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Clinical Pharmacokinetics of Adefovir in Human Immunodeficiency Virus Type 1-Infected Patients

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The pharmacokinetics and bioavailability of adefovir [9-[2-(phosphonomethoxy)ethyl]adenine] were examined at two dose levels in three phase I/II studies in 28 human immunodeficiency type 1-infected patients. The concentrations of adefovir in serum following the intravenous infusion of 1.0 or 3.0 mg/kg of body weight were dose proportional and declined biexponentially, with an overall mean ± standard deviation terminal half-life of 1.6 ± 0.5 h ($n = 28$). Approximately 90% of the intravenous dose was recovered unchanged in the urine in 12 h, and more than 98% was recovered by 24 h postdosing. The overall mean ± standard deviation total serum clearance of the drug (223 ± 53 ml/h/kg; $n = 25$) approximated the renal clearance (205 ± 78 ml/h/kg; $n = 20$), which was significantly higher ($P < 0.01$) than the baseline creatinine clearance in the same patients (88 ± 18 ml/h/kg; $n = 25$). Since adefovir is essentially completely unbound in plasma or serum, these data indicate that active tubular secretion accounted for approximately 60% of the clearance of adefovir. The steady-state volume of distribution of adefovir (418 ± 76 ml/kg; $n = 28$) suggests that the drug was distributed in total body water. Repeated daily dosing with adefovir at 1.0 mg/kg/day ($n = 8$) and 3.0 mg/kg/day ($n = 4$) for 22 days did not significantly alter the pharmacokinetics of the drug; there was no evidence of accumulation. The oral bioavailability of adefovir at a 3.0-mg/kg dose was <12% ($n = 5$) on the basis of the concentrations in serum or 16.4% ± 16.0% on the basis of urinary recovery. The subcutaneous bioavailability of adefovir at a 3.0-mg/kg dose was 102% ± 8.3% ($n = 5$) on the basis of concentrations in serum or 84.8% ± 28.5% on the basis of urinary recovery. These data are consistent with preclinical observations in various species.

Adefovir [9-[2-(phosphonomethoxy)ethyl]adenine; PMEA] is an acyclic analog of dAMP with a phosphonomethylether moiety that is resistant to metabolism. Numerous studies have demonstrated the potent in vitro activity of adefovir against retrovirus replication (11). A similar spectrum of activity has been demonstrated in vivo in mice, cats, and monkeys (2). Unlike acyclovir and the other nucleoside analogs currently used for clinical therapy of viral infections in humans, adefovir does not depend on initial phosphorylation by viral nucleoside kinases to exert its antiviral effect (5). Instead, the drug is phosphorylated to its active form by cellular enzymes. In vitro studies have suggested that the resulting active metabolites are cleared slowly (half-life, 16 to 18 h) from the intracellular space (1).

Data on the pharmacokinetics of adefovir in a number of animal species are available, including mice (16), rats (8, 19), cats (13), and monkeys (3, 4, 10). Plasma adefovir concentrations generally declined in a biexponential manner following intravenous administration, with apparent terminal half-lives ranging from ~0.1 h in mice to 2.0 h in rhesus monkeys. The volume of distribution on the basis of area ranged from 0.3 liters/kg in mice to 1.5 liters/kg in monkeys. The clearance (CL) of adefovir exceeded the theoretical glomerular filtration rate in all species examined. Intramuscular and subcutaneous administration of adefovir gave essentially complete bioavailability, while the oral bioavailability of adefovir was low (ranging from <1% in rhesus monkeys to 11% in rats).

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Preclinical data suggest that the majority of an intravenous adefovir dose is excreted in the urine within 24 h of intravenous administration. The distribution of ^{14}C-adefovir in rats has been evaluated following intravenous administration of 10 mg/kg of body weight (8); the majority of radioactivity recovered at 6 h postdosing was in the urine, while the concentrations in tissue were highest in the kidney (18.7 μg eq/ml). The accumulation of adefovir within the kidney appears consistent with the dose-limiting toxicity of adefovir in rats, namely, nephrotoxicity localized in the proximal tubular cells (14).

Intravenous administration of adefovir has been associated with evidence of activity against human immunodeficiency virus type 1 (HIV-1) in clinical studies, on the basis of surrogate markers (23). This report presents data from three clinical studies on the pharmacokinetics of intravenous adefovir in HIV-1-infected patients. In addition, data on the subcutaneous and oral bioavailabilities of adefovir are presented.

MATERIALS AND METHODS

Patients. All clinical studies were conducted with the informed consent of the patients and the approval of the appropriate institutional review boards. Patients (23 males and 5 females) were selected on the basis of diagnosed HIV-1 infection and normal renal, hepatic, hematologic, and coagulation function (patients presenting with minimal renal, hepatic, or hematologic toxicity were included in the study conducted at the National Institutes of Health). The mean patient age was 36 years (range, 22 to 60 years). The median CD4 cell count was 67 cells per mm^3 (range, 0 to 617 cells per mm^3; $n = 23$). Exclusion criteria included active serious infections (except HIV-1); clinically significant cardiac disease; pregnancy; ongoing therapy with acyclovir, ganciclovir, or foscarnet; or ongoing therapy with any agents with nephrotoxic potential.

Study design. (i) Drug administration. An intravenous formulation of adefovir containing 75 mg of adefovir per ml in a sterile isotonic solution for parenteral administration was obtained from Gilead Sciences, Inc. (Foster City, Calif.). For

intravenous studies, adefovir was infused in 100 ml of 0.9% (normal) saline into a peripheral vein over a 30-min period. For subcutaneous studies, the adefovir formulation (75 mg/ml) was directly injected in up to three divided doses. For oral studies, adefovir was diluted to 30 ml with tap water and was administered orally; this was followed by the oral administration of an additional 100 ml of tap water.

(ii) Pharmacokinetic studies. Adefovir was administered to HIV-1-infected patients at various dose levels by various routes in three clinical protocols.

In study GS-92-201 (study 1) at the National Institutes of Health, adefovir was administered intravenously to 10 patients (7 males and 3 females; mean weight, 75.9 kg; median CD4 count, 114 cells per mm^3) at 1.0 mg/kg and 5 patients (all males; mean weight, 77.2 kg; median CD4 count, 153 cells per mm^3) at 3.0 mg/kg. The study was conducted on an inpatient basis for the first 24 h only and on an outpatient basis thereafter. The concomitant use of antiretroviral drugs (zidovudine, didanosine, or zalcitabine) or investigational agents was not permitted in this study. After a 1-week observation period, four patients receiving drug at the 1.0-mg/kg dose level received additional daily infusions of adefovir for up to 12 weeks, and 2 patients receiving drug at the 3.0-mg/kg dose level received additional daily infusions of adefovir for up to 6 weeks. Blood and urine samples were obtained following the first infusion and following the 22nd infusion of daily therapy (the first infusion of week 4). Five milliliters of blood (approximately 3 ml of serum) was withdrawn from each subject at 0 (preinfusion), 0.5 (end of infusion), 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 12 h postdosing. Blood was allowed to coagulate, and serum was decanted, frozen, and stored at ≤ -20°C until it was analyzed. Urine samples were obtained over the intervals 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 10, and 10 to 12 h postdosing, frozen, and maintained at ≤ -20°C until they were analyzed.

In study GS-92-202 (study 2) at the University of Washington, five patients (four males and one female; mean weight, 73.9 kg) received daily intravenous infusions of adefovir at a dosage of 1.0 mg/kg/day for up to 4 weeks. Three additional patients (all males; mean weight, 76.6 kg) received daily infusions at a dose of 3.0 mg/kg for up to 4 weeks. The study was conducted on an inpatient basis for the first 3 to 4 days and on an outpatient basis thereafter. The median CD4 cell count in these patients was 42 cells per mm^3. Concomitant therapy with zidovudine (three patients) or zalcitabine (one patient) was permitted. Investigational agents were excluded. Blood samples were obtained following the first infusions of weeks 1 and 4 of daily therapy. Five milliliters of blood (approximately 3 ml of serum) was withdrawn from each subject at 0 (preinfusion), 0.5 (end of infusion), 1, 1.5, 2, 3, 4, 6, 8, and 12 h postdosing. Blood was allowed to coagulate, and serum was decanted, frozen, and stored at ≤ -20°C until it was analyzed. No urine samples were collected during the study.

In study GS-92-203 (study 3) at The Johns Hopkins University School of Medicine, five patients (four males and one female; mean weight, 69.4 kg) received adefovir at a 3.0-mg/kg dose by the intravenous, oral, and subcutaneous routes in a three-way crossover design, with a 48-h washout period between administrations. CD4 cell counts were not recorded. The study was conducted on an inpatient basis. Although it was permitted, none of these patients received concomitant therapy with didanosine, zalcitabine, or zidovudine. Therapy with investigational agents was excluded. Patients were fasted from midnight on the night prior to the administration of each dose until 4 h postdosing. Five milliliters of blood (approximately 3 ml of serum) was withdrawn from each subject at 0 (preinfusion), 0.25 (midinfusion), 0.5 (end of infusion), 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h postdosing. Blood was allowed to coagulate, and serum was decanted, frozen, and stored at ≤ -20°C until it was analyzed. Urine samples were collected prior to dosing and over the intervals 0 to 4, 4 to 8, 8 to 12, and 12 to 24 h postdosing.

Analytical procedures. (i) Materials. The adefovir reference standard and the internal standard, 9-[2-(phosphonomethoxy)propyl]adenine (PMPA), were synthesized by Gilead Sciences, Inc. Potassium phosphate, dibasic (anhydrous), sodium dihydrogen phosphate, and sodium hydroxide were obtained from Mallinckrodt (Paris, Ky.). *o*-Phosphoric acid (85%), chloroacetaldehyde, and sodium citrate were from Fisher Scientific (Fair Lawn, N.J.). Tetrabutylammonium dihydrogen phosphate (TBAHP) was obtained from Fluka Chemical Corp. (Ronkonkoma, N.Y.). Acetonitrile, methanol, and deionized water were obtained from Baxter (McGaw Park, Ill.). Pooled normal human serum was obtained from Whittaker (Walkersville, Md.). Pooled normal human urine was obtained from volunteers.

(ii) Determination of adefovir concentrations in serum. The concentrations of adefovir in serum samples were determined under GLP (Good Laboratory Practices) conditions by derivatization and analysis by a validated reverse-phase ion-pairing high-performance liquid chromatography (HPLC) method with fluorescence detection (modified from a published method [17]). Serum (200 μl) was added to 700 μl of internal standard (1.43 μg of PMPA per ml) in a polypropylene centrifuge tube, and the tube was vortexed to mix the contents. The tubes were incubated at 63 ± 2°C for 45 min in a Lab-Line Imperial III Incubator (Baxter) to inactivate the HIV-1 and were allowed to cool. Whatman SAX anion-exchange solid-phase extraction columns (Whatman Lab Division, Clifton, N.J.) were conditioned with 1.0 ml each of methanol, deionized water, and 5 mM potassium phosphate buffer (pH 7.4). Samples were applied and drained slowly, and the columns were rinsed with 1.0 ml of 5 mM potassium phosphate (pH 7.4) and air dried. Adefovir was eluted with 1.0 ml of 60% methanol–40% 200 mM phosphate buffer (pH 2.0). Effluent was neutralized with 0.5 M sodium hydroxide and was evaporated to dryness under reduced pressure with a SpeedVac evaporator (Savant, Farmingdale, N.Y.). Dried samples were reconstituted in 200 μl of derivatization cocktail (43 mM chloroacetaldehyde in 640 mM sodium acetate [pH 4.5]) and were incubated at 90°C for 45 min. The derivatization yield was >80%, and derivatized adefovir was stable for >1 week at room temperature. Samples were evaporated to dryness and reconstituted in 200 μl of mobile phase, and then they were transferred to autoinjector vials for analysis by HPLC. The HPLC system was a Waters Millenium HPLC System (comprising a model 600 pump, a model 600E system controller, a model 717 sample injector, and a model 470 fluorescence detector [Waters Chromatography Div., Milford, Mass.] and an NEC 486/66i Powermate computer). The analytical column was a Zorbax C-18 column (5 μm, 150 by 4.6 mm; Mac Mod Analytical Inc., Chadds Ford, Pa.) equipped with a Brownlee RP-18 Newguard guard column (6.0 by 40 mm; Alltech, Deerfield, N.Y.). The mobile phase was 9% acetonitrile–91% 10 mM phosphate buffer (pH 5.6) containing 5 mM TBAHP. The flow rate was 2.0 ml/min, and the column temperature was 40°C. Detection was achieved by fluorescence with excitation at 254 nm and emission at 424 nm. The retention times on this system were as follows: adefovir, 4.2 min; PMPA, 6.4 min. The method was linear over the range from 250 ng/ml to 15 μg/ml, and the limit of quantitation was 250 ng/ml. The between-run precision and accuracy (expressed as a percent deviation from the nominal value) were <9.4 and <2.0%, respectively, at the limit of quantitation.

(iii) Determination of adefovir concentrations in urine. The concentrations of adefovir in urine samples were determined by derivatization and analysis by a validated reverse-phase ion-pairing HPLC method with fluorescence detection. Aliquots of urine samples (1.0 ml) were incubated at 60°C for 1 h to inactivate virus in a model 11-718-6 dry bath (Fisher Scientific, Rochester, N.Y.) and were allowed to cool. Urine (100 μl) was added to 1.0 ml of internal standard (10 μg of PMPA per ml in deionized water) in a polypropylene centrifuge tube, and the tubes were vortexed to mix the contents. Whatman SAX anion-exchange solid-phase extraction columns (Whatman Lab Division) were conditioned with one column volume (1 ml) each of methanol and deionized water. The samples were applied, and the columns were rinsed with 1.0 ml of water and air dried. Columns were eluted with 250 μl of 720 mM sodium citrate (pH 4.5). The effluents were transferred to autoinjector vials and were derivatized by the addition of 15 μl of 50% (wt/wt) chloroacetaldehyde and incubation at 90°C for 1 h. The HPLC system comprised a model 600 pump, a model 600 E system controller, a model 470 fluorescence detector, and a model 717 automatic sample injector (Waters Chromatography Division, Milford, Mass.). Data were acquired by using Waters ExpertEase Chromatography Software. The HPLC column was a Beckman Ultrasphere ODS-IP (150 by 4.6 mm; Alltech, San Jose, Calif.) equipped with a Hypersil ODS C_{18} (5 μm) guard column (Alltech, Deerfield, N.Y.). The mobile phases were as follows: mobile phase A, 6% acetonitrile and 94% 10 mM phosphate buffer (pH 6) containing 5 mM TBAHP; mobile phase B, 60% acetonitrile and 40% 10 mM phosphate buffer (pH 6) containing 5 mM TBAHP. The step gradient was 100% mobile phase A to 15 min, 100% mobile phase B to 19 min, and then a return to 100% mobile phase A. The run time was 24 min, and the flow rate was 3.0 ml/min. The injection volume was 20 μl, and the column temperature was 30°C. Detection was by fluorescence with excitation at 236 nm and emission at 420 nm. Retention times were as follows: adefovir, 5.5 min; PMPA, 9.2 min. The method was linear over the range from 1.0 to 20 μg/ml, and the limit of quantitation was 1.0 μg/ml. The between-run precision and accuracy (expressed as percent deviation from the nominal value) were <6.1 and <3.0%, respectively, at the limit of quantitation.

Pharmacokinetics and statistical analysis. (i) Pharmacokinetic calculations. The pharmacokinetic parameters for adefovir administered intravenously were assessed by application of the nonlinear curve-fitting software package PCNONLIN (22) by both standard noncompartmental and compartmental methods. The parameters estimated by PCNONLIN included the area under the serum concentration-versus-time curve (AUC) up to the time of the last quantifiable concentration ($AUC_{0-t_{last}}$); the value of AUC extrapolated to infinity ($AUC_{0-\infty}$); the slope of the terminal elimination phase, estimated by linear regression of the log of concentrations (k_e); the half-life of the terminal elimination phase ($0.693/k_e$); the area under the first moment of the serum concentration-versus-time curve extrapolated to infinity; and the mean residence time. The maximum concentration of adefovir in serum (C_{max}) and the time to C_{max} were obtained by observation. Additional parameters were calculated manually. The total serum CL was calculated as dose/$AUC_{0-\infty}$. The steady-state volume of distribution was calculated as the mean residence time × CL. The volume of distribution on the basis of area was calculated as CL/k_e. For the subcutaneous route, percent bioavailability was calculated as $100 \times (AUC_{0-\infty_{subcutaneous}}/AUC_{0-\infty_{intravenous}})$. In the absence of quantifiable concentrations of adefovir in serum, an estimate of the maximum limit of oral bioavailability was calculated as $100 \times (AUC_{0-t_{last,oral}}/AUC_{0-t_{last,intravenous}})$, where $AUC_{0-t_{last,oral}}$ was obtained by extrapolating the lowest quantifiable concentration in serum (the limit of quantitation of the analytical method) over the same time range as the available intravenous data. Further analysis of data on adefovir concentrations in serum after intravenous administration was performed by using a two-compartment model. The model was selected on the basis of standard model selection criteria and examination of scatter plots of the residual error. The resulting pharmacokinetic parameters (data not shown) were not significantly different from those obtained by noncompartmental methods.

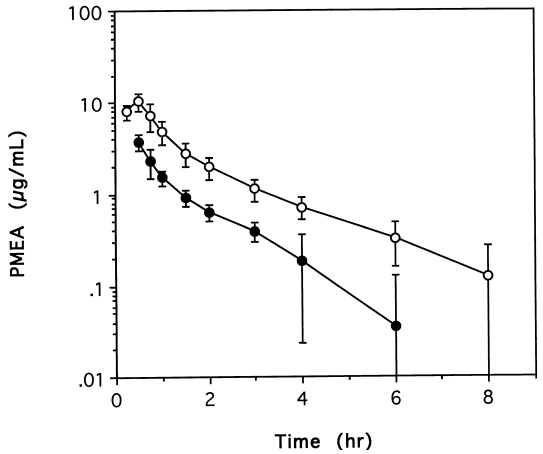

FIG. 1. Effect of dose on mean ± SD concentrations of adefovir (PMEA) following intravenous infusion to HIV-1-infected patients: ●, 1.0 mg/kg (n = 15); ○, 3.0 mg/kg (n = 13).

The cumulative amount of adefovir excreted at the end of each urine collection period (U_{0-t}) was calculated as the sum of the amounts excreted in all previous collection periods. The cumulative percentage of the dose excreted at the end of each collection period was calculated as 100 × (U_{0-t}/dose). When not directly available, the concentration of adefovir in serum at the end of the 24-h urine collection period (C_{24}) was calculated by extrapolation of the last quantifiable concentration in serum (C_{last}) as $C_{last} \times e^{-k_e} \times (24 - t_{last})$. The AUC up to the end of the urine collection period, AUC_{0-24}, was calculated as $AUC_{0-\infty} - (C_{24}/k_e)$. The renal clearance ($CL_R$) of adefovir (in milliliters per hour per kilogram) following intravenous administration was calculated as $(U_{0-24} \cdot 1{,}000)/(AUC_{0-24} \cdot Wt)$, where Wt is the body weight of the patient (in kilograms). Baseline creatinine clearance values (intended to be a measure of the glomerular filtration rate) were determined from creatinine concentrations in serum by using the equation of Cockroft and Gault (6).

Statistical comparisons between pharmacokinetic parameters obtained following the first infusions of weeks 1 and 4 of daily dosing were performed by a paired t test. The clearance of adefovir is essentially accounted for entirely by renal elimination. Comparison of the CL_R of adefovir with baseline creatinine clearance values determined in the same patients provides a measure of tubular secretion. These values were compared within patients by a paired t test. The effect of dose on the pharmacokinetic parameters for adefovir was evaluated by an unpaired t test. A P value of <0.05 was considered significant.

(ii) Protein binding. Binding of adefovir to plasma or serum proteins was evaluated over the concentration range of 0.25 to 25.0 μg/ml by using [^{14}C-8-*adenine*]adefovir (Moravek, Brea, Calif.) in pooled normal human plasma or serum. Duplicate samples were incubated at 37°C for 20 min and were centrifuged through Ultrafree 10,000-molecular-weight-cutoff filters (Millipore, Bedford, Mass.) in a heated centrifuge (approximately 32°C). Samples were corrected for nonspecific binding by comparison with recovery from buffer.

RESULTS

Initial dose studies. The protein binding of adefovir was negligible (<3.0%) over the concentration range from 0.25 to 25.0 μg/ml. Figure 1 compares the mean ± standard deviation (SD) concentrations of adefovir in serum following the initial intravenous administration to HIV-1-infected patients at two dose levels; the corresponding cumulative excretion of adefovir in urine is given in Fig. 2. By using the current analytical methods, no metabolites of adefovir were observed in any of the serum or urine samples analyzed. Concentrations in serum declined biexponentially, with an overall mean terminal half-life of 1.6 ± 0.5 h (n = 28). The maximum concentrations of adefovir observed in serum following intravenous infusion increased proportionally with dose; mean ± SD C_{max}s were 3.77 ± 0.78 and 10.6 ± 2.27 μg/ml after administration of doses of 1.0 and 3.0 mg/kg, respectively.

Table 1 summarizes the noncompartmental pharmacokinetic parameters for adefovir given intravenously over the dose range from 1.0 to 3.0 mg/kg and the overall mean parameters for 28 patients given adefovir by the intravenous route. Analysis of data by a two-compartment model did not significantly alter the derived parameters (data not shown). The observed AUCs were dose proportional; mean ± SD AUCs were 4.61 ± 1.01 and 14.3 ± 2.94 μg · h/ml for doses of 1.0 and 3.0 mg/kg, respectively. The overall mean ± SD urinary recovery of unchanged adefovir at 12 h after administration of an intravenous dose was 89.5% ± 17.7% (n = 20); five patients receiving drug at the 3.0-mg/kg dose level had a urinary recovery of 98.3% ± 18.2% at 24 h postdosing. The overall mean serum CL of the drug (223 ± 53 ml/h/kg; n = 28) approximated CL_R (205 ± 78 ml/h/kg; n = 20), which was more than twofold higher (P < 0.01) than the baseline creatinine clearance in the same patients (88 ± 18 ml/h/kg; n = 25). The terminal elimination half-lives after administration of the 1.0- and 3.0-mg/kg doses were not significantly different (P = 0.38).

Multiple-dose studies. Table 1 compares the major pharmacokinetic parameters for the first infusions of weeks 1 and 4 of daily dosing (in study GS-92-201, data for the first infusion were obtained 1 week prior to the initiation of daily dosing). Mean pharmacokinetic parameters after administration of the 1-mg/kg/day dosage were not significantly different following the 1st and the 22nd infusions. Mean pharmacokinetic parameters after administration of the 3-mg/kg/day dosage were also unchanged by repeated dosing over 22 infusions.

Subcutaneous bioavailability. The mean ± SD concentrations of adefovir observed in serum following intravenous or subcutaneous administration of adefovir at a 3-mg/kg dose are compared in Fig. 3. The two profiles of concentrations of drug in serum were very similar; the C_{max} for the subcutaneous dose was approximately 50% of the C_{max} achieved at the end of infusion for an intravenous dose. The mean ± SD subcutaneous bioavailability of adefovir was 102% ± 8.3% (n = 5) (Table 2). Subcutaneous bioavailability determined from the 24-h urinary recovery of adefovir after intravenous and subcutaneous dosing was 84.8% ± 28.5% (n = 5).

Oral bioavailability. The oral bioavailability of adefovir on the basis of 24-h urinary recovery was variable (mean ± SD, 16.4% ± 16.0% [n = 5]; range, 5.7 to 39.3%). Adefovir did not reach quantifiable concentrations in the serum of patients following oral administration of a 3.0-mg/kg dose. Since the limit of quantitation of the analytical method was relatively high (250 ng/ml), it is possible that the slow absorption of adefovir could have produced a sustained low concentration in serum.

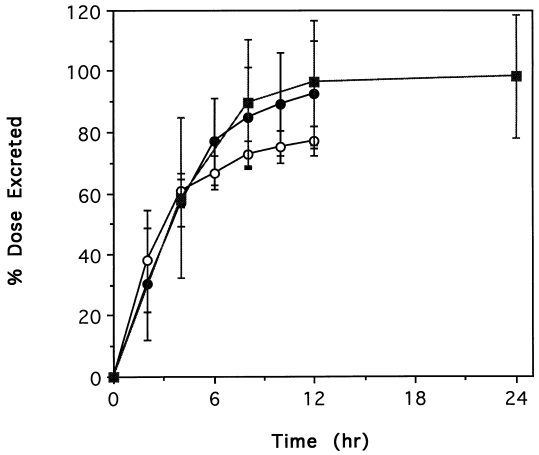

FIG. 2. Cumulative urinary excretion of adefovir following intravenous administration to HIV-1-infected patients (mean ± SD): ●, 1.0 mg/kg (n = 10); ○, 3.0 mg/kg in study 1 (n = 5); ■, 3.0 mg/kg in study 3 (n = 5).

TABLE 1. Pharmacokinetic parameters for adefovir following intravenous administration to HIV-1-infected patients[a]

Infusion and dose (mg/kg)	No. of subjects	Wt (kg)	C_{max} (μg/ml)	$AUC_{0-\infty}$ (μg · h/ml)	CL (ml/h/kg)	CL_R (ml/h/kg)	CL_{CR} (ml/h/kg)	V_{area} (ml/kg)	MRT (h)	V_{ss} (ml/kg)	$t_{1/2}$ (h)	% Recovery in urine at:	
												12 h	24 h
Infusion 1 of week 1													
1.0	15	75.4 (12.3)	3.77 (0.78)	4.61 (1.01)	228 (55)	206 (77)[b]	89 (17)	462 (102)	1.92 (0.57)	418 (81)	1.50 (0.59)	91.5 (18.2)[b]	
3.0	13	72.8 (12.0)	10.6 (2.27)	14.3 (2.94)	220 (52)	205 (84)[b]	88 (20)[b]	513 (113)	1.96 (0.42)	417 (72)	1.68 (0.45)	86.6 (16.3)[b]	98.3 (18.2)[c]
Mean	28	74.2 (12.0)			223 (53)	205 (78)[d]	88 (18)[e]	486 (108)	1.94 (0.50)	418 (76)	1.58 (0.53)	89.5 (17.7)[d]	
Infusion 1 of week 4													
1.0	8		4.03 (2.22)	5.10 (1.53)	219 (63)			452 (109)	1.97 (0.56)	406 (114)	1.55 (0.42)		
3.0	4		12.9 (5.52)	18.2 (4.65)	175 (52)			422 (132)	2.11 (0.32)	367 (108)	1.69 (0.42)		
Mean	12				200 (60)			442 (112)	2.02 (0.48)	393 (109)	1.60 (0.40)		

[a] Values are means (SDs are in parentheses). Abbreviations: CL_{CR}, creatinine clearance; V_{area}, volume of distribution on the basis of area; V_{ss}, volume of distribution at steady state; MRT, mean residence time; $t_{1/2}$, terminal-phase half-life; the other abbreviations are defined in the text.
[b] Mean of 10 patients.
[c] Mean of five patients.
[d] Mean of 20 patients.
[e] Mean of 25 patients.

FIG. 3. Comparison of mean ± SD serum adefovir (PMEA) concentrations following intravenous (●) or subcutaneous (○) administration to five patients at a 3.0-mg/kg dose.

The maximum possible AUCs for these patients were estimated by projecting the minimum quantifiable concentration in serum over the same time range as that for the available intravenous data. The resulting limit of oral bioavailability of adefovir at the 3.0-mg/kg dose was <12% (Table 2).

DISCUSSION

Following intravenous administration, the pharmacokinetics of adefovir were characterized by an apparent biexponential decline in concentrations in serum, with an initial half-life of approximately 15 min. (Fig. 1) and a terminal elimination half-life of approximately 1.5 h. The data were well described by a two-compartment model. The observed terminal elimination half-life presumably reflects the efflux of free adefovir from cells. This relatively short half-life in serum may not reflect the true duration of action of the drug, since the antiviral effect is dependent on the concentrations of the active phosphorylated metabolites of adefovir present within the cell (in vitro half-life, 16 to 18 h) (1). The current analytical methods are relatively insensitive and may preclude observation of a prolonged terminal elimination phase representing the efflux of adefovir derived from intracellular metabolites.

The majority of an intravenous dose of adefovir was recovered unchanged in the urine, and no metabolites of adefovir were detected in clinical samples of urine or serum. No evidence of drug accumulation was seen when adefovir was administered as a once-per-day infusion for 22 days; minor changes in pharmacokinetic parameters observed within patients over the course of the study were not consistent between dose levels and were not apparent in the mean data.

The mean ± SD subcutaneous bioavailability of adefovir was 102% ± 8.3%. This value is consistent with the value (84.8% ± 28.5%) determined from urinary recovery data in the same patients and with preclinical observations. These data suggest that the subcutaneous administration of adefovir may offer an alternative to intravenous infusion.

In the present study, the oral bioavailability of adefovir in HIV-1-infected patients was low (<12% on the basis of undetectable concentrations in serum). Adefovir has low oral bioavailability in a number of species including rats (19), rhesus monkeys (4), and cynomolgus monkeys (10). This appears to be a consequence of the limited intestinal permeation of the phosphonate, which exists as a dianion at physiological pH (pK_{a1} = 2, pK_{a2} = 6.8) (18). In vitro studies with cultured

TABLE 2. Pharmacokinetic parameters for adefovir administered subcutaneously and orally to HIV-1-infected patients[a]

Route[b]	C_{max} (μg/ml)	T_{max} (h)	$AUC_{0-t_{last}}$ (μg · h/ml)	$AUC_{0-\infty}$ (μg · h/ml)	$t_{1/2}$ (h)	% Recovery in urine at 24 h	Bioavailability (%)	Bioavailability based on urine data (%)
Subcutaneous	5.53 (0.48)	0.55 (0.21)		12.5 (2.83)	1.66 (0.17)	103 (57.6)	102 (8.28)	84.8 (28.5)
Oral	<LOQ		<1.4[c]			15.6 (14.3)	<12[c]	16.4 (16.0)

[a] Values are mean (SDs are in parentheses). Abbreviations: LOQ, limit of quantitation of the analytical method (0.25 μg/ml); T_{max}, time to C_{max}; $t_{1/2}$, terminal-phase half-life; the other abbreviations are defined in the text.
[b] Doses of 3.0 mg/kg were given by both routes to five subjects each.
[c] Maximum limit on the basis of projection of the limit of quantitation.

Caco-2 intestinal epithelial cells have demonstrated a low permeation coefficient for adefovir (K_p 1.7×10^{-7} cm/s), similar to other phosphonate nucleotide analogs (20). A bis(pivaloyloxy-methyl) ester prodrug of the phosphonate, bis-POM PMEA, is currently being developed (21). It has greater lipophilicity than adefovir and, hence, a greater ability to cross cell membranes. The resulting prodrug has greater intestinal permeation than the parent drug in vitro and is rapidly converted to adefovir in vivo, leading to significantly greater oral bioavailability than that achieved by adefovir itself (7).

In clinical studies, baseline creatinine clearance values, calculated prior to administration of the first adefovir dose, were used as a measure of the glomerular filtration rate. The CL of adefovir was attributed entirely to renal excretion, and the CL_R adefovir was consistently higher than the baseline creatinine clearance values in the same patients. The excess CL of adefovir above the level of glomerular filtration must have been a consequence of active tubular secretion of the drug in the kidney. Similar renal tubular secretion has been reported for structurally related antiviral nucleotide and nucleoside analogs, including cidofovir (9), stavudine (12), and didanosine (15).

In summary, the pharmacokinetics of intravenous adefovir in 28 HIV-1-infected patients were reproducible and dose independent. Systemic exposure to the drug was proportional to the intravenous dose. The drug was cleared by the kidney and was excreted extensively as unchanged adefovir in the urine. The observed rate of excretion of adefovir may underestimate the true duration of action of the drug. Systemic exposures achieved by subcutaneous and intravenous administration were essentially equivalent, while the oral bioavailability of adefovir was low. The clinical data on the pharmacokinetics of adefovir are consistent with preclinical data.

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