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Pharmacokinetics and Pharmacodynamics in HIV Prevention; Current Status and Future Directions: A Summary of the DAIDS and BMGF Sponsored Think Tank on Pharmacokinetics (PK)/Pharmacodynamics (PD) in HIV Prevention

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Abstract

Thirty years after its beginning, the HIV/AIDS epidemic is still raging around the world. According to UNAIDS, in 2011 alone 1.7M deaths were attributable to AIDS, and 2.5M people were newly infected by the virus. Despite the success in treating HIV-infected people with potent antiretroviral drugs, preventing HIV infection is the key to ending the epidemic. Recently, the efficacy of topical and systemic antiviral chemoprophylaxis (i.e., pre-exposure prophylaxis or "PrEP"), using the same drugs used for HIV treatment, has been demonstrated in a number of clinical trials. However, results from other trials have been inconsistent, especially those evaluating PrEP in women. These inconsistencies may result from our incomplete understanding of pharmacokinetics (PK)/pharmacodynamics (PD) at the mucosal sites of sexual transmission: the male and female gastrointestinal and reproductive tracts. The drug concentrations used in these trials were derived from those used for treatment; however, we still do not know the relationship between the therapeutic and the preventive dose. This article presents the first comprehensive review of the available data in the HIV pharmacology field from animal models to human studies, and outlines gaps, challenges, and future directions. Addressing these pharmacological gaps and challenges will be critical in selecting and advancing future PrEP candidates and strategies with the greatest impact on the HIV epidemic.

Introduction

Over the past 2 years, results from multiple clinical trials have been published evaluating the efficacy of different antiretroviral (ARV)-based strategies for the prevention of human immunodeficiency virus (HIV) transmission (Table 1). Unfortunately, results from these studies have been inconsistent. For example, 1% tenofovir (TFV) vaginal gel demonstrated a 39% reduction in HIV transmission to women in the Phase 2b Centre for the AIDS Programme of Research in South Africa (CAPRISA) 004 study conducted using the coitally related "BAT-24" dosing regimen.[1] In contrast, evaluation of once-daily use of 1% TFV gel was discontinued in the Microbicide Trials Network 003 Vaginal and Oral Interventions to Control the Epidemic (VOICE) trial due to a lack of effect relative to placebo.[2]

Results from trials evaluating oral ARV strategies have been similarly divergent. Daily oral use of Truvada [200 mg emtricitabine (FTC)–300 mg tenofovir disoproxil fumarate (TDF)] was shown to be efficacious in a population of men who have sex with men (MSM) in the Preexposure Prophylaxis (PrEP) Initiative (iPrEX) study,[3] as well as in serodiscordant couples in the Partners PrEP and TDF2 studies.[4–7] Although efficacy results for these studies were statistically significant, the confidence intervals were quite wide, making the determination of efficacy not definitive.

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Table 1. Recent HIV-Prevention Product Efficacy Trial Results

Study	*Product (dosing frequency)*	*Population*	*Efficacy result*
CAPRISA 004[1]	1.0% TFV Gel ("BAT-24")	HIV-negative women	39%, $p = 0.017$; 95% CI: 6–60%
iPrEx[3]	Oral Truvada (daily)	HIV-negative MSM	44%, $p = 0.005$; 95% CI: 15–63%
Partners PrEP[4]	Oral Truvada (daily)	Serodiscordant couples	62%, $p = 0.0003$; 95% CI: 34–78%
	Oral TDF (300 mg) (daily)		73%, $p < 0.0001$; 95% CI: 49–85%
TDF2 Study[7]	Oral Truvada (daily)	HIV-negative women and men	63%, $p = 0.013$; 95% CI: 22–83%
Fem PrEP[5]	Oral Truvada (daily)	HIV-negative women	Early termination
VOICE[2,6]	Oral TFV (daily) 1.0% TFV gel (daily)	HIV-negative women	Early termination

MSM, men who have sex with men.

Conversely, oral TDF/FTC failed to protect HIV-negative women in the FEM-PrEP trial and VOICE.[5,6] Although oral TFV (300 mg TDF) was shown to be efficacious in the Partners PrEP study,[4] its evaluation was discontinued in the VOICE study due to futility.[6]

Possible reasons for these discordant results with the same products in different studies could include differences in dosing regimens, trial designs, trial populations, behavior and sexual practices, and different levels of adherence in the trial populations. Thus, the need for more robust assessments of efficacy potential for next-generation, high-impact, HIV prevention products earlier in clinical development remains a priority for the field.

Although proper preclinical development and clinical safety assessment of ARV-based prevention products have been achieved for a number of products, meaningful evaluation of the efficacy potential of such products, prior to Phase 3 trials, remains elusive. The efficacy of prevention products can be influenced by a number of factors including dose and product exposure, target cell bioavailability, tissue permeation in the compartments of HIV exposure, *in vivo* half-life, protein binding, effects of biological fluids, local immune responses, and perhaps most complex of all, the behaviors affecting consistent use of the product. Unfortunately, typical Phase 2 proof-of-concept study designs based on products for treatment indications are not feasible for prevention products, given the number of participants required to establish the statistical significance of a prevention outcome. Consequently, the prevention field has relied on limited, alternative means of assessing efficacy potential earlier in the drug development pipeline, which include the use of animal models[8] or *ex vivo* tissue explant systems.[9] At best, these systems have provided limited insight to assess safety as well as efficacy potential as influenced by dose, dosing regimen, and *in vivo* properties of the drug candidates. None of the models has yet been conclusively validated against clinical outcomes in human Phase 3 trials. Improving the robustness and accurate assessments of product pharmacokinetics (PK) and pharmacodynamics (PD) in humans and animal models may provide a relevant, useful, and earlier means of identifying safe and effective HIV prevention products.

Determination of *in vivo* ARV concentrations in various compartments after clinical dosing has occasionally been included in early prevention product trials, but typically it has progressed in parallel with larger efficacy studies. Often, it has been considered as a follow-up analysis of samples collected from efficacy studies. In most instances, these samples are plasma and occasionally include peripheral blood mononuclear cells (PBMCs), whole tissue biopsies, or HIV target cells isolated from target mucosal sites. Even vaginal/rectal secretions are rarely collected. Also, when included as part of early clinical evaluation, such trials typically involved sexually abstinent participants, which negated the ability to determine the effects of sexual intercourse on drug levels in the gastrointestinal and genital tract. While these limited PK measurements may be useful in understanding outcomes posttrial, the field needs improved pre-Phase 3 methods to better inform efficacy trial design and more accurately predict efficacy outcomes. The development of more robust human and animal PK/PD assessment systems will provide a better understanding of the relationship between drug concentrations and activity, as well as the effects of dose and drug exposure on clinical outcome.

To define the critical issues relevant to the development of effective PK/PD models for use in HIV prevention, the National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS (DAIDS), Prevention Sciences Program (PSP) at the National Institutes of Health (NIH) in collaboration with the Bill and Melinda Gates Foundation (BMGF) sponsored an Antiretroviral Pharmacology in HIV Prevention Think Tank. This article outlines the major issues addressed at this meeting with particular focus on the details of conventional derivation and use of PK/PD models and data in drug development, the current state of such models and their use in the HIV prevention field, and the PK/PD knowledge and capability gaps in HIV prevention that need to be addressed.

PK/PD in Drug Development

The determination of drug-candidate PK and PD, and the relationships between the two (i.e., a particular concentration of drug *in vivo* yields a specific level of effect/efficacy), is a fundamental component of drug product development. Typically, PK/PD evaluations are initially conducted in animal models. However, PK/PD relationships can be complicated by species differences between animal models and humans [e.g., differences in absorption, distribution, metabolism, and excretion (ADME), plasma to effect site ratios, and protein binding, immune responses]. Consequently, clinical studies are the definitive determination of the PK/PD relationship in humans. Once drug concentrations over a particular time course are derived for specific doses in animals, exposure–response relationships can be analyzed in various ways. Given the lack of a proof of concept outcome in phase 2 PrEP studies, there is increased need to appropriately study PK/PD relationships with prevention products in order to inform development decisions for such products.

This type of analysis is routinely performed for antimicrobials, where a dose-fractionation study design is commonly implemented first. With this approach, a fixed total daily dose of antimicrobial is administered as a single dose or fractionated into smaller doses administered using different dosing intervals. Based on such studies, the antibacterial drugs can be classified according to the correlation between the effect (most often defined as the bacterial count after 24 h of treatment) and three main PK/PD indices: (1) the ratio of the maximal unbound (free) drug concentration (C_{max}) to the minimum inhibitory concentration (MIC) (fC_{max}/MIC), (2) the ratio of the area under the unbound drug concentration–time curve (AUC) to the MIC [f(AUC)/MIC], or (3) the percentage of a 24-h time period that the unbound drug concentration exceeds the MIC (fT > MIC) (Table 2).[10–12] Typically, the approach closest to a linear relationship of drug concentration and effect (generally, a decrease in colony-forming units for bacterial infection) is identified as having the most predictive value for measuring the biological effect of the drug on the pathogen. Although this approach is routinely used to study antimicrobial agents, such an evaluation of anti-HIV drugs would be more challenging given the lack of robust assays for measuring pharmacodynamics in samples from clinical trials.

Importantly, individual drugs (or classes of drugs) with different physicochemical properties or mechanisms of action may display different efficacy outcomes with these alternative means of analysis. Thus, until proven, it should not be assumed that the relationships observed with one particular anti-HIV drug will be the same as another such drug. Until more data are available, it is recommended that each drug in development be individually evaluated by these PK/PD assessment strategies.

There are a number of important variables to consider in the application of these PK/PD assessment strategies as a step toward identifying optimal prevention interventions. First, knowing the amount of free drug and the amount of total drug in the test system is relevant to understanding the outcome. Presumably, a drug that is bound to another molecule in a model system is not available to exert a biological effect, at least not as potently as a free drug. Differences in protein-binding affinity of different ARVs have been well documented[13] and would likely be relevant in the application of such drugs for prevention. Thus, methods of measuring drug concentrations that do not account for free versus total drug concentrations, particularly in the compartments of effect, may lead to biased drug requirement recommendations.

Table 2. Pharmacokinetic/Pharmacodynamic Relationships Used to Predict Antibiotic Efficacy

AUC/MIC	AUC obtained from plotting *in vivo* drug concentrations over a fixed time period, divided by the MIC of drug against a specific pathogen
C_{max}/MIC	The C_{max} measured *in vivo* over a specific time period, divided by the MIC of a drug against a specific pathogen
Time > MIC	The duration of time that *in vivo* drug concentrations are measured to be greater than the MIC of drug against a specific pathogen

AUC, area under the curve; MIC, minimum inhibitory concentration.

Second, it is also important to understand the effects of the immune system on biological outcomes, particularly in the evaluation of drugs targeting infectious agents. If measured effects in an *in vivo* system are a composite of drug effects and innate or adaptive immunity, then assessment of true drug effect alone could be confounded. This issue can complicate results further since immune responses are not typically uniform across different species, or even between individuals of the same species. The use of immune-suppressed, animal-model systems may also complicate effect outcomes. Thus, understanding both protein binding and the immune response in PK/PD model systems is relevant to interpreting the overall findings from such studies. Several different types of immune responses have been described.[14] However, their contribution to the measured efficacy is further complicated by the absence of a clear immune correlate of protection for HIV.

Other variables are also relevant to the application of PK/PD models in drug candidate evaluation. Since a drug distributes to different body sites at different rates, single time-point-based assessments can be misleading. Therefore, it is important to assess exposure across the entire dosing interval using multiple time points. Aside from potentially providing a higher degree of accuracy, assessments over time support the derivation of dosing regimens that are optimized for the duration of effect. This is particularly relevant for the evaluation of chronic, intermittent, or episodic use of oral or topical products. For the purpose of this article, we define episodic as the use of PrEP in an on-demand fashion relative to sexual intercourse. We define chronic dosing as a dose schedule that may include either daily use or sustained release of drugs. Intermittent dosing is the use of a PrEP product for one or more days followed by a variable period of nonuse. In the case of infectious disease indications, studies over time may also be relevant to assessing the risk of resistance since selection of resistance can occur at subtherapeutic concentrations of inhibitory drugs.[15]

It is also important to understand the relationship between drug plasma concentrations (the typical target in PK assessments) and the concentrations in tissue or cell compartments where prevention is mediated. Blood may serve as the means of compartment supply; however, it is not necessarily the case that the amount of drug in plasma at a given time point is the same as that in a particular compartment of effect. This is relevant for an HIV prevention indication (particularly at the earliest time points after dosing), which will rely on specific drug concentrations in compartments of viral exposure—as opposed to a treatment effect, which depends more on chronically established plasma concentrations necessary to suppress ongoing viral replication in the periphery.[16]

When constructing dose-fractionation or escalation studies in these model systems, it is important to be sure that the full dynamic range of effect is evaluated from a drug concentration perspective. Establishing a concentration curve that spans the range of no effect up through maximal effect is critical to dose evaluation and selection. It is also important to understand the elimination kinetics of a drug. The decaying exposure over time could be relevant to medical management of individuals using the product, the understanding of potential risks associated with prescribed product use, as well as potential risks associated with variable adherence. Drug concentrations below the protective target could allow for

HIV infection, and the presence of these suboptimal concentrations in an HIV-positive individual could lead to the *in vivo* selection of resistant virus.

Another major challenge confronting PK assessments in tissue samples for a prevention indication is the fact that tissues themselves are multicompartmental, and only very specific subcompartments are susceptible to HIV infection (e.g., the $CD4^+/CCR5^+$ cell populations). Tissues are comprised of multiple cell types (immune, nonimmune, etc.), blood, and interstitial fluids. However, drug distribution may not be homogeneous throughout these distinct compartments and the cells within them. Thus, the simple homogenization of tissue samples followed by drug concentration determination may not be reflective of the true potential for target cell protection against HIV.

Intratarget cell drug concentrations are particularly important for prodrugs such as TFV, which require intracellular conversion to an active form and to other agents that may be substrates of uptake and efflux transporters. Similarly, drugs that inhibit active HIV replication also need to establish inhibitory intracellular concentrations. In cases of topical dosing, tissue sampling (e.g., genital or rectal tract samples after gel dosing) is further complicated by a risk of cocollecting the dosed drug present in the extracellular fluids associated with these tissue samples. The presence of drug in these fluids may result in an overestimation of drug exposure associated with the tissue sample. However, if a consistent relationship between these fluids and tissues can be documented, and the same sampling method is utilized across preclinical and clinical studies, the concentration obtained may be a viable surrogate of efficacy. Therefore, sample collection and processing methods are extremely relevant in the use of PK/PD model systems and must be carefully controlled and implemented in order to avoid obtaining biased results.

Results from studies involving different sample collection, processing, and analysis procedures may not be readily compared due to the potential variability inherent in the individual methods. The pharmacology of HIV prevention is further complicated since the target cells (such as tissue-based activated $CD4^+/CCR5^+$ lymphocytes) regularly circulate in and out of the compartments of exposure. Thus, the drug needs to be available at all times to those cells entering the compartment to achieve protective levels in those target cells. In the absence of validated processes for isolating cells from tissue, which ensures that intracellular concentrations remain unaltered during laboratory processing, it may be necessary to consider the contribution of blood and extracellular fluid concentrations to the results obtained in the analysis of gross sample homogenates.

The relevance of drug concentrations in specific compartments (or subcompartments) at the site of HIV exposure may be informed by the study of antibiotics in specific compartments and their antimicrobial effects. It is clear from multiple studies that curing of a bacterial infection likely involves more complicated phenomena than simply reaching a specific drug concentration at the site of infection. For example, the presence of drugs in the epithelial lining fluid (ELF) of lung tissue is relevant to the treatment of lower respiratory tract infections since ELF is often the site of extracellular infections.[17] Thus, for a specific indication, the appropriate biological targets for drug measurement cannot be assumed; they must be determined. Once the relevant compartment of effect has been identified, it is necessary to develop methods for specifically measuring exposure to understand the kinetics of penetration in these target subcompartments.[18] For drugs requiring intracellular activation (or drugs that inhibit HIV intracellularly) it will be necessary to determine their concentrations in specific target cells (e.g., activated versus resting $CD4^+/CCR5^+$ T cells), which is particularly complex from an analytical perspective. Moreover, measuring clinical intracellular concentrations of drugs at the time of viral exposure is an even more complex challenge.

Many of the types of assessments described above could be achieved with HIV prevention products through the use of appropriate animal models. Potential advantages of animal models include reasonable and workable sample sizes due to efficient (e.g., 80–100%) infection rates with a fixed number of viral exposures, the ability to conduct diverse virus challenges, and the capacity to comprehensively collect samples. However, animal models for a prevention indication could be more challenging to develop and optimize than those used for a treatment indication. To correlate findings in animal models to humans, it is necessary to understand the complex variables that control drug distribution and metabolism at a specific tissue site, as well as physiological and biological differences between systems. The following section summarizes the details of some of the animal models used to study HIV infection and evaluate candidate-prevention products. The challenges and gaps with these models are also described.

Animal Models for HIV Infection

In vivo *models for efficacy: nonhuman primate (NHP)*

The NHP model is one of the earliest established *in vivo* animal systems used to evaluate the efficacy of oral and topical prevention products. This model allows the assessment of blood/plasma and mucosal fluid/tissue drug concentrations in both cervicovaginal and rectal transmission.[8] The NHP system has some important limitations as an infection model. First, it typically requires challenge with an engineered virus [R5 or X4 tropic env simian/human immunodeficiency virus (SHIV), or RT SHIV]. Thus, viral challenge involves a single virus species, unlike the quasispecies that occur in human exposure, and the virus inoculum in this model is higher than human exposure with sex. Second, primarily due to costs, the number of animals routinely used per study arm is low, limiting the derivation of statistically meaningful results. Finally, viral challenge is not usually achieved in the presence of semen, nor does it involve coitus or coinfection with other sexually transmitted infections. The model has been developed primarily to support challenge with free virus, and most studies do not involve challenge with infected cells.

There are two versions of the vaginal challenge model currently being used, a *multidose* and a *single dose*. The *single-dose* virus challenge can be applied after preliminary treatment of rhesus macaques with medroxyprogesterone (or Depo-Provera), which enhances infection potential,[8] and prior treatment with the test product (e.g., ≥ 30 min before challenge). The *multidose* viral challenge model involves repeated viral exposures at a lower multiplicity of infection over time in pigtailed macaques (no Depo-Provera pretreatment) after application of the test product.[19] Neither of these approaches is a direct parallel to human exposure, and both are potentially complicated by the anatomical, histological, and

physiological differences between the macaque and the human female reproductive tracts. Despite these issues with both NHP models, they have been used in a number of studies to evaluate ARV-based prevention products and strategies (Table 3). Both *single-dose* and *multidose* versions of the challenge model are also available for rectal exposure.

A number of laboratories support the use of the *single-dose* model, and several studies have been reported for a variety of microbicide-candidate products in a variety of formulations.[20–23] This model has been used to examine polyanion-type microbicide products, such as PRO-2000[24] and Carraguard-based formulations.[25] In the case of the polyanion products, low-level efficacy was reported. However, neither of these products proved efficacious in human studies. The Depo-Provera pretreatment serves to render the animals more susceptible to infection (likely due to epithelial and mucus thinning) and synchronizes the menstrual cycle. In this model, reproductive hormones may also influence drug absorption into genital tissues.[26]

Evaluation of HIV prevention products with the *multidose* viral challenge model in pigtail macaques has gained attention. For example, the *multidose* macaque model was used to demonstrate protective effects of both 1% TFV gel and 1% TFV/5% FTC combination gel after vaginal application and subsequent challenge with SHIV.[19] This study demonstrated 100% efficacy for both products. PK evaluations were restricted to plasma concentrations. Although the study demonstrated 100% efficacy, only 76% of plasma samples contained detectable TFV and/or FTC. A subsequent study in the same model examined the window of protection of 1% TFV gel and its relationship to the more relevant drug concentrations in vaginal tissues.[27] This study demonstrated a wide window of protection (~75% efficacy) when gel was applied 3 days before SHIV challenge and suggested that TFV-DP concentrations in vaginal lymphocytes that are above the *in vitro* IC_{95} may be good predictors of protection.[27] Protection in macaques with 1% TFV gel was higher than what was observed in the CAPRISA and VOICE trials, highlighting the contribution of other factors including adherence and inflammation to lack of protection.[2]

More recently, this *low-dose* model was also used to examine the efficacy of orally dosed ARV. In a study involving various oral-dosing regimens of TDF/FTC followed by repeat rectal exposure to virus, protective effects were determined. In a separate set of animals, concentrations of TFV and FTC were measured in rectal fluid, and the active intracellular metabolite of TFV diphosphate (TFV-DP) was measured in rectal tissue and PBMCs after a single dose.[28] Because the PK was performed in a set of animals that was different from the set challenged, PK/PD relationships could not be established. However, several dosing regimens involving intermittent use of the drug were found to be as effective as daily use in protection against rectal challenge. It was concluded that this finding was due to the long intracellular persistence of TFV-DP seen in macaque rectal cells. Further PK/PD correlations in the same animal, and to human study results, are still needed for currently investigated products and beyond.

In vivo *models for efficacy: humanized mouse*

In 2002, a model involving severe combined immunodeficiency (SCID) mice engrafted with human peripheral blood leukocytes was developed in which cell-associated HIV was transmitted through vaginal exposure.[29] Although the *in vivo* relevance of cell-associated HIV transmission in humans is unknown, this model was used to demonstrate the protective effects of several candidate microbicides.[29,30]

Recently, more elaborate humanized mouse infection models have been developed [the RAG-hu mice;[31,32] bone marrow/liver/thymus (BLT) mice[33]]. In the BLT humanized mouse model, SCID mice are implanted with human thymus and liver tissue followed by irradiation and infusion with human $CD34^+$ hematopoietic progenitor cells.[33] This results in immune reconstitution of humanized lymphoid cells in both the female reproductive tract and gastrointestinal tract, which can then be infected with HIV. Although relatively high doses of virus are needed to achieve infection, there is a clear advantage to being able to use clinical HIV isolates for vaginal and rectal challenge, rather than the engineered strains of SHIV used in the NHP model. Advantages of the humanized murine models include the continuous production of human immune cells, which allows their use in long-term experiments, that multiple human donors can be used to produce multiple cohorts of mice, and that multiple prevention (oral and topical) strategies can be evaluated.[33]

Costs to generate humanized mice can rival the costs of NHPs, as they require long periods of time for their production. However, there is a relatively unlimited supply of this model compared to the NHP. The anatomical restrictions of the mouse may limit the volume and dosage forms that can be evaluated with this model. Nonetheless, assays for quantitation of drug in various matrices have been developed and applied to this model,[34] and they may be used more easily for PK/PD assessments. Thus, humanized mouse models offer a potential means of studying prevention efficacy in ways that parallel but also differ from the NHP model. Furthermore, having multiple models for comparison with the human system helps address the limitations associated with them individually.

The relevance of both NHP and humanized mice models to the human system is as yet unclear. Without continued development and characterization, the biological limitations, as well as further benefits, of the models will be difficult to address. Improvements needed to make these models more robust for PK/PD use include (1) characterization and understanding of histological and biological similarities and differences with the human system, (2) optimization and standardization of both sample collection and processing methods, (3) standardization of drug treatment and virus-challenge techniques, (4) understanding if any differences exist in the PK/PD relationship of a given compound compared to humans, and (5) expanding the data set with different drugs.

Human PK/PD Assessments of HIV Prevention Product Candidates

Drug concentrations in human plasma, PBMCs, genital and colorectal tissues, and genital and colorectal fluids have been measured for a number of HIV prevention candidates after clinical dosing. For example, TFV and TFV-DP were measured in plasma, cervicovaginal fluid (CVF), vaginal tissue, and cervical cells after vaginal dosing with 4 ml of 1% TFV gel.[35] High concentrations of TFV and TFV-DP could be found

Table 3. Preclinical Animal Studies with Emtricitabine, Tenofovir, and Tenofovir Disoproxil Fumerate

Drugs and dose	*Interventions*	*Animal model*	*Main findings*
TFV, 20 mg/kg (subcutaneous)	TFV initiated −48 h and continued for 4 weeks	Long-tailed macaques	Full protection
TFV, 30 mg/kg (subcutaneous)	Two doses given at −4 h/+24 h	Rhesus macaques	Full protection
TDF, 0.01–0.02 mg/kg (oral)	TDF initiated −24 h and maintained during virus inoculations	Rhesus macaques	No protection; low doses of TDF
TDF, 10 mg/kg (oral)	Repeated cycles of daily TDF initiated 1–2 days before exposure	Rhesus macaques	Partial efficacy; infection associated with low drug levels
TDF, 22 mg/kg (oral)	Daily or weekly TDF	Rhesus macaques	Infection delayed in treated animals
FTC, 3.5 mg; TDF, 5.2 mg (intraperitoneal)	Daily FTC/TDF initiated 48 h prior to exposure and continued for 7 days	Humanized mice	Complete protection
FTC, 20 mg/kg; TDF, 22 mg/kg; TFV, 22 mg/kg (oral or subcutaneous)	Daily FTC (subcutaneous); daily oral Truvada; daily or intermittent FTC/TFV (subcutaneous)	Rhesus macaques	Risk of infection reduced with FTC and Truvada; no infection with high TFV doses
FTC, 20 mg/kg; TDF, 22 mg/kg (oral)	Intermittent PrEP: fixed dosing and event-driven	Rhesus macaques	Efficacy with two weekly doses of Truvada given at different intervals
FTC, 3.5 mg; TDF, 5.2 mg (intraperitoneal)	Daily FTC/TDF for 7 days	Humanized mice	Protection regardless of the route of exposure
FTC, 20 mg/kg; TDF, 22 mg/kg (oral)	Two doses given 24 h prior and 2 h after virus exposure	Rhesus macaques	Full protection
1% TFV (rectal gel)	Gel applied 15 min to 2 h before exposure	Rhesus macaques	Partial (6/9) protection
1% TFV; 1% TFV and 5% FTC (vaginal gel)	Gel applied 30 min before virus exposure	Pigtail macaques	Full protection
1% TFV (vaginal gel)	Gel applied 4 h before and 4 h after virus exposure (BAT24)	BLT mice	High protection (7/8)
1% TFV gel (vaginal gel)	Gel applied 3 days before virus exposure	Pigtail macaques	Partial (4/6) protection

Adapted from Garcia-Lerma JG and Heneine W: Animal models of antiretroviral prophylaxis for HIV prevention. Curr Opin HIV AIDS 2012;7(6):505–513. Copyright Lippincott Williams & Wilkins.
TFV, tenofovir; TDF, tenofovir disaproxil fumerate; FTC, emtricitabine; PrEP, preexposure prophylaxis.

in the genital tract over 24 h after single and multiple dosing. In a retrospective analysis, both TFV CVF concentrations >1,000 ng/ml and detection of TFV in blood plasma were associated with reduced HIV infection in the CAPRISA 004 study.[36,37] Recently, the MTN 001 study demonstrated that vaginal dosing achieved much lower plasma concentrations and higher vaginal tissue concentrations than those achieved with oral dosing.[38] Based on these results, vaginal dosing with TFV should provide higher concentrations of active drug in the compartment of viral exposure than oral dosing. However, results from the VOICE trial suggest issues beyond the antiviral effect of TFV are relevant to efficacy/effectiveness.

Plasma, genital secretions, and tissue concentrations of TFV, TFV-DP FTC, and FTC- triphosphate (TP) have been similarly evaluated after a single oral dose of TDF/FTC.[39] Interestingly, the investigators noted divergent penetration of FTC and TFV in vaginal/cervical and colorectal tissues. TFV exposure in the female genital tract was similar to blood plasma, whereas TFV exposure in colorectal tissue was 100 times higher. The same pattern was seen with intracellular TFV-DP; concentrations were 100-fold higher in colorectal tissue than in vaginal/cervical tissue. The opposite trend was seen for FTC and FTC-TP; exposure in the female genital tract was approximately five times higher than exposure in colorectal tissue.

Plasma and PBMC drug concentrations have been evaluated in efficacy studies involving TDF/FTC. Generally, drug exposure is lower in those who become infected with HIV than those who remain protected.[3,4] It is difficult to extrapolate findings from one study to another, as different populations of subjects were followed at different time intervals and had different sampling strategies. However, it does appear that the divergent pharmacology of TDF/FTC in mucosal tissues may explain the different rates of efficacy in the face of suboptimal adherence.[40] For example, the iPrEX study demonstrated 44% protection in men who were only sporadically taking their daily doses of TDF/FTC; only 18% of uninfected men in a case-cohort analysis had evidence of daily drug taking behavior, and 52% were using <2 doses of TDF/FTC per week.[41] Yet no efficacy was noted in the FemPrEP study[5] where 20–30% of women had evidence of recent drug taking behavior. Similar to what was noted in macaques,[27] higher colorectal penetration of TFV may not necessitate TDF/FTC's daily use for efficacy in MSM, whereas lower cervical/vaginal penetration may require consistent daily adherence for efficacy.

Recently, investigators have been testing various approaches to *ex vivo* PD assessments in patients receiving HIV prevention products. For example, infection was inhibited after an *ex vivo* HIV challenge of rectal biopsies obtained from men who dosed rectally with 0.25% UC-781 gel.[42] A similar effect was noted with rectal application of 1% TFV gel.[43] Rectal tissue biopsies collected 30 min after single and multiple rectal dosing, as well as a single oral dosing, were analyzed for drug concentrations, and evaluated in the *ex vivo* HIV challenge model.[43] An increased level of protection against HIV challenge correlated with higher tissue concentrations of TFV-DP. In a cell culture model, a significant increase over baseline endogenous anti-HIV activity was noted in CVL from women who had previously applied TFV gel, and this activity correlated with TFV concentration.[44] Moreover, application of a single dose of TFV topically or systemically to ectocervical explants was effective at preventing HIV infection.[45]

Although these *ex vivo* clinical studies have suggested a correlation between drug concentration and efficacy, these methods and models are relatively new and complex and require further development and optimization.[46] As noted earlier, methods for collection and processing of tissue samples are extremely relevant to outcomes in terms of accurate measurement of drug exposure in tissues. Although the typical method for processing tissue samples is homogenization, specific cell isolation may be more informative. However, despite technical feasibility, quantifying intracellular drug concentrations of lipophilic compounds that accurately reflect the original *in vi*vo concentrations at the end of a lengthy cellular isolation process is complex. Moreover, current analytical instrumentation require large numbers (>100,000) of cells. Although these cell numbers can be isolated from 20 biopsies of colorectal tissue, the number of biopsy samples that can be collected from the female genital tract[2–4] is significantly less. Cervical cytobrush sampling also yields small numbers of mononuclear cells.[47] Consequently, tissue collection represents a technical and quantitative challenge to the implementation of these human PD models.

The majority of the *ex vivo* clinical PK/PD effort to date has involved TFV and TDF/FTC. Therefore, the performance of these model systems with drugs that differ in terms of physicochemical properties or mechanisms of action needs to be investigated. Also, the types of PK/PD assessments that have been applied in human systems (i.e., dose, biopsy, *ex vivo* challenge) have not been extensively evaluated in any of the animal model systems available for the study of HIV prevention. Determining if an animal tissue explant system can predict animal efficacy would assist in interpreting and extrapolating human tissue data to clinical outcomes. Expanded study of animal models, which afford more flexibility in terms of use, may provide significant insight into the relationships between drug exposure and biological effect. However, the clinical relevance of animal model data will require a calibration between animal models and humans. It may be that certain correlations are drug specific. The evaluation of more drugs of different classes is essential to understand these relationships and determine the overall utility of animal models as predictors of effective HIV prevention strategies.

Finally, the study of PK and PD in animal or human systems for HIV prevention ideally should go beyond simple correlations of drug concentrations in different compartments with efficacy. Efforts should be made to understand the types of relationships described earlier for the study of antimicrobials (e.g., AUC/MIC, C_{max}/MIC, and time>MIC) for HIV prevention candidates. Different drugs may have different parameter relationships, and understanding those relationships may be crucial to determining the efficacy potential of the drug. The lesson from other systems and indications is that the simple measure of concentration and effect (or the assumption of effect) will likely be inadequate to predict human efficacy outcomes *in vivo*. Multiple and more sophisticated relationships will need to be studied.

Regulatory Aspects of PK/PD

At present, there is no regulatory requirement for animal model data to support the efficacy claims of a product.

Regulatory agencies recognize the utility of animal models in the study of mechanism of action and in efforts to determine dose and dosing regimen, but it is also clear that animal efficacy data are not accepted as representative of efficacy in humans. In the case of HIV prevention, the utility of animal models from the regulatory perspective is dependent on the degree to which the model resembles the human system. Of fundamental importance is whether the animal model is representative of infection in humans. As outlined earlier, we still do not know how current animal infection models correlate to natural HIV infection in humans. Similarly, it is important to demonstrate that the PK profile in the relevant compartment(s) of the animal model can mimic human exposure. It is also essential to demonstrate that a model can be used to characterize drugs with different mechanisms of action and routes of administration. Thus, addressing these issues with available models is important in establishing regulatory significance. Even if models closely parallel human systems, regulators will still require clinical trials.

Therefore, the ultimate benefit of such models resides in their ability to better inform and streamline clinical study designs. Perhaps more relevant for regulatory purposes than animal models is the accurate assessment of product adherence in clinical trials. Understanding actual product use as opposed to prescribed product use is crucial in interpreting the outcomes of clinical trials. Inadequate adherence to product in trials can lead to underestimation of efficacy, and a lack of accurate adherence data can confound the understanding of PK and efficacy outcomes in trials.[48]

Summary

To select those new HIV prevention products capable of having the highest impact on the epidemic, it will be necessary to assess their efficacy potential *prior* to Phase 3 studies with strategies that are more robust and predictive than those used in the past. Thus, with the overarching goal of identifying the most appropriate new products for development and enhancing our ability to interpret results from clinical trials, a better understanding of PK/PD is a key requirement. The joint DAIDS/BMGF Antiretroviral Pharmacology of HIV Prevention Think Tank reached the consensus that one of the most critical needs in the field of HIV prevention is the delineation of the PK relationship between human and animal models of HIV infection. The Think Tank participants also defined a number of key studies that could potentially address this specific need (Table 4).

Table 4. Key Issues and Studies for Pharmacokinetics/Pharmacodynamics in HIV Prevention

1. Complementary, codesigned animal and human studies for PK/PD model development and comparison
2. Dose-ranging, dose-fractionation and time-course studies in humans and animal models
3. PK/PD assessments for defining prevention vs. treatment dose
4. Tissue targets, tissue sampling, process and method definition, optimization, and standardization
5. Expanded PK/PD studies in animal models (humanized mice, NHP)

PK/PD, pharmacokinetics/pharmacodynamics; NHP, nonhuman primates.

A fundamental understanding of the relationship between drug concentration and effect (or potential effect) is critically needed. To generate these data, assessments of antiretroviral PK/PD in animals need to be applied over wider dose ranges, and need to be based on accurate assessment of drug exposure. Discriminating between free (e.g., protein-unbound) and total (e.g., protein-unbound + protein-bound) drug concentrations, as well as accurately assessing drug exposure in subcompartments, will require the development of sophisticated sample collection, processing, and analysis techniques. A wider array of drugs and drug classes needs to be evaluated in these systems. Both animal and human PD models need further development, optimization, and standardization; there needs to be a better understanding of correlations, if any, between these model systems.

NIAID/DAIDS and BMGF are committed to addressing the critical priorities identified at the Think Tank so that our ability in selecting candidate products with the highest potential for impact on the epidemic, as well as understanding results from clinical trials, may be significantly enhanced. Under the auspices of the NIAID/DAIDS Comprehensive Resources for HIV Microbicides and Biomedical Prevention (CRMP) contract, an HIV Pharmacology Best Practices Working Group has been established from a group of interested Think Tank members. This group will focus their initial effort on the key issues described above with an emphasis on planning studies that will attempt to establish a PK/PD relationship between different animal models and between animal models and humans.

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