MAJOR ARTICLE

Single and Multiple Dose Pharmacokinetics of Maraviroc in Saliva, Semen, and Rectal Tissue of Healthy HIV-Negative Men

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Background. Antiretroviral pharmacology in seminal plasma (SP) and rectal tissue (RT) may provide insight into antiretroviral resistance and the prevention of sexual transmission of human immunodeficiency virus (HIV). Saliva may be of utility for noninvasively measuring adherence.

Methods. A pharmacokinetic study was performed in 12 HIV-negative men receiving maraviroc 300 mg twice daily for 8 days. Seven time-matched pairs of blood plasma (BP) and saliva samples were collected over 12 h on day 1 (PK1) and days 7 and 8 (PK2). One RT sample from each subject was collected during PK1 and PK2. Two SP samples were collected from each subject during PK1, and 6 SP samples were collected from each subject during PK2.

Results. SP AUCs were ~50% lower than BP. However, protein binding in SP ranged from 4% to 25%, resulting in protein-free concentrations >2-fold higher than BP. RT AUCs were 7.5- to 26-fold higher than BP. Maraviroc saliva AUCs were ~70% lower than BP, but saliva concentrations correlated with BP ($r^2 = 0.58$).

Conclusions. More pharmacologically available maraviroc was found in SP than BP. High RT concentrations are promising for preventing rectal HIV acquisition. Saliva correlation with BP suggests that this may be useful for monitoring adherence.

Clinical Trials Registration. NCT00775294.

Between 2004 and 2007, the incidence of HIV/AIDS increased 15% in the United States [1]. In men who have sex with men (MSM), this increase was 26%. The highest risk of HIV acquisition occurs with receptive anal intercourse [2], as the rectal mucosa is rich in lymphoid tissue and has a thin epithelium [3].

Data from the Phase III CAPRISA 004 study, which evaluated a topical tenofovir gel formulation for prevention of HIV acquisition in women, not only provide the proof of concept for microbicides but also further evidence that antiretroviral-based prevention strategies can be effective [4]. To date, topical rectal microbicide and vaccine trials have not demonstrated compelling benefit in HIV prevention; therefore, investigations using orally administered antiretrovirals are still necessary [5, 6]. Antiretrovirals can be used for both primary and secondary HIV prevention. Primary prophylaxis prevents acquisition of HIV in an uninfected individual, and secondary prophylaxis reduces the likelihood of an HIV-infected individual transmitting HIV. Primary prevention can be further separated into pre-exposure prophylaxis (PrEP) and post-exposure prophylaxis (PEP). PrEP requires administering antiretrovirals to at-risk individuals prior to a potential HIV exposure, and PEP involves administering antiretrovirals to an HIV-negative individual after a suspected exposure to HIV. Measuring antiretroviral exposure in rectal tissue could assist in selecting drugs and dosing regimens for PrEP and PEP.

HIV transmission modeling has correlated increasing concentrations of HIV RNA in semen to an increasing probability of infection [7]. Despite suppression of HIV

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RNA in blood, HIV RNA can still be detected in genital secretions of up to 8% of HIV-infected men on antiretroviral therapy [8]. Using selected antiretrovirals to target the genital tract and decrease HIV replication in genital secretions has implications for transmission. If semen drug concentrations are high enough, it might be also be possible to deliver a protective amount of drug to a receptive mucosal surface through this route [9].

The ability to monitor drug concentrations has allowed clinicians to make important decisions regarding adherence to antiretroviral regimens. Under most conditions, concentration monitoring requires a blood sample. Saliva sampling has been explored as an alternative to blood sampling and has the advantages of being less invasive and requiring less processing time upon collection [10].

Within the female genital tract, the CCR5 antagonist maraviroc (Celsentri/Selzentry; Pfizer, Inc) achieves very high exposure [11]. The current study was designed to understand exposure of maraviroc in the saliva, seminal fluid, and rectal tissue following single and multiple doses.

METHODS

Study Design and Subject Selection

This 8-day, open-label, pharmacokinetic (PK) study in healthy HIV-negative male volunteers was conducted between July 2008 and May 2009 at the University of North Carolina at Chapel Hill (UNC). Maraviroc tablets and funding for this investigator-initiated study were provided by Pfizer, Inc. The UNC Biomedical Institutional Review Board approved the protocol. All subjects provided written informed consent.

Screening procedures occurred within 42 days of maraviroc dosing. Subjects were eligible to participate if they were healthy males 18–49 years of age having a body mass index between 18 and 30 kg/m^2, with intact genital and gastrointestinal tracts. Subjects were excluded if they had a history of regular alcohol consumption, were currently smoking more than 5 cigarettes per day, had a positive urine drug screen, or had a currently active sexually transmitted disease. Subjects were screened for gonorrhea, chlamydia, trichomonas, syphilis, herpes simplex virus- 2, hepatitis B and C, and HIV. All testing was performed in the McLendon Laboratories of UNC Hospitals and in the UNC Sexually Transmitted Diseases Cooperative Research Center Microbiology Core Lab.

Subjects were excluded for any clinically significant abnormality in the laboratory results or physical examination deemed by the study physician to increase subject risk or compromise study results. Twelve-lead electrocardiogram (ECG) testing was performed per standard Pfizer protocol for healthy volunteer studies with maraviroc, and subjects were excluded if they exhibited a QTc >450 ms [12, 13]. All medications and herbal supplements, with the exception of acetaminophen (up to 1g/day), had to be discontinued at least 7 days prior to study drug dosing until study completion. Subjects were instructed to abstain from all sexual activity and use of intra-rectal products 72 h prior to dosing until study discharge.

Safety laboratory monitoring was performed on days -1, 3 or 4, 6, and follow-up. A full physical examination was performed at screening and at follow-up, and brief physical examinations were performed on days -1 and 6. Urine toxicology screening was performed at screening and on days -1 and 6. Orthostatic blood pressure and pulse measurements were performed at admission and discharge of each visit. ECGs were repeated on days -1, 6, and at follow-up.

Study Visits

Subjects received maraviroc 300 mg orally twice daily on days 1–7 and a single 300-mg dose on the morning of day 8. Subjects followed a low fiber diet for 3 days prior and a clear liquids diet the afternoon prior to the rectal tissue (RT) biopsies, which were performed via flexible sigmoidscopy. Subjects were admitted the evening before day 1 to the UNC TraCS Clinical Translational Research Center (CTRC) and provided a baseline semen sample. Subjects fasted for 2 h before and 4 h after dosing on days 1, 7, and 8. On day 1, paired blood plasma (BP) and saliva samples were obtained at pre-dose, 1, 2, 3, 6, 8, and 12 h after the first dose. Each subject collected 2 semen specimens that time-matched 2 of the 6 post-dose BP samples with a total of 4 subjects assigned to each time point. A single RT biopsy was obtained time-matching one post-dose BP sample with a total of 2 subjects assigned to each time point. Subjects recorded the time of dosing at home and were instructed to take doses without regard to meals. Subjects returned to the CTRC for trough BP and SP sampling and adherence monitoring on days 3 and 5 or on days 4 and 6. Subjects were readmitted to the CTRC in the evening of day 6. On days 7 and 8, BP, saliva, and RT PK sampling identical to day 1 were performed. However, subjects collected 6 semen specimens over days 7–8 that matched the BP sampling time-points. Subjects were discharged after the 12-h PK sample collection on day 8 and returned for safety evaluations 7–10 days after the last dose of maraviroc.

Sample Collection and Processing

Whole blood was obtained using K_2EDTA collection tubes (BD Diagnostics) and centrifuged at 1700 g at 5°C for 10 minutes. Saliva samples were collected and stored without processing. Whole semen samples were allowed to liquefy at room temperature for at least 45 minutes prior to centrifugation at 2500 g at 10°C for 15 minutes. Ten single RT biopsies were collected using Radial Jaw® 4 Large Capacity Forceps (Boston Scientific), pooled into a single cryovial, and snap frozen. Rectal biopsy sites were rinsed with a solution containing simethicone 40 mg (simethicone oral suspension 40 mg/0.6 mL, Major Pharmaceuticals) diluted in 500 mL sterile water for irrigation prior to collection. All specimens were stored at −80°C until analysis.

Maraviroc was extracted from 100 μL BP, 200 μL saliva, and 200 μL SP using solid phase extraction (SPE) with Bond Elut-Varian C-18, 100 mg, 1CC cartridges. For saliva and SP, acetonitrile protein precipitation was performed prior to SPE. Maraviroc was extracted from ~25 mg of homogenized rectal tissue by acetonitrile protein precipitation. Extraction recoveries were ≥85% for RT and BP and ≥74% for all other matrices. Variability in extraction recovery was <10%.

SP protein binding was determined by incubating 300 μL of SP pooled by subject from PK2 in duplicate at 37°C for 16 h in rapid equilibrium dialysis cartridges (Rapid Equilibrium Dialysis Device System, Thermo Scientific; Thermo Scientific RED Device Inserts, Thermo Scientific Part No: 89809; reusable Teflon base plate, Thermo Part No: 89811), followed by protein precipitation. Validation of equilibrium dialysis was performed according to FDA guidelines [14].

Maraviroc concentrations were analyzed using validated methods on an Agilent 1200 series High Performance Liquid Chromatography System and an 1100 MSD (Agilent Technologies, New Castle, Delaware). The Agilent 1100 MSD instrument was used in positive ESI spray mode, with a source temperature of 350°C. Analytes were separated on a Zorbax Eclipse XDB column (4.6 × 50 mm, 1.8 μm) with a frit (4.6 mm, 0.2 μm) (Agilent Technologies) using a gradient mobile phase method. The m/z was 514.3 for maraviroc and 309.1 for the internal standard alprazolam. The quantification ranges of the assays were 1–1000 ng/mL for BP, saliva, and SP, and 7-7000 ng/g for rectal tissue. Intra- and inter-day accuracy and precision was ≤15% and ≤10%, respectively, for all matrices. All methods were validated as mandated by the industry guidance set by the US DHHS, FDA, and CDER [14].

Data Analysis

BP, saliva, SP, and RT pharmacokinetic parameters were estimated using noncompartmental methods (Phoenix WinNonlin; Pharsight). The maximum concentration (Cmax) was determined visually, and Tmax was defined at Cmax. Exact sample collection times were used in the analysis. The area under the plasma concentration-time curve within the dosing interval (AUC_{12h}) was estimated using the log-linear trapezoidal method, and visual curve stripping was performed for estimation of the terminal elimination slope. C_{12hb} was obtained from the intermediate analysis output calculating AUC_{12h}. For PK2 individual time concentration profiles were created using the six samples collected on days 7 and 8, and by supposition, the concentration at 12 h post-dose was used as the concentration at time zero. Previous investigations have determined that sampling frequency does not affect SP concentrations of antiretrovirals [15]. To estimate SP PK parameters for PK1, and RT PK parameters for PK 1 and PK2, a composite approach was used by analyzing geometric mean concentration data. Composite profiles for PK1 SP, PK1 RT and PK2 RT were created using geometric mean concentrations and times at each time point, and samples were grouped using the closest nominal time. A rectal tissue density of 1.04 g/mL was used to convert ng/g to ng/mL [16]. To compare SP and RT exposure to BP, SP:BP and RT:BP AUC_{12h} ratios were calculated for days 1 and 7/8. To describe multidose accumulation in BP, SP, and RT, PK2:PK1 AUC_{12h} ratios were calculated.

Descriptive statistics were generated by SAS Institute, Inc software version 9.1.3 (Cary, NC). Demographic data and pharmacokinetic parameters are presented as median (range). Geometric mean ratios (GMR) with 90% confidence intervals (90% CI) are presented for PK1 saliva vs BP, PK2 saliva vs BP, and PK2 SP vs BP. For the ratios that included a composite profile (PK1 SP vs BP, PK1 RT vs BP, and PK2 RT vs BP), the composite parameter value was divided by the corresponding geometric mean BP. Spearman correlation was performed across all paired data.

The percent of protein-unbound maraviroc was calculated by subtracting the percent protein-bound from 100%. The unbound trough concentration of drug in SP was calculated conservatively: the minimum C_{12h} in SP from PK2 was multiplied by the minimum percent protein-unbound derived from the RED cartridge analytical method. The protein-unbound GMRs were calculated similarly by using the reported unbound fraction in BP (24%) [17].

RESULTS

Subject Demographics, Disposition, and Safety

Thirty-two men screened for this study: 14 were enrolled, and 12 completed. Of the 14 subjects, one subject withdrew due to grade 2 arthralgia on day 3, and the second subject was withdrawn due to positive urine toxicology for amphetamines. These 2 subjects did not contribute demographic or PK data. Median (range) age of the 12 participants was 22 (20–42) years, weight was 81.3 (62–92.4) kg, and body mass index was 25.1 (20.2–29.1) kg/m^2. Eight of the 12 subjects were Caucasian/white, 3 were African American/black, and 1 identified as mixed race (African/European).

Subjects tolerated the study medication well. Most adverse events (AEs) were mild in severity, and no serious AEs were reported. The most frequently reported AEs were headache (25%), GI disturbance (25%), dizziness (17%), and dry mouth (17%). One subject reported mild joint pain and gum sensitivity that resolved by follow-up. Three subjects were unable to produce a semen specimen within the allotted time near the end of the multiple dose study visit, one of whom reported testicular pain (related to sampling; unrelated to maraviroc) and missed 2 samples. RT sampling was well tolerated. Three subjects reported spotting on toilet tissue and a small amount of blood on stool immediately following the procedure, resolving within a few hours.

BP, Saliva, Semen and Rectal Tissue Pharmacokinetics

Figures 1 and 2 depict the BP, saliva, SP, and RT concentrations for all subjects at day 1 and day 7/8, respectively. For SP PK1, 3 samples were collected for T = 1, 5 for T = 2, 3 T = 3, and 4 for T = 6, T = 8, and T = 12. For SP PK2, 3 subjects were unable to collect all 6 samples. In 2 of these subjects, the terminal elimination slope could not be modeled, and therefore their PK parameters could not be resolved. For RT PK1, 2 samples were collected for each time point. For RT PK2, 2 samples were collected at all time-points, with the exception of 1 collected at T = 6 and 3 collected at T = 8. Maraviroc was detected in all biological matrices after the first dose. RT concentrations exceeded BP concentrations at all time-points on both days 1 and 7/8. On day 1 SP concentrations were lower than BP up to 6 h post-dose; after 6 h, they were similar. Saliva concentrations remained detectable throughout the dosing interval in all collected post-dose samples, but concentrations were lower than BP. On day 7/8, similar SP, BP, and saliva PK were observed. Spearman rank correlation coefficients indicated that BP concentrations correlated with saliva (rho = 0.76, $P < .0001$) and SP (rho = 0.74, $P < .0001$). BP concentrations did not correlate with rectal tissue (rho = 0.21, $P = .33$). All BP, saliva, and SP C_{12h} samples collected on days 3–6 had detectable maraviroc concentrations suggesting that subjects were compliant with the study drug regimen (data not shown).

Table 1 summarizes the PK parameters for each matrix. After single and multiple doses, median AUC_{12h}, C_{max}, and C_{12h} were highest in RT, and C_{max} was higher in BP than saliva and SP. For PK1 and PK2, AUC_{12h} and C_{12h} were lower in saliva than BP and SP.

Table 2 summarizes accumulation ratios (PK2:PK1) for each matrix over time. The maraviroc accumulation ratio in BP was 1.4, in saliva was 1.7, in SP was 1.6, and in RT was 4.9. Maraviroc exposures in SP, saliva, and rectal tissue were compared with BP using the following ratios: SP:BP, SAL:BP, RT:BP. For PK1, the saliva C_{12h} and AUC_{12h} were 51% and 72% lower than in BP, respectively. After PK2, the saliva C_{12h} and AUC_{12h} were 60% and 66% lower than in BP. SP PK1 C_{12h} and AUC_{12h} were 2% and 55% lower than in BP, respectively. SP PK2 C_{12h} and AUC_{12h} were 32% and 44% lower than in BP, respectively. For rectal tissue, the PK1 C_{12h} and AUC_{12h} were 51.7 and 7.5 times higher than in BP, respectively. The rectal tissue PK2 C_{12h} and AUC_{12h} were 87.6 and 26.2 times higher, respectively, than in BP.

SP protein binding ranged from 3.6% to 24.8% with a median of 8.9%. The protein binding in blood plasma is reported to be 76% [17]. The unbound trough concentration (C_{12h}) in SP was conservatively calculated to be at least 28-fold higher than the protein-free IC90 for maraviroc (14.2 ng/mL vs 0.5 ng/mL). Although total drug concentrations are lower in SP compared with BP, protein-free trough concentrations in SP are ~2.1-fold higher than protein-free trough concentrations in BP, and protein-free exposures in SP are ~1.8-fold higher than protein-free exposures in BP.

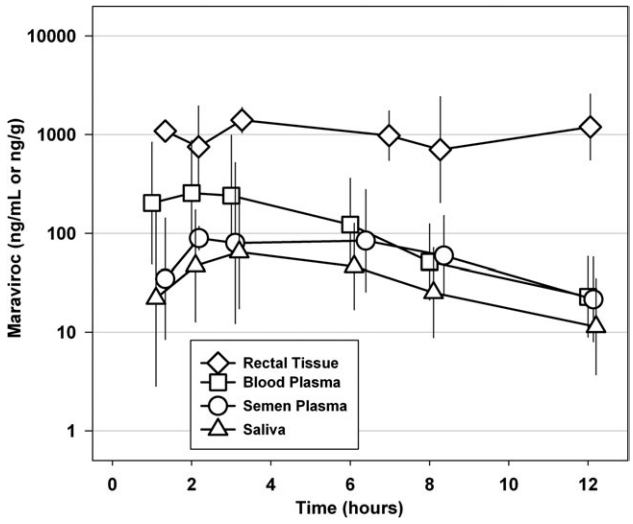

Figure 1. Maraviroc concentrations after a single dose (PK1)-geometric mean (90%CI) maraviroc concentrations in rectal tissue (*diamonds*), BP (*squares*), SP (*circles*), and saliva (triangles) on study day 1. Times are staggered to improve visualization of the data. Geometric mean sampling times are used for SP and RT.

DISCUSSION

R5 HIV is responsible for >95% of sexually transmitted new infections [18, 19]. Such viruses require CCR5 binding for entry and infection of mononuclear cells. Since maraviroc blocks CCR5 binding, its use could be a significant addition to

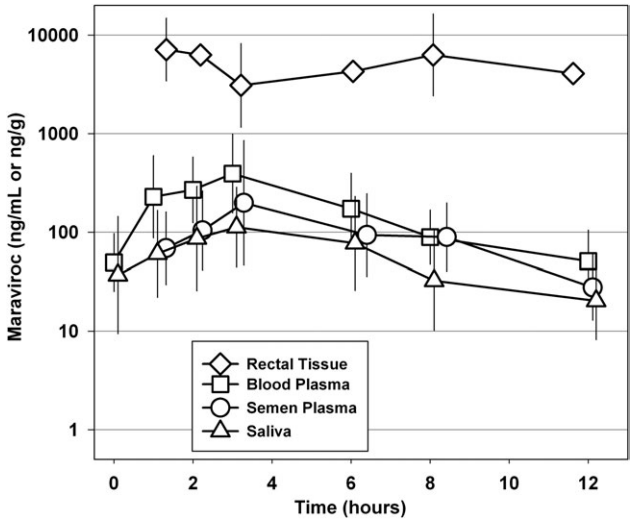

Figure 2. Maraviroc concentrations after multiple doses (PK2)-maraviroc concentrations in rectal tissue (*diamonds*) and SP (*circles*) on study day 7 and 8 and in BP (*squares*) and saliva (*triangles*) on study day 8. Times are staggered to improve visualization of the data. Geometric mean sampling times are used for SP and RT.

Table 1. Median (Range) Maraviroc Pharmacokinetic Parameters in Blood Plasma, Saliva, Seminal Plasma, and Rectal Tissue After Single (PK1) and Multiple Dosing (PK2)

	Blood plasma	Saliva	Seminal plasma	Rectal tissue
PK1 (Single dose)				
C_{max} (ng/mL or ng/g)	412 (155-690)	82.4 (37.7–199)	89.0[a]	1399[a]
T_{max} (h)	1.6 (1.0–6.0)	3.0 (1.0–6.0)	2.2[a]	3.3[a]
C_{12h} (ng/mL or ng/g)	20.5 (7.2–51.8)	11.7 (3.9–37.2)	22.3[a]	1186[a]
AUC_{12h} (ng*h/mL or ng*h/g)	1680 (510–3015)	483 (198–943)	700[a]	11622[a]
PK2 (Multiple Dose)				
C_{max} (ng/mL or ng/g)	522 (282–1402)	186 (58.6–325)	180 (90.2–664)[b]	7119[a]
T_{max} (h)	3.0 (1.0–6.0)	3.0 (1.0–6.1)	3.5 (2.4–6.3)[b]	1.3[a]
C_{12h} (ng/mL or ng/g)	52.4 (22.8–102)	24.3 (7.3–42.7)	38.2 (18.9–74.9)[b]	4466[a]
AUC_{12h} (ng*h/mL or ng*h/g)	2086 (1477–4372)	827 (252–1298)	1123 (633–2087)[b]	57326[a]

NOTE. PK1 seminal plasma, PK1 rectal tissue, and PK2 rectal tissue data were analyzed as composite concentration-time profiles. Blood plasma and saliva parameters for PK2 were calculated using data from study day 8. Seminal plasma ($N = 10$) and rectal tissue parameters for PK2 were calculated using data from study days 7 and 8. AUC_{12h} and C_{12h} could not be determined for 2 subjects' PK2 seminal plasma due to missing samples and were excluded.

[a] Composite profiles.

[b] $N = 10$.

antiretroviral prevention strategies. Additionally, maraviroc carries relatively few adverse events that occur in low frequency, making it a potentially favorable candidate [17]. This study was designed to assess the pharmacologic plausibility of using orally dosed maraviroc for primary (RT) and secondary (SP) prevention in men and to explore alternate methods for clinical trials adherence measures (saliva).

Understanding antiretroviral RT pharmacokinetics is essential in developing appropriate strategies to prevent rectal acquisition of HIV. Compared to BP, RT exposures of maraviroc were 7.5 times higher after a single dose and 26 times higher after multiple doses. Currently, the concentration of maraviroc needed to prevent HIV is not known. However, colorectal tissue explant data demonstrate that a maraviroc concentration of 500 ng/mL prevented 85% of infections after incubating the tissue for at least 3 h [20]. Our data show that a single maraviroc dose achieves an average concentration (AUC_{12h}/12 h) 2-fold higher than this concentration. This quick penetration of maraviroc into the rectal tissue may be due to a highly vascularized mucosa and interstitial trapping. Our findings are also consistent with radio-imaging studies in rodents which demonstrated 40–50 fold higher exposures of radiolabeled maraviroc in the gastrointestinal tract (including GALT lymphatics) when administered intravenously [21]. The accumulation in RT after multiple dosing is 4 times greater than in BP (4.1 vs 1.29). This suggests that mechanisms other than distribution from BP are responsible for this accumulation. We hypothesize that these high exposures may in part be due to the elimination of maraviroc (30% of the dose is fecally eliminated) and mucus trapping of drug [22]. The use of composite profiles in this study was employed due to the nature of sampling limitations for SP after a single dose and RT. Geometric means of the concentrations and times for these composites best represented the data visually compared with means or medians.

Table 2. Relative Exposure of Maraviroc in Saliva, Seminal, and Rectal Tissue to Blood Plasma and Day 8:Day 1 Accumulation Ratios

	Blood plasma	Saliva	Seminal plasma	Rectal tissue
PK1 (Single Dose)				
C_{12h}	ref	0.49 (0.35–0.68)	0.98[a]	51.75[a]
AUC_{12h}	ref	0.28 (0.23–0.35)	0.45[a]	7.48[a]
PK2 (Multiple Dose)				
C_{12h}	ref	0.40 (0.29–0.54)	0.68 (0.48–0.95)	87.55[a]
AUC_{12h}	ref	0.34 (0.27–0.43)	0.56 (0.44–0.70)	26.24[a]
PK2 : PK1				
C_{12h}	2.23 (1.78–2.78)	1.81 (1.37–2.38)	1.57[a]	3.77[a]
AUC_{12h}	1.41 (1.18–1.68)	1.74 (1.38–2.19)	1.65[a]	4.93[a]

NOTE. C_{12h} and AUC_{12h} ratios for saliva to BP (Saliva:BP), SP to BP (SP:BP) ($N = 10$), and rectal tissue to BP (RT:BP). A tissue density of 1.04 g/mL was used to compare rectal tissue concentrations to BP concentrations. Accumulations ratios comparing PK2 (day 8) C_{12h} and AUC_{12h} to PK1 (day 1) C_{12h} and AUC_{12h}. Geometric mean ratios (90% CI) are presented, except for parameters based on composite profiles.

[a] Composite profile used.

This study was not designed to evaluate the terminal elimination of maraviroc in the rectal mucosa. This study characterized the pharmacokinetics of the unperturbed rectal mucosa. Bowel preparations were not used prior to the biopsy procedure, since hyperosmolar enemas can shift a significant amount of water into the lumen of the colon and cause epithelial sloughing [23]. However, future studies assessing the impact of bowel preparations will be important, as they can be commonly (up to 60% of MSM) used prior to anal intercourse [24] and may increase the risk of HIV transmission [2]. A study by Hendrix et al is ongoing to better assess preferences and the effects of various enemas on the integrity of the rectal mucosa (http://clinicaltrials.gov/ct2/show/NCT00696618).

Previous data in the female genital tract demonstrated that maraviroc concentrated 3-fold in cervicovaginal fluid (CVF), and protein binding in CVF was 10% of that in BP [11]. Overall, this study found that total SP concentrations were lower than BP. Maraviroc is the first antiretroviral to demonstrate a discrepancy between male and female genital tract exposures. Although the explanation for this finding is not unknown, these data caution against extrapolating genital tract pharmacology data generated in one sex to the opposite sex.

Our finding of total maraviroc exposure in SP being lower than BP is consistent with other antiretrovirals based on BP protein binding. A correlation can be visualized between the relative exposures of antiretrovirals (as measured by SP:BP AUC ratios) and BP protein binding (Figure 3). As BP protein binding decreases, there is greater amount of protein-unbound drug available to distribute into physiological compartments.

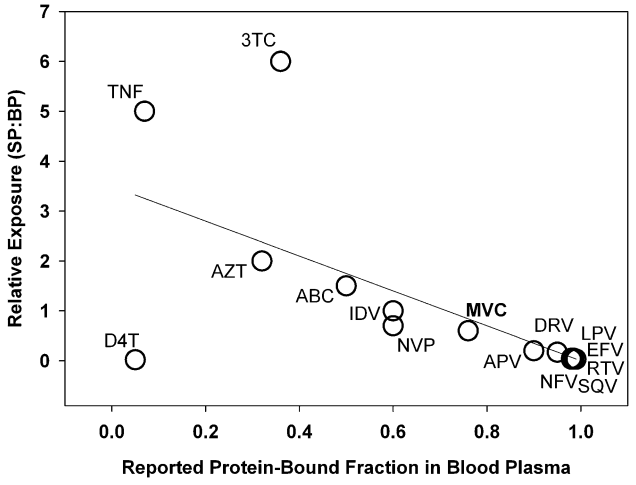

Figure 3. Relative antiretroviral exposure in the male genital tract versus protein binding in blood plasma–the relative exposure (SP:BP) of 15 antiretrovirals in the male genital tract negatively correlates with their respective reported protein binding in blood plasma (rho = −.59; $P = .02$). Tenofovir (TNF), Stavudine (D4T), Lamivudine (3TC), Zidovudine (AZT), Abacavir (ABC), Indinavir (IDV), Nevirapine (NVP), Maraviroc (MVC), Amprenavir (APV), Darunavir (DRV), Nelfinavir (NFV), Lopinavir (LPV), Efavirenz (EFV), Ritonavir (RTV), Saquinavir (SQV). [25, 26]

Approximately 76% of maraviroc is bound to albumin in BP, leaving 24% available for pharmacologic activity. However, albumin concentrations in SP are ~1/35 of that in BP [27]. This investigation determined that unbound SP concentrations were ~2-fold higher than BP concentrations, and at least 28-fold higher than the protein-free IC90 for maraviroc (0.5 ng/mL). To evaluate maraviroc's role in secondary prevention, the ability of maraviroc to fully suppress HIV replication in the male genital tract will need to be confirmed in animal models or longitudinal investigations in HIV-infected men.

Finally, saliva was investigated as a potential approach to adherence testing. The AUC and C12h of maraviroc in saliva were ~30%, and 40%–50% of that of BP, respectively. Of the biological matrices investigated, saliva concentrations correlated with BP concentrations (rho = 0.76, $P < .001$), suggesting that saliva could be used for real-time adherence monitoring, especially in prevention trials.

In summary, this is the first study to evaluate antiretroviral exposure in the rectal mucosa, and the first to measure maraviroc and its protein binding in SP. These data provide pharmacologic plausibility of maraviroc's use in primary and secondary HIV prevention. The quick penetration and sustained concentrations of maraviroc in the rectal mucosa are desirable characteristics. The unbound concentrations in semen are higher than in blood and could be effective in suppressing R5 tropic HIV replication in the male genital tract. Future investigations will determine if the concentrations in rectal tissue and semen can prevent HIV acquisition and fully suppress viral shedding. Saliva sampling is a feasible noninvasive method of monitoring drug adherence. Despite intensive sampling and scheduling challenges for multiple precisely timed rectal biopsies and semen samples, our data demonstrate that these studies can be performed efficiently and safely.

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