AAC Journals.ASM.org

Pharmacokinetic Modeling of Lamivudine and Zidovudine Triphosphates Predicts Differential Pharmacokinetics in Seminal Mononuclear Cells and Peripheral Blood Mononuclear Cells

Julie B. Dumond,[a] Kuo H. Yang,[a] Racheal Kendrick,[a]* Y. Sunila Reddy,[a]* Angela D. M. Kashuba,[a,b] Luigi Troiani,[b] Arlene S. Bridges,[a]* Susan A. Fiscus,[b] Alan Forrest,[c] Myron S. Cohen[b]

UNC Eshelman School of Pharmacy, Division of Pharmacotherapy and Experimental Therapeutics, Chapel Hill, North Carolina, USA[a]; School of Medicine, Division of Infectious Diseases, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA[b]; School of Pharmacy and Pharmaceutical Sciences, Department of Pharmacy Practice, State University of New York, University at Buffalo, Buffalo, New York, USA[c]

The male genital tract is a potential site of viral persistence. Therefore, adequate concentrations of antiretrovirals are required to eliminate HIV replication in the genital tract. Despite higher zidovudine (ZDV) and lamivudine (3TC) concentrations in seminal plasma (SP) than in blood plasma (BP) (SP/BP drug concentration ratios of 2.3 and 6.7, respectively), we have previously reported lower relative intracellular concentrations of their active metabolites, zidovudine triphosphate (ZDV-TP) and lamivudine triphosphate (3TC-TP), in seminal mononuclear cells (SMCs) than in peripheral blood mononuclear cells (PBMCs) (SMC/PBMC drug concentration ratios of 0.36 and 1.0, respectively). Here, we use population pharmacokinetic (PK) modeling-based methods to simultaneously describe parent and intracellular metabolite PK in blood, semen, and PBMCs and SMCs. From this model, the time to steady state in each matrix was estimated, and the results indicate that the PK of 3TC-TP and ZDV-TP in PBMCs are different from the PK of the two in SMCs and different for the two triphosphates. We found that steady-state conditions in PBMCs were achieved within 2 days for ZDV-TP and 3 days for 3TC-TP. However, steady-state conditions in SMCs were achieved within 2 days for ZDV-TP and 2 weeks for 3TC-TP. Despite this, or perhaps because of it, ZDV-TP in SMCs does not achieve the surrogate 50% inhibitory concentration (IC_{50}) (as established for PBMCs, assuming SMC IC_{50} = PBMC IC_{50}) at the standard 300-mg twice-daily dosing. Mechanistic studies are needed to understand these differences and to explore intracellular metabolite behavior in SMCs for other nucleoside analogues used in HIV prevention, treatment, and cure.

Ongoing viral replication in the male genital tract poses challenges for HIV prevention, treatment, and cure. From a prevention standpoint, virus in semen remains a major vector of HIV transmission; in serodiscordant heterosexual couples, early initiation of antiretroviral (ARV) treatment in the infected partner, with maintained suppression of peripheral HIV RNA, can reduce the rate of transmission by 96%, compared to delaying ARV treatment until later in the course of disease (1). The single linked HIV transmission event in the HPTN052 early treatment arm was from an infected male to his uninfected female partner within approximately 29 days of starting ARV therapy (1, 2). Additionally, to date, evidence strongly suggests that early, effective antiretroviral treatment limits the size of the viral reservoir, providing the best chance of HIV eradication (3). Finally, ongoing viral replication has been demonstrated in sanctuary sites, such as the central nervous system, gut-associated lymphoid tissue, and the genital tract, where antiretroviral concentrations may differ from those seen systemically (reviewed in reference 4). Since HIV eradication will not be possible with ongoing viral replication in sanctuary compartments, it is important to select ARVs with optimized pharmacokinetic (PK) and pharmacodynamic profiles to control viral replication in the male genital tract, as well as other sanctuary sites.

We have previously published a noncompartmental pharmacokinetic analysis of zidovudine (ZDV) and lamivudine (3TC) in blood plasma (BP) and seminal plasma (SP), and their active triphosphate analogs zidovudine triphosphate (ZDV-TP) and lamivudine triphosphate (3TC-TP) in peripheral blood mononuclear cells (PBMCs) and seminal mononuclear cells (SMCs) (5). With this unique, rich data set, our goals for this analysis were as follows: (i) to create a PK model to describe the distribution of ZDV and 3TC in BP and SP, (ii) to describe the rate of formation and elimination of ZDV-TP and 3TC-TP in SMCs in relation to PBMCs, and (iii) to estimate the time to steady state and theoretical effective concentrations in SMCs.

MATERIALS AND METHODS

Study design. The data used to develop this model were extracted from a previously published study of 13 HIV-infected men receiving 300 mg of ZDV and 150 mg of 3TC twice daily as part of their ARV regimen (5). This study's protocol was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill. Sample collection was comprised of one intense sampling period and three sparse sampling periods. During the intense sampling period, blood was collected

Received 14 May 2015 Returned for modification 22 June 2015
Accepted 20 July 2015
Accepted manuscript posted online 3 August 2015
Citation Dumond JB, Yang KH, Kendrick R, Reddy YS, Kashuba ADM, Troiani L, Bridges AS, Fiscus SA, Forrest A, Cohen MS. 2015. Pharmacokinetic modeling of lamivudine and zidovudine triphosphates predicts differential pharmacokinetics in seminal mononuclear cells and peripheral blood mononuclear cells. Antimicrob Agents Chemother 59:6395–6401. doi:10.1128/AAC.01148-15.

Address correspondence to Julie B. Dumond, jdumond@unc.edu.
* Present address: Racheal Kendrick, Quintiles, Inc., Overland Park, Kansas, USA; Y. Sunila Reddy, Gilead Sciences, Foster City, California, USA; Arlene S. Bridges, Agilent Technologies, Santa Clara, California, USA.
J.B.D. and K.H.Y. contributed equally to this work.

doi:10.1128/AAC.01148-15

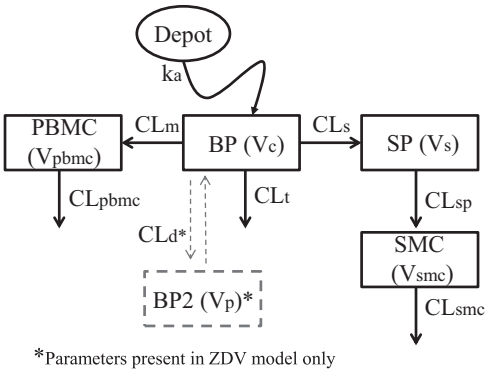

FIG 1 Schematic of lamivudine (3TC) and zidovudine (ZDV) pharmacokinetic models. The models differ only in the addition of a second blood plasma (BP) compartment for ZDV, shown in the box outlined by gray dashed lines. For 3TC, BP was described using one compartment with first-order absorption (k_a, absorption rate constant) and first-order elimination (CL_t, clearance; defined as total body clearance [CL_{tt}] minus clearance from blood to seminal plasma [CL_s] minus clearance from blood to PBMCs [CL_m]; $CL_t = CL_{tt} - CL_s - CL_m$). For ZDV, BP was described as using two compartments (CL_d, distributional clearance), with the same absorption and elimination parameters. Drug transferred from BP to seminal plasma (SP) via the clearance parameter CL_s, which is defined as total body clearance (CL_{tt}) multiplied by the fraction to semen (F_s) ($CL_s = CL_{tt} \times F_s$). Transfer of drug to these compartments was assumed to be unidirectional. Disposition in SP was described as a one-compartment system with first-order clearance (CL_s) to the seminal mononuclear cells (SMCs) and seminal volume (V_s). Drug transferred from SP to SMCs via the clearance parameter CL_{sp} and SMCs was described as a one-compartment system with first-order clearance (CL_{smc}) and volume (V_{smc}). Drug transferred from BP to peripheral blood mononuclear cells (PBMCs) via the clearance parameter CL_m, which is defined as total body clearance (CL_{tt}) multiplied by the fraction metabolized (F_m) ($CL_m = CL_{tt} \times F_m$). Disposition in PBMCs was described as a one-compartment system with first-order clearance (CL_{pbmc}) and volume (V_{pbmc}).

predose and 0.5, 1, 2, 4, 6, 8, and 12 h postdose, and semen was collected predose and 12 h postdose. The sparse sampling periods were conducted within the following 2 weeks, and in this period, one semen sample and one blood sample were collected at 3, 6, and 9 h postdose. ZDV and 3TC were measured in blood and seminal plasma, while ZDV-TP and 3TC-TP were measured in PBMCs and SMCs using validated liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) methods (6, 7).

Model development. Each drug and its metabolite were comodeled, using the first-order conditional estimation with interaction method in NONMEM version 7.3 (ICON Development Solutions, Hanover, MD). R version 3.1.0 (https://www.r-project.org) was used for data management and graphical analysis. Model selection was based on decreases in objective function and the likelihood ratio test (alpha = 0.05), goodness-of-fit plots, and visual predictive checks of predicted concentrations versus observed concentrations. All concentrations were converted to nanograms/milliliter, using an estimate for the volume of mononuclear cells of 282 fl/cell (8), and concentrations were log transformed for modeling purposes.

One- and two-compartment models were evaluated for each parent drug's disposition in BP, and then the PBMC compartments were added to describe triphosphate concentrations. Once this model was stabilized, the SP, and then the SMC compartments were added in a sequential manner. Several methods of linking the matrices were tested, including one-way and two-way transit, fractional clearances, and nonlinear disposition. Within each modeling step, parameters were initially added to the model with interindividual variability (IIV) and then evaluated for contribution to model fit. Initial estimates were based on biological plausibility and model parameter exploration in Berkeley Madonna 8.3.18 (University of California at Berkeley, Berkeley, CA). Berkeley Madonna is an easy-to-use, flexible, general differential equation solver with graphics capabilities, allowing one to determine the effects of various values of model parameters on model predictions compared to the observed data. Certain unidentifiable model parameters, such as the fraction of drug transferred to PBMCs and SP, were fixed to biologically plausible values, using this program (9). Each matrix in each model used a separate proportional-error term to describe residual variability, and as these samples were collected at the same time, correlation between these error terms was estimated. Finally, interoccasion variability was tested for inclusion on relevant param-

TABLE 1 Estimated model parameters for zidovudine (ZDV) and lamivudine (3TC)

Parameter(s)[a]	Geometric mean value for parameter (95% CI or CV%)[b]	
	ZDV	3TC
Fixed effects		
k_a (1/h)	1.20 (0.68 to 10.7)	1.26 (0.857 to 2.36)
CL_{tt} (liter/h)	231 (199 to 299)	31.3 (25.9 to 34.8)
V_c (liter)	69.5 (31.2 to 368)	138 (113 to 155)
CL_{pbmc} (10^{-2} liter/hour)	1.86 (1.63 to 2.08)	2.44 (1.73 to 3.16)
CL_{sp} (10^{-3} liter/hour)	7.83 (7.03 to 8.84)	0.303 (0.214 to 0.435)
CL_{smc} (10^{-4} liter/hour)	450 (332 to 606)	0.680 (0.470 to 1.17)
V_p (liter)	190 (124 to 991)	
CL_d (liter/hour)	60.4 (22.1 to 78.2)	
F_m	1×10^{-4} (fixed)	0.1 (fixed)
F_s	1×10^{-4} (fixed)	0.01 (fixed)
Interindividual variability (CV%)		
k_a		0.548 (0.548 to 52.1)
CL_{tt}		21.6 (7.04 to 27.1)
V_c	162.2 (22.2 to 217)	17.8 (3.81 to 26.2)
CL_{pbmc}	17.4 (0.224 to 26)	45.3 (19.8 to 60.6)
CL_{sp}		20.3 (0.167 to 26.8)
CL_{smc}	61.1 (0.707 to 77.3)	59.7 (41.2 to 70.4)
CL_{tt} and V_c (covariance, 10^{-2})		3.84 (0.420 to 6.80)
Interoccasion variability (CV%)		
k_a		118.7 (86 to 158)
CL_{tt}	49.3 (0.265 to 62.4)	5.00 (1.25 to 25.3)
V_c		2.50 (0.706 to 25.8)
k_a and CL_{tt} (covariance, 10^{-2})		5.61 (11.0 to 16.3)
k_a and V_c (10^{-2})		2.08 (−12.6 to 19.0)
CL and V_c (10^{-3})		1.10 (−0.680 to 63.0)
Residual variability (CV%)		
BP	43.0 (32.9 to 63.5)	29.9 (24.0 to 32.5)
PBMCs	29.9 (22.3 to 38.1)	56.2 (41.4 to 73.9)
SP	57.5 (44.7 to 70.3)	41.4 (32.9 to 49)
SMCs	30.7 (22.7 to 43.9)	41.5 (19.5 to 64.5)
BP and PBMCs (covariance, 10^{-2})	1.42 (−8.80 to 10.6)	3.16 (−1.85 to 8.63)
BP and SP (10^{-2})	24.7 (0.236 to 35.4)	2.84 (−0.814 to 8.64)
SP and PBMCs (10^{-2})	0.96 (−17.2 to 15.4)	23.3 (9.64 to 30.4)
BP and SMCs (10^{-2})	11.0 (−10.4 to 13.8)	−6.99 (−12.4 to 8.22)
PBMCs and SMCs (10^{-2})	5.93 (−1.82 to 11.6)	−13.7 (−30.6 to 28.6)
SP and SMCs (10^{-2})	14.1 (−7.66 to 18.5)	−10.5 (−24.1 to 17.0)

[a] Parameters are depicted in Fig. 1 and defined in the Fig. 1 legend and in the text. V_p, volume of distribution in the peripheral compartment.
[b] Values are presented as geometric means with bootstrapped 95% confidence intervals (95% CI) or coefficient of variance (CV%).

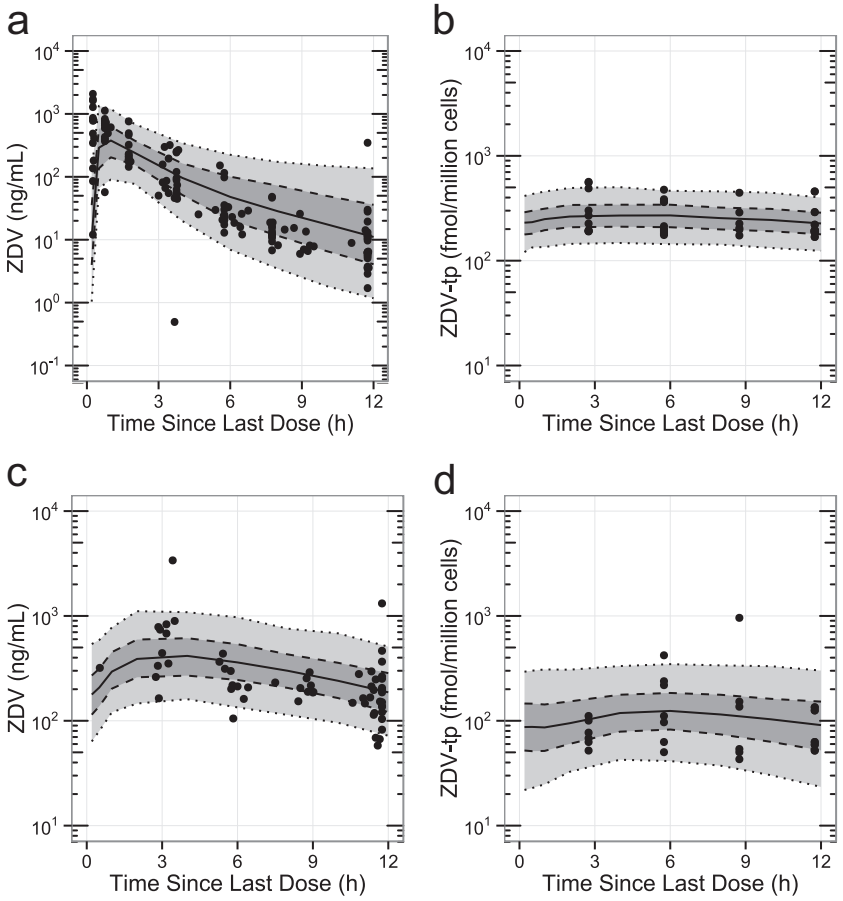

FIG 2 Visual predictive checks for zidovudine (ZDV) (a and c) and zidovudine triphosphate (ZDV-tp) (b and d) in blood plasma (a), PBMCs (b), seminal plasma (c), and seminal mononuclear cells (d). The solid line is the median of the simulated data set, the dashed lines encompassing the dark gray region represent the 75th and 25th percentiles of the simulations, and the dotted lines encompassing the light gray regions represent the 95th and 5th percentiles of the simulations. The black dots are the observed data points.

eters, as subjects had contributed samples on multiple occasions. Due to the small number of relatively homogenous subjects (all men, equal numbers of African-Americans and Caucasians, age range from 28 to 48 years), covariate analysis of demographic descriptors that could influence pharmacokinetics was not performed. Bootstrap analysis (100 replicates) was used to validate parameter estimates and model stability.

Simulations for visual predictive checks, estimation of time to steady state, and approach to theoretical 50% inhibitory concentrations (IC_{50}s) were performed in NONMEM 7.3. The IC_{50}s were obtained from the literature (10) and prescribing information (11) and are as follows: 115 ng/ml for 3TC, 554 fmol/10^6 cells for 3TC-TP, 13 ng/ml for ZDV, and 269 fmol/10^6 cells for ZDV-TP. Concentrations provided in the original references as molar units were converted using a standard assumption of cellular volume of 282 fl/cell as described above. Simulations for visual predictive checks assumed that all observations were collected during the same occasion.

RESULTS

Final model and validation. The final models for each drug are shown in Fig. 1. Briefly, 3TC in BP was best described using one compartment, while ZDV in BP was best described using two compartments (central and peripheral), both with first-order absorption and first-order elimination. For both drugs, a total systemic clearance (CL_{tt}) was estimated, and the BP clearance (CL_t) was defined as the total systemic clearance minus the clearance to the PBMCs ($CL_m = CL_{tt} \times F_m$ where CL_m is clearance from blood to PBMCs and F_m is the fraction metabolized), minus the clearance to the SP ($CL_s = CL_{tt} \times F_s$ where CL_s is clearance to semen and F_s is the fraction to semen). CL_m and CL_s include formation of triphosphate metabolite (three-step phosphorylation) and entry into the monocytic compartments. PBMC pharmacokinetics were described with a single compartment; SP pharmacokinetics were described with a compartment linked by a first-order clearance parameter to a compartment describing the SMC concentrations. Triphosphates exited both the SMC and PBMC compartments with first-order clearance terms.

Table 1 lists the model parameter values for each drug, and the nonparametric 5 to 95% bootstrap confidence intervals for each parameter. Exponential error models were used to describe interindividual variability, and proportional error models were used to describe residual variability. Interoccasion variability was placed on the absorption rate constant (k_a), CL_{tt}, and volume of distribution in the central compartment (V_c) for 3TC and on CL_{tt} for ZDV.

Figures 2a to d and 3a to d show the visual predictive checks for ZDV/ZDV-TP and 3TC/3TC-TP, respectively, in the four matrices. The solid lines indicate the prediction median, and the shaded region is the 90% confidence interval. The data points are the observations used to develop the model. These graphs indicate that each model does a reasonable job of describing the observed data.

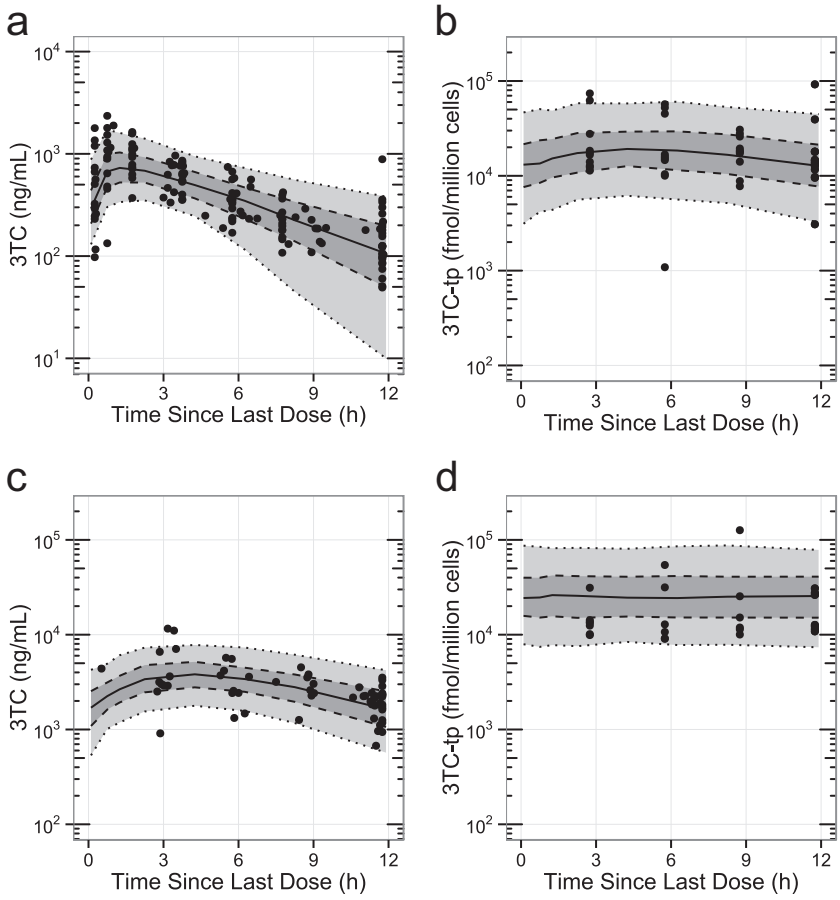

FIG 3 Visual predictive checks for lamivudine (3TC) (a and c) and lamivudine triphosphate (3TC-tp) (b and d) in blood plasma (a), PBMCs (b), seminal plasma (c), and seminal mononuclear cells (d). The solid line is the median of the simulated data set, the dashed lines encompassing the dark gray region represent the 75th and 25th percentiles of the simulations, and the dotted lines encompassing the light gray regions represent the 95th and 5th percentiles of the simulations. The black dots are the observed data points.

Figures 4a to d and 5a to d show the simulated concentration-time profiles as each drug approaches steady state from a first dose in each matrix. As expected for the parent drugs, given their short BP and SP half-lives, steady state is achieved quickly, after approximately 2 doses (1 day). For the longer-lived triphosphate metabolites, we observed differences in approach to steady state between PBMCs and SMCs and between 3TC-TP and ZDV-TP. As previously observed, the ZDV-TP exposures in SMCs were 36% of those in PBMCs, and here, the time to steady state was 30% shorter (2 days, based on median simulation values) compared to PBMCs (3 days). The estimated half-lives for ZDV-TP in PBMCs and SMCs were 20 h and 14 h, respectively. For 3TC-TP, although PBMC and SMC exposures were similar at steady state, the approach to steady state was quite different. In PBMCs, the 3TC-TP time to steady state was 3 days (similar to ZDV-TP in PBMCs). However, the SMC concentrations accumulated over 20 days before reaching steady-state conditions. The estimated half-lives for 3TC-TP in PBMCs and SMCs were 5 h and 159 h, respectively.

Also shown in Fig. 4 and 5 are the IC_{50}s for wild-type HIV in BP and PBMCs. Since the targets in SP and SMCs are unknown, BP and PBMC values were used as surrogates, assuming the same general relationship between parent and metabolite concentrations (12, 13). Median 3TC and 3TC-TP concentrations achieve and exceed IC_{50}s in all matrices with the first dose. ZDV is well above the targets in BP and SP, but median predicted concentrations in PBMCs and SMCs do not reach the surrogate target. Assuming dose linearity for ZDV and ZDV-TP, simulations of higher ZDV doses (data not shown) suggest that 450 to 600 mg twice daily would be required for at least half of the simulated profiles to reach the PBMC target at steady state and that 750 to 900 mg twice daily would be required for at least half of the simulated profiles to reach the SMC target at steady state.

DISCUSSION

The penetration of the parent drugs studied here, 3TC and ZDV, into SP has been studied by several groups, and consistently found to exceed concentrations in BP (reviewed in reference 14). Their active intracellular metabolite concentrations are less well studied, but work thus far indicates that the presence of the parent drug in the SP does not guarantee corresponding high levels of phosphorylation (5). Although penetration of parent drug into the SP can be predicted by plasma protein binding (15), the correlates and predictors of phosphorylation in SMCs are currently unknown. The phosphorylation process is driven by stepwise kinase activity, and the competition between the drug triphosphate and the endogenous nucleotide that it mimics (dCTP for 3TC and deoxythy-

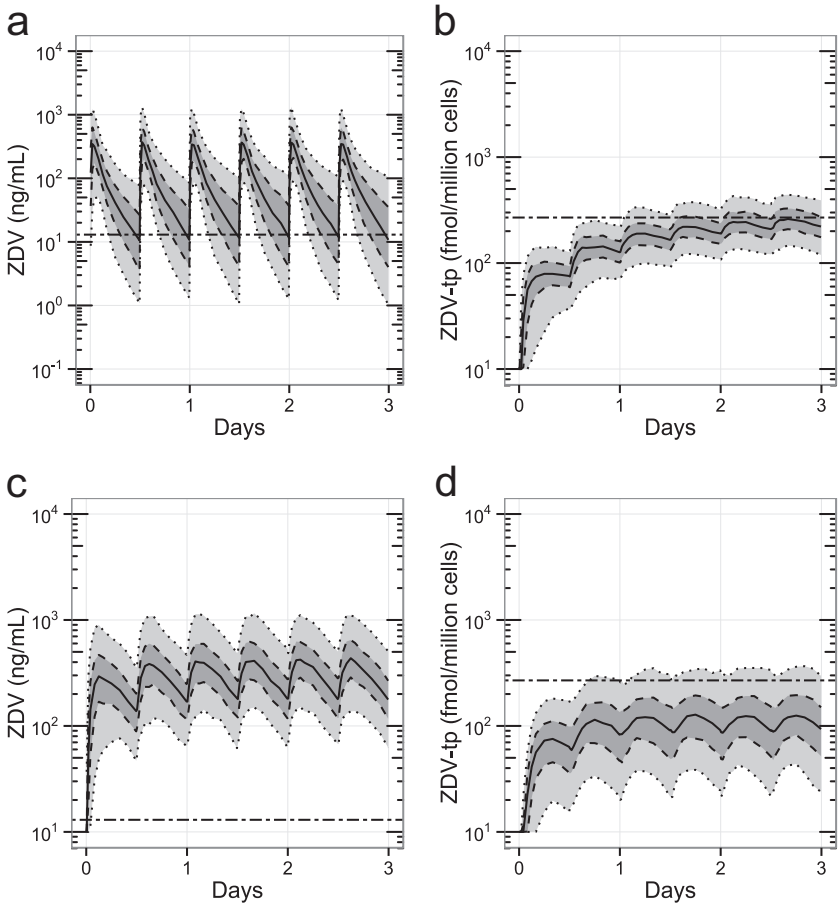

FIG 4 Simulations from the first dose to steady state for zidovudine (ZDV) (a and c) and zidovudine triphosphate (ZDV-tp) (b and d) in blood plasma (a), PBMCs (b), seminal plasma (c), and seminal mononuclear cells (d). The solid line is the median of the simulated data set, the dashed lines encompassing the dark gray region represent the 75th and 25th percentiles of the simulations, and the dotted lines encompassing the light gray regions represent the 95th and 5th percentiles of the simulations. The dashed-and-dotted horizontal line references literature IC_{50} values in each matrix (13 ng/ml for ZDV and 269 fmol/10^6 cells for ZDV-tp).

midine triphosphate for ZDV) for incorporation into the viral DNA produced by reverse transcriptase drives efficacy (16). The anabolic process for the triphosphates is fairly well described in PBMCs (16) but is largely unknown in SMCs. Our work suggests that these basic cellular processes differ in ways that result in different PK profiles in SMCs for nucleoside reverse transcriptase inhibitors and that each agent with clinical relevance must be studied to elucidate individual mechanisms. Differences in cellular activation state (17), the specific cell populations that comprise the mononuclear cell pool in semen and blood (18), drug metabolism (i.e., ZDV glucuronidation) (19), and kinase activities (16) may contribute to these differences. For both PBMCs and SMCs, the catabolic process for the triphosphate metabolites is not well described, although previous modeling work combining *ex vivo* and *in vivo* studies of 3TC-TP in PBMCs suggests that catabolism of 3TC-TP in PBMCs is less likely to be a critical determinant of disposition and more likely to be dependent on the total 3TC parent and metabolite pools within the cell (20).

Using antiretrovirals that achieve suppressive concentrations in the genital tract are the best chance to prevent transmission and limit reservoir establishment early in infection. Although ZDV-TP in SMCs achieves steady state rapidly, its median concentrations are well below the PBMC IC_{50}. Consistent with this, ZDV-resistant viral transmission has been reported (21). Conversely, 3TC-TP has a very long half-life in SMCs, potentially related to the differences in pharmacology, cell composition, and overall pool of 3TC and its metabolites in SMCs detailed above, and yet concentrations are well above the IC_{50}. It is currently unknown whether these *in vitro* IC_{50} estimates in PBMCs are meaningful in semen, but they do provide some insight into relevant concentrations. 3TC remains a mainstay of therapy in the fixed-dose combinations used extensively in the developing world, with zidovudine a potential alternative to tenofovir (22). As HIV-infected patients in the developing world are more likely to be infected for longer prior to beginning antiretrovirals, thus allowing the establishment of the viral reservoir, the ability of these drugs to suppress virus in reservoir compartments is paramount to achieving cure.

Tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) have largely replaced 3TC and ZDV as the nucleoside backbone of HIV treatment in the United States, although 3TC is included in the recommended first-line regimen of 3TC-abacavir-dolutegravir (Triumeq; ViiV Healthcare, Research Triangle Park, NC) for antiretroviral-naive patients able to safely receive abacavir (23). To our knowledge, FTC and its triphosphate have not been studied in SP or SMCs; evidence in the female genital tract suggests

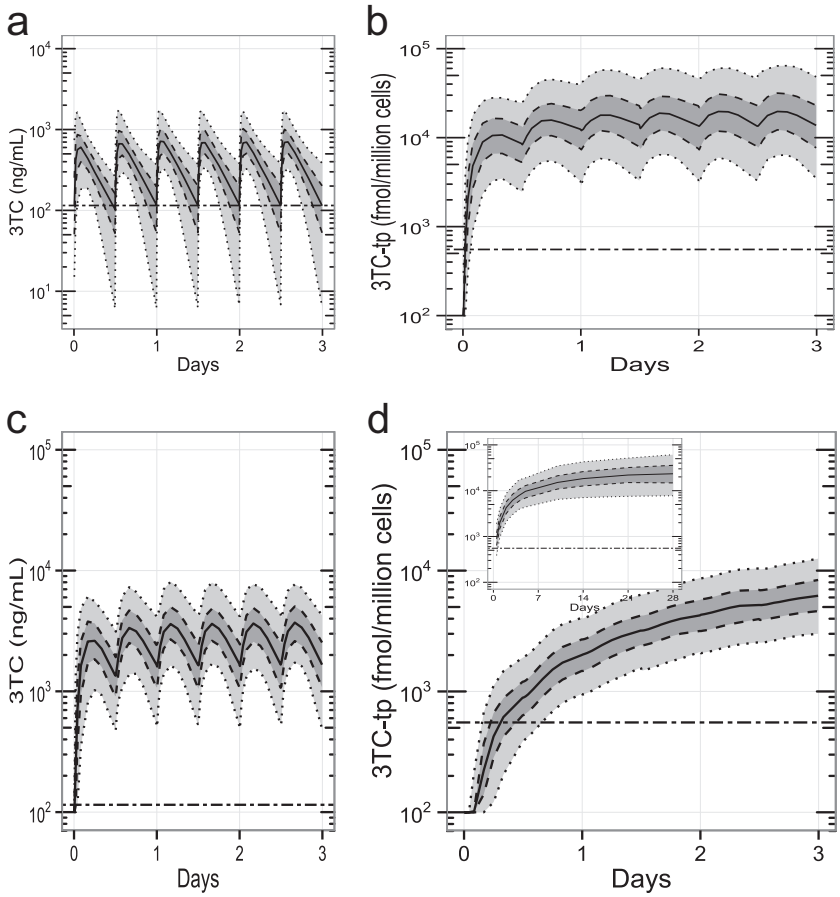

FIG 5 Simulations from the first dose to steady state for lamivudine (3TC) (a and c) and lamivudine triphosphate (3TC-tp) (b and d) in blood plasma (a), PBMCs (b), seminal plasma (c), and seminal mononuclear cells (d). The inset in panel d shows the full approach of 3TC-tp to steady state over 28 days in seminal mononuclear cells. The solid line is the median of the simulated data set, the dashed lines encompassing the dark gray region represent the 75th and 25th percentiles of the simulations, and the dotted lines encompassing the light gray regions represent the 95th and 5th percentiles of the simulations. The dashed-and-dotted horizontal line references literature IC_{50} values in each matrix (115 ng/ml for 3TC and 554 fmol/10^6 cells for 3TC-tp).

that parent FTC exposure would be similar to 3TC (24), but behavior of the triphosphate in SMCs is unknown. The model developed for 3TC could be applied to FTC disposition in the male genital tract, if such data were available. The penetration of TDF into SP has been studied by several groups (25, 26), but only one paper has examined its active diphosphate form in SMCs (27). Vourvahis and colleagues found 9-fold-higher concentrations of the diphosphate in SMCs at a first dose of TDF monotherapy, and 17-fold-higher concentrations in SMCs at steady state; in SMCs, these concentrations exceed the proposed IC_{90} in PBMCs (16 fmol/10^6 cells) (28) by at least 10-fold. Models have not been applied to these data, to our knowledge; tenofovir alafenamide, an alternate formulation of tenofovir designed specifically to increase PBMC tenofovir diphosphate concentrations while decreasing BP tenofovir concentrations, will soon be approved in the United States and is likely to replace the tenofovir disoproxil fumarate formulation in the developed world (29, 30). The models developed here could also be applied to PBMC and SMC tenofovir diphosphate concentrations in subjects receiving these formulations. Our group plans to generate these data for FTC, tenofovir disoproxil fumarate, and tenofovir alafenamide in the future.

The ability to study viral dynamics and reservoir formation in the genital tract, in concert with pharmacology, has advanced substantially since this work was performed (2000 to 2003), and thus, such virologic data are not available to pair with this pharmacokinetic data for a full exploration of mechanistic implications. Given these findings of differing pharmacology in SP and SMCs between nucleosides, determining the pharmacologic mechanisms of phosphorylation and predictors of ARV behavior for clinically used agents in concert with viral dynamics in the male genital tract is necessary for optimizing drug therapy for HIV treatment as prevention and eradication.

ACKNOWLEDGMENTS

The original clinical study was supported by an investigator-initiated grant to A. D. M. Kashuba from GlaxoSmithKline. J. B. Dumond is supported in part by grant K23-AI093156. A. D. M. Kashuba is supported in part by grants AI111891, AI096138, MH094177, AI103390, AI073146, AI111936, and AI116276-01, and A. D. M. Kashuba and K. H. Yang are supported in part by the UNC Center for AIDS Research (P30AI050410) and CARE (U19AI096113). A. D. M. Kashuba and M. S. Cohen are supported in part by grant 4R37DK049381.

REFERENCES

1. **Cohen MS, Chen YQ, McCauley M, Gamble T, Hosseinipour MC, Kumarasamy N, Hakim JG, Kumwenda J, Grinsztejn B, Pilotto JH, Godbole SV, Mehendale S, Chariyalertsak S, Santos BR, Mayer KH, Hoffman IF, Eshleman SH, Piwowar-Manning E, Wang L, Makhema J, Mills LA, de Bruyn G, Sanne I, Eron J, Gallant J, Havlir D, Swindells S, Ribaudo H, Elharrar V, Burns D, Taha TE, Nielsen-Saines K, Celentano D, Essex M, Fleming TR.** 2011. Prevention of HIV-1 infection with early antiretroviral therapy. N Engl J Med **365:**493–505. http://dx.doi.org/10.1056/NEJMoa1105243.
2. **Ping L-H, Jabara CB, Rodrigo AG, Hudelson SE, Piwowar-Manning E, Wang L, Eshleman SH, Cohen MS, Swanstrom R.** 2013. HIV-1 transmission during early antiretroviral therapy: evaluation of two HIV-1 transmission events in the HPTN 052 prevention study. PLoS One **8:**e71557. http://dx.doi.org/10.1371/journal.pone.0071557.
3. **Ananworanich J, Dube K, Chomont N.** 2015. How does the timing of antiretroviral therapy initiation in acute infection affect HIV reservoirs? Curr Opin HIV AIDS **10:**18–28. http://dx.doi.org/10.1097/COH.0000000000000122.
4. **Palmer S, Josefsson L, Coffin JM.** 2011. HIV reservoirs and the possibility of a cure for HIV infection. J Intern Med **270:**550–560. http://dx.doi.org/10.1111/j.1365-2796.2011.02457.x.
5. **Dumond JB, Reddy YS, Troiani L, Rodriguez JF, Bridges AS, Fiscus SA, Yuen GJ, Cohen MS, Kashuba AD.** 2008. Differential extracellular and intracellular concentrations of zidovudine and lamivudine in semen and plasma of HIV-1-infected men. J Acquir Immune Defic Syndr **48:**156–162. http://dx.doi.org/10.1097/QAI.0b013e31816de21e.
6. **Pereira AS, Kenney KB, Cohen MS, Hall JE, Eron JJ, Tidwell RR, Dunn JA.** 2000. Simultaneous determination of lamivudine and zidovudine concentrations in human seminal plasma using high-performance liquid chromatography and tandem mass spectrometry. J Chromatogr B Biomed Sci Appl **742:**173–183. http://dx.doi.org/10.1016/S0378-4347(00)00162-6.
7. **Rodriguez JF, Rodriguez JL, Santana J, Garcia H, Rosario O.** 2000. Simultaneous quantitation of intracellular zidovudine and lamivudine triphosphates in human immunodeficiency virus-infected individuals. Antimicrob Agents Chemother **44:**3097–3100. http://dx.doi.org/10.1128/AAC.44.11.3097-3100.2000.
8. **Simiele M, D'Avolio A, Baietto L, Siccardi M, Sciandra M, Agati S, Cusato J, Bonora S, Di Perri G.** 2011. Evaluation of the mean corpuscular volume of peripheral blood mononuclear cells of HIV patients by a Coulter Counter to determine intracellular drug concentrations. Antimicrob Agents Chemother **55:**2976–2978. http://dx.doi.org/10.1128/AAC.01236-10.
9. **Shivva V, Korell J, Tucker IG, Duffull SB.** 2013. An approach for identifiability of population pharmacokinetic-pharmacodynamic models. CPT Pharmacometrics Syst Pharmacol **2:**e49. http://dx.doi.org/10.1038/psp.2013.25.
10. **Duan C, Poticha D, Stoeckli TC, Petropoulos CJ, Whitcomb JM, McHenry CS, Kuritzkes DR.** 2001. Inhibition of purified recombinant reverse transcriptase from wild-type and zidovudine-resistant clinical isolates of human immunodeficiency virus type 1 by zidovudine, stavudine, and lamivudine triphosphates. J Infect Dis **184:**1336–1340.
11. **ViiV Healthcare.** 2013. Combivir (lamivudine and zidovudine) (tablets 150 mg/300 mg) full U.S. prescribing information. ViiV Healthcare, Research Triangle Park, NC.
12. **Fletcher CV, Kawle SP, Kakuda TN, Anderson PL, Weller D, Bushman LR, Brundage RC, Remmel RP.** 2000. Zidovudine triphosphate and lamivudine triphosphate concentration-response relationships in HIV-infected persons. AIDS **14:**2137–2144. http://dx.doi.org/10.1097/00002030-200009290-00010.
13. **Kakuda TN, Page LM, Anderson PL, Henry K, Schacker TW, Rhame FS, Acosta EP, Brundage RC, Fletcher CV.** 2001. Pharmacological basis for concentration-controlled therapy with zidovudine, lamivudine, and indinavir. Antimicrob Agents Chemother **45:**236–242. http://dx.doi.org/10.1128/AAC.45.1.236-242.2001.
14. **Else LJ, Taylor S, Back DJ, Khoo SH.** 2011. Pharmacokinetics of antiretroviral drugs in anatomical sanctuary sites: the male and female genital tract. Antivir Ther **16:**1149–1167. http://dx.doi.org/10.3851/IMP1919.
15. **Nicol MR, Kashuba AD.** 2010. Pharmacologic opportunities for HIV prevention. Clin Pharmacol Ther **88:**598–609. http://dx.doi.org/10.1038/clpt.2010.189.
16. **Anderson PL, Kakuda TN, Lichtenstein KA.** 2004. The cellular pharmacology of nucleoside- and nucleotide-analogue reverse-transcriptase inhibitors and its relationship to clinical toxicities. Clin Infect Dis **38:**743–753. http://dx.doi.org/10.1086/381678.
17. **Gao WY, Agbaria R, Driscoll JS, Mitsuya H.** 1994. Divergent anti-human immunodeficiency virus activity and anabolic phosphorylation of 2′,3′-dideoxynucleoside analogs in resting and activated human cells. J Biol Chem **269:**12633–12638.
18. **Anderson PL, Zheng JH, King T, Bushman LR, Predhomme J, Meditz A, Gerber J, Fletcher CV.** 2007. Concentrations of zidovudine- and lamivudine-triphosphate according to cell type in HIV-seronegative adults. AIDS **21:**1849–1854. http://dx.doi.org/10.1097/QAD.0b013e3282741feb.
19. **Anderson PL, Noormohamed SE, Henry K, Brundage RC, Balfour HH, Jr, Fletcher CV.** 2000. Semen and serum pharmacokinetics of zidovudine and zidovudine-glucuronide in men with HIV-1 infection. Pharmacotherapy **20:**917–922. http://dx.doi.org/10.1592/phco.20.11.917.35263.
20. **Zhou Z, Rodman JH, Flynn PM, Robbins BL, Wilcox CK, D'Argenio DZ.** 2006. Model for intracellular lamivudine metabolism in peripheral blood mononuclear cells ex vivo and in human immunodeficiency virus type 1-infected adolescents. Antimicrob Agents Chemother **50:**2686–2694. http://dx.doi.org/10.1128/AAC.01637-05.
21. **Erice A, Mayers DL, Strike DG, Sannerud KJ, McCutchan FE, Henry K, Balfour HH, Jr.** 1993. Brief report: primary infection with zidovudine-resistant human immunodeficiency virus type 1. N Engl J Med **328:**1163–1165. http://dx.doi.org/10.1056/NEJM199304223281605.
22. **World Health Organization.** 2013. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. World Health Organization, Geneva, Switzerland. http://www.who.int/hiv/pub/guidelines/arv2013/download/en/.
23. **US Department of Health and Human Services Panel on Antiretroviral Guidelines for Adults and Adolescents.** 8 April 2015. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. US Department of Health and Human Services, Washington, DC. http://aidsinfo.nih.gov/guidelines/html/1/adult-and-adolescent-treatment-guidelines/0. Accessed 6 June 2015.
24. **Dumond JB, Yeh RF, Patterson KB, Corbett AH, Jung BH, Rezk NL, Bridges AS, Stewart PW, Cohen MS, Kashuba AD.** 2007. Antiretroviral drug exposure in the female genital tract: implications for oral pre- and post-exposure prophylaxis. AIDS **21:**1899–1907. http://dx.doi.org/10.1097/QAD.0b013e328270385a.
25. **Ghosn J, Chaix ML, Peytavin G, Rey E, Bresson JL, Goujard C, Katlama C, Viard JP, Treluyer JM, Rouzioux C.** 2004. Penetration of enfuvirtide, tenofovir, efavirenz, and protease inhibitors in the genital tract of HIV-1-infected men. AIDS **18:**1958–1961. http://dx.doi.org/10.1097/00002030-200409240-00014.
26. **Lowe SH, van Leeuwen E, Droste JA, van der Veen F, Reiss P, Lange JM, Burger DM, Repping S, Prins JM.** 2007. Semen quality and drug concentrations in seminal plasma of patients using a didanosine or didanosine plus tenofovir containing antiretroviral regimen. Ther Drug Monit **29:**566–570. http://dx.doi.org/10.1097/FTD.0b013e31811fef29.
27. **Vourvahis M, Tappouni HL, Patterson KB, Chen YC, Rezk NL, Fiscus SA, Kearney BP, Rooney JF, Hui J, Cohen MS, Kashuba AD.** 2008. The pharmacokinetics and viral activity of tenofovir in the male genital tract. J Acquir Immune Defic Syndr **47:**329–333. http://dx.doi.org/10.1097/QAI.0b013e3181632cc3.
28. **Seifert SM, Glidden DV, Meditz AL, Castillo-Mancilla JR, Gardner EM, Predhomme JA, Rower C, Klein B, Kerr BJ, Guida LA, Zheng JH, Bushman LR, Anderson PL.** 2015. Dose response for starting and stopping HIV preexposure prophylaxis for men who have sex with men. Clin Infect Dis **60:**804–810. http://dx.doi.org/10.1093/cid/ciu916.
29. **Mills A, Crofoot G, Jr, McDonald C, Shalit P, Flamm JA, Gathe J, Scribner A, Shamblaw D, Saag M, Cao H, Martin H, Das M, Thomas A, Liu HC, Yan M, Callebaut C, Custodio J, Cheng A, McCallister S.** 2015. Tenofovir alafenamide versus tenofovir disoproxil fumarate in the first protease inhibitor-based single tablet regimen for initial HIV-1 therapy: a randomized phase 2 study. J Acquir Immune Defic Syndr **69:**439–445. http://dx.doi.org/10.1097/QAI.0000000000000618.
30. **Sax PE, Wohl D, Yin MT, Post F, DeJesus E, Saag M, Pozniak A, Thompson M, Podzamczer D, Molina JM, Oka S, Koenig E, Trottier B, Andrade-Villanueva J, Crofoot G, Custodio JM, Plummer A, Zhong L, Cao H, Martin H, Callebaut C, Cheng AK, Fordyce MW, McCallister S, GS-US-292-0104/0111 Study Team.** 2015. Tenofovir alafenamide versus tenofovir disoproxil fumarate, coformulated with elvitegravir, cobicistat, and emtricitabine, for initial treatment of HIV-1 infection: two randomised, double-blind, phase 3, noninferiority trials. Lancet **385:**2606–2615. http://dx.doi.org/10.1016/S0140-6736(15)60616-X.