

Plasma (predose and 4- and 8 h postdose on day 0) and female genital or male RFs at 8 h postdose were collected for RPV PK analysis. Paired plasma and genital or RF samples were then collected on days 1, 3, 7, 11 (plasma only), 14, 21, 28, 42, 56, and 84 postdose.

Paired tissue biopsies were taken from the pericervical vaginal fornix in women or the rectal mucosa in men at days 7 and 14, 14 and 28, or 28 and 56, depending on the study phase (**Table 1**).

Administered product. RPV-LA (formulation G001), available as a nanoparticle suspension with a concentration of 300 mg/ml, was given by i.m. injection into either buttock (300 mg—1 ml and 600 mg—2 ml) or both buttocks (1,200 mg—two 2 ml injections) on day 0.

Sample collection

Plasma. At each scheduled time point, 6 ml whole blood was collected into lithium heparin Vacutainer vials (BD Biosciences, San Jose CA) and immediately placed on wet ice inside a light-protective container to prevent photodegeneration before centrifugation at 1,000*g* for 10 min at 4 °C (within 2 h). Plasma aliquots were stored in light-protective polypropylene tubes at −20 °C until analysis.

CVF. Female participants collected samples by aspiration of vaginal secretions using a self-inserted disposable, sterile plastic volumetric device (Rovumeter, University of North Carolina School of Pharmacy)—a syringe-like device 135 mm long, with a constant outer diameter of 8 mm and a blunt, rounded distal end at which a 5-mm opening enables sample aspiration on applying suction to the plunger. The undiluted aspirate was weighed, then chilled on wet ice in a light-protective polypropylene cryovial, before storage at −80 °C until analysis.

During study phase III on days 0 (before dose), 28, and 56, CVL samples were collected, by aspiration after lavage of the cervix and vaginal vault with 10 ml of normal saline, in order to determine antiviral activity (PD analysis). Therefore, at these study visits, direct aspiration of fluid was not performed in order to maximize the sample yield collected during the lavage process; instead, undiluted CVF was collected using Schirmer Tear-Test blotting paper strips (Intervet, Roseland, NJ) applied to the high vaginal mucosa with a vaginal speculum in place. The strips were weighed both before and after collection of the sample to enable calculation of the volume of adsorbed fluid, and the strips were placed in a light-protective cryovial for storage at −80 °C until analysis.

Rectal fluid. Samples for RF PK analysis were collected from male participants, after evacuating bowels, by adsorption onto Weck Cel cellulose spears (EYETEC; Network Medical Products, North Yorkshire, UK) that were placed in contact with apposed rectal mucosal surfaces (using a proctoscope) for at least 120 s; dry and wet weights were used to calculate the volume of fluid collected, and spears were placed inside light-protective cryovials for storage at −80 °C until analysis.

Tissue. Vaginal tissue was collected by biopsy; after insertion of the speculum, a 3 × 3 × 1 mm specimen was obtained by Sarratt biopsy forceps (Stericom, Chesham, UK), and samples were stored within 30 min in a light-protective cryovial at −80 °C until analysis.

Rectal biopsies were obtained by the same methodology described above (following the insertion of a proctoscope) from a site in the rectum proximal to the dentate line to avoid highly innervated tissue.

Analytical methods

PK. RPV concentrations in all matrices were quantified by validated high-pressure liquid chromatography–mass spectrometry using a Thermo triple quadrupole TSQ Ultra mass spectrometer (Thermo Electron Corporation, Hemel Hempstead, UK) operating in the positive ionization mode (selected reaction monitoring).[2,17] Full methodological validation is beyond the scope of this article and is provided in a separate publication.

In brief, a stable isotope–labeled internal standard (^{13}C-d4-Rilpivirine; 20 µl, 80 ng/ml) was added to plasma, CVF, and tissue (VT and RT) samples (100 µl per sample), followed by extraction with protein precipitation (acetonitrile/water; 5:1 vol/vol) and was quantified using an RPV-spiked plasma calibration curve (0·5–400 ng/ml; 100 µl per calibrator level; in duplicate).

Due to its acidic (pH 4–5) and viscous nature, CVF was diluted 1:4 with a known volume of phosphate-buffered saline (1 mmol/l; adjusted to pH 4.5 with orthophosphoric acid) in order to create a homogeneous matrix and to improve pipetting accuracy. A 100-µl aliquot was then transferred to glass tubes, and the relevant dilution factor was recorded and imputed into the analytical software.

For tissue biopsies, the weight of tissue (in milligrams) was recorded before extraction. Tissue biopsies were transferred to a MINILYS tissue homogenizer (Bertin Technologies, Bordeaux, France) and Precellys–Keramik kit (Bertin Technologies) containing 0·5-ml tubes prefilled with 14-mm ceramic beads, and made up to a volume of 100 µl with blank plasma.

Inter- and intra-assay precision and accuracy for quality control (QC) samples at low (LQC), medium (MQC), and high (HQC) concentrations in plasma were <15%. The percentage recovery (internal standard normalized) of RPV from plasma (≥96%) was shown to be consistent, precise, and reproducible. Furthermore, the percentage recovery of RPV from direct CVF aspirates (~90%) and rectal tissue (RT) (~96%) using protein precipitation was equivalent to that of plasma, thus demonstrating that drug-free plasma serves as a suitable pseudo-matrix for quantification of RPV in these matrices.

RPV concentrations in RF were quantified using a plasma calibration curve spiked (50 µl; in duplicate) onto Weck Cel or PVA polyvinyl alcohol-based spears and extracted by liquid–liquid extraction (hexane/ethyl acetate; 80:20 vol/vol). The calibration curve was linear over the 0.025–20 ng/sample. Inter- and intra-assay precision and accuracy for all QC concentrations were between 3 and 11%. The percentage recovery (internal standard normalized) of RPV absorbed onto Weck Cel or PVA-based spears, after liquid–liquid extraction, was ≥80%, and the effect of the sample matrix was minimal (<5% interference) when evaluated by spiking and postcolumn infusion experiments.

RPV concentrations in CVL were quantified using an RPV-spiked CVL calibration curve (100 µl per calibrator level) and extracted by protein precipitation (acetonitrile/water; 5:1 vol/vol). RPV-free CVL (for spiking purposes) was obtained at baseline from the subjects undergoing CVL

sampling and, subsequently, pooled. The CVL calibration curve was linear over 0.05–20 ng/ml.

RPV concentrations in all matrices were expressed as nanograms/milliliter. Tissue homogenate and rectal/vaginal fluid samples absorbed onto ophthalmic spears were quantified using a nanogram/sample calibration curve in order to account for variations in tissue weight and fluid volumes. RPV concentrations in tissue (expressed as nanograms/milliliter) were calculated by converting x mg of tissue to a volume assuming a tissue density of 1.05 g/ml.

PD. To determine the effect of RPV genital-tract concentrations on HIV replication *in vitro*, CVL samples were collected at baseline and on days 28 and 56 from women who received either 300 or 1,200 mg doses, during the third study phase. The antiviral activity of CVL samples was assessed against HIV-1_{BaL} challenge of TZM-bl cells as previously described.[7,10] In brief, TZM-bl cells were plated at 3×10^4/well and incubated overnight before exposure to approximately 10^3 $TCID_{50}$ HIV-1_{BaL} in the presence of undiluted CVL or control buffer (normal saline containing 200 µg/ml bovine serum albumin) in triplicate wells. At 48 h postinfection, the inoculum was removed by washing once with 200 µl phosphate-buffered saline; cells were lysed in 100 µl luciferase cell culture lysis reagent (Promega, Madison, WI), and cell lysates were stored at −80 °C until they were assessed for luciferase activity using a luciferase assay buffer (Promega).

Data analysis. The calculated parameters for plasma and genital-tract RPV were maximum observed concentration (C_{max}), the area under the concentration–time curve from day 0 to 84 (AUC_{84d}), and the concentration measured at 84 days after the observed i.m. dose (C_{84d}).

All PK parameters were calculated using actual blood sampling times and noncompartmental modeling techniques (WinNonlin Phoenix (version 6·1; Pharsight, Mountain View, CA).

Dose proportionality was assessed by comparing dose-normalized (to 300 mg) and log-transformed PK data (AUC and C_{max}) using an analysis of variance and pairwise comparisons. In addition, a regression analysis of individual data based on the model $y = \alpha\,\text{dose}^{\beta}$ was applied, where y is either AUC or C_{max}, and β is the slope. A value of 1 for β indicates perfect dose proportionality.

Descriptive statistics, including GMs and 90% CIs, were calculated for all parameters.

Ratios of compartmental-to-systemic drug concentrations were calculated for each PK parameter (C_{max}, AUC_{84d}, C_{84}) and at all time points over the course of 84 days. The effects of gender, body weight, BMI, age, and ethnicity on systemic (plasma) and compartmental (female genital-tract) PK were evaluated using univariate and multivariate linear regression analyses. PK parameters were log transformed and dose normalized (to a dose of 300 mg). Variables were included in a full multivariate regression model if $P < 0.1$, and backward elimination ($P < 0.1$) was used to identify the most important predictors. Colinearity diagnostics were undertaken for expected interacting variables. All statistical analyses were performed using SPSS (version 20.0; IBM, New York, NY).

For the PK/PD correlation (CVL RPV concentration vs. HIV inhibition *in vitro*), a nonparametric Spearman's correlation was calculated using GraphPad Prism software (version. 6; GraphPad Prism, La Jolla, CA).

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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AUTHOR CONTRIBUTIONS

A.G.A.J., L.J.E., P.M.M.M., D.J.B., B.C.H., B.G.G., and M.B. designed the research. A.G.A.J., Z.K., L.R.-N., C.J.H., and M.B. performed the research. L.J.E., P.M.M.M., D.E., D.J.B., and B.C.H. analyzed the data. A.G.A.J., L.J.E., P.M.M.M., and M.B. wrote the manuscript L.R.-N. performed the research.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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