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Oral, Subcutaneous, and Intramuscular Bioavailabilities of the Antiviral Nucleotide Analog 9-(2-Phosphonylmethoxyethyl) Adenine in Cynomolgus Monkeys

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Intravenous, subcutaneous, intramuscular, and oral pharmacokinetics of the antiretroviral nucleotide analog [9-(2-phosphonylmethoxyethyl)adenine] (PMEA) were examined in a crossover study with four cynomolgus monkeys using ^{14}C-labelled drug at 10 mg/kg of body weight (20 μCi/kg). Plasma radioactivity declined biexponentially following intravenous administration. Radiochromatography of plasma revealed an absence of PMEA metabolites. Intramuscular and subcutaneous bioavailabilities of PMEA were (means ± standard deviation) 126% ± 30% and 101% ± 25%, respectively, supporting the clinical utility of these routes. The oral bioavailability of PMEA in this species (4.0% ± 1.0%) appeared to be limited by intestinal permeability and is likely to be equally low in humans.

9-(2-Phosphonylmethoxyethyl)adenine (PMEA) (Fig. 1) is an acyclic nucleotide analog with potent and selective antiretroviral activity in vitro and in vivo (3). PMEA is currently being evaluated in clinical trials with patients infected with human immunodeficiency virus. Alternative drugs approved for clinical therapy of human immunodeficiency virus infection, such as the nucleoside analogs 3′-azido-2′,3′-dideoxythymidine (zidovudine) (7), 2′,3′-dideoxyinosine (ddI) (9), 2′,3′-dideoxycytidine (ddC) (11), and 2′,3′-didehydro-3′-deoxythymidine (d4T) (8) are orally available and hence are compatible with chronic therapy. However, the oral bioavailabilities of zidovudine and ddI are limited by metabolic transformation, and systemic exposure to these drugs varies with the route of administration. In contrast, PMEA appears to be metabolically stable (2, 12, 14), and hence the route of administration would not be expected to affect its bioavailability in vivo.

The pharmacokinetics of intravenous PMEA have been examined in mice (12) at 25 to 500 mg/kg of body weight by using high-performance liquid chromatography (HPLC) with fluorescence derivatization and in rhesus monkeys (4) at 250 mg/kg by using HPLC with UV detection. Levels of PMEA in plasma displayed a monoexponential decline in mice, while a biexponential decline was observed in rhesus monkeys, with a terminal half-life of 2.0 h. The reported clearance value in rhesus monkeys (1.6 liter/h/kg) was calculated incorrectly from absolute dose. The true mean total body clearance (CL_{tot}) was 0.56 liter/h/kg and was significantly less than the value reported for a 25-mg/kg dose in the same species (5). Extravascular pharmacokinetics of PMEA have been examined in two species. Intramuscular bioavailability was 160% in the rhesus monkey (4), while oral bioavailability in this species was less than 1%. Oral bioavailability of PMEA in rats (14) was 7.8% at a 30-mg/kg dose, on the basis of urinary recovery of intact PMEA determined by HPLC with fluorescence derivatization. PMEA was excreted primarily by renal clearance in mice and rats, and the localization of PMEA in the kidney of the rhesus monkey suggests a similar fate.

In the present study, the pharmacokinetics of intravenous, intramuscular, subcutaneous and oral PMEA were compared following administration of radiolabelled drug to cynomolgus monkeys, using a dose (10 mg/kg) closer to the anticipated clinical range. The study was intended to resolve the apparent discrepancies in literature reports of PMEA pharmacokinetics and to provide a solid rationale for the use of the subcutaneous or intramuscular route in the clinic.

PMEA (lot 1965-BL-3) was obtained from Raylo Chemicals (Edmonton, Canada). [^{14}C-8-adenine]PMEA (lot 102-012-053; specific activity, 53 mCi/mmol) was obtained from Moravek Biochemicals (Brea, Calif.). Two male and two female cynomolgus monkeys (*Macaca fascicularis*) weighing 3.7 ± 1.5 kg (mean ± standard deviation [SD]) were used for the study. Monkeys were provided food (Purina Monkey Chow 5038) and water ad libitum during the course of the study. Intramuscular ketamine HCl was used as needed to sedate animals during administration or sample collection. No data are available on any potential pharmacokinetic interaction of PMEA with ketamine. However, there are no known pharmacokinetic interactions of ketamine with clinically used drugs (20), and ketamine has been shown to have no hemodynamic effect in the cynomolgus monkey (19). Each animal received 10 mg of PMEA per kg (20 μCi of [^{14}C]PMEA per kg) by four routes in a four-way crossover study design with a 2-week washout period between doses. The drug was administered by intravenous, subcutaneous, and intramuscular administration, and by oral gavage. PMEA was injected intravenously as a bolus over less than 30 s. Blood samples (1 to 2 ml) were withdrawn at 5, 15, and 30 min and at 1, 2, 4, 6, 12, 24, 36, 48, 72, and 96 h after administration and collected into heparinized tubes. All blood samples were processed for plasma immediately, and aliquots (0.1 ml) were oxidized with a Tri-Carb sample oxidizer (Packard, Meriden, Conn.). Total ^{14}C was determined in oxidized samples by scintillation counting. No quenching was observed over the range of 200 to 100,000 dpm/ml. The limit of quantitation was 40 ng/ml of PMEA (200 dpm/ml). Individual samples were analyzed by HPLC with radioactive flow detection on a system designed to separate PMEA from potential metabolites, including the phosphorylated form PMEAp (details of the chromatographic system are shown in Fig. 3).

The pharmacokinetic parameters for PMEA were assessed by noncompartmental methods with statistical moment analy-

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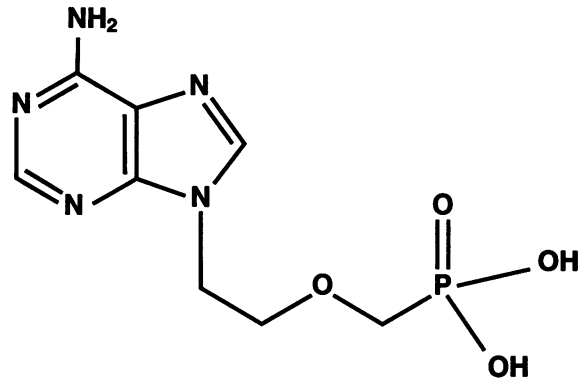

FIG. 1. Structure of PMEA.

sis (21) or compartmental methods using the commercial nonlinear curve-fitting software packages ULTRAFIT (6) and PCNONLIN (18). For noncompartmental analysis, the area under the plasma concentration-time curve up to the time of the last quantifiable concentration ($AUC_{0-t_{last}}$) was calculated by the linear trapezoidal method, and the slope of the terminal elimination phase (k_e) was estimated by linear regression. The value of AUC extrapolated to infinity ($AUC_{0-\infty}$) was calculated as $AUC_{0-t_{last}} + C_{last}/k_e$, where C_{last} is the last quantifiable concentration in plasma. The area under the first moment of the concentration-versus-time curve up to the time of the last quantifiable concentration ($AUMC_{0-t_{last}}$) was calculated for noncompartmental analysis by the linear trapezoidal method. The value of AUMC extrapolated to infinity ($AUMC_{0-\infty}$) was calculated as $AUMC_{0-t_{last}} + t_{last} C_{last}/k_e + C_{last}/k_e^2$. The mean residence time (MRT) was calculated as $AUMC_{0-\infty}/AUC_{0-\infty}$, and the CL_{tot} was calculated as dose/$AUC_{0-\infty}$. The steady-state volume of distribution (V_{ss}) was calculated as $CL_{tot}MRT$. The half-life of the terminal elimination phase was calculated as $0.693/k_e$. The maximum drug concentration (C_{max}) and time to C_{max} were obtained by visual inspection. The mean absorption time for subcutaneous, intramuscular, and oral routes was defined as the difference between the MRT determined for these routes and that determined following intravenous injection ($MRT_{route} - MRT_{intravenous}$). Bioavailability for the subcutaneous route was calculated as $100(AUC_{0-\infty\ subcutaneous}/AUC_{0-\infty\ intravenous})$ for individual monkeys. Bioavailability for the intramuscular route was calculated in a similar manner. A clear elimination phase was not discernible following oral administration, and bioavailability for this route was calculated by using AUC values extrapolated to 6 h.

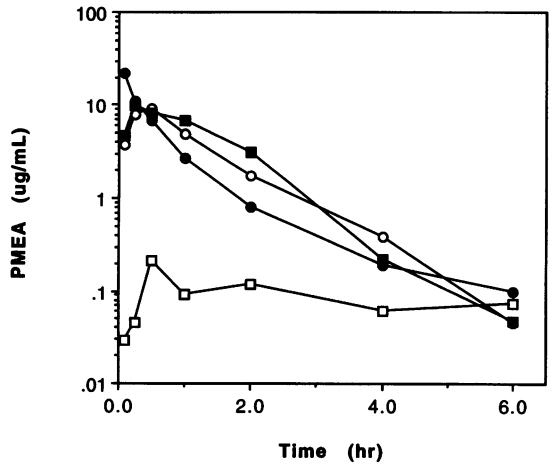

FIG. 2. [^{14}C]PMEA in plasma following administration to cynomolgus monkeys at 10 mg/kg (●, intravenous injection; ○, subcutaneous injection; ■, intramuscular injection; □, oral gavage).

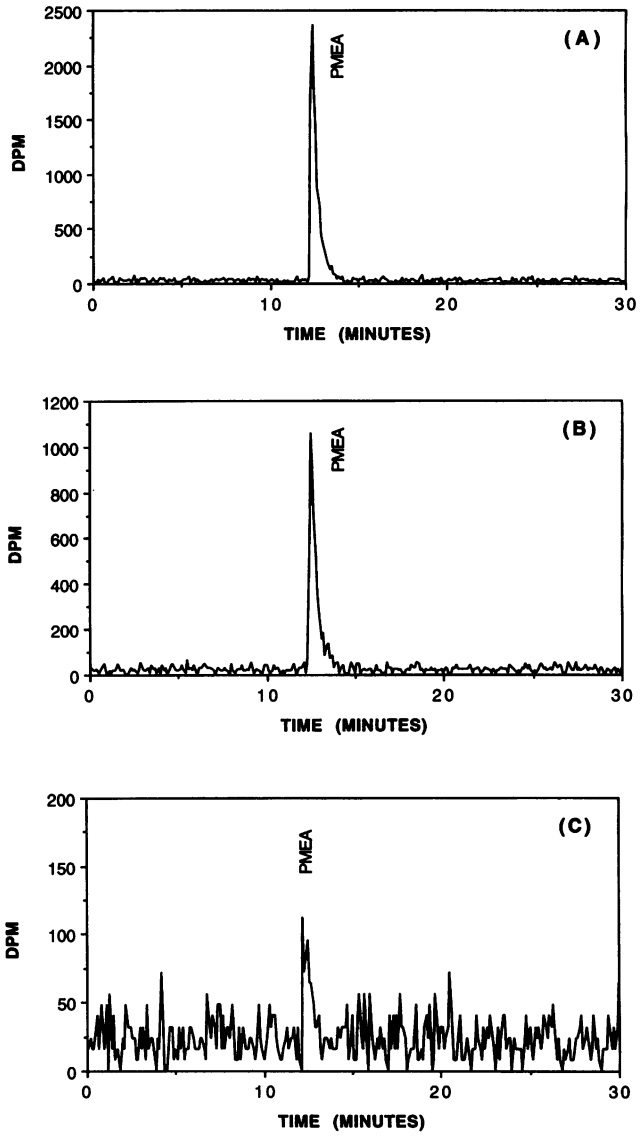

FIG. 3. Radiochromatograms of plasma samples obtained following intravenous administration of [^{14}C]PMEA. (A) [^{14}C]PMEA standard; (B) plasma at 5 min postdose; (C) plasma at 1 h postdose. Radiochromatography was performed by direct injection of plasma (50 to 200 μl) onto a Vydac C18 column (5-μm pore size, 250 by 4.6 mm; The Separation Group, Hesperia, Calif.). The mobile phase was initially isocratic 25 mM potassium phosphate buffer, pH 6.0, for 5 min, followed by a linear gradient to 65% acetonitrile in 25 mM potassium phosphate buffer, pH 6.0, over 15 min. Detection was by ^{14}C radioactivity using a Radiomatic Flo/One Beta model 525 (Packard, Meriden, Conn.). PMEAp elutes at 15 min on this system.

Compartmental analysis was performed by nonlinear curve fitting using a biexponential model. A biexponential model was selected on the basis of the model discrimination criteria of Akaike (1) and Schwartz (15), together with examination of

TABLE 1. Pharmacokinetic parameters following intravenous administration of [^{14}C-8-adenine]PMEA at 10 mg/kg[a]

Type of analysis	$AUC_{0-\infty}$ (mg · h/ml)	$AUMC_{0-\infty}$ (mg · h2/ml)	MRT (h)	CL_{tot} (liters/h/kg)	V_{ss} (liters/kg)	k_e (h^{-1})	Terminal $t_{1/2}$[b] (h)
Noncompartmental	11.5 ± 3.6	9.8 ± 2.6	0.87 ± 0.11	0.93 ± 0.27	0.81 ± 0.30	0.69 ± 0.06	1.0 ± 0.1
Biexponential	11.5 ± 3.5	8.7 ± 3.1	0.75 ± 0.07	0.94 ± 0.27	0.69 ± 0.18	0.70 ± 0.06	1.0 ± 0.1

[a] Values are means ± SD for four animals.
[b] $t_{1/2}$, half-life.

scatter plots of the weighted residual error. Data for concentration in serum were analyzed by fitting to the equation $Cp(t) = Ae^{-\alpha t} + Be^{-\beta t}$, where $Cp(t)$ is the concentration in plasma at time t, A and B are the zero time intercepts of the two exponential terms, and α and β are the rate constants of the corresponding terms. From the resulting equation parameters, the value of $AUC_{0-\infty}$ was calculated directly as $A/\alpha + B/\beta$. The corresponding biexponential value of the area under the first moment of the concentration-time curve ($AUMC_{0-\infty}$) was calculated as $A/\alpha^2 + B/\beta^2$. The mean residence time, CL_{tot}, and V_{ss} were calculated as for noncompartmental values. The slope of the terminal elimination phase was represented by β.

Mean plasma [^{14}C]PMEA levels for intravenous, subcutaneous, intramuscular, and oral administration are compared for the four routes in Fig. 2. Radiochromatography of selected plasma samples verified that all radioactivity present was attributable to unchanged [^{14}C]PMEA (Fig. 3). These data confirm earlier reports that PMEA is not readily metabolized in mice (12) or rats (14) on the basis of HPLC analysis of urine. Radioactivity levels beyond 1 h postdose were too low to detect by radiochromatography. Total [^{14}C]PMEA concentrations in plasma following intravenous administration declined in a biexponential fashion. Analysis of the data by nonlinear curve fitting gave results similar to those obtained by noncompartmental methods (Table 1). The terminal phases achieved following subcutaneous or intramuscular administration were equivalent to that observed for intravenous PMEA.

Noncompartmental pharmacokinetic parameters are compared for the subcutaneous, intramuscular, and oral routes in Table 2. The mean ± SD total body clearance of PMEA following intravenous administration was 0.84 ± 0.22 liters/h/kg. This is significantly higher than literature values for glomerular filtration rate (0.088 liters/h/kg) in cynomolgus monkeys (10), indicating that renal tubular secretion potentially plays a role in the elimination of PMEA in this species. The observation that CL_{tot} of PMEA does not exceed the predicted renal blood flow of 2.0 liters/h/kg in the cynomolgus monkey, calculated as 25% of the cardiac output (19), is further evidence of a lack of nonrenal clearance. The mean ± SD V_{ss} calculated from statistical moment analysis was 0.65 ± 0.19 liters/kg. This value is significantly smaller than the volume of distribution (1.9 liters/kg) reported previously for PMEA in rhesus monkeys (4). However, the reported value was calculated by using the intercept of the terminal exponential phase, the accuracy of which may have been limited by a relatively insensitive UV detection method.

The apparent terminal elimination half-life following intravenous injection in cynomolgus monkeys was 1.0 h, and systemic PMEA levels were undetectable beyond 12 h at this dose level (<200 dpm/ml). This relatively rapid elimination may not reflect the true duration of action of the drug, since in vitro cellular uptake studies have previously indicated that PMEA and its phosphorylated metabolites have a prolonged (>8-h) intracellular half-life (17). The same studies have shown that intracellular concentrations of PMEA achieved are approximately 1,000-fold lower than those present outside the cell. The slow efflux of a small intracellular pool of PMEA in vivo would be very difficult to detect with the current analytical methodology.

The presence of two distinct phases in the decline of PMEA plasma levels following intravenous administration agrees with observations with rhesus monkeys (4, 5) and suggests that the previous studies with mice (12) may not have had sufficient analytical power to detect the true terminal phase.

Pharmacokinetic parameters for subcutaneous and intramuscular routes were not significantly different from those following intravenous PMEA. The observed bioavailability for the subcutaneous and intramuscular routes were (means ± SD) 101% ± 25% and 126% ± 30%, respectively. The slightly greater bioavailability of intramuscular PMEA compared with that from an intravenous injection is probably a result of errors inherent in extrapolating the terminal phase. These data suggest that either route may offer a more convenient alternative to intravenous infusion in a clinical setting without significantly altering the pharmacokinetics and hence the efficacy of the drug.

The absolute oral bioavailability of PMEA (mean ± SD) was 4.0% ± 1.0%. Although this value is imprecise in the absence of a defined terminal phase, it is consistent with published observations for higher doses of PMEA in rhesus

TABLE 2. Noncompartmental pharmacokinetic parameters following administration of [^{14}C-8-adenine]PMEA at 10 mg/kg

Route[a]	$AUC_{0-\infty}$ (mg · h/ml)	$AUMC_{0-\infty}$ (mg · h2/ml)	MRT (h)	MAT[b] (h)	C_{max} (liters/kg)	T_{max} (h)	Terminal $t_{1/2}$[d] (h)	Bioavailability (%)
Subcutaneous	12.5 ± 3.25	15.0 ± 6.68	1.14 ± 0.28	0.38 ± 0.29	9.43 ± 1.72	0.44 ± 0.13	0.79 ± 0.24	101 ± 25
Intramuscular	16.5 ± 3.19	20.7 ± 6.70	1.24 ± 0.18	0.49 ± 0.20	10.1 ± 1.99	0.33 ± 0.14	0.70 ± 0.07	126 ± 30
Oral	0.53 ± 0.26[e]	1.30 ± 0.50[e]	2.72 ± 0.66	1.94 ± 0.69	0.28 ± 0.33	1.50 ± 1.29		4.0 ± 1.0

[a] Values are means ± SD for four animals for the subcutaneous and oral routes. For the intramuscular route, the values are means ± SD for three animals, since data for one animal in the intramuscular leg of the study were excluded from calculations because of inadvertent sampling from a vein in the same anatomical region as the site of injection.
[b] MAT, mean absorption time.
[c] T_{max}, time to C_{max}.
[d] $t_{1/2}$, half-life.
[e] Values are extrapolated to 6 h only.

monkeys (5) and rats (14), suggesting that the drug may have low oral bioavailability in humans. PMEA is highly water-soluble at physiological pH (>200 mg/ml) and appears refractory to metabolism in all species examined to date (12, 14). PMEA is not a substrate for deaminases isolated from bovine intestine (13). In addition, the relatively low permeability coefficient determined for PMEA diffusion through Caco-2 cell monolayers (16) would seem to support the hypothesis that oral absorption of PMEA is limited by low permeability across the intestinal epithelium rather than by metabolic degradation. A similar low oral bioavailability is likely for PMEA in humans.

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