Pharmacokinetic/pharmacodynamic investigation of single dose oral maraviroc in the context of HIV-1 pre exposure prophylaxis (PrEP)

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Conflict of interests

Abstract

To investigate harmacokinetics/pharmacodynamics of single-dose maraviroc 300mg in HIV-1 exposure compartments.

Maraviroc concentrations in blood, secretions (vaginal, urethral, oral, rectal) and tissue (vaginal, rectal) were measured and ex vivo challenge performed in 54 healthy volunteers to study protection from HIV-infection.

Maraviroc Cmax occurred within 4h in most compartments. Concentrations from 4-72h were above IC90 in all compartments, range 15-8095ng/mL. Mean AUC_{0-72} compartment-to-plasma ratios were highest in the rectum (45-819) and urethra (144) compared with the female genital tract (1.6-4.8) and saliva (0.2). No sex differences in AUC0-72 or Cmax were observed. No ex vivo protection from HIV-1BaL occurred in rectal or vaginal tissue.

Despite high and sustained concentrations, single-dose maraviroc was not protective against ex-vivo challenge of vaginal/ rectal tissue.

Introduction:

Daily and on demand pre-exposure prophylaxis (PrEP) to prevent HIV transmission with oral tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) has shown efficacy in clinical trials but maybe limited by renal/bone toxicity and emerging drug resistance globally .[1-3]

Maraviroc is a CCR5 co-receptor antagonist approved for the treatment of CCR5-tropic HIV-infection and has many desirable characteristics for PrEP[4]; it prevents virus entry into the host cell, is rapidly absorbed into cervicovaginal and rectal tissues (RT)[5,6] and is not recommended for first-line therapy, hence resistance is rare[7].

Evidence that maraviroc could prevent HIV vaginal transmission was provided for maraviroc in humanized RAG-hu mice[8] and macaques[9]. However, results for rectal transmission are less promising: neither macaque10[9] or ex vivo challenge of human rectal mucosa following single dosing showed protection from infection[11]. Human colorectal explants do show protection (IC_{80}) at concentrations of 500ng/mL and this concentration[12], is achieved in RT and vaginal tissue (VT) within two hours (h) of a single 300mg dose[5,6].

This study evaluated the pharmacokinetics (PK) and pharmacodynamics (PD) of a single dose (300mg) of maraviroc for 72h in multiple biological compartments in men and women and evaluated *ex vivo* protection from HIV-1$_{BaL}$ in the vagina and in the rectum.

MATERIALS AND METHODS

The study was approved by the National Research Ethics Service (13/LO/0147; www.clinicaltrialsregister.eu/ctr-search/trial/2012-003778-16/GB.). All subjects provided written informed consent.

Study design

HIV-negative men and women with no sexually transmitted infections were randomised in this open-label, PK/PD trial to one of five arms: control arm (A) and four intervention arms (B-E). Controls had two sets of PD samples taken one month apart. Subjects in intervention arms received a single dose of maraviroc 300mg on two occasions one month apart. Staggered sampling was undertaken at time points between 0-72h after dosing according to randomisation arm (Figure 1).

Collected samples were: blood, saliva (by Salivette®), rectal fluid (RF, by Weck-cel sponges; Weck-Cel surgical spear; Medtronic Ophthalmic, Jacksonville) vaginal fluid (VF, self-collected using a Rovumeter aspiration device (Recipe Pharmaceuticals, Munich), male urethral fluid (UF, by an absorptive swab, 2mmx5mmx2mm; Hunt Developments, London), and VT and RT (by Sarratt biopsy forceps obtaining five 3mmx3mmx1mm-biopsies; excess faeces were removed, VT/RT was stored for PK analysis (-80°C) or placed in 100μL PBS and transported immediately (median time 30 min) to the laboratory on ice for ex-vivo PD assays.

PK analysis

Bioanalytical method validation was carried out in accordance with FDA guidelines[12]

- **Maraviroc concentrations in plasma, saliva, vaginal secretions (direct aspirate) and tissues**

Drug concentrations were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS)[12]. Briefly, plasma, saliva, VF aspirates (diluted 1:2 with 1mM phosphate buffered saline) and VT samples were extracted by protein precipitation (in acetonitrile/water 5:1 v/v). Prior to extraction, tissue weight (mg) was recorded and converted to a volume (mL) assuming a tissue density of

1.05g/mL, and made up to 100µL with drug-free plasma. Tissues were homogenised using a MINILYS homogenizer (Bertin Technologies, Bordeaux) and Precellys–Keramik kit (Bertin Technologies, Bordeaux) containing 0.5mL tubes prefilled with 14mm ceramic beads. The calibration curve ranged between 2.5-2500ng/mL; a low calibration range (0.25-10ng/mL) was used for samples below the limit of quantification (LLQ). Inter/intra-assay precision and accuracy were <15%.

- **Maraviroc concentrations in vaginal, rectal and urethral fluid**

Drug was extracted from the sponges with a mixture of methanol/0.1% ammonium hydroxide. Internal standard maraviroc d6 (20µL; 100ng/mL) and 1mL of tert-butyl methyl ether (TBME) were added and samples tumbled (60min). The solvent phase was transferred to clean glass tubes and evaporated to dryness under a nitrogen stream and reconstituted in acetonitrile/water (100µL; 70:30 v/v). The volume of fluid on each sponge was predetermined by subtracting the weight of the "dry" sponge prior to sample collection. The calibration curve (0.02–75ng/sample) was constructed by spiking maraviroc plasma calibration standards (50µL; in duplicate) onto cellulose-based Weck-cel sponges.

Maraviroc concentrations were expressed as ng/mL of plasma or secretions/tissue. Tissue homogenate, RF and UF samples were quantified using an ng/sample calibration curve and converted to ng/mL by adjusting for recorded tissue and fluid volumes.

PD analysis

Susceptibility to HIV infection was assessed using an *ex-vivo* challenge model[14]. Reproducibility of results obtained with this model has been shown to be consistent among different laboratories.[15] VT and RT biopsies were cut in explants and exposed in duplicates to R5-tropic HIV-1$_{BaL}$ (10^4 TCID$_{50}$/mL) for 2h. A negative control of infection was included. Explants were washed four times with PBS to remove unbound virus. Rectal explants were transferred onto gelfoam rafts[14] (Welbeck Pharmaceuticals, UK), and vaginal explants onto a fresh tissue culture plate. Tissue explants were cultured for 15 days. Approximately 50% of the culture supernatants were harvested every two to three days, and both cultures were re-fed with fresh media in the absence of drug.

Viral replication was measured as p24 concentration (HIV-1 p24 ELISA, Zeptometrix Corporation, Buffalo) in culture supernatant at day 3,7,11,15.

Statistical analysis.

Pharmacokinetic analysis:

Area under the curve 0-72 hours (AUC_{0-72}), maximum concentration (C_{max}), concentration at 72h post-dose (C_{72}), time to maximum concentration (T_{max}), and half-life in plasma, saliva and genital tract (RF, RT and UF for males and VF, VT and RF for females) were determined utilising the sparse sampling option of WinNonlin Phoenix (version 6.1; Pharsight Corporation, Mountain View), calculating mean parameter values and 95% confidence intervals (95%CI; for AUC_{0-72} and C_{max} only) by naïve pooling. Compartment-to-plasma ratios ($parameter_{COMP}/parameter_{Plasma}$) were derived. Concentrations below the assay limit of quantification (LLQ) were expressed as LLQ/2. Due to differential maraviroc protein binding between plasma and genital tract[15], the unadjusted *in vitro* IC_{90} of 0.50ng/mL (un-IC_{90}) was used for comparisons in all compartments.

Differences in maraviroc AUC_{0-72} and C_{max} between males and females for plasma, saliva and RF were evaluated using a pairwise z-test. Similarly, differences in maraviroc plasma AUC_{0-72} and C_{max} compared to other compartments were assessed for males and females separately but with a Bonferroni correction for multiple comparisons.

PD analysis:

p24 AUC between days 3- 15 of culture ($p24\text{-}AUC_{3-15}$) were estimated with the non-cumulative viral antigen concentrations at the supernatant harvest days using the log-linear trapezoidal method (Prism, GraphPad, San Diego). p24 concentrations and $p24\ AUC_{3-15}$ among maraviroc-treated and untreated controls were compared using a Kruskal-Wallis test (Prism, GraphPad, San Diego).

Results

Demographics and safety

Fifty-eight subjects (30 male, 28 female) were included in the analysis (Table 1). The study drug was well-tolerated with no adverse effects reported.

PK of maraviroc in compartments

PK results are illustrated in Table 1c and Figure 1a/b. Maraviroc C_{max} was reached within 4h post-dose in all compartments, except for RF, where T_{max} was 48h in males and 36h in females. C_{72} were above the un-IC_{90} in RF (8095ng/ml), RT (79ng/ml) and UF (38ng/ml) in males and in RF (6549ng/ml), VF (aspirate: 7ng/ml, swab: 15ng/ml) and VT (19ng/ml) in females, with no gender differences seen in Cmax or AUC_{0-72} (P>0.05 for all).

Plasma

Concentrations remained above the un-IC_{90} for 24h in nine/10 subjects, and after72h 8/12subjects were above this threshold. The average terminal elimination half-life was 12h for males and 19h for females.

Plasma concentrations correlated with saliva (r^2=0.755, females; r^2=0.815, males, P=<0.0001), VT (r^2=0.661; P<0.0001), VF swabs (r^2=0.297; P=0.0004), UF (r^2=0.244; P=0.0008) and RT (r^2=0.114; P=0.0203) but not with RF (r^2=0.0007; P=0.868, females; r^2=0.00578; P=0.632, males) or VF direct aspirate (r^2=0.0923; P=0.0637).

Saliva

C_{max} in saliva exceeded un-IC_{90} in 13/13 subjects. Saliva concentrations were undetectable for 7/13 at 48h, and 11/12 at 72h. The AUC_{0-72} maraviroc$_{Saliva}$/maraviroc$_{Plasma}$ ratio was approximately 0.2 in males and females and constant over 72h.

Rectal

The average AUC_{0-72} maraviroc$_{RF}$/maraviroc$_{Plasma}$ ratio was 819 (males) and 737 (females), increasing over time as RF concentrations accumulated (C_{72}=8094ng/mL, males; C_{72}=6548ng/mL, females) and plasma concentrations declined (maraviroc$_{RF}$/maraviroc$_{Plasma}$ ratios >1000 after 24h).

C_{max} in RT was reached at 4h and was ~8-fold higher than plasma (P<0.0001). RT exceeded plasma concentrations throughout the 72h despite a rapid decline between 48-72h (C_{48}=1039ng/mL vs C_{72}=79ng/mL). The overall AUC_{0-72} maraviroc$_{RT}$/maraviroc$_{Plasma}$ ratio was 45. RF and RT significantly correlated in males (r^2= 0.617; P<0.0001).

Vagina

C_{max} in VF was reached at 4h using direct aspiration and using Weck-cel sponges. Concentrations between the two techniques correlated (r^2=0.467, P<0.0001) but were higher using Weck-cell sponges. In directly aspirated VF, maraviroc concentrations were lower than plasma during the first 12h (maraviroc$_{VF}$/maraviroc$_{Plasma}$=0.48 at 4h), suggesting a delay in drug absorption into the female genital tract.

VT C_{max} was reached at 4h and maraviroc$_{VT}$/maraviroc$_{Plasma}$ accumulation was 2.5 (P<0.01). The overall AUC_{0-72} maraviroc$_{VT}$/maraviroc$_{Plasma}$ was 4.8 (P<0.0001). VF and VT concentrations (swab only) correlated weakly (r^2=0.182; P=0.0208).

Urethra

In urethral swabs, maraviroc concentrations were detectable above the LLQ of the assay in 5/6 subjects 2h post-dose and all were detectable and above the un-IC$_{90}$ by 4h.. AUC_{0-72} was significantly higher in the urethra compared to plasma (maraviroc$_{UF}$/maraviroc$_{Plasma}$=144; P<0.0001).

Prophylactic efficacy of MVC against rectal and vaginal transmission

No protection from single dose maraviroc was observed in rectal explants after 15 days of culture (Figure 1c). p24-Cmax reached in the control arm after 15 days of *ex-vivo* culture was one log greater in rectal explants than in VT. In vaginal explants, a non-significant reduction in p24 was observed in samples collected 2h post-dose (Figure 1d), however this effect was lost by 4h post-dose.

No differences in p24 AUC_{3-15} were observed for RT and VT between control and treated arms indicating that single dose maraviroc did not affect viral replication kinetics of HIV-1$_{BaL}$ in VT and RT (Figure S1). No correlation between day 15 p24 concentrations and maraviroc level in any compartment was observed.

Discussion

We showed that a single oral dose of 300mg maraviroc results in high concentrations at multiple HIV transmission sites, with no differences between men and women. C_{max} were higher than plasma in all

sites except saliva and VF aspirate. Maraviroc persisted longest in RT, RF and UF resulting in high RT-to-plasma ratios towards the end of the sampling interval, as plasma concentrations declined. VT/VF concentrations remained above the un-IC_{90} for 48h. Differences in maraviroc concentrations between directly aspirated versus VF swabs may be due, in part, to the practical limitations of the collection methods (e.g. under or overestimation of the fluid volume, presence of air bubbles) or may be attributed to physiological differences between the two matrices (e.g. variation in protein content and composition); for example, swab samples may contain a greater proportion of plasma transudate and exfoliate from the vaginal wall. Both RF and UF showed high C_{max} and high PK variability, which may partially reflect excretion of unchanged drug, as approximately 25% and 8% of maraviroc is eliminated via faeces and urine, respectively. This is the first report of antiretroviral drug concentrations in the urethra and the good penetration into this key site of HIV acquisition is reassuring. The close correlation of saliva and plasma maraviroc concentrations suggests that saliva sampling may have a role in monitoring adherence.

Despite its favourable PK, single dose maraviroc did not provide protection from HIV-1$_{BaL}$ infection using an *ex-vivo* challenge model. Consistent with the histological and immunological differences between the rectum and vagina[17], maximum p24 concentrations were one log greater in rectal explants than in VT. The non-significant reduced infection rate at 2h post-dose observed in VT is physiologically possible and requires further investigation.

Our *ex-vivo* results concur with a lack of protection from HIV-1 observed following multiple oral dosing of maraviroc in macaques[10] and a single dosing study in humans using RT only[11]. However, more frequent repeat dosing in mucosal tissue explant studies has shown a significant increase of anti-HIV activity[13] and daily dosing may facilitate maraviroc protection further[18].

The overall lack of prophylactic efficacy may reflect drug concentrations below the threshold required to block *ex-vivo* infection in these transmission sites, which are densely populated with CCR5 expressing cells. This is supported by the greater ease with which rectal tissue was infected compared to vaginal tissue [14] and may suggest further exploration of maraviroc as PrEP in women. Maraviroc also increases mucosal CCR5+ T cells trafficking from the systemic compartment[10] and changes efflux drug transporter expression, which may negatively impact *ex-vivo* protective efficacy[10].

Currently the *ex-vivo* challenge of mucosal explants remains the only practical way to address PD, aside from large phase III trials and provides an important tool for risk reduction of late stage failure when selecting drug strategies for large studies.

In conclusion, we show that the high and sustained concentrations of maraviroc achieved in tissues following a 300mg single maraviroc dose, are not sufficient to prevent rectal or vaginal HIV transmission using ex-vivo challenge.

ACKNOWLEDGMENTS

References:

1. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, Goicochea P et al ,the iPrEx Study Team. 2010. Preexposure chemoprophylaxis for HIV prevention in men who have sex with men. N. Engl. J. Med. 363:2587–2599.

2. Molina JM, Capitant C, Spire B, Pialoux G et al ANRS IPERGAY Study Group. On-Demand Pre exposure Prophylaxis in Men at High Risk for HIV-1 Infection. N Engl J Med. 2015 Dec 3;373(23):2237-46

3. Mulligan K et al. Effects of emtricitabine/tenofovir on bone mineral density in HIV-negative persons in a randomized, double-blind, placebo-controlled trial: DXA results from iPrEx. Clin Infect Dis, 2015

4. Perry CM. 2010. Maraviroc: a review of its use in the management of CCR5-tropic HIV-1 infection. Drugs 70:1189–1213.

5. Dumond JB, Spacek MB, Heidt PE, Cohen MS, Kashuba AD. 2011. Single and multiple dose pharmacokinetics of maraviroc in saliva, semen, and rectal tissue of healthy HIV-negative men. J. Infect. Dis. 203:1484–1490.

6. Dumond JB, Patterson KB, Pecha AL et al. 2009. Maraviroc concentrates in the cervicovaginal fluid and vaginal tissue of HIV-negative women. J.Acquir. Immune Defic. Syndr. 51:546–553.

7. Frentz D, Boucher CA,van de Vijver DA. 2012.Temporal changes in the epidemiology of transmission of drug-resistant HIV-1 across the world. AIDS Rev. 14:17–27.

8. Neff CP, Ndolo T, Tandon A, Habu Y, Akkina R. 2010. Oral pre exposure prophylaxis by anti-retrovirals raltegravir and maraviroc protects against HIV-1 vaginal transmission in a humanized mouse model. PLoS One 21:e15257. doi:10.1371/journal.pone.0015257.

9. Veazey RS, Springer MS, Marx PA, Dufour J, Klasse PJ, JP M. 2005. Protection of macaques from vaginal SHIV challenge by an orally delivered CCR5 inhibitor. Nat. Med. 11:1293–1294.

10. Massud I[1], Aung W, Martin A, Bachman S et al. Lack of prophylactic efficacy of oral maraviroc in macaques despite high drug concentrations in rectal tissues. J Virol. 2013 Aug;87(16):8952-61.

11. Coll J, Moltó J, Boix J, Gómez-Mora E et al. 2015. Single oral dose of maraviroc does not prevent ex-vivo HIV infection of rectal mucosa in HIV-1 negative human volunteers. AIDS 23;29(16):2149-54

12. FDA. Guidance for Industry, Bioanalytical Method Validation. US Food and Drug Administration.Draft Guidance September 2013; http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm368107.pdf

13. Else L, Watson V, Tjia J, Hughes A, Siccardi M, Khoo S, Back D.2010. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. J Chromatogr B Analyt Technol Biomed Life Sci. 2010 Jun 1;878(19):1455-65

14. Herrera C[1], Cranage M, McGowan I, Anton P, Shattock RJ. Colorectal microbicide design: triple combinations of reverse transcriptase inhibitors are optimal against HIV-1 in tissue explants. AIDS. 2011 Oct 23;25(16):1971-9. doi: 10.1097/QAD.0b013e32834b3629.

15. Richardson-Harman N, Lackman-Smith C, Fletcher PS, Anton PA, Bremer JW, Dezzutti CS, Elliott J, Grivel JC, Guenthner P, Gupta P, Jones M, Lurain NS, Margolis LB, Mohan S, Ratner D, Reichelderfer P, Roberts P, Shattock RJ, Cummins JE, Jr. (2009) Multisite comparison of

anti-human immunodeficiency virus microbicide activity in explant assays using a novel endpoint analysis. J Clin Microbiol 47: 3530-9

16. Dorr P, Westby M, Dobbs S, et al. Maraviroc (UK-427,857), a potent, orally bioavailable, and selective small-molecule inhibitor of chemokine receptor CCR5 with broad-spectrum anti-human immunodeficiency virus type 1 activity. Antimicrob Agents Chemother 2005;49:4721–4732.
17. Poles, M. A., Elliott, J. Taing, P., Anton, P. A., & Chen, I. S. A preponderance of CCR5(+) CXCR4(+) mononuclear cells enhances gastrointestinal mucosal susceptibility to human immunodeficiency virus type 1 infection. J Virol. 75(18), 8390-8399 (2001).
18. Gulick R, Wilkin TJ, Chen Y, et al. HPTN 069/ACTG 5305: phase II study of maraviroc-based regimens for HIV PrEP in MSM. Conference on Retroviruses and Opportunistic Infections (CROI), February 22-25, 2016, Boston. Abstract 103.

Table 1a: Study design

No. men	4	7	6	8	5
No. women	6	5	6	4	7
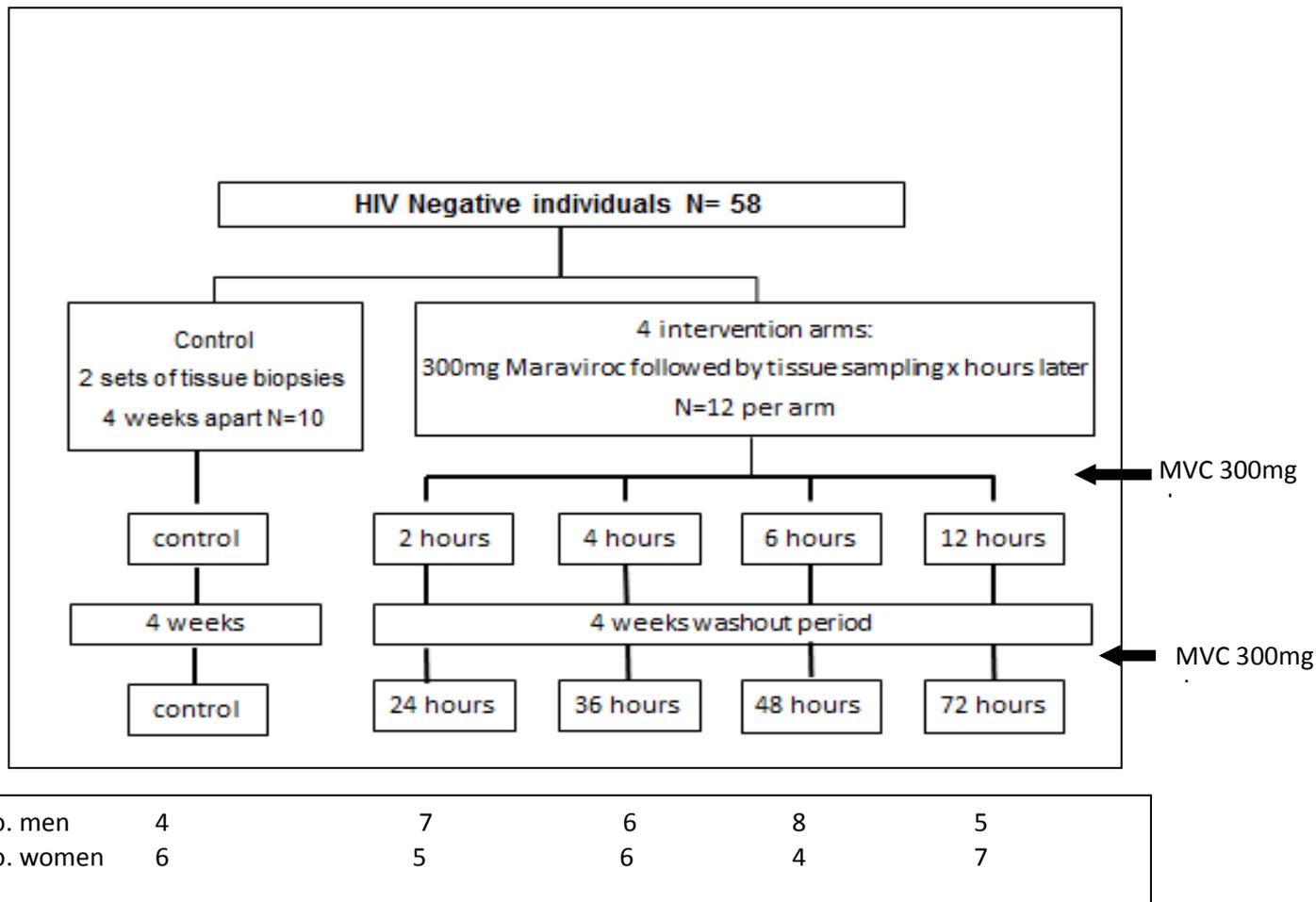
Table 1b: Demographic and baseline characteristics

	Total N=58
Demographics	
Age in years mean (SD	32 (10.66)
Gender, n (%)	
Female	28 (48%)
Male	30(52%)
Ethnicity n (%)	
White	38 (65%)
Black	16 (27%)
Other	4 (8%)
Weight (kg) mean (SD)	73.84 (14.44)
BMI kg/m^2 mean (SD)	24.73 (4.00)

SD: Standard deviation

Baseline characteristics were summarised as the mean and standard deviation (continuous normally distributed variables), median and interquartile range (non-normally distributed variables), and as frequency and percentage (categorical variables)

Table 1c. Male and female pharmacokinetic parameters in all compartments following a single dose of maraviroc 300 mg. P values in bold type are significantly different to the plasma compartment.

	Male				Female			
Compartment	AUC$_{0-72}$ (ng.h/ml)	95%CI	Ratio (vs. plasma)	p value (z test)	AUC$_{0-72}$ (ng.h/ml)	95%CI	Ratio (vs. plasma)	p value (z test)
Plasma	1212	830-1594			1353	876-1829		
Saliva	220	170-269	0.18	**<0.0001**	285	137-433	0.21	**<0.0001**
Rectal Fluid	991868	396703-1587033	818.51	**<0.01**	996496	367801-1625191	736.75	**<0.01**
Rectal Tissue	53950	31012-76888	44.52	**<0.0001**				
Urethra	173965	96354-251576	143.56	**<0.0001**				
Vaginal Fluid (aspirate)					2182	1211-3153	1.61	>0.05
Vaginal Fluid (swab)					5134	3774-6493	3.80	**<0.0001**
Vaginal Tissue					6537	5052-8023	4.83	**<0.0001**
Compartment	C$_{max}$ (ng/ml)	95%CI	Ratio (vs. plasma)	p value (z test)	C$_{max}$ (ng/ml)	95%CI	Ratio (vs. plasma)	p value (z test)
Plasma	141	46-236			242	43-441		
Saliva	30	11-48	0.21	<0.05	32	21-44	0.13	<0.05
Rectal Fluid	26165	352-51977	185.78	<0.05	45654	-2722-94031	188.40	>0.05
Rectal Tissue	1174	779-1569	8.33	**<0.0001**				
Urethra	22156	1993-42320	157.32	<0.05				

Vaginal Fluid (aspirate)					115	2-228	0.48	>0.05
Vaginal Fluid (swab)					395	184-605	1.63	>0.05
Vaginal Tissue					611	444-779	2.52	**<0.01**

Number of samples (%) below the assay limit of quantification (LLQ): Plasma – 3/94 (3%); Saliva – 20/94 (21%); Rectal Fluid – 0/82 (0%); Rectal Tissue – 0/44 (0%); Urethra – 5/47 (11%); Vaginal Fluid, aspirate – 2/41 (5%); Vaginal Fluid, swab – 1/41 (2%); Vaginal Tissue – 9/42 (21%)

Figure 1.Pharmacokinetic (1a/1b) /Pharmacodynamic (1c/1d) profile in multiple tissue compartments in HIV-negative men (1a/1c) and women (1b/1d) following a single dose of maraviroc 300 mg orally.


1a. Maraviroc drug levels in men

1b. Maraviroc drug levels in men
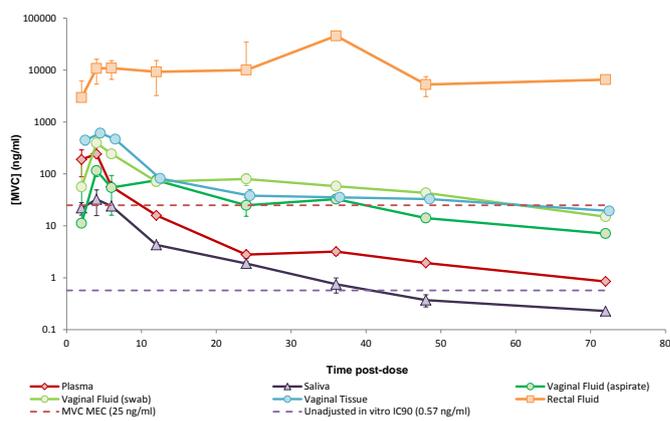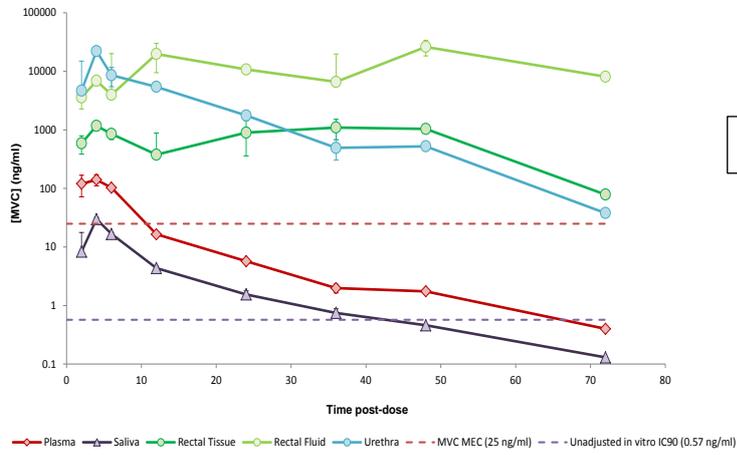
1c and 1d: HIV protection using ex vivo challenge
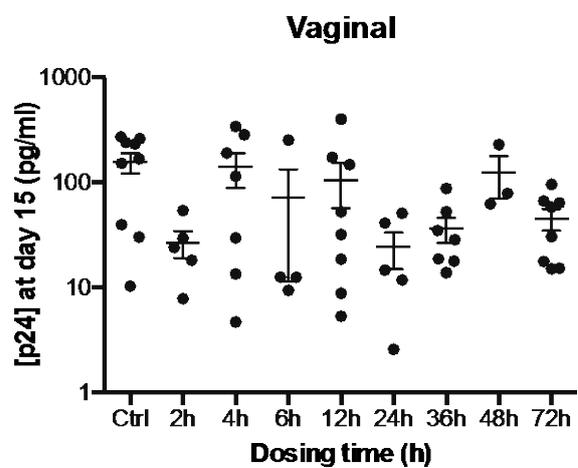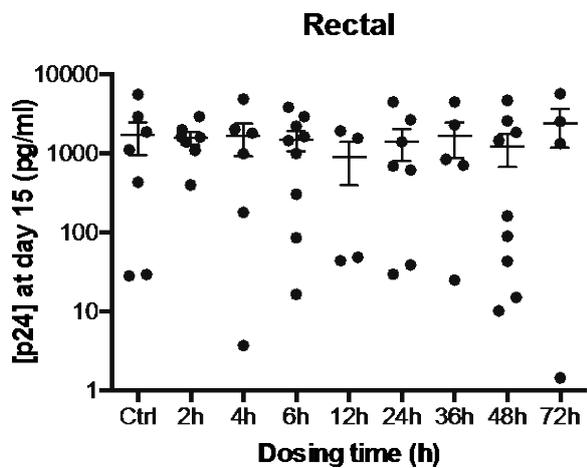
Rectal

Vaginal

Legend figure 1: PK data are expressed as mean (sem).Tissue explants were exposed 2 h to virus and then washed four times with PBS prior to transfer to gelfoam rafts for rectal explants and to fresh culture plates for vaginal explants. Explants were kept in culture for 15 days. The concentrations of p24 in the harvested supernatants were quantified by ELISA at days 3, 7, 11 and 15 of culture. The p24 concentrations at day 15 (c, d) for rectal and vaginal explants, respectively, are shown for the control and each dosing arm. Points represent the mean of duplicates for each participant, and lines the mean (+/- SEM) of each arm.

Figure S1. Pharmacodynamic profile in multiple tissue compartments in HIV-negative men (1a) and women (1b) following a single dose of maraviroc 300 mg orally.
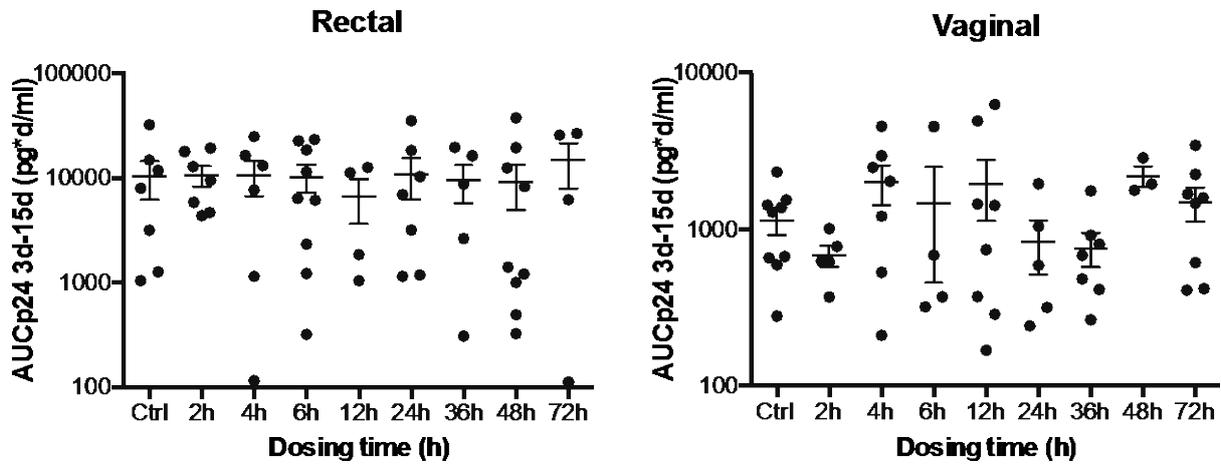
Legend figure S1: Tissue explants were exposed 2 h to virus and then washed four times with PBS prior to transfer to gelfoam rafts for rectal explants and to fresh culture plates for vaginal explants. Explants were kept in culture for 15 days. The concentrations of p24 in the harvested supernatants were quantified by ELISA at days 3, 7, 11 and 15 of culture. The p24 AUC_{3d-15d} (a, b) for rectal and vaginal explants, respectively, are shown for the control and each dosing arm. Points represent the mean of duplicates for each participant, and lines the mean (+/- SEM) of each arm.