Lack of Pharmacokinetic Interaction between Amdoxovir and Reduced- and Standard-Dose Zidovudine in HIV-1-Infected Individuals[▽]

Selwyn J. Hurwitz,^{1,2} Ghazia Asif,^{1,2} Emilie Fromentin,^{1,2}
Phillip M. Tharnish,² and Raymond F. Schinazi^{1,2*}

Center for AIDS Research, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30322,¹ and Veterans Affairs Medical Center, Decatur, Georgia 30033²

Received 25 August 2009/Returned for modification 23 October 2009/Accepted 19 December 2009

Amdoxovir (AMD_X) inhibits HIV-1 containing the M184V/I mutation and is rapidly absorbed and deaminated to its active metabolite, β -D-dioxolane guanosine (DXG). DXG is synergistic with zidovudine (ZDV) in HIV-1-infected primary human lymphocytes. A recent *in silico* pharmacokinetic (PK)/enzyme kinetic study suggested that ZDV at 200 mg twice a day (b.i.d.) may reduce toxicity without compromising efficacy relative to the standard 300-mg b.i.d. dose. Therefore, an intense PK clinical study was conducted using AMD_X/placebo, with or without ZDV, in 24 subjects randomized to receive oral AMD_X at 500 mg b.i.d., AMD_X at 500 mg plus ZDV at 200 or 300 mg b.i.d., or ZDV at 200 or 300 mg b.i.d. for 10 days. Full plasma PK profiles were collected on days 1 and 10, and complete urine sampling was performed on day 9. Plasma and urine concentrations of AMD_X, DXG, ZDV, and ZDV-5'-O-glucuronide (GZDV) were measured using a validated liquid chromatography-tandem mass spectrometry method. Data were analyzed using noncompartmental methods, and multiple comparisons were performed on the log-transformed parameters, at steady state. Coadministration of AMD_X with ZDV did not significantly change either of the plasma PK parameters or percent recovery in the urine of AMD_X, DXG, or ZDV/GZDV. Larger studies with AMD_X/ZDV, with a longer duration, are warranted.

Nucleoside reverse transcriptase inhibitors (NRTI) remain the backbone of current HIV therapy, in which they are combined with protease inhibitors (PI), nonnucleoside reverse transcriptase inhibitors (NNRTI), integrase inhibitors, or entry/fusion inhibitors (11, 12, 32, 42). Since existing combinatorial regimens cannot eradicate infection due to the compartmentalization of the virus and its latent properties, chronic therapy will remain the standard of care for the foreseeable future (44, 45). Highly active antiretroviral therapy (HAART) regimens have limitations, primarily due to toxicities and/or the emergence of drug-resistant HIV strains (39). Therefore, the development of safe and effective drug combinations that can not only inhibit both wild-type and resistant strains of HIV-1 but also prevent or retard future resistance development needs to be a continued focus in HIV drug development. Amdoxovir [(–)- β -D-2,6-diaminopurine dioxolane (AMD_X or DAPD)] is a guanosine nucleoside analogue being developed for the treatment of HIV-1 infections (23, 31, 47). AMD_X is in advanced phase 2 clinical development under a U.S. Food and Drug Administration Investigational New Drug Application and has been administered safely to over 200 persons in seven human phase I/II trials (23, 31, 36, 47, 50). AMD_X is a prodrug which undergoes rapid oral absorption in humans and other species and is deaminated by the ubiquitous enzyme adenosine deaminase to 9-(β -D-1,3-dioxolan-4-yl)guanine (DXG) (4, 16,

35). DXG is phosphorylated to its active triphosphate form, DXG-triphosphate (DXG-TP), which is a potent inhibitor of wild-type and drug-resistant forms of HIV-1 (16, 33) and a modest inhibitor of hepatitis B virus in human hepatocytes (5, 43, 51). Drug-resistant HIV mutants susceptible to DXG include viruses containing M184V/I and thymidine analog mutations (TAMs) (M41L, D67N, K70R, L210W, T215Y/F, and K219Q/E) and the 69SS double insert (24, 25, 33). The decay half-life of the active metabolite DXG-TP was ~16 h in activated primary human lymphocytes and ~9 h (or 27 h if including a 48-h time point) in humans, suggesting that twice a day (b.i.d.) dosing should provide adequate therapeutic coverage (26, 30). Cellular toxicity studies suggested that AMD_X and DXG did not affect the levels of mitochondrial DNA in human hepatoma cells (HepG2 cells) treated for 14 days at 10 μ M, and there was no increase in lactic acid production in these cells (9). Resistance *in vitro* develops slowly and is associated with a K65R or L74V mutation (20, 38, 49). Viruses containing the K65R mutation show moderate cross-resistance to zalcitabine, didanosine, adefovir, and lamivudine (3TC) but increased sensitivity to zidovudine (ZDV) (38). An *in vitro* study demonstrated that ZDV alone selected for a mixture of K70K/R mutations at week 25 and that AMD_X alone selected for a mixture of K65R and L74V mutations at week 20. However, when AMD_X and ZDV were used in combination in HIV-infected primary human lymphocytes, no drug-resistant mutations were detected through week 28 (40). Therefore, the inclusion of ZDV in combination with AMD_X may prevent or delay the emergence of these mutations. Coincubation of ZDV and DXG with phytohemagglutinin (PHA)-stimulated human peripheral blood mononuclear (PBM) cells did not result in

* Corresponding author. Mailing address: Veterans Affairs Medical Center, Medical Research 151H, 1670 Clairmont Road, Decatur, GA 30033. Phone: (404) 728-7711. Fax: (404) 728-7726. E-mail: rschina@emory.edu.

[▽] Published ahead of print on 28 December 2009.

TABLE 1. Geometric means (% CV) and statistical analysis of pharmacokinetic parameters for AMDX on day 10

Group or comparison	Median T_{max} (h)	C_{max} (ng/ml)	AUC_{τ} (ng · h/ml)	CL/F (liters/h/kg)	$t_{1/2}$ (h)	AUC_{τ} ratio (day 10/day 1)
1 (AMDX; $n = 6$)	2	499 (52.9)	1,326 (60.9)	5.49 (65.0)	1.4(38.9)	0.95 (97.4)
2 (AMDX + ZDV at 200 mg; $n = 6$)	2.5	649 (51.7)	1,730 (79.0)	4.48 (76.6)	1.7 (5.24)	0.99 (65.8)
3 (AMDX + ZDV at 300 mg; $n = 6$)	2.5	714 (33.7)	2,090 (48.5)	3.81 (59.5)	1.1 (41.4)	0.67 (57.9)
Multiple comparisons of ratios of geometric means (P values in Tukey-Kramer modified t test)						
1 vs 2		0.77 (0.57)	0.77 (0.71)	1.23 (0.83)	0.76 (0.54)	0.96 (0.99)
1 vs 3		0.70 (0.37)	0.64 (0.39)	1.44 (0.57)	1.24 (0.69)	1.42 (0.65)
2 vs 3		0.91(0.93)	0.83 (0.84)	1.18 (0.89)	1.63 (0.17)	1.49 (0.56)

decreased phosphorylation of either NRTI at physiologically relevant concentrations (26).

ZDV is a commonly used NRTI in many HAART regimens (3, 10, 17), and the single-dose plasma pharmacokinetics (PK) of ZDV following intravenous and oral administration in HIV-infected individuals is well described (1, 14, 22, 37, 52). ZDV treatment is limited by toxic side effects, including nausea and malaise, as well as serious bone marrow cytotoxicities, such as anemia and neutropenia (6, 41, 46). The bone marrow cytotoxicities of ZDV are believed to be associated with mitochondrial damage and correlate with ZDV-monophosphate (ZDV-MP) levels (48). The current approved dose for ZDV is 300 mg b.i.d. However, Barry et al. demonstrated that a reduced dose of ZDV, 100 mg three times a day (t.i.d.), produced similar cellular levels of ZDV-TP, which mediates antiviral effects, while significantly decreasing ZDV plasma concentrations and intracellular levels of ZDV-MP (2). The Thai national guidelines for the management of HIV recommend that the ZDV dose be reduced from 300 to 200 mg b.i.d. for patients weighing less than 60 kg, which has resulted in fewer side effects and improved long-term tolerability without evidence of reduced efficacy (7, 8, 34). A PK and enzyme kinetic simulation study was conducted by superimposing the population PK of ZDV (37, 52) over the distribution of enzyme kinetic parameters derived from a population of treatment-naïve HIV-1-positive subjects (28, 29) to test the hypothesis that thymidylate kinase (TMPK), the rate-limiting enzyme of ZDV phosphorylation, may be oversaturated at clinical doses (19, 27). The *in silico* study suggested that the current ZDV dose could be lowered from 300 to 200 mg b.i.d. for subjects with body weights more typical of Western populations to reduce toxicities while maintaining adequate ZDV-TP concentrations. However, lowering the dose further was predicted to produce a more steep decrease in ZDV-TP levels.

A proof-of-concept clinical study was performed in which 24 HIV-1-infected subjects not currently receiving antiretroviral therapy were randomized to receive either AMDX at 500 mg b.i.d., ZDV at 200 or 300 mg b.i.d., or AMDX at 500 mg plus ZDV at 200 or 300 mg b.i.d. for 10 days, with full PK profiles collected on day 1 (first dose), day 10 (steady state), and predose on day 5 and with urine collection at days 9 to 10 to determine drug-drug interactions between ZDV and AMDX/DXG as a prelude to a larger phase II study.

MATERIALS AND METHODS

Materials. AMDX, DXG, and ZDV reference standards were obtained from RFS Pharma, LLC; 5'-O-glucuronide of ZDV (GZDV) was obtained from Toronto Research Chemicals, Toronto, Canada. 2,6-Diaminopurine-2'-deoxyriboside (DPD) and 2'-deoxyadenosine (2'-dA) were obtained from Sigma. 2'-Deoxycoformycin (DCF) was purchased from Waterstone Technology (Carmel, IN). Solvents for high-performance liquid chromatography (HPLC) analyses were obtained from Fischer Scientific (Fair Lawn, NJ).

Clinical samples. The study protocol was approved by the following ethics committees in Argentina: Facultad de Medicina, Universidad de Buenos Aires, Comité Independiente de Ética en Investigación (CIEI-FM-UBA), and Administración Nacional de Medicamentos, Alimentos y Tecnología Médica (ANMAT). Written informed consent was obtained from all subjects. Hispanic HIV-infected volunteers (12 males and 12 females) not receiving antiretroviral therapy, with plasma HIV-1 RNA viral loads (VLs) of $\geq 5,000$ copies/ml and CD4⁺ cell counts of ≥ 200 cells/mm³, were enrolled. The mean age was 34 years (range, 21 to 52 years), and the mean body weight was 68 kg (range, 50.1 to 92.6 kg). All 24 randomized subjects who received a drug(s) completed the study and were included in the PK analysis. Subjects were randomized to receive AMDX at 500 mg b.i.d. or AMDX at 500 mg plus ZDV at 200 or 300 mg twice daily from days 1 to 9, with one dose on day 10. In each arm, subjects were randomized 3:1 to receive AMDX or placebo. Blood samples were drawn after the first dose on day 1, at 0, 0.5, 1, 2, 4, 6, 8, 10, and 12 h, and on day 10, at 0, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, and 48 h, following the last dose, and predose on day 5 and were collected in DCF-containing EDTA tubes to prevent deamination of AMDX into DXG after sample collection. Blood samples were centrifuged and stored at -80°C before being assayed using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Safety was evaluated by determining the proportion of grade 3 or greater adverse events per treatment, using the AIDS Clinical Trials Group (ACTG) toxicity grading scale, and antiviral activity (change in plasma HIV-1 RNA [$\log_{10}$ VL]) was determined daily (36).

LC-MS/MS assay. Plasma concentrations of AMDX, DXG, and ZDV were measured simultaneously using a validated LC-MS/MS assay (18). Briefly, plasma samples were extracted using a solution of methanol containing 250 ng ml⁻¹ DPD (internal standard). After centrifugation, the supernatants were evaporated to dryness. The residue was reconstituted with 2 mM ammonium formate (pH 4.8) and 0.04 mM 2'-dA and filtered using a Costar Spin-X microcentrifuge tube filter. Five microliters of the filtrate was directly injected into the chromatographic system.

Likewise, the amounts of AMDX, DXG, ZDV, and GZDV in urine were also measured using LC-MS/MS. The separation was performed on a Betabasic-18 column (100 mm by 1 mm by 3- μm particle size; Thermo Scientific, Waltham, MA), using a Dionex Packing Ultimate 3000 modular LC. The mobile phases consisted of 2 mM ammonium formate buffer, pH 4.8, containing 0.04 mM 2'-dA and 6% methanol, increased to 90% after 8.5 min, for 4.5 min. The column temperature was kept constant at 30°C, and the flow rate was 50 $\mu\text{l min}^{-1}$. A TSQ Quantum Ultra triple-quadrupole mass spectrometer (Thermo Electron Corp.) with an electrospray ionization source was operated in positive mode. Selected reaction monitoring (SRM) mass transitions (m/z) used were 253.2/151.1 for AMDX, 254.2/152.1 for DXG, 268.2/127.1 for ZDV, 444/127.1 for GZDV, and 267.2/151.1 for the internal standard, DPD. The assay was linear from 2 to 3,000 ng ml⁻¹ for AMDX and DXG and from 2 to 5,000 ng ml⁻¹ for ZDV for the plasma and from 1 to 500 $\mu\text{g ml}^{-1}$ with 100-fold dilution for all of the analytes, including GZDV, for the urine analysis.

TABLE 2. Geometric means (% CV) and statistical analysis of pharmacokinetic parameters for DXG administered as AMDX on day 10

Group or comparison	Median T_{max} (h)	C_{max} (ng/ml)	AUC_{τ} (ng · h/ml)	CL/F (liters/h/kg)	$t_{1/2}$ (h)	AUC_{τ} ratio (day 10/day 1)	% DXG _{urine} in τ	% AMDX _{urine} in τ
1 (AMDX; $n = 6$)	1.3	1,545 (44.4)	6,834 (18.2)	0.88 (19.0)	17.6 (46.8)	1.18 (28.8)	25.3 (8.3)	1.1 (136)
2 (AMDX + ZDV at 200 mg; $n = 6$)	1.5	1,315 (39.5)	6,225 (41.6)	0.95 (44.8)	14.7 (46.0)	0.91 (34)	26.2 (26.2)	3.6 (64.7)
3 (AMDX + ZDV at 300 mg; $n = 6$)	1.8	1,908 (14.1)	7,658 (16.9)	0.91 (39.0)	14.8 (43.6)	1.19 (35.9)	27.7 (21.3)	3.85 (66.5)
Multiple comparisons of ratios of geometric means (P values in Tukey- Kramer modified t test)								
1 vs 2		1.18 (0.70)	1.10 (0.82)	0.93 (0.92)	1.19 (0.77)	1.30 (0.36)	0.97 (0.95)	0.53 (0.34)
1 vs 3		0.81 (0.54)	0.89 (0.75)	0.98 (0.99)	1.19 (0.77)	0.99 (1.00)	0.92 (0.73)	0.50 (0.28)
2 vs 3		0.69 (0.17)	0.81 (0.40)	1.04 (0.97)	1.00 (1.00)	0.76 (1.00)	0.95 (0.89)	0.94 (0.99)

Measurement of ADA activity in plasma. Adenosine deaminase (ADA) levels in the plasmas of subjects were measured using a commercially available assay kit from Diazyme Laboratories, Poway, CA.

PK and statistical analyses. Noncompartmental PK analysis was performed on plasma concentrations of AMDX, DXG, and ZDV on day 1 (first dose) and day 10 (steady state), using Kinetica (version 5.0; Thermo Fisher Scientific Inc., Waltham, MA) and assuming extravascular drug input. Statistical analysis of percent recovery of AMDX, DXG, ZDV, and GZDV in the urine at steady state was also performed. Areas under the concentration-time curve between doses (AUC_{τ} , where $\tau = 12$ h) were used to calculate relative drug accumulation ($AUC_{\tau, \text{day 10}}/AUC_{\tau, \text{day 1}}$). Oral clearance (CL/F) on day 1 was measured, noting that AUC_{total} on day 1 is theoretically equivalent to AUC_{τ} on day 10, assuming a steady state (21). Maximum concentration of drug in serum (C_{max}) values were reported as actual values. Since NRTI are used for the chronic treatment of HIV-1, detailed statistical analysis was performed on the PK parameters measured at steady state (day 10). Normal probability plots and Shapiro-Wilk tests for normality were performed on the nontransformed and natural log-transformed CL/F values for AMDX ($n = 18$ subjects), DXG ($n = 18$), and ZDV ($n = 16$), using the univariate procedure of SAS (version 9.2; SAS, Cary, NC). P values for the Shapiro-Wilk test were as follows, for nontransformed and natural log-transformed CL/F values, respectively: for AMDX, 0.02 versus 0.55; for DXG, 0.10 versus 0.78; and for ZDV, 0.19 versus 0.60 (lower P values indicate increased deviations from normality). Therefore, further statistical analysis assumed log-normally distributed PK parameters. Geometric means and % coefficients of variation [%CV = $\sqrt{(e^{\text{var}} - 1) \times 100}$; var = variance of the log-normalized parameter], C_{max} , time to C_{max} (T_{max}), AUC_{τ} , the half-life ($t_{1/2}$) for day 10, AUC_{total} , the ratio $AUC_{\tau, \text{day 10}}/AUC_{\tau, \text{day 1}}$, and the percentage of AMDX recovered in urine in one dose interval at steady state, as AMDX and DXG (% AMDX_{urine} in τ and % DXG_{urine} in τ , respectively), are summarized in

Tables 1 and 2, respectively, together with P values for multiple paired comparisons performed on the log-transformed parameters, using the Tukey Student t test with the Kramer modification for unbalanced designs, using the GLM procedure of SAS (version 9.2). P values of <0.05 were considered statistically significant. The steady-state PK parameters of ZDV (Table 3) included T_{max} , C_{max} /mg, AUC_{τ} /mg, CL/F, $t_{1/2}$, AUC_{τ} ratio for day 10 to day 1, and the percentage of the ZDV dose recovered in urine in one dose interval, as GZDV and ZDV (% GZDV_{urine} in τ and % ZDV_{urine} in τ , respectively). C_{max} and AUC parameters for ZDV were normalized to dose to allow comparisons to be performed relative to the pooled ZDV monotherapy cohorts ($n = 4$) and groups receiving ZDV with AMDX. Correlations between ADA activity in plasma and C_{max} and AUC_{τ} were assessed by linear regression.

RESULTS

PK of AMDX and DXG. Plasma concentrations of AMDX and DXG on day 1 (first dose) and day 10 (steady state) (mean $\pm$ standard deviation [SD]) versus time were plotted for each cohort (Fig. 1A and B, respectively). AMDX was rapidly absorbed and deaminated to DXG following oral administration on both days. Plasma concentrations of AMDX were similar between day 1 and steady state. No correlation was noted between plasma concentration and C_{max} of either AMDX or DXG and ADA levels in plasma ($r^2 < 0.2$) (data not shown).

TABLE 3. Geometric means (% CV) and statistical analysis of pharmacokinetic parameters for ZDV on day 10

Group or comparison	Median T_{max} (h)	C_{max} (ng/ml/mg)	AUC_{τ} (ng · h/ml/mg)	CL/F (liters/h/kg)	$t_{1/2}$ (h)	AUC_{τ} ratio (day 10/day 1)	% GZDV _{urine} in τ	% ZDV _{urine} in τ
1 (ZDV; $n = 4$)	0.5	7.58 (59.6)	9.62 (42.6)	1.39 (40.2)	2.2 (30.2)	1.21 (7.6)	61.15 (19.1)	6.24 (36.3)
2 (ZDV at 200 mg + AMDX; $n = 6$)	0.5	4.34 (101)	6.51 (29.9)	2.34 (34.4)	2.5 (24.5)	1.04 (29.4)	61.87 (23.0)	5.88 (54.4)
3 (ZDV at 300 mg + AMDX; $n = 6$)	0.5	6.74 (43.8)	8.77 (17.3)	1.80 (30.9)	2.4 (13.1)	0.86 (33.4)	62.89 (17.2)	6.74 (43.8)
Multiple comparisons of ratios of geometric means (P values in Tukey-Kramer modified t test)								
1 vs 2		1.75 (0.38)	1.48 (0.13)	0.60 (0.08)	0.90 (0.72)	1.16 (0.67)	0.99 (1.00)	1.06 (0.98)
1 vs 3		1.18 (0.92)	1.10 (0.87)	0.78 (0.49)	0.91 (0.77)	1.41 (0.16)	0.97 (0.97)	0.93 (0.96)
2 vs 3		0.673 (0.54)	0.74 (0.21)	1.30 (0.39)	1.01 (1.00)	1.21 (0.47)	0.98 (0.99)	0.87 (0.86)

FIG. 1. Plasma concentrations (ng/ml; mean $\pm$ SD) of AMDX (open symbols) and DXG (closed symbols) by cohort following the administration of 500 mg of AMDX b.i.d. following the initial dose on day 1 (A) and following the day 10 dose (B). Subjects received AMDX alone ($\circ$) or with ZDV at 200 mg ($\triangle$) or 300 mg ($\square$) b.i.d.

The noncompartmental parameters (geometric means and %CV) of AMDX and DXG for subjects taking AMDX with and without ZDV, together with the respective P values summarizing Tukey's paired comparison t tests of each parameter, are reported in Tables 1 and 2, respectively. The $T_{\max}$ values for AMDX and DXG were similar on days 1 and 10 and were generally between 1 and 3 h (Fig. 1 and 2). The geometric mean plasma $C_{\max}$ of DXG ranged from 1,272 to 1,398, compared to 514 to 892 ng/ml for AMDX, on day 1 and from 1,315 to 1,908 versus 499 to 714 ng/ml, respectively, on day 10. The intersubject variability in $C_{\max}$ between cohorts at steady state was from 33.7 to 52.9% and from 14.1 to 44.4% for AMDX and DXG, respectively. AMDX declined more rapidly than DXG, with the geometric mean $t_{1/2}$ ranging from 1.3 to 1.6 h and from 2.5 to 2.9 h for AMDX and

DXG, respectively, on day 1. A much longer $t_{1/2}$ of decay was noted between 12 and 48 h for DXG (day 10), with geometric mean values ranging from 14.7 to 17.6 h. The corresponding geometric mean percentage of dose recovered in urine in one dose interval was 25.3 to 27.7% as DXG and 1.1 to 3.9% as AMDX. No significant differences were detected in the plasma or urine PK parameters for AMDX and DXG ($P > 0.05$) between cohorts receiving AMDX.

PK of ZDV. Plasma concentrations of ZDV on day 1 and at steady state (mean $\pm$ SD) versus time were plotted (log-linear scale) for each cohort (Fig. 2A and B, respectively). ZDV was rapidly absorbed and demonstrated a median $T_{\max}$ of 0.5 to 0.75 h postdosing for all regimens. The noncompartmental PK parameters of ZDV are summarized in Table 3, together with the

FIG. 2. Plasma concentrations (ng/ml; mean $\pm$ SD) of ZDV on day 1 (A) and at steady state (B) by cohort. ●, ZDV at 200 mg ($n = 2$); ■, ZDV at 300 mg ($n = 2$); ▲, ZDV at 200 mg plus AMDX at 500 mg b.i.d. ($n = 6$); ▼, ZDV at 300 mg plus AMDX at 500 mg b.i.d. ($n = 6$).

respective P values summarizing Tukey's paired comparison t tests of each parameter between cohorts. The geometric mean $C_{\max}$ on day 1 ranged from 4.34 to 7.58 ng/ml per mg of dose. ZDV was eliminated with a geometric mean $t_{1/2}$ ranging from 2.2 to 2.5 h on day 10. The resulting geometric mean $AUC_{12\text{ h}}/\text{mg}$ on day 10 for the various cohorts ranged from 6.5 to 7.6. AUC_{τ} accounted for 97.5 and 100% of AUC_{total} for ZDV on days 1 and 10, respectively. The geometric mean ratios of $AUC_{12\text{ h}}$ on day 10 to that on day 1 ranged from 0.86 to 1.21, suggesting limited dose accumulation, as expected due to the relatively short $t_{1/2}$ of ZDV. The geometric mean percentages of dose recovered in urine in one dose interval at steady state, as GZDV and ZDV, were 61 to 63% and 5.9 to 6.7%, respectively, between cohorts.

DISCUSSION

The feasibility of developing an AMDX and ZDV coformulation is currently being explored, since this combination demonstrates synergy *in vitro* and at least additivity *in vivo* (36) against HIV-1 and prevents the selection of K65R mutation/TAMs in primary human lymphocytes (40). A previous simulation study suggested that the ZDV dose may be reduced from 300 mg b.i.d. to 200 mg b.i.d. to limit toxicity without compromising cellular levels of ZDV-TP, which is responsible for ZDV efficacy (27). Drug-drug interactions are often problematic and particularly important in the management of HIV-1 infection (13). There is no *a priori* reason to expect a drug interaction between AMDX and ZDV, since they use

different phosphorylation pathways (thymidine kinase and 5'-nucleotidase, respectively). However, it was important to confirm the lack of interaction prior to performing larger studies. Additionally, PK assessment of ZDV at a reduced dose compared to the standard dose, alone and in combination with AMDX, was also appropriate in this context. Therefore, it was prudent to perform a proof-of-concept study to assess whether any clinically significant PK interactions occur between ZDV and AMDX or its metabolite, DXG.

The first-dose and steady-state PK of AMDX/DXG were similar to those reported in a previous study in which PK measurements were performed on days 1 and 15, using the same dose of AMDX for 15 days, in treatment-naïve and experienced subjects (47). The lack of correlation between ADA levels in the plasma and $C_{\max}$ of AMDX and DXG was expected, since ADA is not confined to blood cells (e.g., erythrocytes) but is also expressed at high levels in the GI tract and liver (35). Therefore, deamination of AMDX to DXG could also occur before it reaches the systemic circulation. The $C_{\max}$ of AMDX and DXG was reached within 1 to 2 h following dosing, suggesting rapid oral absorption and conversion of AMDX to DXG *in vivo* by adenosine deaminase. The variability in AMDX concentrations tended to be higher at early sampling time points (0.5 h), probably due to different absorption and conversion rates of AMDX to DXG. The intersubject variability of DXG plasma concentrations was relatively small compared to that for AMDX. Since DXG triphosphate is the active form of the drug and AMDX is essentially nontoxic at clinically relevant concentrations, AMDX is an acceptable pro-drug of DXG. Concentrations of DXG were higher and declined slower than those of AMDX, and they exhibited a long $t_{1/2}$ on day 10 which was evident only after 12 h postdosing, indicative of multiexponential decay in plasma.

The PK of ZDV demonstrated similar interindividual variability and parameters to those in previously reported studies (1, 2, 37, 52). Plasma concentrations were similar on days 1 and 10, as expected, due to the relatively short $t_{1/2}$ of ZDV in plasma. Multiple comparisons using analysis of variance (ANOVA) failed to detect significant differences in noncompartmental plasma or urine PK parameters at steady state (including $C_{\max}/F$, CL/F , or % recovered in urine as ZDV or GZDV) for ZDV administered alone or with AMDX (Table 3). However, the PK parameters of ZDV demonstrated relatively large interindividual variance and were limited by the small sample size.

The geometric mean steady-state (day 10) trough plasma concentrations of DXG were between 75 and 121 ng/ml for all cohorts, which exceeded the *in vitro* anti-HIV-1 (wild type) 50% effective concentration (EC_{50}) of $0.25 \pm 0.17 \mu\text{M}$ (equivalent to $63 \pm 43 \text{ ng/ml}$), measured using PHA-stimulated PBM cells (20), or $0.05 \pm 0.02 \mu\text{M}$ ($12 \pm 4 \text{ ng/ml}$), measured using human cord blood mononuclear cells (25). The activity of NRTI results from concentrations of the NRTI-TP in activated $CD4^+$ lymphocytes (42). The cellular $t_{1/2}$ of ZDV-TP in activated PBM cells is 3 to 4 h, while the human $t_{1/2}$, measured in a clinical study wherein ZDV was administered at 300 mg b.i.d., was $\sim 6 \text{ h}$ (2). Therefore, little dose accumulation is expected for ZDV-TP given every 12 h. However, the terminal $t_{1/2}$ of DXG-TP in PHA-stimulated PBM cells was about 16 h (26) and may be higher due to replacement of cellular

DXG-TP by DXG entering from the plasma, given the prolonged terminal plasma $t_{1/2}$ of DXG. Therefore, some dose accumulation of DXG-TP is expected in PBM cells, which could produce a higher potency than that suggested by trough levels of DXG in plasma.

Urine collection on day 9 (steady state) demonstrated that the main AMDX metabolite recovered in the urine was DXG ($\sim 28\%$), with small amounts of AMDX ($\sim 4\%$). Multiple comparisons using ANOVA failed to detect any significant differences in plasma or urine noncompartmental PK parameters at steady state (including $C_{\max}$, CL/F , and % recovered in urine as AMDX or DXG) for AMDX/DXG when AMDX was administered alone or with ZDV at 200 or 300 mg b.i.d. (Tables 1 and 2).

In this clinical study, ZDV at 200 and 300 mg b.i.d. and placebo produced mean changes in VL from baseline of -0.69 , -0.55 , and $+0.1 \log_{10}$, respectively (36), which were similar to those for previous monotherapy trials with ZDV at 300 mg b.i.d. (15). AMDX monotherapy at 500 mg b.i.d. produced a VL change of $-1.09 \log_{10}$, which was similar to what was observed with treatment-naïve subjects in a previous monotherapy study (47). AMDX with ZDV at 200 and 300 mg b.i.d. produced VL changes of -1.69 and $-2.00 \log_{10}$, respectively. AMDX with 200 mg of ZDV was significantly more potent than monotherapy with AMDX ($P = 0.021$), but the difference in mean $\log_{10}$ VL decline between AMDX regimens with 200 and 300 mg ZDV b.i.d. was not statistically significant. These results suggest an additive or synergistic activity between AMDX and ZDV. There was also a decrease in the variability in VL in subjects taking ZDV with AMDX compared to those taking AMDX alone, which could have resulted from the complementary resistance patterns of these NRTI (36).

In summary, there were no significant PK drug-drug interactions between ZDV and either AMDX or DXG in plasma or in the urine during coadministration of AMDX and ZDV. This PK study, together with associated antiviral and tolerability data (36), suggests that the combination of ZDV with AMDX warrants further study and that dose reduction strategies for ZDV might be beneficial in maintaining efficacy and limiting toxicity (41). Longer and larger studies with coformulated AMDX-ZDV are warranted to confirm and extend the results of the current study.

ACKNOWLEDGMENTS

This work was supported in part by the Emory Center for AIDS Research (CFAR), by NIH grants 5P30-AI-1241980 and 5R37-AI-041980, and by the Department of Veterans Affairs.

We thank ACLIRES-Argentina for performing the clinical study and Judy Mathew, Steve Coats, and Tony Whitaker for performing the ADA studies and for generously providing the standards and samples for our analyses. We also acknowledge all subjects who participated in this study for their time and dedication.

R. F. Schinazi is the founder and major shareholder of RFS Pharma, LLC, and the inventor of AMDX and may receive royalties from future sales of AMDX. RFS Pharma provided no funding to Emory University/VAMC.

REFERENCES

- Acosta, E. P., L. M. Page, and C. V. Fletcher. 1996. Clinical pharmacokinetics of zidovudine. An update. *Clin. Pharmacokinet.* 30:251–262.
- Barry, M. G., S. H. Khoo, G. J. Veal, P. G. Hoggard, S. E. Gibbons, E. G. Wilkins, O. Williams, A. M. Breckenridge, and D. J. Back. 1996. The effect of zidovudine dose on the formation of intracellular phosphorylated metabolites. *AIDS* 10:1361–1367.

3. Carpenter, C. C., D. A. Cooper, M. A. Fischl, J. M. Gatell, B. G. Gazzard, S. M. Hammer, M. S. Hirsch, D. M. Jacobsen, D. A. Katzenstein, J. S. Montaner, D. D. Richman, M. S. Saag, M. Schechter, R. T. Schooley, M. A. Thompson, S. Vella, P. G. Yeni, and P. A. Volberding. 2000. Antiretroviral therapy in adults: updated recommendations of the International AIDS Society—USA Panel. *JAMA* **283**:381–390.
4. Chen, H., R. F. Schinazi, P. Rajagopalan, Z. Gao, C. K. Chu, H. M. McClure, and F. D. Boudinot. 1999. Pharmacokinetics of (–)-beta-D-dioxolane guanine and prodrug (–)-beta-D-2,6-diaminopurine dioxolane in rats and monkeys. *AIDS Res. Hum. Retroviruses* **15**:1625–1630.
5. Chin, R., T. Shaw, J. Torresi, V. Sozzi, C. Trautwein, T. Bock, M. Manns, H. Isom, P. Furman, and S. Locarnini. 2001. In vitro susceptibilities of wild-type or drug-resistant hepatitis B virus to (–)-beta-D-2,6-diaminopurine dioxolane and 2'-fluoro-5-methyl-beta-L-arabinofuranosyluracil. *Antimicrob. Agents Chemother.* **45**:2495–2501.
6. Collier, A. C., S. Bozzette, R. W. Coombs, D. M. Causey, D. A. Schoenfeld, S. A. Spector, C. B. Pettinelli, G. Davies, D. D. Richman, J. M. Leedom, et al. 1990. A pilot study of low-dose zidovudine in human immunodeficiency virus infection. *N. Engl. J. Med.* **323**:1015–1021.
7. Cressey, T. R., G. Jourdain, M. J. Lallemand, S. Kunkeaw, J. B. Jackson, P. Musoke, E. Capparelli, and M. Mirochnick. 2005. Persistence of nevirapine exposure during the postpartum period after intrapartum single-dose nevirapine in addition to zidovudine prophylaxis for the prevention of mother-to-child transmission of HIV-1. *J. Acquir. Immune Defic. Syndr.* **38**:283–288.
8. Cressey, T. R., P. Leenasirimakul, G. Jourdain, Y. Tawon, P. O. Sukranchana, and M. Lallemand. 2006. Intensive pharmacokinetics of zidovudine 200 mg twice daily in HIV-1-infected patients weighing less than 60 kg on highly active antiretroviral therapy. *J. Acquir. Immune Defic. Syndr.* **42**:387–389.
9. Cui, L., R. F. Schinazi, G. Gosselin, J. L. Imbach, C. K. Chu, R. F. Rando, G. R. Revankar, and J. P. Sommadossi. 1996. Effect of beta-enantiomeric and racemic nucleoside analogues on mitochondrial functions in HepG2 cells. Implications for predicting drug hepatotoxicity. *Biochem. Pharmacol.* **52**:1577–1584.
10. Darbyshire, J., M. Foulkes, R. Peto, W. Duncan, A. Babiker, R. Collins, M. Hughes, T. Peto, and A. Walker. 2000. Immediate versus deferred zidovudine (AZT) in asymptomatic or mildly symptomatic HIV infected adults. *Cochrane Database Syst. Rev.* **2000**:CD002039.
11. de Clercq, E. 1996. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) for the treatment of human immunodeficiency virus type 1 (HIV-1) infections: strategies to overcome drug resistance development. *Med. Res. Rev.* **16**:125–157.
12. Deeks, S. G., M. Smith, M. Holodniy, and J. O. Kahn. 1997. HIV-1 protease inhibitors. A review for clinicians. *JAMA* **277**:145–153.
13. Department of Health and Human Services (DHHS) Panel on Antiretroviral Guidelines for Adults and Adolescents. 2008. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. DHHS, Washington, DC. <http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf>.
14. Dudley, M. N. 1995. Clinical pharmacokinetics of nucleoside antiretroviral agents. *J. Infect. Dis.* **171**(Suppl. 2):S99–S112.
15. Eron, J. J., S. L. Benoit, J. Jemsek, R. D. MacArthur, J. Santana, J. B. Quinn, D. R. Kuritzkes, M. A. Fallon, and M. Rubin. 1995. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 CD4⁺ cells per cubic millimeter. North American HIV Working Party. *N. Engl. J. Med.* **333**:1662–1669.
16. Feng, J. Y., W. B. Parker, M. L. Krajewski, D. Deville-Bonne, M. Veron, P. Krishnan, Y. C. Cheng, and K. Borroto-Esoda. 2004. Anabolism of amdoxovir: phosphorylation of dioxolane guanosine and its 5'-phosphates by mammalian phosphotransferases. *Biochem. Pharmacol.* **68**:1879–1888.
17. Fischl, M. A., D. D. Richman, D. M. Causey, M. H. Grieco, Y. Bryson, D. Mildvan, O. L. Laskin, J. E. Groopman, P. A. Volberding, R. T. Schooley, et al. 1989. Prolonged zidovudine therapy in patients with AIDS and advanced AIDS-related complex. AZT Collaborative Working Group. *JAMA* **262**:2405–2410.
18. Fromentin, E., G. Asif, A. Obikhod, S. J. Hurwitz, and R. F. Schinazi. 2009. Simultaneous quantification of 9(β-D-1,3-dioxolan-4-yl)guanine, amdoxovir and zidovudine in human plasma by liquid chromatography-tandem mass spectrometric assay. *J. Chromatogr. B* **877**:3482–3488.
19. Furman, P. A., J. A. Fyfe, M. H. St. Clair, K. Weinhold, J. L. Rideout, G. A. Freeman, S. N. Lehrman, D. P. Bolognesi, S. Broder, H. Mitsuya, et al. 1986. Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase. *Proc. Natl. Acad. Sci. U. S. A.* **83**:8333–8337.
20. Furman, P. A., J. Jeffrey, L. L. Kiefer, J. Y. Feng, K. S. Anderson, K. Borroto-Esoda, E. Hill, W. C. Copeland, C. K. Chu, J. P. Sommadossi, I. Liberman, R. F. Schinazi, and G. R. Painter. 2001. Mechanism of action of 1-beta-D-2,6-diaminopurine dioxolane, a prodrug of the human immunodeficiency virus type 1 inhibitor 1-beta-D-dioxolane guanosine. *Antimicrob. Agents Chemother.* **45**:158–165.
21. Gibaldi, M., and D. Perrier. 1982. *Pharmacokinetics*, 2nd ed., vol. 15. Marcel Dekker, New York, NY.
22. Gitterman, S. R., G. L. Drusano, M. J. Egorin, and H. C. Standiford. 1990. Population pharmacokinetics of zidovudine. The Veterans Administration Cooperative Studies Group. *Clin. Pharmacol. Ther.* **48**:161–167.
23. Gripshover, B. M., H. Ribaud, J. Santana, J. G. Gerber, T. B. Campbell, E. Hogg, B. Jarocki, S. M. Hammer, and D. R. Kuritzkes. 2006. Amdoxovir versus placebo with enfuvirtide plus optimized background therapy for HIV-1-infected subjects failing current therapy (AACTG A5118). *Antivir. Ther.* **11**:619–623.
24. Gu, Z., M. A. Wainberg, N. Nguyen-Ba, L. L'Heureux, J. M. de Muys, T. L. Bowlin, and R. F. Rando. 1999. Mechanism of action and in vitro activity of 1',3'-dioxolanylpurine nucleoside analogues against sensitive and drug-resistant human immunodeficiency virus type 1 variants. *Antimicrob. Agents Chemother.* **43**:2376–2382.
25. Gu, Z., M. A. Wainberg, P. Nguyen-Ba, L. L'Heureux, J. M. de Muys, and R. F. Rando. 1999. Anti-HIV-1 activities of 1,3-dioxolane guanine and 2,6-diaminopurine dioxolane. *Nucleosides Nucleotides* **18**:891–892.
26. Hernandez-Santiago, B. I., A. Obikhod, E. Fromentin, S. J. Hurwitz, and R. F. Schinazi. 2007. Cellular pharmacology of 9-(beta-D-1,3-dioxolan-4-yl) guanine and its lack of drug interactions with zidovudine in primary human lymphocytes. *Antivir. Chem. Chemother.* **18**:343–346.
27. Hurwitz, S. J., G. Asif, N. M. Kivel, and R. F. Schinazi. 2008. 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. *Antimicrob. Agents Chemother.* **52**:4241–4250.
28. Jacobsson, B., S. Britton, Q. He, A. Karlsson, and S. Eriksson. 1995. Decreased thymidine kinase levels in peripheral blood cells from HIV-seropositive individuals: implications for zidovudine metabolism. *AIDS Res. Hum. Retroviruses* **11**:805–811.
29. Jacobsson, B., S. Britton, Y. Tornevik, and S. Eriksson. 1998. Decrease in thymidylate kinase activity in peripheral blood mononuclear cells from HIV-infected individuals. *Biochem. Pharmacol.* **56**:389–395.
30. Kewn, S., L. H. Wang, P. G. Hoggard, F. Rousseau, R. Hart, J. P. MacNeela, S. H. Khoo, and D. J. Back. 2003. Enzymatic assay for measurement of intracellular DXG triphosphate concentrations in peripheral blood mononuclear cells from human immunodeficiency virus type 1-infected patients. *Antimicrob. Agents Chemother.* **47**:255–261.
31. Margolis, D. M., A. L. Mukherjee, C. V. Fletcher, E. Hogg, D. Ogata-Arakaki, T. Petersen, D. Rusin, A. Martinez, and J. W. Mellors. 2007. The use of beta-D-2,6-diaminopurine dioxolane with or without mycophenolate mofetil in drug-resistant HIV infection. *AIDS* **21**:2025–2032.
32. Merrill, D. P., M. Moonis, T. C. Chou, and M. S. Hirsch. 1996. Lamivudine or stavudine in two- and three-drug combinations against human immunodeficiency virus type 1 replication in vitro. *J. Infect. Dis.* **173**:355–364.
33. Mewshaw, J. P., F. T. Myrick, D. A. Wakefield, B. J. Hooper, J. L. Harris, B. McCreedy, and K. Borroto-Esoda. 2002. Dioxolane guanosine, the active form of the prodrug diaminopurine dioxolane, is a potent inhibitor of drug-resistant HIV-1 isolates from patients for whom standard nucleoside therapy fails. *J. Acquir. Immune Defic. Syndr.* **29**:11–20.
34. Ministry of Public Health of Thailand. 2005. National guidelines for the clinical management of HIV infection in children and adults. Ministry of Public Health, AIDS Division, Department of Communicable Disease Control, Nonthaburi, Thailand.
35. Moriwaki, Y., T. Yamamoto, and K. Higashino. 1999. Enzymes involved in purine metabolism—a review of histochemical localization and functional implications. *Histol. Histopathol.* **14**:1321–1340.
36. Murphy, R. L., N. M. Kivel, C. Zala, C. Ochoa, P. Tharnish, J. Mathew, M. L. Pascual, and R. F. Schinazi. Antiviral activity and tolerability of amdoxovir in combination with zidovudine in a randomized double-blind placebo-controlled study in HIV-1 infected persons. *Antivir. Ther.*, in press.
37. Panhard, X., M. Legrand, A. M. Taburet, B. Diquet, C. Goujard, and F. Mentre. 2007. Population pharmacokinetic analysis of lamivudine, stavudine and zidovudine in controlled HIV-infected patients on HAART. *Eur. J. Clin. Pharmacol.* **63**:1019–1029.
38. Parikh, U. M., D. L. Koontz, C. K. Chu, R. F. Schinazi, and J. W. Mellors. 2005. In vitro activity of structurally diverse nucleoside analogs against human immunodeficiency virus type 1 with the K65R mutation in reverse transcriptase. *Antimicrob. Agents Chemother.* **49**:1139–1144.
39. Pereira, C. F., and J. T. Paridaen. 2004. Anti-HIV drug development—an overview. *Curr. Pharm. Des.* **10**:4005–4037.
40. Rapp, K. L., M. Ruckstuhl, and R. F. Schinazi. 2007. The combination of zidovudine and amdoxovir prevents the selection of thymidine analogue mutations in primary human lymphocytes. *abstr. 117. Abstr. 16th Int. HIV Drug Resist. Workshop*, 12 to 16 June 2007, Barbados, West Indies.
41. Richman, D. D., M. A. Fischl, M. H. Grieco, M. S. Gottlieb, P. A. Volberding, O. L. Laskin, J. M. Leedom, J. E. Groopman, D. Mildvan, M. S. Hirsch, et al. 1987. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N. Engl. J. Med.* **317**:192–197.
42. Schinazi, R. F., B. I. Hernandez-Santiago, and S. J. Hurwitz. 2006. Pharmacology of current and promising nucleosides for the treatment of human immunodeficiency viruses. *Antiviral Res.* **71**:322–334.
43. Seigner, B., C. Pichoud, P. Martin, P. Furman, C. Trepo, and F. Zoulim.

2002. Inhibitory activity of dioxolane purine analogs on wild-type and lamivudine-resistant mutants of hepadnaviruses. *Hepatology* **36**:710–722.
44. **Siliciano, J. D., and R. F. Siliciano.** 2004. A long-term latent reservoir for HIV-1: discovery and clinical implications. *J. Antimicrob. Chemother.* **54**: 6–9.
45. **Siliciano, R. F.** 2005. Scientific rationale for antiretroviral therapy in 2005: viral reservoirs and resistance evolution. *Top. HIV Med.* **13**:96–100.
46. **Spiga, M. G., D. A. Weidner, C. Trentesaux, R. D. LeBoeuf, and J. P. Sommadossi.** 1999. Inhibition of beta-globin gene expression by 3'-azido-3'-deoxythymidine in human erythroid progenitor cells. *Antiviral Res.* **44**:167–177.
47. **Thompson, M. A., H. A. Kessler, J. J. Eron, Jr., J. M. Jacobson, N. Adda, G. Shen, J. Zong, J. Harris, C. Moxham, and F. S. Rousseau.** 2005. Short-term safety and pharmacodynamics of amdoxovir in HIV-infected patients. *AIDS* **19**:1607–1615.
48. **Tornevik, Y., B. Ullman, J. Balzarini, B. Wahren, and S. Eriksson.** 1995. Cytotoxicity of 3'-azido-3'-deoxythymidine correlates with 3'-azidothymidine-5'-monophosphate (AZTMP) levels, whereas anti-human immunodeficiency virus (HIV) activity correlates with 3'-azidothymidine-5'-triphosphate (AZTTP) levels in cultured CEM T-lymphoblastoid cells. *Biochem. Pharmacol.* **49**:829–837.
49. **White, K. L., N. A. Margot, T. Wrin, C. J. Petropoulos, M. D. Miller, and L. K. Naeger.** 2002. Molecular mechanisms of resistance to human immunodeficiency virus type 1 with reverse transcriptase mutations K65R and K65R+M184V and their effects on enzyme function and viral replication capacity. *Antimicrob. Agents Chemother.* **46**:3437–3446.
50. **Won, S. Y., and H. A. Kessler.** Amdoxovir. *In* The use of antibiotics: a clinical review of antibacterial, antifungal and antiviral drugs, 6th ed., in press. The Bath Press, Avon, United Kingdom.
51. **Ying, C., E. De Clercq, W. Nicholson, P. Furman, and J. Neyts.** 2000. Inhibition of the replication of the DNA polymerase M550V mutation variant of human hepatitis B virus by adefovir, tenofovir, L-FMAU, DAPD, penciclovir and lobucavir. *J. Viral Hepat.* **7**:161–165.
52. **Zhou, X. J., L. B. Sheiner, R. T. D'Aquila, M. D. Hughes, M. S. Hirsch, M. A. Fischl, V. A. Johnson, M. Myers, and J. P. Sommadossi.** 1999. Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. *Antimicrob. Agents Chemother.* **43**:121–128.