Development of an Optimized Dose for Coformulation of Zidovudine with Drugs That Select for the K65R Mutation Using a Population Pharmacokinetic and Enzyme Kinetic Simulation Model[†]

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In vitro selection studies and data from large genotype databases from clinical studies have demonstrated that tenofovir disoproxil fumarate and abacavir sulfate select for the K65R mutation in the human immunodeficiency virus type 1 polymerase region. Furthermore, other novel non-thymine nucleoside reverse transcriptase (RT) inhibitors also select for this mutation *in vitro*. Studies performed *in vitro* and in humans suggest that viruses containing the K65R mutation remained susceptible to zidovudine (ZDV) and other thymine nucleoside antiretroviral agents. Therefore, ZDV could be coformulated with these agents as a “resistance repellent” agent for the K65R mutation. The approved ZDV oral dose is 300 mg twice a day (b.i.d.) and is commonly associated with bone marrow toxicity thought to be secondary to ZDV-5'-monophosphate (ZDV-MP) accumulation. A simulation study was performed *in silico* to optimize the ZDV dose for b.i.d. administration with K65R-selecting antiretroviral agents in virtual subjects using the population pharmacokinetic and cellular enzyme kinetic parameters of ZDV. These simulations predicted that a reduction in the ZDV dose from 300 to 200 mg b.i.d. should produce similar amounts of ZDV-5'-triphosphate (ZDV-TP) associated with antiviral efficacy (>97% overlap) and reduced plasma ZDV and cellular amounts of ZDV-MP associated with toxicity. The simulations also predicted reduced peak and trough amounts of cellular ZDV-TP after treatment with 600 mg ZDV once a day (q.d.) rather than 300 or 200 mg ZDV b.i.d., indicating that q.d. dosing with ZDV should be avoided. These *in silico* predictions suggest that 200 mg ZDV b.i.d. is an efficacious and safe dose that could delay the emergence of the K65R mutation.

Current first-line highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV-1) infections combines two nucleoside reverse transcriptase inhibitors (NRTI) together with either a protease inhibitor or a non-NRTI (18, 19, 54). These drug combinations have markedly decreased mortality and morbidity from HIV-1 infections in the developed world (11). Existing therapeutic modalities cannot eradicate HIV-1 infection because of the compartmentalization of the virus and its latent properties (80, 81). Therefore, chronic therapy remains the standard of care for the foreseeable future. HAART regimens are selected in part to minimize cross-resistance and thereby delay the emergence of resistant viruses. However, all regimens eventually fail, due primarily to a lack of adherence to strict regimens, delayed toxicities, and/or the emergence of drug-resistant HIV-1 strains (68). Thus, it is a major imperative to develop regimens that delay, prevent, or attenuate the onset of resistance for second-line treatments for infected individuals who have already demonstrated mutations in the systemic circulation. The occurrence of common resistance mutations, including thymine analog mutations (specifically, D67N, K70R, T215Y, and

T219Q), K65R, and M184V, needs to be the continued focus in the drug development of HIV-1 NRTI (79).

Data from large genotype databases demonstrated an increased incidence of the K65R mutation from 0.8% in 1998 to 3.8% in 2003, presumably as a result of the increased use of K65R-selecting drugs (33, 76). This mutation produces a single amino acid change from lysine to arginine in the HIV-1 reverse transcriptase (RT) gene. The *in vitro* selection of K65R, accompanied with moderate resistance, has been demonstrated for nonthymine NRTI, including abacavir sulfate (ABC), tenofovir disoproxil fumarate (TDF), zalcitabine, didanosine, adefovir dipivoxil and lamivudine (3TC), β -D-2',3'-dideoxy-2',3'-dideoxy-5-fluorocytidine (D-d4FC, dextelvucitabine; Reverset), and β -D-(2R,4R)-1,3-dioxolane guanosine (DXG) (39, 76, 88, 89). The guanosine nucleoside prodrug of DXG, amdoxovir [AMDX; (–)- β -D-2,6-diaminopurine dioxolane (DAPD)] (12, 25, 31), is being developed by RFS Pharma, LLC, primarily for the second-line treatment of HIV-1 infections (35, 52). The advantages of DXG include an increased sensitivity to M184V/I strains *in vitro* and activity against thymine analog mutations that may have been selected during previous antiretroviral therapy and 69SS double insert (36, 37, 55). DXG is synergistic with several NRTI, including zidovudine (ZDV), 3TC, and nevirapine (36). *In vitro* studies with HIV-1 in culture with MT-2 cells demonstrated a slow onset of resistance to DXG that was associated with the K65R mutation (66, 67, 89). An *in vitro* study demonstrated that ZDV alone selected for a mixture of K70K/R mutations at week 25, and

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DAPD alone selected for a mixture of the K65R and L74V mutations at week 20. However, when DAPD and ZDV were used in combination in HIV-infected primary human lymphocytes, no drug-resistant mutations were detected through week 28 (71). Furthermore, mechanistic studies demonstrated that K65R mutants remained susceptible to thymine NRTI, including ZDV and stavudine (8, 29, 38, 39, 66). Regimens containing TDF in combination with lamivudine and abacavir have demonstrated high failure rates due to the emergence of drug-resistance mutations, including K65R. A composite analysis of data from those regimens revealed a success rate of 86% when the regimen contained ZDV compared to 62% when it did not. Furthermore, no K65R mutations were observed in subjects on regimens containing ZDV, suggesting that ZDV has value in preventing selection for this mutation (33). A previous study has also demonstrated a reduced selection of the K65R mutation when ZDV was added to a regimen containing ABC (50). Therefore, the coformulation of ZDV with K65R-mutation-selecting drugs is warranted (33, 53, 69), since ZDV has the potential to serve as a "resistance repellent" agent for the K65R mutation. The addition of ZDV may not be appropriate if it competes for rate-limiting enzyme phosphorylation with other NRTI in the HAART regimen. However, the enzyme used for the rate-limiting phosphorylation step of ZDV differs from those of TDF, ABC, and DXG (3, 4, 20, 22–25, 30, 41, 55, 60, 85).

ZDV was the first antiretroviral drug tested in the clinic initially as a monotherapy drug and later as a component of HAART regimens (11, 17, 26) and was approved as a generic formulation in September 2005 by the United States Food and Drug Administration. Like other NRTI, ZDV undergoes three intracellular phosphorylation steps to form the active ZDV-5'-triphosphate (ZDV-TP), which inhibits wild-type HIV-1 RT with a median inhibitory concentration of about 0.035 μ M (72). The single-dose plasma pharmacokinetics of ZDV have been well described in HIV-1-infected individuals following intravenous and oral administration (1, 10, 20, 34, 65, 78, 90).

ZDV treatment is limited by toxic side effects, including nausea and malaise, as well as serious bone marrow cytotoxicities, including anemia and neutropenia (15, 73, 82). The bone marrow cytotoxicities of ZDV are believed to be associated with mitochondrial damage and correlate with the ZDV-5'-monophosphate (ZDV-MP) content (87). The current approved oral dose of ZDV in most of the world is 300 mg twice a day (b.i.d.). A double-blind, parallel group multicenter study involving 474 HIV-infected patients, comparing ZDV monotherapy daily doses of 400, 800, and 1,600 mg, was published in 1992 (62). A dose-dependent statistically significant increase in the incidence of anemia and leucopenia ($P = 0.008$), neutropenia ($P = 0.0005$), and neuropathy ($P = 0.03$) was observed, and the percentage of subjects who failed to complete the study due to side effects was dose related (21%, 31%, and 32% for the 400-, 800-, and 1,600-mg daily doses of ZDV, respectively). Although there was a trend toward fewer cases of AIDS dementia complex, it was not statistically significant. It was concluded that lower ZDV doses reduced toxicity, and doses >400 mg to 600 mg a day offered no clinical advantage (62). Furthermore, a study by Barry et al. (7) reported that a reduced dose of 100 mg ZDV three times a day (t.i.d.) produced similar amounts of cellular ZDV-TP, which mediates its anti-

viral effect, with significantly decreased ZDV plasma concentrations and intracellular amounts of ZDV-MP, lending support to enzymatic studies that suggest thymidylate kinase (TMPK) may be oversaturated at clinical doses (30). There are conflicting reports regarding the clinical relevance of the saturation of TMPK at clinical ZDV doses. Fletcher et al. reported higher average amounts of cellular ZDV-TP and decreased variance (0.62 nM and 32% coefficient of variation [CV] versus 0.76 nM and 16% CV, respectively) when ZDV doses were adjusted to maintain a target plasma concentration (27), suggesting that variability between subjects in pharmacokinetics is important. However, the accumulation of ZDV-TP is further complicated, since phosphorylation is cell cycle dependent and the fraction of dividing cells may vary between HIV-infected individuals (48, 49, 59, 86). Once activated, cells from different hosts may have various TMPK activities (49). Given the large variation in cellular ZDV-TP concentrations measured in peripheral blood mononuclear (PBM) cells (a CV of approximately 100%) (2, 28), large clinical trials would be needed to statistically demonstrate whether ZDV phosphorylation is saturable. A potentially useful approach could be to merge the enzyme kinetic data, which are best characterized in vitro, with the in vivo-derived population pharmacokinetic parameters of ZDV in HIV-infected individuals, in an in silico simulation study. This approach would allow large populations of virtual subjects to be simulated and compared with actual clinical data.

The results of Barry et al. (7) suggest the potential for dose saturation of ZDV phosphorylation; moreover, in vitro data indicate that virus containing the K65R mutation is more sensitive to ZDV (8, 50, 66, 67). Furthermore, a regimen of 600 mg ZDV once a day (q.d.) was recently shown to produce a less-pronounced and slower onset of viral depletion than was observed for the standard regimen of 300 mg b.i.d. (77). Therefore, the objectives of this study were to develop a population pharmacokinetic and pharmacodynamic model that combines population pharmacokinetic parameters and population variance in cellular enzyme levels in HIV-1-infected individuals, to determine whether dose saturation of ZDV-TP is supported mechanistically, to develop an optimal dosage regimen of ZDV for coformulation as a K65R-resistant repellent with DAPD and other NRTI when the initial load of K65R is low, and to assess whether the outcomes of the 600 mg ZDV q.d. trial were predictable based on plasma pharmacokinetics and enzyme dynamic data derived in vitro. Since ZDV reduces the overall viral load by <1 log, it was considered prudent to target the cellular contents of ZDV-TP similar to those observed presently in the clinic.

MATERIALS AND METHODS

The structure of the population pharmacokinetics and the cellular pharmacology models used in this study are summarized in Fig. 1.

Population pharmacokinetics of ZDV. Population characteristics and pharmacokinetic parameters are summarized in Table 1. The two-compartment model of Zhou et al. (90) was fitted using data from 175 individuals who received 200 mg ZDV t.i.d. However, only 93 subjects were used to model ZDV in the absence of nevirapine, which produced a pharmacokinetic interaction with ZDV in that study. Drug absorption was approximated as zero order, since insufficient data were available to characterize the absorption phase, and subjects were divided into groups of fast and slow absorbers. Fast absorbers (41.7% of individuals) absorbed ZDV over 0.25 h, while slow absorbers (the remainder) absorbed ZDV

FIG. 1. Overall schematic representation of the pharmacokinetic and viral dynamic model of ZDV phosphorylation.

TABLE 1. Population demographics and pharmacokinetic parameters of oral ZDV used for the simulation study

Population parameter ^a	Estimate (% CV)	95% confidence interval and/or range ^b
Zhou parameters		
Age (yr)	39.2 (20)	20.5–60.8*
Body wt (kg)	73.9 (22)	41–109*
CL/F (liters/h)	127.2 (4.9)	114.9–139.1
wt-CL/F (liters/h/kg)	0.93 (35.7)	0.28–1.58, 0–4*
age-CL/F (liters/h/yr)	6.52 (19.9)	3.97–9.07
V_{ss} (liters)	463.8 (17.4)	306.2–625.0
wt- V_{ss} (liters/kg)	9.83 (28.9)	4.26–15.4
ϕ	0.610 (46.4)	0.0553–1.165
CL_d (liters/h)	27.0 (15.2)	19.0–35.0
$\omega^2_{CL/F}$	0.0703 (28.2)	0.0315–0.109
R_{V_1/V_2}	0.374 (15.1)	0.263–0.485
σ^2	0.261 (9.2)	0.216–0.308
b	1.56 (8.3)	1.311–1.809
Panhard parameters		
k_a (h^{-1})	2.66	1.38–5.12
CL/F (liters/h)	195	148–256
V/F (liters)	344	220–537
$\omega^2_{CL/F}$	0.446	
σ^2	0.1102	0.0801–0.151
a (ng/ml)	120	

^a See references 90 and 65 for the Zhou and Panhard parameters, respectively. Weight-CL/F (wt-CL/F), age-CL/F, and wt- V_{ss} were used to calculate CL/F and V_{ss}/F using body weight in the equations $CL/F = 127 + 0.93 \times (\text{body weight} - 70)$, for individuals <30 years old, and $CL/F = 127 + 0.93 \times (\text{body weight} - 70) + 6.52 \times (\text{age} - 25)$, for individuals ≥ 30 years old. CL_d is the intercompartment clearance between compartments 1 and 2. R_{V_1/V_2} is the ratio V_1/V_2 . b is the factor by which residual variance is boosted during the absorption phase (<2 h). k_a is a first-order absorption rate constant. V/F and V_1/F are the volumes of the plasma compartment for one- and two-compartment models, respectively. F is the fraction (unknown) of the oral dose absorbed.

^b Ranges are indicated by an asterisk.

over 1.57 h. The systemic clearance (CL/F; liters/h) was related to the covariate's age (years) and body weight (kg) using the following equations. For individuals <30 years old, the equation $CL/F = 127 + 0.93 \times (\text{body weight} - 70)$ was used; otherwise, the equation $CL/F = 127 + 0.93 \times (\text{body weight} - 70) + 6.52 \times (\text{age} - 25)$ was used. The steady-state volume of distribution (V_{ss}/F ; liters) was related to body weight using the equation $V_{ss}/F = 464 + 9.83 \times (\text{body weight} - 70)$. Similarly, the volume of the central compartment (V_1/F ; liters) is calculated using a constant fitted ratio of V_1/V_{ss} ($R_{V_1/V_{ss}}$) using the equation $V_1/F = R_{V_1/V_{ss}} \times V_{ss}/F$. The volume of the peripheral tissue compartment (V_2/F) was calculated by using the ratio R_{V_1/V_2} (Table 1).

Both CL/F and V_{ss}/F were assumed to follow log-normal distributions, with variance equal to $\omega^2_{CL/F}$ and $\phi \times \omega^2_{CL/F}$, respectively, where ϕ is the fixed ratio between variances. The residual/interindividual variance was modeled as $\epsilon_{ij} \times b \times C_p$, where ϵ_{ij} (the error term) is normally distributed with a mean of 0 and a variance of σ^2 , the factor b is a constant parameter that is assumed equal to 1 after the absorption phase (>2 h) and allows for a greater variability during the drug absorption phase, and C_p is the plasma concentration of ZDV. Given the limited number of subjects, large number of covariates, and lack of a reported variance-covariance matrix, the available data might not have supported the successful estimation of all potentially important covariate effects and covariances. It is also possible that linking intersubject variances in CL/F and V/F could result in a reduced overall variability in the simulation. The covariate distributions from Zhou et al. (90) were used unchanged in the simulation.

The plasma pharmacokinetics of ZDV are characterized by a biexponential decay after intravenous injection (9). However, some pharmacokinetic studies have utilized a one-compartment model to describe ZDV disposition (65), in part due to some obscuring of the distribution phase by the absorption phase. Panhard et al. fitted a one-compartment model in 10 infected individuals receiving ZDV alone (65). The residual/interindividual variance was described using the combined proportional and additive error model $\sigma^2_{total} = \sigma^2 \times (a + C_{ij})$, where a is a fitted constant and C_{ij} is the predicted plasma concentration. Since much of the variance occurs during drug absorption, this approach may tend to overestimate variance in the postabsorption phase, resulting in an overpredicted variability of ZDV-MP and -TP. The parameters for both pharmacokinetic models are presented in Table 1.

Accumulation of ZDV and its nucleotides in human PBM cells. Plasma and cytosolic concentrations of ZDV were assumed to equilibrate instantaneously, since ZDV is not appreciably protein bound and equilibration between the plasma and cytoplasm is achieved rapidly due to the action of equilibrative

TABLE 2. Population enzyme kinetic parameters for cellular metabolism of ZDV used for the simulation study

Parameter ^a	Median	Range (IQ range) ^b	Reference
$V_{\max,TK_1}$ vs ZDV ($\mu\text{mol mg}^{-1} \text{min}^{-1}$)	35	0–300 (13–65)	48
K_{m,TK_1} vs ZDV (μM)	3		30
$V_{\max,TMPK}$ vs T ($\mu\text{mol mg}^{-1} \text{min}^{-1}$)	53	0–723 (22–116)	49
$K_{m,TMPK}$ vs ZDV-MP (μM)	7.6		45
Protein per 10^6 cells (mg)	0.053		4, 43 ^c
Relative TMPK efficiency (ZDV-MP/ dT -MP) (%)	1		45
Activated lymphocytes (in vivo) (%)	5		40
Projected cell area (μm^2)	80		84
$k_{MP} = k_{DP,MP}$ (h^{-1})	0.28	None (constant)	86
$\omega^2 V_{\max,TK_1}$	1.42		48
$\omega^2 V_{\max,TMPK}$	0.995		49

^a $V_{\max}$ values were constrained to be less than the maximal value observed in noninfected individuals, who tend to have higher activities than infected individuals. T is time (min). Projected cell area was used to calculate the aqueous volume of 0.4 picoliters for an activated lymphocyte, assuming a specific gravity of 1.06, spherical geometry, and 70% water. ω^2 refers to the variance of a log-normal distribution.

^b IQ, interquartile.

^c Means of 0.040 (43) and 0.066 (4) were determined previously.

nucleoside transporters present on the cell membranes of lymphocytes (5, 70). The initial intracellular phosphorylation step of ZDV is catalyzed by cellular thymidine kinase (TK). The primary enzyme for 5'-monophosphorylation to ZDV-MP is TK_1 , which is located primarily in the cytosol of cells in the S phase. However, mitochondrial TK (TK_2) has also been shown to phosphorylate ZDV in cultured monocytes/macrophages that do not express TK_1 but to a much lesser degree (3, 4, 22, 23, 30, 60). The K_m (concentration at 50% of the maximal metabolism rate) of ZDV versus TK_1 is 3 μM (23, 30), and the activity of TK_1 versus ZDV is 0.6 of the activity versus thymidine. TMPK catalyzes the subsequent phosphorylation to ZDV-5'-diphosphate (ZDV-DP) and is rate limiting with a K_m of 7.6 to 8 μM versus that of ZDV-MP (30, 45). Distributions describing the maximal rates of conversion of the phosphorylation of ZDV ($V_{\max,TK_1}$) and ZDV-MP ($V_{\max,TMPK}$), respectively, were estimated using data from previous enzymatic studies conducted on PBM cells isolated from cohorts of infected individuals (48, 49). These and other studies have reported decreased average levels of TK_1 and TMPK in the in vitro-activated cells of individuals treated with ZDV for more than 6 months. Therefore, the distribution of TK_1 and TMPK activities was calculated using the TK_1 activities of 24 HIV-positive individuals not previously exposed to AZT (48) and 27 HIV-positive individuals with various exposures to ZDV (49), respectively. Since TK_1 and TMPK are cell cycle dependent (32), the PBM cells of infected individuals were stimulated ex vivo using phytohemagglutinin (PHA) (48, 49), followed by lysing and measurement of the enzyme activities. The activities of TK_1 were measured using ZDV (20 μM), which is a higher concentration than that used in vivo but is sufficient to saturate the enzyme, allowing the capacity of the enzyme ($V_{\max,TK_1}$) to be measured. TMPK activities were measured using thymidine-MP as a substrate (50 μM). $V_{\max,TMPK}$ was calculated by assuming a TMPK efficiency of 1% of that of ZDV-MP relative to thymidine-MP (45). The relatively slow $V_{\max}$ of ZDV-MP is believed to be related to steric hindrance in the binding of ZDV-MP to TMPK (51). $V_{\max,TMPK}$ and $V_{\max,TK_1}$ were assumed to be distributed in a log-normal manner. To ensure that $V_{\max}$ values remained in the physiological range, maximal values were constrained to be less than the largest values reported for noninfected individuals in the study, since the values were higher in noninfected than in HIV-infected individuals (48, 49). Units of the apparent $V_{\max}$ were converted to μmol of ZDV-DP/h/liter, using an average of two reported measurements of the protein content of PHA-stimulated PBM cells (0.053 mg/ 10^6 cells) (4, 43). The aqueous volume of activated lymphocytes was calculated using a mean projected cell surface area of 80 μm^2 (84), assuming a specific gravity of 1.06, 70% water, and spherical geometry. This volume was then used to convert pmol/ 10^6 cells to μM . The parameters used for modeling the cellular phosphorylation and dephosphorylation of ZDV are presented in Table 2. It was assumed that PHA stimulates about 40% of the lymphocyte population in vitro (86).

The cytoplasmic accumulation of ZDV-MP is described by the equation $d\text{ZDV-MP}/dt = C_1 \times V_{\max,TK_1}/(C_1 + K_{m,TK_1}) + \text{ZDV-DP} \times k_{DP,MP} - \text{ZDV-MP} \times k_{MP} - V_{\max,TMPK} \times \text{ZDV-MP}/(\text{ZDV-MP} + K_{m,TMPK})$, where C_1 is the concentration of ZDV in cells (at equilibrium, $C_1 = C_p$), $k_{DP,MP}$ is the rate of dephosphorylation of ZDV-DP to ZDV-MP, k_{MP} is the rate of dephosphorylation

of ZDV-MP to ZDV, and $V_{\max,TMPK}$ is the maximal rate of conversion of the phosphorylation of ZDV-MP to DP.

The final phosphorylation step to active ZDV-TP is catalyzed by nucleoside diphosphate kinase, is not rate limiting under physiological conditions, and takes place too rapidly to be easily characterized in vitro (86). Considering the area-under-the-curve ratio of ZDV-TP to ZDV-MP in vivo ($0.42 \pm 0.42:0.52 \pm 0.32$ [pmol/ 10^6 cells/h $\pm$ the standard error] for a 100-mg dose and $0.61 \pm 0.81:0.56 \pm 0.57$ for a 300-mg dose, respectively [7]), a constant 1:1 ratio of ZDV-TP to -DP was assumed. $k_{DP,MP}$ and k_{MP} were calculated using steady-state data from an in-vitro study of ZDV nucleotides measured in PHA-stimulated PBM cells following a 4-h incubation with various concentrations of ZDV (86). Thus, at steady state, the rate of accumulation of ZDV-MP [$(C_1 \times V_{\max,TK_1})/(C_1 + K_{m,TK_1})$] equals the rate of decomposition of ZDV-MP to ZDV ($\text{ZDV-MP} \times k_{MP}$), allowing k_{MP} to be calculated as the regression slope of $C_1 \times V_{\max,TK_1}/(C_1 + K_{m,TK_1})$ versus ZDV. Likewise, the first-order decomposition rate of ZDV-DP to ZDV-MP was calculated as the regression slope of $V_{\max,TMPK} \times \text{ZDV-MP}/(\text{ZDV-MP} + K_{m,TMPK})$ versus ZDV-DP (86).

The accumulation of ZDV-DP and ZDV-TP (ZDV-DP,TP) was described by the equation $d\text{ZDV-DP,TP}/dt = V_{\max,TMPK} \times \text{ZDV-MP}/(\text{ZDV-MP} + K_{m,TMPK}) - \text{ZDV-DP} \times k_{DP,MP}$, where $d(\text{ZDV-DP,TP})/dt$ is the rate of change in the total cellular concentration of ZDV-DP and -TP.

The cellular contents of the various nucleotides of ZDV in activated cells were used to estimate the ZDV nucleotide contents of cells in vivo, assuming an in-vivo-activated cell fraction of 8% (40) and that phosphorylation in active cells is minimal in nonactivated cells (86). It was previously shown that the ratio between ZDV-MP, -DP, and -TP was similar in resting and PHA-stimulated PBM cells, while the absolute concentrations were 60 to 150 times higher in stimulated cells (86). Thus, assuming an 8% stimulation of PBM cells in vitro, activated PBM cells would contribute between 84 and 92% of the total ZDV nucleotides in humans. The average percentage (88%) was used to calculate the total amounts of ZDV-MP, -DP, and -TP using the simulated amounts in activated cells. Plasma concentrations of ZDV and cellular amounts of ZDV-MP were compared with data from existing clinical studies (7, 28, 65, 90).

Computer simulations. Monte Carlo population pharmacokinetic and enzyme dynamic simulations were conducted for 5,000 individuals per simulated dose regimen, using Trial Simulator version 2.2.1 (Pharsight Corp., Mountain View, CA), which utilizes a fifth-order Runge-Cutta algorithm for numerical integration. This program allows customized differential equations, together with probability distributions for each parameter in the equations, to be entered. The simulated results were then analyzed using routines in the S-Plus computer program (version 6.0 professional; Insightful Corp., Seattle, WA) embedded in the Trial Simulator software. The medians, 25th and 75th percentiles versus time of ZDV concentrations in plasma, and cytoplasmic content of ZDV-MP and ZDV-TP were calculated. Three simulations of 5,000 individuals each were performed to ensure reproducibility of the output (<5% differences in median and interquartile ranges).

RESULTS

The outline of the model is shown in graphic form in Fig. 1. Simulations were performed using both pharmacokinetic models at ZDV doses of 50, 100, 200, and 300 mg b.i.d. and 600 mg q.d. Median and interquartile ranges of plasma ZDV and cellular ZDV-MP and -TP were plotted for the 200 and 300 mg b.i.d. doses (Fig. 2 to 4). Box plots of between-dose peak and trough amounts of ZDV-TP (pmol/ 10^6 cells) (median and interquartile ranges) for all doses using the one- and two-compartment population pharmacokinetic models are shown in Fig. 5A and B, respectively. The levels of ZDV and ZDV-MP, -DP, and -TP essentially achieved steady state within 48 h. Plasma concentrations were assumed to increase linearly with dosage in both models. The two-compartment model (90) produced higher maximum concentration ($C_{\max}$) values, a more gradual terminal slope, and a smaller variance after the absorption phase ($t > 2$ h) than the one-compartment model (Fig. 2). Likewise, peak amounts of cellular ZDV-MP were higher and terminal decline slopes were slower for the two-compartment model than the one-compartment model (Fig.

FIG. 2. Simulated plasma concentrations of ZDV ($n = 5,000$) for the 200- and 300-mg doses (gray and black, respectively) according to the models of Zhou et al. (median, solid line; 25th and 75th percentiles [p_{25} and p_{75}], dashed lines) and Panhard et al. (median, dotted and dashed line; p_{25} and p_{75} , dotted lines) over 60 h (65, 90).

3). Both models predicted a noticeable increase in predicted ZDV plasma concentrations and amounts of ZDV-MP when doses were increased from 200 to 300 mg b.i.d.

A high degree of overlap of the peak amounts of ZDV-TP was predicted using the two-compartment model of Zhou et al. as well as the one-compartment model of Panhard et al. for ZDV-TP at the 200- and 300-mg b.i.d. doses (98% and 97%,

respectively; Fig. 4 and Fig. 5A and B). The respective peak amounts of ZDV-TP at 300 mg b.i.d. were 0.061 pmol/ 10^6 cells (interquartile range, 0.031 to 0.12) and 0.062 pmol/ 10^6 cells (interquartile range, 0.032 to 0.12) for one- and two-compartment models, respectively. However, the one-compartment model predicted lower trough amounts of ZDV-TP between doses (Fig. 4). Comparison of the median peak amounts of

FIG. 3. Simulated cellular levels of ZDV-MP per 10^6 cells ($n = 5,000$) for the 200- and 300-mg doses (gray and black, respectively) according to the models of Zhou et al. (median, solid line; 25th and 75th percentiles [p_{25} and p_{75}], dashed lines) and Panhard et al. (median, dotted and dashed line; p_{25} and p_{75} , dotted lines) over 60 h (65, 90).

FIG. 4. Predicted levels of ZDV-TP per 10^6 cells ($n = 5,000$) for the 200- and 300-mg doses (gray and black, respectively) according to the models of Zhou et al. (median, solid line; 25th and 75th percentiles [p_{25} and p_{75}], dashed lines) and Panhard et al. (median, dotted and dashed line; p_{25} and p_{75} , dotted lines) over 60 h (65, 90).

ZDV-TP at steady state in the dose range from 50 to 300 mg b.i.d. with linear predictions using median peak values of the 50- and 100-mg b.i.d. doses suggests some saturation of the phosphorylation to ZDV-TP, demonstrated by the lower-than-expected median peak ZDV-TP amount at 300 mg b.i.d. Furthermore, the simulation based on the two-compartment model suggested higher amounts of cellular ZDV-TP (Fig. 5A) than the one-compartment model (Fig. 5B). A less-than-linear increase in between-dose minimal (trough) amounts of ZDV-TP was suggested by the simulation using the two-compartment model (Fig. 5A) that was not evident for the one-compartment simulation (Fig. 5B). Both simulations predicted reduced peak and trough amounts of cellular ZDV-TP after treatment with 600 mg ZDV q.d. than with 300 or 200 mg ZDV b.i.d.

DISCUSSION

Pharmacokinetic and pharmacodynamic simulations are useful tools for consolidating all available drug information into a usable form and are gaining favor in the pharmaceutical industry to design clinical trials, since they allow detailed analyses of dosage regimens *in silico* before the actual studies are conducted (13, 21, 56, 57, 63, 64). Rosario et al. recently utilized clinical trial simulations to streamline the phase 2a development of the CCR5 receptor blocking agent maraviroc (75). Furthermore, Hurwitz et al. recently developed a pharmacodynamic model of viral depletion for use with lamivudine (46).

The objective of this study was to superimpose previously reported population pharmacokinetic parameters together with distribution summaries of the cellular enzyme kinetics of ZDV metabolism derived from HIV-1-infected individuals, who were not previously treated with ZDV, using a simulation model. Activated $CD4^+$ lymphocytes are the dominant sub-

strates for HIV-1 infection and could be a significant site for the selection of the K65R mutant virus. The model was then used to predict the accumulation of ZDV nucleotides in activated $CD4^+$ lymphocytes versus dose regimen. Predicted concentrations of ZDV-TP in activated $CD4^+$ PBM cells may be useful for possible incorporation into a virus pharmacokinetic-pharmacodynamic model that relates virus depletion profiles versus time and dose of administration (46, 47).

Simulations were repeated using a one- and a two-compartment population pharmacokinetic model, with different error structures (65, 90). The higher C_{max} values and smaller variance after the absorption phase predicted by the two-compartment model of Zhou et al. (90) compared to the one-compartment model may result in part from the different variance structures with a boosted variance during the absorption phase in the two-compartment model. Furthermore, the more gradual terminal slope was expected from the two-compartment model, which predicts a more gradual elimination rate in the latter part of the dosing interval.

The peak cellular levels of ZDV-MP (associated with bone marrow toxicity) predicted by the two-compartment model were higher and the terminal elimination slightly slower than those predicted by the one-compartment model in accordance with the plasma concentration-versus-time profiles of ZDV (Fig. 2). Peak amounts of ZDV-MP were higher in the simulation compared to those reported by Barry et al. in 10 HIV-infected subjects given 300 mg ZDV b.i.d. (2.25 ± 1.5 [mean $\pm$ standard deviation] pmol/ 10^6 cells; $n = 10$). Calculation of the ZDV-MP levels assumed a stimulated fraction of PBM cells in humans of 8%. Therefore, ZDV-MP levels in activated cells are expected to be much larger and would exceed the K_m value versus TMPK ($7.6 \mu M$) for much of the dose interval.

Simulations using the one- and two-compartment pharmacokinetic models predicted a high degree of overlap in cellular

FIG. 5. Box plots (medians and interquartile ranges; whisker plots are p10 to p90 ranges) of peak (checked) and trough (shaded) levels of ZDV-TP in lymphocytes in vivo following ZDV doses of 50, 100, 150, 200, and 300 mg b.i.d. and 600 mg q.d., using the pharmacokinetic model of Zhou et al. (A) and Panhard et al. (B), respectively. Prediction lines were derived by a regression of median values for the 50- and 100-mg b.i.d. doses.

ZDV-TP between the 200- and 300-mg b.i.d. doses (97% and 98%, respectively) (Fig. 4). Peak amounts of predicted ZDV-TP at the 300-mg b.i.d. dose were 0.061 pmol/10⁶ cells (interquartile range, 0.031 to 0.12) and 0.062 pmol/10⁶ cells (interquartile range, 0.032 to 0.12), respectively, and were comparable to clinical observations from the study by Barry et al. of 10 HIV-infected individuals given 300 mg ZDV b.i.d. (0.07 ± 0.09 [mean ± standard deviation] pmol/10⁶ cells; *n* = 10) (7). The corresponding terminal half-lives for ZDV-TP estimated using the median simulated curves were 9.8 and 5.4 h, respectively, compared to 6.5-h half-life estimated using the mean curve for the same dose regimen (7). Interestingly, similar peak amounts of ZDV-TP were reported for the 100- and 300-mg b.i.d. doses in the same study. The more rapid terminal decline in cellular ZDV-TP observed for the lower dose could result from a declining replacement of decaying cellular ZDV-TP from the phosphorylation of ZDV entering cells when concentrations of ZDV are low. Similarly, the more rapid decline in ZDV-TP is expected for the one-compartment

model, since it predicts a more rapid terminal elimination of ZDV from the plasma. A decreased replacement in ZDV-TP could also explain the more rapid cellular half-life for ZDV-TP reported in vitro (<2.5 h) (86) than in vivo observations (7 to 10 h) (2, 28, 74, 83), since cellular half-lives are typically measured in vitro after cells are resuspended in medium in the absence of extracellular ZDV.

The higher median ZDV-TP levels predicted by the two-compartment model (Fig. 5A) compared to those of the one-compartment model (Fig. 5B) could be related to the higher predicted plasma *C*_{max} values of ZDV. Comparisons of the median peak amounts of ZDV-TP at steady state, in the dose range of 200 to 300 mg b.i.d., with predictions using a linear regression of median peak values of the 50- and 100-mg b.i.d. doses suggested at least partial saturation of the phosphorylation to ZDV-TP. The less-than-linear increase in the between-dose trough ZDV-TP concentration predicted using the two-compartment model (Fig. 5A) compared with the one-compartment simulation (Fig. 5B) may be explained in part by the more rapid decline in ZDV-TP during the terminal phase of the dosing interval. ZDV-TP acts as a competitive inhibitor and chain terminator during reverse transcription. Therefore, the trough concentrations of ZDV-TP are of critical importance, since CD4⁺ cells are maximally sensitive to HIV infection when ZDV-TP concentrations are lowest. Both models suggested that similar peak amounts of ZDV-TP follow 600-mg and 300-mg b.i.d. doses, which could result from enzyme saturation and/or a lack of accumulation of ZDV-TP resulting from the prolonged dose interval. The median peak amounts of ZDV-TP predicted for the 600-mg q.d. dose were 0.054 and 0.057 pmol/10⁶ cells, using the two- and one-compartment pharmacokinetic models, respectively, and the corresponding median trough amounts of ZDV-TP were 0.03 and 0.0033 pmol/10⁶ cells, respectively. The trough amounts of ZDV-TP were comparable with those reported by Flynn et al. at the same dose when coadministered with 3TC in 27 HIV-infected adolescents: 0.045 pmol/10⁶ cells (95% confidence interval, 0.035 to 0.065; *n* = 27) and 0.01 pmol/10⁶ cells (95% confidence interval, 0.01 to 0.02), respectively (28).

Both pharmacokinetic models produced similar overall conclusions for ZDV phosphorylation. However, the population enzymology component of the model was limited by the availability of only one data set each, describing the phosphorylation potential (*V*_{max}) for TK₁ and TMPK, respectively, in the PBM cells of infected subjects following ex vivo stimulation (48, 49). These studies indicated substantial variances in the respective *V*_{max} values between HIV-infected individuals. A decrease in TK₁ and TMPK activities in the PBM cells of infected individuals was reported in individuals treated with ZDV for more than 6 months. However, the significance of this finding is not clear, since statistically different cellular amounts of ZDV-TP were not demonstrated in a study of subjects exposed to ZDV over a similar time interval (6, 44). ZDV-MP levels tended to be high, while amounts of ZDV-TP agreed closely with in vivo amounts. However, in silico predictions depend on the model structure together with parameter estimates and their associated variance structures. An average estimate for the aqueous cell volume in CD4⁺ cells was used in these simulations. In reality, cell volumes in an activated lymphocyte population vary according to stage in the cell cycle.

The primary substrates for HIV infection are activated T-CD4⁺ lymphocytes. In most studies, the ZDV-TP content is measured in PBM cells obtained by centrifugation using a Ficoll gradient without further enriching for activated CD4⁺ cells (7, 28, 83). Since TK₁ and TMPK expressions are cell cycle dependent, consideration of data from these measurements underestimates the ZDV-TP content in activated lymphocytes. In this study, the ratio of activated PBM cells was assumed to be constant. However, the activated lymphocyte fraction tends to be elevated in untreated HIV-infected individuals and may decrease toward more normal levels once the infection is stabilized using HAART (59). Therefore, it may be useful for future studies to measure the amounts in activated cells if feasible or to provide a cell cycle distribution together with the ZDV-TP content. The parameter distributions were from different studies, so statistical correlations between parameter covariates could not be assessed. The large variances in enzyme levels between infected individuals make it difficult to estimate the ratios of activities between TK₁ (producing ZDV-MP) and TMPK (producing ZDV-DP) (48, 49). This simulation relied on the interplay between the various parameters, and it is possible that there could be a compounding or compensation in the overall error associated with the variance parameters.

The Thai national guidelines for the management of HIV recommend that ZDV doses be reduced from 300 to 200 mg b.i.d. in patients weighing less than 60 kg, which has resulted in fewer side effects and improved long-term tolerability without evidence of reduced efficacy (58). A pharmacokinetic study conducted in Thailand demonstrated similar plasma concentrations in subjects weighing less than 60 kg treated at the 200-mg b.i.d. dose, compared with a historical data set from heavier subjects administered 300 mg b.i.d. (16). Although the oral clearance of ZDV may be reduced in individuals with body weights <60 kg (10, 90), other studies have failed to demonstrate a linear relationship between body weight and ZDV clearance (78). Furthermore, the wide interindividual variance in cellular TK₁ and TMPK (45, 48) suggests that plasma concentrations of ZDV alone would be insufficient to predict levels of the active ZDV-TP. The *in silico* findings of this study suggest that the current ZDV dose could also be lowered from 300 to 200 mg b.i.d. in subjects with body weights more typical of Western populations to reduce toxicities and still maintain adequate ZDV-TP concentrations. However, it would not be prudent to decrease the dose to levels much lower than 200 mg b.i.d., as depicted in Fig. 5A and B, since a more linear decrease was predicted for median peak and trough amounts of ZDV-TP between 50 to 200 mg b.i.d. but not for 300 mg b.i.d. However, the simulations assumed ZDV was not coadministered with agents that alter its metabolism. Therefore, ZDV doses may need modification when coadministered with protease inhibitors, including nevirapine which may induce glucuronidation and/or reduce absorption (65, 90), resulting in lower plasma concentrations. The NRTI TDF, 3TC, and DAPD are not known to affect ZDV metabolism (42, 61).

TDF is administered q.d. in accordance with the long cellular half-life of tenofovir-DP (>60 h). Although the addition of ZDV to q.d. TDF should be beneficial, a coformulation with ZDV would result in a less-effective ZDV response. The half-lives of DXG-TP and carbovir-TP (the active metabolite of

ABC) are ~16 h and 12 to 24 h, respectively (42, 79). DAPD is administered b.i.d., while ABC demonstrated similar efficacy when administered q.d. or b.i.d. (14). Therefore, ZDV may be a candidate for coformulation at an optimal b.i.d. dose regimen with an NRTI such as DAPD, the prodrug of DXG or ABC.

Based on these *in silico* results, an intensive pharmacokinetic phase 2 clinical study of 24 HIV-1-infected subjects receiving 200 or 300 mg ZDV b.i.d. in combination with 500 mg DAPD b.i.d. for 10 days has recently been completed and supports our *in silico* findings that 200 mg ZDV b.i.d. produces a similar antiviral response as the approved ZDV dose of 300 mg b.i.d. without any untoward effects (61). The data generated from this clinical study will be important for positioning DAPD coformulated with 200 mg ZDV b.i.d. in advanced phase 2/3 studies. Furthermore, the utility of a lower effective dose of ZDV without its associated toxicity, particularly bone marrow toxicity, would be beneficial in future HAART combinations when used with drugs that select for the K65R mutation.

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