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Pharmacokinetics of Antiretrovirals in Mucosal Tissue

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Abstract

Introduction—In the absence of an HIV vaccine or cure, antiretroviral (ARV) based prevention strategies are being investigated to reduce HIV incidence. These prevention strategies depend on achieving effective drug concentrations at the site HIV exposure which is most commonly the mucosal tissues of the lower gastrointestinal tract and the female genital tract.

Areas covered—This article collates all known data regarding drug exposure in these vulnerable mucosal tissues, and reviews important mechanisms of ARV drug distribution. Research papers and abstracts describing antiretroviral pharmacokinetics in the female genital tract and lower gastrointestinal mucosal tissues available in MEDLINE® or presented at scientific conferences prior to December 2014 are reviewed in detail. Important influences on ARV mucosal tissue distribution, including protein binding, active drug transport, and endogenous hormones, are also reviewed.

Expert opinion—ARVs exhibit highly variable pharmacokinetics in mucosal tissues. In general, antiretroviral exposure is higher in the lower gastrointestinal tract compared to the female genital tract, but concentrations required for protective efficacy are largely unknown. The expected site of HIV exposure represents an important consideration when designing and optimizing antiretroviral based prevention strategies.

Keywords

antiretroviral; mucosal tissue; HIV prevention; pharmacokinetics

1. Introduction

As of the end of 2013, 35 million people worldwide were living with HIV/AIDS.(1) Highly active antiretroviral treatment (HAART) strategies effectively control HIV's progression into AIDS, restoring life expectancy and quality of life to that of an uninfected person. Nonetheless, of the 32.6 million HIV infected persons living in lower and middle-income countries, approximately 64% (20.9 million) do not have access to HAART.(1) Therefore, prevention strategies are needed to contain the epidemic.

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In the absence of a licensed vaccine, the use of antiretrovirals for HIV prevention has been investigated. The utility of one of these approaches, treatment as prevention (TasP), was recently established in the landmark HPTN052 study where it was found that consistent suppression of blood plasma HIV RNA in HIV infected subjects using early HAART reduced transmission to their uninfected partners by greater than 95%.⁽²⁾ This study demonstrated the highest degree of protection of any HIV prevention trial. In the United States, less than 50% of HIV infected patients on HAART exhibit a suppressed viral load.⁽³⁾ This is an important limitation of the TasP approach and warrants further exploration into alternative HIV prevention strategies in uninfected individuals.

In 2012, Truvada[®], a fixed dose combination tablet of two nucleoside reverse transcriptase inhibitors tenofovir disoproxil fumarate and emtricitabine, received FDA approval to be used as part of an HIV prevention package in high risk individuals. Truvada[®] received this licensing on the basis of a 44-75% decrease in HIV incidence in 3 clinical trials studying its use in diverse study populations including: serodiscordant heterosexual couples (Partner's PrEP) and other high risk heterosexual individuals (TDF2), and men who have sex with men (MSM; iPrEX).⁽⁴⁻⁶⁾ Subsequently, a study investigating Truvada[®] pre exposure prophylaxis (PrEP) in IV drug users demonstrated 49% protection.⁽⁷⁾ Truvada[®] is the first relatively discreet, user-controlled HIV prevention option available to women. Yet, clinical trials in women have exhibited mixed results: two large phase III trials, FEM-PrEP and VOICE, failed to show HIV prevention efficacy for tenofovir taken with or without emtricitabine.^(8, 9) These results were explained by the low degree of adherence to the daily regimens by study subjects. Subsequently, observational analyses have directly linked the effectiveness of Truvada[®] for PrEP with adherence.^(10, 11) However, the degree of adherence required for protection may be contingent on the site of HIV exposure as evidenced by recent data demonstrating only two doses per week of Truvada[®] reduced HIV incidence by up to 90% in the MSM population of iPrEX (10): a level of adherence that was also seen in FEM PrEP and VOICE.^(8, 9)

The most common mode of HIV transmission is through sexual activity, whereby mucosal tissues are the primary sites of HIV exposure. For TasP approaches to be successful, drug concentrations in infected individuals must be sufficient to suppress viral replication and shedding in the local anatomical sites associated with transmission, and may be necessary at high concentrations in the mucosal fluid serving as the viral source of transmission.⁽¹²⁾ Conversely, PrEP approaches rely on drug concentrations in an uninfected individual being sufficient to prevent viral entry, integration or replication in HIV target cells in mucosal tissue and/or regional lymph nodes at the time of exposure.⁽¹³⁾ Consequently, when considering the utility of TasP and PrEP for global reduction in HIV incidence, a thorough understanding of antiretroviral pharmacology in mucosal tissues becomes exceedingly important to ensure that optimal drugs, doses, and dosing schedules have been selected. For this purpose, the present review will examine antiretroviral pharmacokinetics in the mucosal tissue types most commonly involved in HIV transmission –the lower gastrointestinal tract and the female genital tract.

2. Methods

Research papers and abstracts describing antiretroviral pharmacokinetics in the female genital tract and lower gastrointestinal mucosal tissues published in the MEDLINE® database from January 2000 to December 2014 were identified using the following search terms: HIV, antiretroviral, pharmacokinetics, cervicovaginal fluid, cervical tissue, vaginal tissue, cervix, vagina, rectal fluid, rectal tissue, and rectum. Individual drug names were also included in the search terms. All original articles and relevant reviews discussing antiretroviral concentrations in the mucosal fluid or tissue of the gastrointestinal and female genital tract for any commercially available antiretrovirals or antiretroviral currently under investigation were reviewed in detail. Abstracts presented at scientific conferences prior to December 2014 were also included in this review.

3. Mucosal Tissue Pharmacokinetic Considerations

Mucosal tissues represent a particularly dynamic microenvironment which is subject to a number of regulatory pathways including paracrine, autocrine and endocrine signaling. Understanding the pharmacologic factors guiding drug distribution and how these are regulated within the tissue compartment is important for interpreting mucosal tissue pharmacokinetic data. In the subsections below, we describe three of these factors: protein binding, membrane drug transporter activity, and endogenous hormones.

3.1 Protein Binding

Protein binding is an important consideration in the pharmacokinetic profile of antiretrovirals, because only the unbound fraction (ie free drug) is available for pharmacologic activity. The extent of protein binding may affect a compound's ability to exit the vasculature and penetrate into the interstitial tissue space.(14) The primary proteins responsible for binding antiretrovirals are albumin and α 1 acid glycoprotein (AAG).(15) The extent of binding to these proteins is highly variable amongst the antiretroviral classes.(16) For instance, most protease inhibitors are more than 90% bound to AAG; while the non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz is more than 99% bound to albumin.(16) Importantly, the concentration of albumin and AAG are much lower in mucosal fluid relative to blood plasma. In one investigation of cervical mucus, the mean (SD) concentration of albumin and AAG was 73 (45) and 6.6 (3.6) mg/L, respectively. Both of these values are less than 1% of the concentration found in blood plasma.(17) For one antiretroviral, maraviroc, protein binding is reduced 10-fold in the female genital tract: from approximately 76% in blood plasma to approximately 7.6% in cervicovaginal secretions.(18, 19) These findings imply that a higher degree of pharmacologic activity might be achieved in mucosal fluid relative to plasma given the same total drug concentration in the two matrices.

Alteration in protein binding can affect pharmacodynamic markers such as the 50% inhibitory concentration (IC_{50}), and confound pharmacokinetic (PK)/pharmacodynamic (PD) analyses that extrapolate inhibitory quotients (the ratio between drug concentration at the end of the dosing interval and level of drug resistance of the viral isolate), to clinical trial data.(16) For example, the protease inhibitor SC-52151 failed to show a clinical effect

despite achieving an adequate concentration in the plasma.(20) This was later shown to be due to AAG binding, which reduced the activity of the drug by 17-fold in MT-2 cells.(21) Kempf et al. found that by including just 10% fetal calf serum in cell culture media, lopinavir was 96% protein bound, thus providing proof of concept for in vitro manipulation to produce better pharmacodynamic estimates.(22) A recent in vitro investigation in CD4+ T cells isolated from peripheral blood mononuclear cells (PBMCs) found that increasing the extracellular concentration of human serum albumin (HSA) from 1 to 80 mg/mL resulted in reduced intracellular concentrations of approximately 8-fold for efavirenz (at a concentration of 5ng/mL), 1.7-fold for raltegravir (at a concentration of 5ng/mL), and 2.3-fold for etravirine (at a concentration of 2ng/mL).(23) Thus careful consideration of the extent to which a drug is protein bound is critical for translating in vitro data into the in vivo system.

3.2 Drug Transporters

Transporter proteins are located on cell membranes of tissues such as the colorectal and genital tract mucosa, and are highly involved in the uptake and efflux of drugs. Several antiretrovirals are substrates of drug transporters within the ATP binding cassette (ABC) and solute carrier (SLC) families.(24) Recent efforts have been directed at elucidating the role of mucosal tissue transporters in the distribution of drugs to the female genital and lower gastrointestinal tracts. Zhou et al. investigated the mRNA expression of several ABC efflux transporters as well as SLC and SLCO uptake transporters in human vaginal and ectocervical tissue.(25) Liver tissue was used as a reference standard due to abundant expression of the drug transporters investigated.(26) The efflux transporters MRP1 and MRP4 were highly expressed (50-100% of reference tissue) in cervicovaginal tissue, while MRP2 and the uptake transporters, OAT1 and OAT3, were less than 2% of the reference tissue. This analysis didn't include information regarding location of these transporters within the epithelium, which is important to interpret the data correctly. For instance, abundant expression of efflux transporters in the squamous epithelium adjacent to the lumen would increase elimination of drug substrates from the tissue. However efflux transporters located within the basal lamina (the most interior layer of the epithelium), would increase transport into the squamous epithelial layer and lead to accumulation of drug substrates.

Nicol et al. addressed this by quantifying the mRNA and protein expression of 3 efflux (MDR1, MRP2, MRP4) and 3 uptake (OAT1, OAT3 and OATP1B1) transporters in cervical, vaginal, and colorectal tissue using Real-Time PCR and immunohistochemistry techniques.(27) Zhou's findings agree with Nicol's analysis which revealed consistent expression of the ABC transporters at different levels in each tissue type. MDR1 and MRP2 mRNA expression in female genital mucosal tissue was found to be 3 to 4-fold higher than colorectal tissue ($p < 0.001$). Conversely, MRP4 mRNA in colorectal tissue was 2-fold higher than female genital mucosal tissue ($p < 0.05$). While diffuse MDR1 protein expression was seen throughout the vaginal and ectocervical tissue, expression appeared to be localized to the luminal surface in endocervical and colorectal tissue. Diffuse MRP2 protein expression was observed in the epithelia of all tissue types. Finally, MRP4 protein expression appeared diffuse throughout the vaginal squamous epithelium and colorectal columnar epithelium. However, positively stained lymphocytes and monocytes appeared to be localized to the

luminal surface of the colorectal epithelium. The influx transporters OAT1, OAT3, and OAT1B1, exhibited a lower degree of expression in all tissues compared with efflux transporters: <25% of all mucosal tissue samples exhibited quantifiable expression and all detectable tissue samples were <0.1% of expression compared to kidney tissue (used as a reference due to its abundant expression of uptake transporters).(26, 27) These data suggest that efflux transporters (MDR1, MRP2, and MRP4) play a role in drug distribution in female genital and colorectal tissues.

Table 1 summarizes the drug transporter affinity for available antiretrovirals. Recent modeling work has determined that being a substrate of MRP1 positively correlates with female genital tract tissue concentrations; whereas being a substrate for MRP4 negatively correlates with tissue concentration.(28) The negative correlation between tissue penetration and MRP4 substrate status is in support of the findings that MRP4 expression appears diffuse throughout the female genital tract thus decreasing accumulation in the tissue.

Finally, the expression of efflux transporters appears to be regulated by inflammatory cytokines released in response to infection or injury.(29, 30) Pro-inflammatory mediators like IL-6, IL-1 β and INF- γ have been shown to decrease the expression of BCRP and MDR1(30-32). Since mucosal inflammation may be consequence of HIV infection(33), it is possible that stage of HIV infection may influence antiretroviral drug distribution. Investigation with the antibiotic linezolid (an MDR1 substrate) has shown altered exposure in soft tissues correlated with degree of inflammation.(34) However, the effect of local inflammation on antiretroviral drug distribution to mucosal tissues is yet to be determined.

3.3 Endogenous Hormones

Another important consideration is the effect of endogenous hormones on drug distribution to mucosal tissues. Hormonal and physiological variations occurring during the follicular, ovulatory and luteal phases of the menstrual cycle may alter certain pharmacokinetic parameters such as protein binding and oxidative hepatic metabolism.(35) In cultured epithelial cells of the female genital tract, estradiol and progesterone were found to regulate the intracellular concentration of tenofovir diphosphate.(36) Additionally, MDR1 expression in the female genital mucosa appears to be subject to hormonal regulation with highest expression correlating with the luteal phase of the menstrual cycle(37) and lowest expression in postmenopausal women.(27) However, the literature describing the clinical implications of these physiological alterations for antiretrovirals is extremely limited. Sheth et al. investigated trough cervicovaginal concentrations of tenofovir, emtricitabine, and atazanavir in 20 HIV infected women and found no correlation between cervicovaginal fluid concentration and menstrual cycle.(38) Likewise, cervical raltegravir trough concentrations in 9 healthy volunteers demonstrated no correlation with phase of menstrual cycle.(39) However, the extensive between-subject variability in raltegravir cervical tissue concentrations (coefficient of variation=195.7%) makes it difficult to conclude that menstrual cycle does not impact drug distribution to the female genital tract. No investigation to date has explored the impact of hormonal changes on drug distribution within the colorectal tissue.

4. Mucosal Tissue Pharmacokinetics

The distribution of antiretroviral agents has been widely investigated in the female genital tract by measuring drug concentrations in cervicovaginal fluid. Cervicovaginal fluid allows for more intensive pharmacokinetic sampling within individuals and overcomes some of the challenges posed by tissue biopsies. Distribution of antiretrovirals in the mucosal fluid of the lower gastrointestinal tract has not been widely studied. One study that measured drug concentration of dolutegravir in rectal fluid found that self-collected rectal fluid swabs were an unreliable surrogate for drug exposure in the rectal tissue because of large within- and between-subject variability.(40)

When evaluating mucosal tissue pharmacokinetics, a ratio between drug exposure in the compartment relative to drug exposure in the blood plasma is commonly calculated to correct for plasma drug exposure with different drug combinations and dosing strategies. The ratio can be calculated with either single sampling times or by area under the concentration time curve (AUC). Utilizing the AUC approach minimizes spurious results due to lag times in distribution to peripheral compartments. Figure 1 reports the penetration ratios for each antiretroviral in the female genital or lower gastrointestinal tissue and includes ratios derived with AUCs (when available) or concentrations at the end of the dosing interval (C_{tau}). A summary table of the methods and results of antiretroviral pharmacokinetic studies in the female genital mucosal tissues up to 2011 can be found in a review by Else and colleagues.(41)

4.1 Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

Combination NRTIs form the backbone of most HIV treatment regimens. These agents require intracellular phosphorylation to their active metabolites, which compete with the host's endogenous nucleotides for incorporation into the elongating proviral DNA strand. In general, agents within this class exhibit a low degree of protein binding (<50%) and hepatic biotransformation.(42) Upon phosphorylation the intracellular active moieties are trapped within the cell membrane, and only parent compounds are readily detectable in extracellular fluid spaces such as blood plasma, cervicovaginal fluid, and seminal plasma. Currently one of the most commonly used NRTIs, tenofovir, is being investigated for PrEP in a 1% gel formulation. This section will review the mucosal tissue pharmacokinetics of orally and topically administered NRTIs.

Dumond et al first described the median (IQR) cervicovaginal fluid to blood plasma AUC_{0-tau} ratios following single and multiple doses of 7 NRTIs in HIV infected patients initiating or changing antiretrovirals for HIV treatment. These are listed by rank order for the single dose in Table 2. Following the single dose, all agents were detected in cervicovaginal fluid within 4 hours and reached peak concentrations within 6 hours following a single dose.(43) Of the NRTIs only the older agents, didanosine and stavudine, exhibited cervicovaginal fluid to blood plasma ratios of less than 20%. Subsequently, tenofovir and emtricitabine were shown to exhibit 2-26 times higher cervicovaginal fluid exposure compared to blood plasma exposure when measured after a single dose over 14 days. The terminal half-life in plasma was estimated to be 47 hours for tenofovir and 49 hours for emtricitabine, and in cervicovaginal fluid, it was estimated to be 71 hours for

tenofovir and 40 hours for emtricitabine.(44) Since these half-lives are similar to previously published emtricitabine triphosphate (FTCtp) and tenofovir diphosphate (TFVdp) data(45), this extended elimination phase was thought to reflect clearance and dephosphorylation of intracellular FTCtp and TFVdp.

The formation of TFVdp and FTCtp have been well characterized in the female genital tract following a single dose.(44, 46, 47) The most recent of these studies measured parent and intracellular concentrations at multiple time points over 48 hours across a 4-fold dosing range (150 to 600mg for tenofovir disoproxil fumarate and for 100 to 400mg emtricitabine). (46) While the time to peak concentrations (Tmax) of tenofovir and emtricitabine is achieved within 6 hours in cervical and vaginal tissues, the formation of the active metabolites are delayed, with a median Tmax of 24 hours for TFVdp and 12 hours for FTCtp.(46) Although TFVdp was detected in vaginal tissue for up to 14 days following a single fixed dose combination tablet of tenofovir disoproxil fumarate 300mg and emtricitabine 200mg, FTCtp was only detected for up to 2 days.(44) Louissaint et al. confirmed the persistence of TFVdp in vaginal tissue after a single dose, and calculated a terminal half-life of 53 hours.(47) This tissue half-life is consistent with TFVdp's 49.7 hour in vitro half-life in resting PBMC cultures.(45)

Multiple pharmacokinetic investigations suggest differential distribution of tenofovir and emtricitabine between mucosal tissues types, which has important implications for antiretroviral based prevention strategies. Pharmacokinetic analysis investigating exposure at 6 to 48 hours following a single dose found 10-fold higher TFVdp exposure in colorectal tissue compared to female genital mucosal tissue.(46) However, FTCtp was 170 times higher in the female genital mucosal tissue compared to the rectal tissue. When these compounds were measured over 14 days following a single dose, tenofovir and TFVdp rectal tissue AUC_{0-14days} remained were 60-100 times higher than female genital tract tissue.(44, 47) The difference in the concentration gradient between these two studies is likely due to the delayed accumulation of TFVdp in rectal tissue (median Tmax 48 hours) compared to female genital tract tissue (median Tmax=24 hours).(46) Emtricitabine and FTCtp AUC_{0-14days} were approximately 10 times higher in female genital tract tissue than colorectal tissue.(44)

Topical tenofovir 1% gel has been investigated for application in both the female genital tract and the colorectum. Once instilled, TFVdp can be detected in tissues within 0.5 hours. (48, 49) A randomized cross-over PK study found that a 40mg dose of topical tenofovir in the vagina achieved more than a 100-fold higher tenofovir and TFVdp concentrations in vaginal and cervical tissue compared to the standard 300mg oral dose.(50) Maximum blood plasma concentrations following daily vaginal application of the gel was approximately 1% of the maximum concentration (Cmax) following daily oral tenofovir 300mg (median Cmax=3.9 vs 332ng/mL, respectively). Thirty minutes after application, a separate study found that the gel yielded TFVdp concentrations in colorectal tissue 6 times higher than concentrations achieved after an oral dose(49) Maximum blood plasma concentrations following 7 daily rectal administrations of the gel were ~3% of the exposure following a single dose of oral tenofovir 300mg (median Cmax=8.61 vs 252ng/mL). Rectal administration also led to detectable tenofovir concentrations in vaginal fluid: vaginal fluid

C_{max} was approximately 2.5 logs lower than colorectal fluid but approximately 2-fold higher compared to vaginal fluid C_{max} following oral dosing.(51)

4.2 Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

The NNRTIs constitute several first line antiretroviral therapies in combination with NRTIs. By binding non-competitively to the reverse transcriptase enzyme, they inhibit transcription of viral RNA. Delavirdine, efavirenz and nevirapine are first generation NNRTIs while rilpivirine and etravirine are second generation. The penetration of most NNRTIs into the female genital tract is limited.(14) In a study by Min et al. cervicovaginal fluid:blood plasma ratios were 0.83 for nevirapine, 0.48 for delavirdine, and < 0.08 for efavirenz.(52) These penetration ratios demonstrate an inverse relationship with the degree of protein binding exhibited by each agent (nevirapine=60% bound, delavirdine=98% bound, and efavirenz 99.5%).(53-55) The penetration of these agents into the colorectal tissue has not been studied.

In the DIVA02 study of 12 HIV-1 infected women, the etravirine median (IQR) cervicovaginal fluid:blood plasma ratio was 1.2 (0.5-4.8).(56) Despite high (99%) protein binding, etravirine's low molecular mass and lipophilic structure may be responsible for the increased mucosal tissue penetration. A separate study in 12 HIV-negative men determined that after a single dose of etravirine, rectal tissue exposure was ~15-fold higher than blood plasma: with repeated dosing, rectal tissue exposure was ~9-fold higher.(57)

The pharmacokinetics of oral rilpivirine have not been investigated in mucosal tissues. However, interest has grown for the long acting parenteral formulation of the drug, both for PrEP and to increase adherence in HIV treatment. Mucosal distribution with this formulation has been investigated in HIV-negative men and women at doses of 150mg, 300mg, 600mg and 1200mg given intramuscularly (IM).(58) In men, at the 600mg dose the geometric mean (90% CI) rectal tissue:plasma ratio was 0.98(0.85-1.11) on day 7 while in women, the geometric mean (90% CI) vaginal tissue:plasma ratio was 0.72(0.4-1.05) on day 7. The authors concluded that the compartmental rilpivirine concentrations were proportional to the dose administered. A planned phase II study of the long acting injectable formulation of rilpivirine TMC278LA will evaluate the 1200 mg dose for chemoprophylaxis.(59)

Dapivirine is also being developed as a topical microbicide formulated in intravaginal rings (IVRs), gels and films. In 24 HIV-negative women, concentrations attained in the lower female genital tract 28 days after placement of an IVR were 5 log units higher than maximum concentrations achieved in the plasma over the 28 day period.(60) Further, there was a linear relationship (p=0.0157) between tissue concentration and protective effect.(61) The FAME-02 study in 60 HIV-negative women that compared the PK/PD and safety of gel (0.05%) and film (1.25mg) formulations of dapivirine found that both formulations achieved low and comparable plasma concentrations (302.47 pg/mL for gel compared to 227.13 pg/mL for film). However tissue concentrations of dapivirine from the film were four times lower than concentrations from the gel, and this correlated with greater infectivity in the ex-vivo challenge experiments with vaginal and cervical biopsies.(62) These novel formulations may be used to help achieve protective mucosal drug exposure with decreased systemic exposure and reduced dosing frequency.

4.3 Integrase Inhibitors (INSTIs)

INSTIs represent the newest class of antiretrovirals with the first agent in class, raltegravir, approved in 2007. This was followed by elvitegravir in 2012 and dolutegravir in 2013. As a class, these agents are potent, well tolerated, and exhibit lower drug interaction potential than protease inhibitors (PIs) and NNRTIs. Glucuronidation is the primary route of metabolism for raltegravir and dolutegravir, while elvitegravir is primarily metabolized by the CYP450 enzyme system. For this reason some have been proposed as second generation PrEP strategies. To date, raltegravir and dolutegravir have data available on drug penetration into mucosal tissues.

Raltegravir exhibits the lowest degree of protein binding (83%) within this class.(63) Data in HIV-negative and HIV-infected premenopausal women have shown median (IQR) raltegravir cervicovaginal fluid:blood plasma steady state AUC_{0-12hr} ratios of 0.93 (0.4, 1.7) and 4.4 (1.5, 7.2), respectively.(64) The DIVA trial investigated raltegravir trough concentrations in the blood plasma and cervicovaginal fluid of 14 HIV infected women.(65) The median (IQR) cervicovaginal fluid:blood plasma ratio was 2.3 (1.4-4.1). Of the trial's three study subjects with bacterial vaginosis, two exhibited cervicovaginal fluid:blood plasma ratios that were 4 to 16-fold greater than the median, suggesting that inflammation may increase raltegravir exposure in the female genital tract. Raltegravir is a substrate for the MDR1 efflux transporter (66) which is highly expressed in the female genital tract and generally attenuated in the presence of inflammatory mediators, therefore accumulation of raltegravir in inflamed female genital mucosal tissue is biologically plausible.

Conversely, dolutegravir exposure in female genital tract tissues and cervicovaginal fluid was found to be <10% of blood plasma.(67) Dolutegravir is much more extensively protein bound (98%) than raltegravir which could potentially explain this low degree of distribution.(68) Despite this, 94% of samples collected from the female genital tract were above dolutegravir's protein adjusted IC_{90} of 64ng/mL(67). Since cervicovaginal fluid concentrations of AAG (the major serum protein responsible for binding dolutegravir) are approximately 1% of blood plasma(17), dolutegravir exposure in the female genital tract may be sufficient despite the compound's poor penetrative capacity.

In the lower gastrointestinal tract, raltegravir was found to rapidly distribute after a single dose, reaching concentrations in the terminal ileum which exceeded plasma concentrations within 2 hours and peak concentrations within 4 hours.(69) After a single 400mg dose, the highest concentrations were achieved in the terminal ileum followed by the splenic flexure, and the rectal tissue ($AUC_{composite} = 594, 258, \text{ and } 143 \text{ ng} \cdot \text{hr} \cdot \text{mL}^{-1}$, respectively). Under steady-state conditions with 400mg dosed twice daily, the splenic flexure exhibited the highest exposure with an $AUC_{composite}$ of $2240 \text{ ng} \cdot \text{hr} \cdot \text{mL}^{-1}$ where the $AUC_{composite}$ was $788 \text{ ng} \cdot \text{hr} \cdot \text{mL}^{-1}$ in both the terminal ileum and the rectum. A single dose, dose ranging pharmacokinetic study investigating 200 to 800mg or raltegravir in healthy women found exposure in the lower gastrointestinal tract is approximately 25-fold greater than in the female genital tract, and colorectal exposure is similar between women and men.(70) Dolutegravir was found to poorly distribute to the rectal tissue of healthy males, with a median $AUC_{composite}$ penetration ratio of 0.17.(40) Despite low rectal exposure relative to

blood plasma, 100% of concentrations from the lower gastrointestinal mucosa were above the protein adjusted IC₉₀.(40)

Cabotegravir is an investigational INSTI, which has a similar chemical structure to dolutegravir.(71) It is formulated as a long acting injectable nanoparticle suspension for intramuscular administration in HIV treatment and prevention.(71) Cabotegravir exhibits plasma pharmacokinetics which support 4 and 8 week dosing intervals. Both dosing intervals are currently under investigation in phase IIb studies for HIV treatment.(72) Similar to dolutegravir, a 400mg IM dose of cabotegravir was found to exhibit cervical/vaginal and colorectal penetration ratios of 0.16-0.19 and 0.08, respectively, 2-8 weeks after a single injection.(73) While median rectal tissue concentrations at 4 and 12 weeks following a 400mg split IM injection were below the protein adjusted IC₉₀ (16.6ng/mL), the median concentration in cervical and vaginal tissue was above this value at the week 4 and 12 visit, respectively.(73) Recently, Radzio et al. demonstrated 100% protection in macaques vaginally challenged with biweekly SHIV for up to 20 weeks after the last of 3 monthly 50mg/kg injections.(74) This study found low penetration to the mucosal compartment similar to humans with cervicovaginal and rectal fluid:plasma AUC ratios of 0.16 and 0.38, respectively. Andrews et al. also demonstrated 100% protection in macaques that were rectally challenged with weekly SHIV for up to 20 weeks following two monthly 50mg/kg injections and 5 weeks following a single injection.(75) In the single injection study, plasma concentration $1 \times \text{PAIC}_{90}$ correlated with 97% protection whereas plasma concentrations $< \text{PAIC}_{90}$ resulted in 75% protection. Following plasma pharmacokinetic modeling and simulation, the group determined that an 800mg split IM dose administered every 3 months would be carried forward into clinical PrEP trials.(76)

4.4 Protease Inhibitors (PIs)

PIs inhibit the protease enzyme responsible for post translational cleavage of viral proteins. These compounds generally have large molecular weights and exhibit extensive protein binding and hepatic biotransformation via the CYP450 system.(77) These pharmacologic complexities, coupled with their post integration mechanism of action, have resulted in this class being generally overlooked for HIV prevention strategies. Therefore the literature regarding protease inhibitors' mucosal tissue pharmacokinetics is limited. Investigation of the cervicovaginal secretions of HIV infected women receiving stable HAART revealed that indinavir and darunavir exhibit the highest degree of female genital tract penetration among the protease inhibitors. Median (range) trough cervicovaginal fluid:blood plasma ratios of 3.8 (0.99-10) and 4.64(0.8-26.93) have been reported for indinavir.(52, 78) For darunavir the median (IQR) AUC cervicovaginal:blood plasma ratio is 1.5 (1.0-1.6).(79) Amprenavir, ritonavir, atazanavir, lopinavir, and saquinavir (listed in order of rank from highest to lowest) all exhibit ratios of < 1 .(43, 52, 78) Of all the protease inhibitors indinavir exhibits the lowest degree of protein binding (54-70%), which possibly accounts for its high penetrative capacity.(80)

Only darunavir and ritonavir have been studied in the lower gastrointestinal mucosal tissues of HIV-negative men. Following a single dose, darunavir and ritonavir were rapidly detected (within 1 hour), reaching peak concentrations by 5 hours.(57) Under steady state conditions,

the rectal tissue: blood plasma $AUC_{0-12\text{hour}}$ ratio was ~ 2.7 for darunavir and 27 for ritonavir. (57)

The potency and high genetic barrier to resistance exhibited by protease inhibitors, make them favorable HIV treatment options. It has been previously shown that viral replication can persist in lymphoid tissue despite effective HAART therapy and that this persistence may correlate with decreased antiretroviral exposure. (81, 82) Since gut associated lymphoid tissue may serve as a potential reservoir of persistent viral replication in patients, tissue penetration could be a theoretical consideration in designing ARV regimens, similar to the CNS Penetration-Effectiveness (CPE) score proposed by the CHARTER Group. (83) However, unlike the CPE score, increased mucosal tissue penetration have not yet been linked to more favorable outcomes.

4.5 Entry inhibitors

Maraviroc is a chemokine receptor antagonist that prevents the virus from binding to the CCR-5 co-receptor on CD4+ T cells. Currently, it is the only clinically approved agent in its class. Maraviroc concentrates in the female genital tract. (19) In a study of 12 healthy volunteer women, $AUC_{0-12\text{h}}$ in cervicovaginal fluid was 3645 (1785–7505) $\text{ng}\cdot\text{h}\cdot\text{mL}^{-1}$ after a single 300 mg dose. After twice daily dosing for seven days, cervicovaginal fluid and vaginal tissue $AUC_{0-12\text{h}}$ was 7500 (3078–9090) $\text{ng}\cdot\text{h}\cdot\text{mL}^{-1}$ and 4857 $\text{ng}\cdot\text{h}\cdot\text{mL}^{-1}$, respectively. (19) High concentrations of maraviroc were found in the rectal tissue of HIV-negative men both after single and multiple dosing at 300 mg doses. (84) Composite AUC ratios in the rectal tissue were 7.5 (single dose) to 26-fold (multiple dose) higher than blood plasma. The maraviroc accumulation ratio from single to multiple dosing in plasma and rectal tissue was 1.4 and 4.9, respectively, which suggests an additional mechanism of increased drug exposure in colorectal tissue. Since maraviroc's bioavailability is 23% (85), it is likely that local, luminal concentrations of maraviroc passing through the gastrointestinal tract are contributing to increased rectal tissue exposure. A recent phase 1, single-dose pharmacokinetic study also found a 10-fold higher exposure of maraviroc in rectal tissue as compared to cervicovaginal tissue. (70) Since maraviroc is a substrate for the efflux transporter MDR1, and this transporter demonstrates 5.5-fold higher mRNA expression in vaginal tissue (27), it may be responsible for the differential penetration between female genital tract tissue and colorectal tissue.

Maraviroc has also been incorporated into silicone elastomer vaginal rings for use as a topical microbicide in combination with dapivirine. This system that contains 100 mg maraviroc and 25 mg dapivirine was recently studied as part of a phase I clinical trial in 24 HIV-negative women who wore the ring for 28 days. While dapivirine was detected in all 24 cervical tissue samples (mean concentration of 1.6 $\mu\text{g}/\text{mL}$), only 4 of 24 (16.67%) samples exhibited detectable concentrations of maraviroc. (61) This low release of maraviroc has resulted in re-formulation efforts, which are ongoing. (86)

5. Expert Opinion

Antiretroviral based prevention has previously demonstrated a high level of effectiveness in the context of mother-to-child transmission with nevirapine and zidovudine. (87) These

prevention approaches have been explored for their potential to offer efficacious, relatively discreet, user-controlled HIV prevention options to adults at high risk of HIV acquisition. Because HIV is primarily transmitted through mucosal fluids, and early infection occurs within mucosal tissues, characterizing pharmacokinetic behavior within these peripheral compartments is important for optimizing PrEP and TasP initiatives.

Given that the PrEP end user population is healthy adults, the ideal PrEP agent would have minimal side effects, a low drug interaction potential, and allow for infrequent or as-needed dosing. Oral dosing formulations for two NRTIs (tenofovir and emtricitabine), raltegravir and maraviroc all exhibit excellent capacity to penetrate into mucosal tissue with concentrations that either match or exceed blood plasma concentrations with single and multiple doses. These agents' penetrative ability coupled with relatively uncomplicated drug interaction and side effect profiles make them good candidates for PrEP. Also, alternative antiretroviral formulations, such as 1% tenofovir gel and the dapivirine ring, are designed to achieve high local tissue concentrations and low plasma exposure. These formulations offer intermittent or reduced frequency dosing options.

In general NNRTIs and PIs exhibit a low degree of penetration into tissue. While complicated drug interaction and adverse events profiles impede their use in PrEP, NNRTIs and PIs are widely used in HIV treatment. Drugs within these classes that demonstrate extensive penetration, like darunavir and etravirine, may provide some theoretical benefit to TasP approaches.

Lastly, the pharmacokinetic data demonstrating preferential distribution of certain antiretrovirals, like tenofovir, into the colorectal mucosa relative to the female genital mucosa strongly support the notion that the site of HIV exposure determines the degree of protection achieved by a PrEP regimen. This knowledge can be used to tailor PrEP strategies to the target end user. For instance future PrEP trials or marketing initiatives directed at women are more likely to benefit from rigorous adherence counseling and intervention given that the female genital tract exhibits a smaller pharmacokinetic buffer for missed doses. In contrast, investigations of intermittent Truvada[®] PrEP regimens for MSM is strongly supported by pharmacokinetic data. This concept was recently confirmed with the success of the IPERGAY trial, which investigated an intermittent dosing regimen before and after sex in MSM.(88)

Understanding the pharmacokinetics of antiretrovirals in the mucosal tissue represents only one aspect of optimizing antiretroviral use for HIV prevention. A thorough characterization of target concentrations required for protection and subsequently well-defined PK/PD relationships represents another important aspect of defining dosing strategies.(89) However, this has been inconsistently defined for antiretroviral prevention. One reason is the lack of consensus on appropriate preclinical models of infection for defining concentration response relationships. The limitations of in vitro and ex vivo models have been reviewed in detail elsewhere (90, 91), but include a lack of tissue architecture and relevant microenvironment, and the use of clinically appropriate virus and viral inoculum.

Using clinical trial data from the CAPRISA004 study Karim et al. determined that women with cervicovaginal fluid tenofovir concentrations of >1000ng/mL exhibited a higher degree of protection (74%) than those with <1000ng/mL (14%).(92) Whether this target tenofovir concentration is also required for oral dosing strategies remains to be determined. Using data generated from the iPrEx study, Anderson et al. have proposed an EC₉₀ concentration of 16 fmol TFVdp/million PBMCs with oral Truvada[®] dosing in MSM.(93) However the concentrations used in this approach were derived from cryopreserved PBMCs which can lose up to 60% of TFVdp compared to traditional methods of cell isolation.(93, 94) Therefore, this estimate likely underestimates the true effective concentration of TFVdp in PBMCs that results in colorectal tissue protection. Given the pharmacokinetic differences between the female genital and lower gastrointestinal tracts, it's also unlikely that these results can be extrapolated to a female population.

One recent preclinical and clinical approach to determine the concentration vs response relationship for tenofovir and emtricitabine measured intracellular metabolites (TFVdp and FTCtp) relative to competitive natural substrates of reverse transcriptase (deoxyadenosine triphosphate; dATP and deoxycytidine triphosphate; dCTP).(95, 96) When combining these data into a mathematical model to simulate mucosal tissue exposure achieved by various dosing strategies, 80% protection from HIV transmission was predicted for the female genital tract with daily Truvada[®] dosing and 90% protection was predicted for the lower gastrointestinal tract with 2 doses per week. These predictions are in close agreement with observational data from clinical trials.(10, 11)

In the absence of well-defined efficacy target concentrations, important inferences can still be made from the available mucosal tissue pharmacokinetic data. Although it's not clear which anatomical locations should be preferentially targeted to reduce HIV transmission (eg mucosal tissues, regional lymph nodes), antiretrovirals with mucosal tissue concentrations above the viral IC₉₀ may be considered preferable for both PrEP and TasP. Since the extent of antiretroviral mucosal tissue penetration is unpredictable, tissue pharmacokinetic data are essential for optimizing dosing strategies for HIV prevention.

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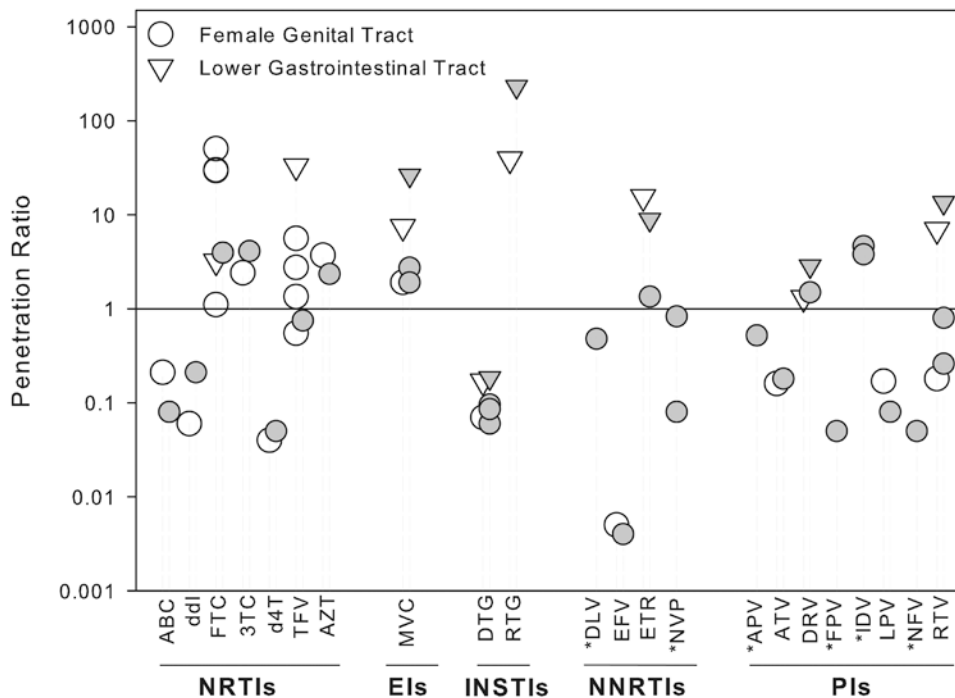
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Highlights

1. Antiretroviral distribution to mucosal tissues is governed by a number of complex factors including lipophilicity, protein binding, and active transport.
2. The degree of antiretroviral mucosal tissue distribution is highly variable between and within antiretroviral therapeutic drug classes (Figure 1).
3. As a drug class, nucleoside reverse transcriptase inhibitors (NRTIs) achieve higher exposure in the mucosal tissues compared to non-nucleoside reverse transcriptase (NNRTIs) and protease inhibitors (PIs) (Figure 1).
4. In general antiretrovirals exhibit higher exposure in the lower gastrointestinal mucosal tissue compared to the female genital mucosal tissue.
5. This article elucidates some important knowledge gaps in the field of mucosal tissue pharmacokinetics which warrant further study to better characterize, including: 1) drug transporters and their regulation in mucosal tissues 2) the effect of hormonal regulation and inflammation on mucosal tissue distribution and 3) mucosal pharmacodynamic targets for HIV prevention.



*Reported ratio calculated from matched trough concentrations rather than AUC.

Figure 1. Antiretroviral Female Genital and Lower Gastrointestinal Tract Penetration Ratios

This figure reports the mucosal tissue or fluid: blood plasma area under the concentration time curve (AUC) ratios for 22 antiretrovirals stratified by class. Both single dose (open symbols) and steady state (closed symbols) are reported when available. The line of unity (mucosal tissue/fluid AUC=blood plasma AUC) is denoted by the solid reference line. In general penetration ratios tend to be higher in the lower gastrointestinal tract for most antiretrovirals. As a class nucleoside reverse transcriptase inhibitors (NRTIs) and the entry inhibitor (EI), maraviroc(MVC), tend to demonstrate a high degree of penetration (>1) into the female genital and lower gastrointestinal tracts whereas non nucleoside reverse transcriptase inhibitors (NNRTIS) and protease inhibitors (PIs) tend to exhibit low penetration (<1). ABC=abacavir, ddI=didanosine, FTC=emtricitabine, 3TC=lamivudine, d4T=stavudine, TFV=tenofovir, AZT=Zidovudine, DTG=dolutegravir, RTG=raltegravir, DLV=delavirdine, EFV=efavirenz, ETR=etravirine, NVP=nevirapine, APV=amprenavir, ATV=atazanavir, DRV=darunavir, FPV=fosamprenavir, IDV=indinavir, LPV=lopinavir, NFV=nelfinavir, RTV=ritonavir, INSTIs=integrase strand transfer inhibitors.

Table 1
Antiretroviral Drugs Are Substrates for Drug Transporters (24, 68)

Class	Antiretroviral	Drug Transporter
	Abacavir	P-gp BCRP MRP4
	Emtricitabine	MRP1
NRTIs	Lamivudine	BCRP OCT1 OCT2
	Tenofovir DF	P-gp
	Zidovudine	MRP4 BCRP OAT1 OAT2 OAT3 OAT4 CNT1 CNT3 ENT2
INSTIs	Dolutegravir	P-gp, BCRP
	Raltegravir	P-gp
	Atazanavir	P-gp MRP1 MRP2
PIs	Darunavir	P-gp OATP1A2 OATP1B1
	Lopinavir	P-gp MRP1 MRP2 OATP1A2 OATP1B1
EIs	Maraviroc	P-gp

BCRP=breast cancer resistance protein, DF=disoproxil fumarate, EIs=Entry inhibitors, INSTIs=integrase strand transfer inhibitors, NRTIs=nucleoside reverse transcriptase inhibitors, PIs=protease inhibitors

Table 2
Distribution in Cervicovaginal Fluid after Single and Multiple Doses

Class	Antiretroviral	Number	CVF:BP AUC _{0-12h} Median (Interquartile Range)			Reference
			Single Dose	Multiple Dose	Multiple Dose	
NRTIs	Zidovudine	3	3.71 (1.13, 6.04)	2.35 (1.21-21.2)	(43)	
	Lamivudine	13	2.41 (1.09, 15.48)	4.11 (2.3-5.94)	(43)	
	Tenofovir	10	1.35 (0.0023, 4.47)	0.75 (0.37-6.45)	(43)	
	Emtricitabine	3	1.11 (0.91, 11.00)	3.95 (1.87-6.71)	(43)	
	Abacavir	5	0.21 (0.08, 0.70)	0.08 (0.08-0.13)	(43)	
	Didanosine	2	0.06 (0.06, 1.31)	0.21 (0.01, 0.4)	(43)	
	Stavudine	3	0.04 (0.007, 0.35)	0.05 (0-0.12)	(43)	
	Efavirenz	6	0.005 (0.001, 0.008)	0.004 (0.001, 0.006)	(43)	
	Nevirapine	7	Not available	0.83 (0.43, 1.58)	(52)	
	Etravirine	12	Not available	1.2 (0.5, 4.8)	(56)	
	Raltegravir	6	Not available	4.4 (1.5, 1.7)	(64)	
	Dolutegravir	8	0.07 (0.05, 0.18)	0.06 (0.04, 0.11)	(67) ^d	
PIs	Darunavir	8	Not Available	1.5 (1.0-1.6)	(79)	
	Ritonavir	12	0.18 (0.005, 0.42)	0.26 (0.11, 1.69)	(43)	
	Lopinavir	4	0.17 (0.003, 0.3)	0.08 (0.03, 1.28)	(43)	
	Atazanavir	8	0.16 (0.1, 0.79)	0.18 (0.05, 0.66)	(43)	

^dConcentration data from HIV negative subjects

AUC=area under the concentration time curve, BP=blood plasma, CVF=cervicovaginal fluid, INSTIs=integrase strand transfer inhibitor, NRTI=nucleoside reverse transcriptase inhibitor, NNRTI=nonnucleoside reverse transcriptase inhibitor, PK=pharmacokinetics, PIs=protease inhibitor