Age-Related Differences in Pharmacokinetics of Phosphonoformate in Cats

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Received 15 September 1989/Accepted 13 February 1990

Phosphonoformate (PFA) is a simple PP_i analog which inhibits the activities of a variety of viral DNA polymerase, RNA polymerase, and reverse transcriptase enzymes. PFA is a topical and parenteral treatment for human herpesvirus infections and is currently in phase I trials for treatment of acquired immunodeficiency syndrome. Pharmacokinetic properties of PFA in young (growing) and adult specific-pathogen-free cats were compared. Mean PFA clearance from plasma was twofold higher in young cats (7.52 ml/min per kg of body weight) than in adult cats (3.70 ml/min per kg). Higher PFA clearance from plasma observed in young cats may result from higher renal clearance or enhanced accumulation of PFA in bone tissue of young versus adult cats. No plasma protein binding of PFA was observed. Mean oral bioavailability was 35% in young cats. These data indicate that age-related differences in PFA clearance from plasma occur in cats.

Phosphonoformate (PFA), or Foscarnet, is a simple PP_i analog (Fig. 1) which reportedly inhibits the enzymatic activities of a variety of DNA and RNA polymerases and reverse transcriptases in vitro (14, 20, 21, 23, 28-30, 36-38). PFA has been administered topically to treat herpes simplex virus type 2 infections and intravenously to treat cytomegalovirus infections in humans (2, 6, 15, 17, 18, 21, 24, 39). Phase I trials are currently in progress to evaluate the efficacy of PFA for treatment of acquired immunodeficiency syndrome (AIDS) (1, 7, 9, 16, 21).

Pharmacokinetic studies of PFA with mice, monkeys, dogs, and pigs have demonstrated PFA half-lives of 0.7, 1.2, 2.0, and 3.6 h, respectively (20). PFA has no known metabolites, is excreted in urine, and accumulates in bone (15).

Cats infected with feline leukemia virus, a retrovirus, are important models for AIDS research and antiretrovirus drug testing. Parallels between human immunodeficiency virus and feline leukemia virus include immunodeficiency; predispositions to bacterial, viral, protozoal, and fungal infections; neoplastic disorders; anemia; thrombocytopenia; and leukopenia (4, 5, 10, 12, 13, 19).

Objectives of the present investigation were to define the pharmacokinetic parameters of PFA, including plasma clearance, half-life, and oral bioavailability, in young and adult cats. Pharmacokinetic parameters of PFA will be used to determine dosage regimens for in vivo trials evaluating the efficacy of PFA for the prevention and treatment of feline leukemia virus infection.

MATERIALS AND METHODS

Cats. For this study, three 1-year-old and three 10-week-old specific-pathogen-free cats were obtained from the specific-pathogen-free colony at The Ohio State University.

Drug. Trisodium phosphonoformate hexahydrate (PFA) was provided by Astra Läkemedel AB, Södertälje, Sweden. PFA was solubilized at 40 mg/ml in sterile saline solution for

intravenous (i.v.) studies and in sterile water for oral studies. The pHs were adjusted to 7.4 with 1 N HCl. The PFA solutions were sterilized by passage through a 40- μ m-pore-size filter.

Dosage. Food was withheld from cats overnight before each study. Three 1-year-old cats received 40 mg of PFA in saline per kg of body weight as a single rapid i.v. bolus injection. In a crossover study, three young cats were given PFA i.v. and orally. Two 10-week-old cats received 40 mg of PFA per kg as a single rapid i.v. bolus injection, and one 10-week-old cat received 400 mg of PFA per kg orally. At 14 weeks of age, the two cats previously given an i.v. dose of PFA received 400 mg of PFA per kg orally and the cat previously given PFA orally received 40 mg of PFA per kg as a single rapid i.v. bolus injection.

Blood collection. Venous blood samples were collected in heparin-treated tubes 1 min before and at 2, 10, 20, 30, 45, 60, 120, 180, and 240 min after PFA dosing. The 2-min sample was omitted and additional samples were collected at 360 and 480 min for cats that received an oral dose. Blood samples were centrifuged (380 $\times$ g) for 10 min, and plasma was collected and frozen until analysis.

Determination of PFA concentrations in plasma. PFA standards (160, 80, 40, 20, 10, and 5 μ g/ml) were prepared in drug-free, heparinized, specific-pathogen-free cat plasma. Standards and test plasma samples were diluted 1:4 with 0.001 M pyrophosphoric acid (75 μ l of plasma per 300 μ l of pyrophosphoric acid), transferred to micropartition tubes (Millex-HA; Millipore Corp., Bedford, Mass.), and centrifuged (6,200 $\times$ g) for 20 min. Filtrates were transferred to 300- μ l sample vials for analysis.

Concentrations of PFA in plasma were determined by reverse-phase high-performance liquid chromatography by the method of Pettersson et al. (22), with an amperometric detector (model 5100A; Environmental Sciences Associates, Inc.). The analytical column was a Pecosphere C18 cartridge column (3 by 3 μ m; The Perkin-Elmer Corp., Norwalk, Conn.). The guard cell and the analytical cell were placed in series after the analytical column. The guard cell was set at +0.80 V; detector 1 was set at +0.75 V, gain 3, and a

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FIG. 1. Concentration-time curve showing PFA concentrations in plasma (means $\pm$ standard deviations) for three adult cats and three young cats given 40 mg of PFA per kg as a single i.v. injection.

response time of 4 s; and detector 2 was set at +1.10 V, gain 16, and a response time of 5 s. The mobile-phase (1% 0.001 M pyrophosphoric acid–15% methanol, in 0.001 M tetrahexylammonium hydrogensulfate) flow rate was 1 ml/min. Sample injection volume and analysis time were 5 μ l and 4 min, respectively. The retention time for PFA was 3.08 min.

PFA peak heights were measured. Peak heights of PFA external standards in plasma (160, 80, 40, 20, 10, and 5 μ g/ml) were employed to construct a linear regression curve which was used to calculate sample PFA concentrations in plasma. Internal standards were not used.

PFA protein binding. The percentage of protein binding of PFA was calculated by comparing PFA concentrations obtained from 200 μ g of PFA per ml in buffer versus 200 μ g of PFA per ml in plasma after centrifugation through micropartition tubes.

Pharmacokinetic analysis. Pharmacokinetic analysis was based on a two-compartment model using the equation $C_t = Ae^{-\alpha t} + Be^{-\beta t}$, where C_t is concentration in plasma at time t in micrograms per milliliter. A and B are the y intercepts and α and β are the slopes of the distribution and elimination phases, respectively. Area under the concentration-time curve from 0 h to infinity ($AUC_{0-\infty}$) was calculated from $A/\alpha + B/\beta$. Area under the first moment of the concentration-time curve (AUMC) was determined from $A/\alpha^2 + B/\beta^2$. Total body clearance (CL_T) was computed from $dose_{i.v.}/AUC$. Volume of distribution of the central compartment was calculated from $dose_{i.v.}/(A + B)$. The steady-state volume of distribution was derived from $dose_{i.v.}$ ($AUMC/AUC^2$). The mean residence time after intravenous administration was computed from $AUMC/AUC$. Elimination half-life was calculated from $0.693/\beta$. Calculations for oral dosing included $AUC_{0-\infty}$ computed by the trapezoidal method with extrapolation from the last measured concentration to infinity, maximum concentration in serum, and percentage of oral bioavailability from the formula $[(AUC_{p.o.}/AUC_{i.v.}) \times 100]$ ($dose_{i.v.}/dose_{p.o.}$) (11). Pharmacokinetic parameters were estimated by the Hartley modification of the Gauss-Newton method of least squares by using PCNONLIN (Statistical Consultants, Inc., Louisville, Ky.) (35). Sample concentrations were assigned equal weights.

Statistical analysis. Significant differences between young and adult cats were determined by the Mann-Whitney U test (32).

TABLE 1. Pharmacokinetic parameters^a for a single i.v. dose of PFA (40 mg/kg) in adult and young cats^b

Age of cats	A (μ g/ml)	α (per min, 10^3)	B^c (μ g/ml)	β (per min, 10^3)	$AUC_{0-\infty}^c$ (μ g $\cdot$ min/ml, 10^3)	CL^c (ml/min per kg)	V_1 (ml/kg)	V_{ss} (ml/kg)	$t_{1/2}$ (min)
Adult ($n = 3$)	202 $\pm$ 88	73.7 $\pm$ 25.5	68.3 $\pm$ 16.3	8.11 $\pm$ 0.72	11.1 $\pm$ 2.1	3.70 $\pm$ 0.78	158 $\pm$ 45	363 $\pm$ 111	85.4 (78.5–93.6)
Young ($n = 3$)	162 $\pm$ 25	78.7 $\pm$ 35.9	26.7 $\pm$ 19.2	8.71 $\pm$ 6.62	5.4 $\pm$ 0.9	7.52 $\pm$ 1.36	221 $\pm$ 60	915 $\pm$ 791	71.6 (46.8–418.0)

^a A , Zero time intercept for α phase; α , distribution phase; B , zero time intercept for β phase; β , elimination phase; $AUC_{0-\infty}$, area under the concentration-time curve from 0 h to infinity; CL , clearance; V_1 , volume of distribution in the central compartment; V_{ss} , volume of distribution at steady state; $t_{1/2}$, half-life.

^b Values are means $\pm$ standard deviations except those for $t_{1/2}$ which are median and range.

^c Differences between adult and young cats were statistically significant at $P < 0.05$.

FIG. 2. Concentration-time curve showing PFA concentrations in plasma (means $\pm$ standard deviations) for three young cats given 400 mg of PFA per kg as a single oral dose.

RESULTS

Standard curves. Correlation coefficients for standard PFA curves obtained by linear regression analyses ranged from 0.9956 to 0.9996 for concentrations between 5 and 160 $\mu\text{g/ml}$.

PFA protein binding. No differences in PFA concentration were observed when 200 μg of PFA per ml was added to buffer or plasma and centrifuged through a micropartition tube, indicating that PFA does not bind to feline plasma proteins.

Predose samples. Negative-control, predose plasma samples showed no detectable PFA concentrations.

i.v. administration. Figure 1 compares mean concentrations of PFA in plasma over time for PFA administered as a single i.v. dose to adult versus young cats. Mean PFA concentrations in plasma after an i.v. dose of 40 mg of PFA per kg were higher in adult cats than in young cats at all time points. Table 1 summarizes pharmacokinetic parameters for PFA administered as a single i.v. bolus to adult and young cats. Mean values for B and $\text{AUC}_{0-\infty}$ were higher in adult cats (68.3 $\mu\text{g/ml}$ and 11.1 $\mu\text{g} \cdot \text{min/ml}$ [10^3], respectively) than in young cats (26.7 $\mu\text{g/ml}$ and 5.4 $\mu\text{g} \cdot \text{min/ml}$ [10^3], respectively) ($P < 0.05$). On the other hand, mean plasma clearance was higher in young cats (7.52 ml/min per kg) than in adult cats (3.70 ml/min per kg) ($P < 0.05$). Median plasma half-lives were 85.4 and 71.6 min in adult and young cats, respectively.

Oral administration. Figure 2 shows the mean concentrations of PFA in plasma over time for 400 mg of PFA per kg administered to young cats as a single oral dose. Pharmacokinetic parameters for PFA given orally were as follows: $\text{AUC}_{0-\infty}$, $1.83 \pm 0.57 \mu\text{g} \cdot \text{min/ml}$ (10^3); maximum concentration in serum, $51.9 \pm 10.3 \mu\text{g/ml}$; time to maximum concentration in serum, $135 \pm 98.4 \text{ min}$; and oral bioavailability, $35 \pm 14\%$ (all values are means $\pm$ standard deviations).

DISCUSSION

Since the discovery of human immunodeficiency virus as the etiologic agent of AIDS (3, 8), the search for effective antiretrovirus compounds has accelerated. PFA inhibits a variety of animal and human retroviruses in vitro (14, 20, 21, 23, 28–30, 36–38) and is currently being evaluated in phase I trials for treatment of AIDS (1, 7, 9, 17, 21). In addition to having antiretrovirus activity, PFA is effective against herpes simplex virus type 2 and cytomegalovirus infections (2,

6, 15, 17, 18, 21, 24, 28). This is an important consideration, because herpesvirus infections are frequently encountered in AIDS patients (31).

This study was aimed at the characterization of age-related differences in the pharmacokinetics of PFA using an animal model. The feline model was chosen because it allowed collection of serial blood samples and because of its relative low cost and its susceptibility to retrovirus infection (26). PFA reportedly has no metabolites and is principally excreted in urine (15). Mean oral bioavailability of PFA in young cats was 35%, indicating that oral dosing may be feasible. Oral bioavailability varies with species, with values of 10% for dogs and 95% for rabbits (21, 25). Means for maximum concentration of drug in serum and time to maximum concentration of drug in serum for a single oral dose of PFA (400 mg/kg) were 51.9 $\mu\text{g/ml}$ and 135 min, respectively. Variations between animals in times to maximum concentration of drug in serum may have resulted from differences in gastric emptying. Six human immunodeficiency virus-positive patients showed oral PFA absorption of 12 to 22% (mean, 17.9%) (34). PFA clearance from plasma was twofold higher in young cats than in adult cats, and median plasma PFA half-lives for the two age groups were similar (Table 1). Mean plasma PFA clearance values for young and adult cats were 7.52 and 3.70 ml/min per kg, respectively. Because PFA clearance for adult cats exceeded the glomerular filtration rate ($2.94 \pm 0.32 \text{ ml/min per kg}$) (27) and there are no known metabolites, it is assumed that PFA is rapidly distributed to bone, where it is sequestered or slowly eliminated or both. Similar effects have been reported for other species (15, 20) and in humans, in which cases up to 20% of available PFA was not excreted in the urine 7 days after the end of PFA administration (33).

Differences in clearance between adult and young cats may be due to dissimilarities in renal clearance or to greater uptake of PFA by bone tissue of young animals, in which there is bone growth in addition to remodeling. Interestingly, in a separate study of young cats treated with high doses of PFA (1,000 mg/kg per day) for 14 days (C. L. Swenson, S. E. Weisbrode, L. A. Nagode, K. A. Hayes, C. L. Steinmeyer, and L. E. Mathes, *Calcif. Tissue Int.*, in press), we have documented rickets-like bone lesions characterized by widened growth plates, increased osteoid, and failure of mineralization. Helgstrand et al. report that approximately 30% of the available PFA accumulates in the bones of young mice, while less than 10% accumulates in the bones of adult mice (15). Similar effects may occur in cats, contributing to bone lesions. Histologically, renal tubules and glomeruli of 10- to 14-week-old cats are not fully developed, suggesting that greater renal clearance due to decreased reabsorption may be a factor in younger animals. Additional studies are necessary to clarify this point. Our data indicate that higher dose rates of PFA would be required to achieve concentrations in plasma in young animals equal to those in adult animals. Since PFA is currently in phase I trials for AIDS, this could have important implications for pediatric AIDS patients.

ACKNOWLEDGMENTS

We acknowledge the support provided by The Center for Retrovirus Research and The Comprehensive Cancer Center of The Ohio State University in the performance of this study. The project has been funded, at least in part, by Public Health Service contract NO1-AI-62525 from the Developmental Therapeutics Branch, AIDS Program, National Institute of Allergy and Infectious Diseases.

PFA was kindly provided by Astra Läkemedel AB, Södertälje, Sweden.

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