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**

Robin Shattock, Carolina Herrera, Juan Manuel Tiraboschi, Laura Else, Deirdre Egan, Alieu Amara have no conflict of interests. Laura Dickenson is supported by PreDiCT-TB and has received a travel bursary from Gilead Sciences. Marta Boffito has received travel and research grants from and has been advisor for Janssen, Roche, Pfizer, ViiV, Bristol-Myers Squibb, Merck Sharp & Dohme, Boehringer Ingelheim, Mylan, Cipla, and Gilead. Julie Fox has received travel and research grants from Janssen, Gilead, ViiV and Bristol-Myers Squibb. David Back and Saye Khoo have received an educational grant from Gilead and honoraria from Gilead for lectures and Advisory Boards. Since completing the study Akil Jackson now works for Gilead Sciences.

**Abstract**

To investigate the pharmacokinetics/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 AUC0-72 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 PrEP4; 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 rare7.

Evidence that maraviroc could prevent HIV vaginal transmission was provided for maraviroc in humanized RAG-hu mice8 and macaques9. However, results for rectal transmission are less promising: neither macaque109 or ex vivo challenge of human rectal mucosa following single dosing showed protection from infection11. Human colorectal explants do show protection (IC80) at concentrations of 500ng/mL and this concentration12, is achieved in RT and vaginal tissue (VT) within two hours (h) of a single 300mg dose5,6.

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-1BaL in the vagina and in the rectum.

**MATERIALS AND METHODS**

The study was approved by the National Research Ethics Service (13/LO/0147). 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 guidelines12

* + **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 model14. 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-1BaL (104 TCID50/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 rafts14 (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 the 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 (AUC0-72), maximum concentration (Cmax), concentration at 72h post-dose (C72), time to maximum concentration (Tmax), 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 AUC0-72 and Cmax only) by naïve pooling. Compartment-to-plasma ratios (parameterCOMP/parameterPlasma) 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 tract15, the unadjusted *in vitro* IC90 of 0.50ng/mL (un-IC90) was used for comparisons in all compartments.

Differences in maraviroc AUC0-72 and Cmax between males and females for plasma, saliva and RF were evaluated using a pairwise z-test. Similarly, differences in maraviroc plasma AUC0-72 and Cmax 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-AUC3-15) 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 AUC3-15 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 Cmax was reached within 4h post-dose in all compartments, except for RF, where Tmax was 48h in males and 36h in females. C72 were above the un-IC90 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 AUC0-72 (P>0.05 for all).

**Plasma**

Concentrations remained above the un-IC90 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 (r2=0.755, females; r2=0.815, males, P=<0.0001), VT (r2=0.661; P<0.0001), VF swabs (r2=0.297; P=0.0004), UF (r2=0.244; P=0.0008) and RT (r2=0.114; P=0.0203) but not with RF (r2=0.0007; P=0.868, females; r2=0.00578; P=0.632, males) or VF direct aspirate (r2=0.0923; P=0.0637).

**Saliva**

Cmax in saliva exceeded un-IC90 in 13/13 subjects. Saliva concentrations were undetectable for 7/13 at 48h, and 11/12 at 72h. The AUC0-72 maravirocSaliva/maravirocPlasma ratio was approximately 0.2 in males and females and constant over 72h.

**Rectal**

The average AUC0-72 maravirocRF/maravirocPlasma ratio was 819 (males) and 737 (females), increasing over time as RF concentrations accumulated (C72=8094ng/mL, males; C72=6548ng/mL, females) and plasma concentrations declined (maravirocRF/maravirocPlasma ratios >1000 after 24h).

Cmax 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 (C48=1039ng/mL vs C72=79ng/mL). The overall AUC0-72 maravirocRT/maravirocPlasma ratio was 45. RF and RT significantly correlated in males (r2=0.617; P<0.0001).

**Vagina**

Cmax in VF was reached at 4h using direct aspiration and using Weck-cel sponges. Concentrations between the two techniques correlated (r2=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 (maravirocVF/maravirocPlasma=0.48 at 4h), suggesting a delay in drug absorption into the female genital tract.

VT Cmax was reached at 4h and maravirocVT/maravirocPlasma accumulation was 2.5 (P<0.01). The overall AUC0-72 maravirocVT/maravirocPlasma was 4.8 (P<0.0001). VF and VT concentrations (swab only) correlated weakly (r2=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-IC90 by 4h.. AUC0-72 was significantly higher in the urethra compared to plasma (maravirocUF/maravirocPlasma=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 AUC3-15 were observed for RT and VT between control and treated arms indicating that single dose maraviroc did not affect viral replication kinetics of HIV-1BaL 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. Cmax 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-IC90 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 Cmax 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-1BaL infection using an *ex-vivo* challenge model. Consistent with the histological and immunological differences between the rectum and vagina17, 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 macaques10 and a single dosing study in humans using RT only11. However, more frequent repeat dosing in mucosal tissue explant studies has shown a significant increase of anti-HIV activity13 and daily dosing may facilitate maraviroc protection further18.

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 compartment10 and changes efflux drug transporter expression, which may negatively impact *ex-vivo* protective efficacy10.

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.

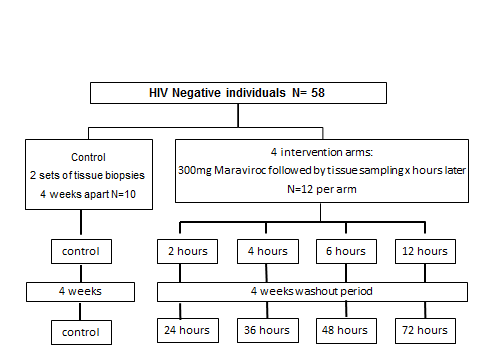
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**Table 1a: Study design**



MVC 300mg dose

MVC 300mg dose

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/m2 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** | **AUC0-72 (ng.h/ml)** | **95%CI** | **Ratio (vs. plasma)** | **p value (z test)** | **AUC0-72 (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** | **Cmax (ng/ml)** | **95%CI** | **Ratio (vs. plasma)** | **p value (z test)** | **Cmax (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

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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 AUC3d-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.