Critical Need for Appropriate Mucosal Sample Collection to Determine Relational Animal Pharmacokinetic–Pharmacodynamic Models in HIV Prevention

Leslie Marshall,¹ James Cummins,¹ Hans Spiegel,² James Turpin,¹ Fulvia Veronese,¹ and Angela Kashuba,³ on behalf of the HIV Prevention Pharmacology Best Practices Working Group

EDITOR: A critical need in the field of HIV prevention is the implementation of studies designed to delineate the pharmacokinetic (PK) and pharmacodynamic (PD) relationship between humans and animal models of HIV infection. This need was identified at the Antiretroviral Pharmacology in HIV Prevention Think Tank, which was supported by the Division of AIDS (DAIDS) at the National Institute of Allergy and Infectious Disease (NIAID) in collaboration with the Bill and Melinda Gates Foundation (BMGF). Key issues identified by think tank participants included (1) route of administration, (2) drug concentrations in target mucosal tissues, (3) use of isolated cells vs. tissue homogenates for analyte detection, (4) dose ranging and fractionation studies, (5) virus inoculum for PD studies, and (6) use of *ex vivo* explants as a PK-PD surrogate. All of these issues have been explored in a critical review published concurrently in this journal.¹ In particular, dose ranging, time course, and dose fractionation data are needed to determine whether predictive or relational PK-PD models can be established that inform dose selection in proposed human studies.² To address this issue, a second meeting of a panel of experts in the field of HIV Prevention Pharmacology was convened in Washington, D.C. in 2012, which was also supported by DAIDS/NIAID in collaboration with BMGF. The objective of the meeting was to facilitate the development of protocols, linked by common design parameters, to support PK-PD studies in relevant animal models of HIV transmission. Two animal models were chosen for exploring the drug PK-PD relationship in efficacy studies of HIV prevention. Keeping the common design parameters in mind, the panel developed two protocols for humanized bone marrow-liver-thymus (BLT) mouse and nonhuman primate (NHP) studies to address these key issues. The design of these studies was informed by a gap analysis conducted by panel members based on available PK data from murine and NHP prevention studies.

Classically, NHPs infected with simian immunodeficiency virus (SIV) or simian-human immunodeficiency virus (SHIV) have been used as models that mimic specific pathogenic aspects of human HIV infection.³ More recently, the humanized BLT mouse model has been used for assessing PK-PD relationships due to the availability of larger numbers of animals at a cost equivalent to that of the NHPs.⁴ BLT mice are engrafted with human cells that can be productively infected with HIV and exhibit human HIV disease progression features such as circulating CD4 cell depletion and HIV-associated pathogenesis in the gut-associated lymphoid tissue (GALT).⁵ Although many studies have established the BLT model for measuring efficacy of HIV prevention,^{6–10} a major gap identified by the expert panelists was a lack of extensive PK analysis in this model. Therefore, panel members concluded that further analysis was required to establish the BLT mouse as a relevant model to establish initial PK and PD parameters to inform subsequent studies. Based on its wide usage in both preclinical animal efficacy studies and human clinical trials for HIV prevention, the prodrug tenofovir disoproxil fumarate (TDF) was selected for the studies in BLT mice and NHPs. Taking into account the significant differences between the two models that influence study design and conduct, planned experiments will incorporate dose ranging and fractionation along with PK sampling across comprehensive time points in blood/plasma, vaginal/rectal fluids and biopsies, and isolated target cells (e.g., peripheral blood mononuclear cells or tissue-derived mononuclear cells).

Pharmacokinetic evaluation of drug concentration and distribution at primary sites of viral exposure throughout the genital and gastrointestinal tracts over time, along with efficacy measures at controlled concentrations, can provide essential information for determining an optimal prevention dose regimen with which to move into clinical studies.¹ However, consideration of the mechanism of action of study

¹Preclinical Microbicide and Prevention Research Branch, Prevention Sciences Program, Division of AIDS, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland.

²HJF-DAIDS, a Division of The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Contractor to National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland.

³Division of Pharmacotherapy and Experimental Therapeutics, Eshelman School of Pharmacy, and UNC Center for AIDS Research, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina.

drugs and the collection of the appropriate cells and/or tissues are essential for making these determinations. Drugs that act intracellularly, such as tenofovir (TFV), must enter the cell and undergo metabolism to the fully active moiety tenofovir-diphosphate (TFV-DP). Analytical methods have been established for determining intracellular concentrations of TFV-DP in HIV target cells^{11,12}; however, it is unclear if recovery of sufficient numbers of cells in the target tissue from the BLT mouse or NHP models can be achieved.¹⁰ In addition, it is unclear if drug concentration in tissue homogenates correlates with intracellular results from cells isolated from primary sites of viral exposure throughout the genital and gastrointestinal tracts. Therefore, a goal of the comparative studies is to determine if there is a consistent, predictable relationship between the intracellular concentration of metabolized drug and drug levels in more easily accessible tissue homogenates. In addition to the mechanism of action, there are other factors that must be taken into account for the development of experimental designs that adequately address the PK and PD relationships between drug concentration and efficacy. These factors include metabolism of the compound, retention of the compound in cells and/or tissues, and varied behavior of the compound in different mucosal sites. Clinical data regarding the PK for antiretrovirals as therapy for HIV infection have previously and will continue to inform prevention studies. However, an efficacious dose for treatment of an HIV-infected individual may not be the same dose that is optimally protective in HIV noninfected individuals, thus an *a priori* assumption of equivalence may not be correct.

To identify the difference between doses necessary for HIV suppression in a therapeutic setting versus the dose necessary for protection against HIV infection in a prevention setting, clinical pharmacologist panel members recommended extensive dose ranging and fractionation studies in both the humanized BLT mouse and NHP models, similar to what is regularly performed for antimicrobials. Initial dose ranging studies in BLT mice will use a predicted efficacious dose based on published results combined with lower and higher doses.^{6,9,10} Each of the doses will be fractionated into once, twice, and three times daily regimens providing the total predicted efficacious dose. The goal is to identify the correlation between the effect, in this case HIV replication as determined by reduced viral RNA or p24 levels, and three main PK/PD indices: (1) the ratio of the maximal free drug concentration (C_{max}) to the minimum inhibitory concentration (MIC) [fC_{max}/MIC], (2) the ratio of the area under the curve (AUC) to the MIC [$f(AUC)/MIC$], or (3) the percentage of a 24-h time period that free drug concentrations exceed the MIC [$fT > MIC$]. As discussed in the comprehensive review, the approach closest to a linear relationship of drug concentration and effect is identified as having the most predictive value for measuring the biological effect on the pathogen.¹ Assuming a similar correlation exists in NHPs the resulting data will allow optimization of the proposed NHP protocol to both minimize the numbers of animals used and verify the PK-PD relationship between the BLT and NHP models. The objective of this effort will be to provide the key pharmacological studies needed to establish predictive and/or relational PK-PD models between mice and NHPs. The resulting models will be applied to known outcomes in clinical trials and will be used to determine whether the same relationship is valid in humans. Importantly, as discussed above, individual drugs with

different physicochemical properties and mechanisms of action may exhibit different outcomes with these types of analyses. Therefore, these studies aim to create a framework of analyses using TDF as a well-established case study in HIV prevention needed for application to other drugs.

The studies discussed above require mucosal sampling from multiple compartments (e.g., increased numbers and types at key time points) in both animal models, and represent a significant step toward addressing similarities and/or differences in the PK-PD relationship between preclinical models. However, determining whether preclinical PK-PD models can predict clinical PK-PD associations will require that equivalent data are obtained from human clinical studies. Thus, the realistic bridging between animals and humans will involve a similar approach with intense sampling in human clinical trials. The panel recommended that increased cell and tissue sampling with accompanying PK assessments be incorporated into future human studies. The members of the HIV Prevention Pharmacology BPWG strongly emphasized that these intensive PK assessments should be conducted within Phase I and before Phase II/III evaluation of candidate prevention products. They also encouraged the incorporation of mucosal tissue sampling and PK evaluations in ongoing human studies whenever possible in order to acquire those data that are critical for interpreting clinical outcomes in HIV prevention trials.

Acknowledgments

The authors wish to thank Stephen Becker and the Bill and Melinda Gates Foundation for providing assistance and logistical support in coordinating the meeting, the panel members for reviewing the manuscript and providing critical comments, and Anabel Lowry, Lester Freeman [Contractor, Henry M. Jackson Foundation (HJF)-DAIDS], and Glenn Swartz (Contractor, Advanced Biosciences Laboratories) for logistical assistance.

The Relational Animal PK Protocol Meeting was funded in part with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under contract no. HHSN272200800014C. L.M. wrote the first draft of the article. All authors and panel members contributed to the final version of the article and approved it.

The following individuals participated as panelists at the DAIDS/BMGF Relational Animal PK Protocol Meeting: Peter Anderson (University of Colorado), Peter Anton (UCLA), James Cummins (NIAID/DAIDS), Gustavo Doncel (CONRAD/East VA Medical School), J. Victor Garcia-Martinez (University of North Carolina at Chapel Hill), Alan Forrest (State University of New York, University at Buffalo), Walid Heneine (CDC), Angela Kashuba (University of North Carolina at Chapel Hill), Leslie Marshall (NIAID/DAIDS), Nicola Richardson-Harman (Alpha StatConsult), Keith Rodvold (University of Illinois at Chicago), Lisa Rohan (Magee-Womens Research Institute), Joseph Romano (NWJ Group, LLC), Hans Spiegel (HJF-DAIDS), James Turpin (NIAID/DAIDS), and Fulvia Veronese (NIAID-DAIDS).

Author Disclosure Statement

No competing financial interests exist.

References

1. Romano J, Kashuba A, Becker S, Cummins J, Turpin J, and Veronese F on Behalf of the Antiretroviral Pharmacology in HIV Prevention Think Tank Participants: 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. *AIDS Res Hum Retroviruses* 2013; [Epub ahead of print]; DOI: 10.1089/aid.2013.0122.
2. Mahmood I: Application of allometric principles for the prediction of pharmacokinetics in human and veterinary drug development. *Adv Drug Deliv Rev* 2007;59(11):1177–1192.
3. Van Rompay KK: The use of nonhuman primate models of HIV infection for the evaluation of antiviral strategies. *AIDS Res Hum Retroviruses* 2012;28(1):16–35.
4. Akkina R: New generation humanized mice for virus research: Comparative aspects and future prospects. *Virology* 2013;435(1):14–28.
5. Denton PW and Garcia JV: Mucosal HIV-1 transmission and prevention strategies in BLT humanized mice. *Trends Microbiol.* 2012;20(6):268–274.
6. Denton PW, Estes JD, Sun Z, *et al.*: Antiretroviral pre-exposure prophylaxis prevents vaginal transmission of HIV-1 in humanized BLT mice. *PLoS Med* 2008;5(1):e16.
7. Denton PW, Krisko JF, Powell DA, *et al.*: Systemic administration of antiretrovirals prior to exposure prevents rectal and intravenous HIV-1 transmission in humanized BLT mice. *PLoS One* 2010;5(1):e8829.
8. Denton PW, Othieno F, Martinez-Torres F, *et al.*: One percent tenofovir applied topically to humanized BLT mice and used according to the CAPRISA 004 experimental design demonstrates partial protection from vaginal HIV infection, validating the BLT model for evaluation of new microbicide candidates. *J Virol* 2011;85(15):7582–7593.
9. Denton PW, Olesen R, Choudhary SK, *et al.*: Generation of HIV latency in humanized BLT mice. *J Virol* 2012;86(1): 630–634.
10. Wahl A, Swanson MD, Nochi T, *et al.*: Human breast milk and antiretrovirals dramatically reduce oral HIV-1 transmission in BLT humanized mice. *PLoS Pathog* 2012;8(6): e1002732.
11. King T, Bushman L, Kiser J, *et al.*: Liquid chromatography-tandem mass spectrometric determination of tenofovir-diphosphate in human peripheral blood mononuclear cells. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006;843(2): 147–156.
12. Bushman LR, Kiser JJ, Rower JE, *et al.*: Determination of nucleoside analog mono-, di-, and tri-phosphates in cellular matrix by solid phase extraction and ultra-sensitive LC-MS/MS detection. *J Pharm Biomed Anal* 2011;56(2):390–401.

Address correspondence to:

Leslie Marshall

Preclinical Microbicide and Prevention Research Branch

Prevention Sciences Program

DAIDS, NIAID, NIH

6700B Rockledge Drive, Room 5224

Bethesda, Maryland 20892

E-mail: marshallj@niaid.nih.gov