Oral Bioavailability of the Antiretroviral Agent 9-(2-phosphonylmethoxyethyl)adenine (PMEA) from Three Formulations of the Prodrug Bis(pivaloyloxymethyl)-PMEA in Fasted Male Cynomolgus Monkeys

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The bioavailability of PMEA from three oral formulations of the prodrug bis(POM)-PMEA has been evaluated in fasted male cynomolgus monkeys. The formulations examined included a hydroxypropyl-β-cyclodextrin (HPBCD) complex, a PEG based cosolvent solution, and an aqueous suspension. Oral formulations containing ^{3}H-bis(POM)-PMEA were compared to intravenous ^{3}H-PMEA at 10.9 mg-eq/kg in a crossover study in four monkeys, with a 7 day washout period. No intact bis(POM)-PMEA or monoester were detected in plasma. Bioavailabilities of PMEA from the prodrug were 24.7 ± 6.5%, 27.3 ± 12.3% and 22.2 ± 15.6% for the HPBCD complex, PEG solution and aqueous suspension, respectively. The oral bioavailability of PMEA from bis(POM)-PMEA was not limited by dissolution rate of the prodrug. Data for the PEG cosolvent solution and suspension indicate that the prodrug could potentially be formulated as a soft gelatin capsule or a tablet.

KEY WORDS: PMEA; prodrug; oral bioavailability; formulation; monkey; antiviral.

INTRODUCTION

9-(2-phosphonylmethoxyethyl)adenine (PMEA) (Fig. 1) is an acyclic nucleotide analog with potent and selective inhibitory activity *in vitro* and *in vivo* against retrovirus replication (for a review see reference 1). The antiviral activity of intravenous PMEA is currently being evaluated in clinical trials in patients infected with human immunodeficiency virus (HIV). Alternative drugs used clinically for the treatment of HIV-infection, such as the nucleoside analog 3′-azido-2′,3′-dideoxythymidine (AZT), are orally available (2) and hence are compatible with chronic therapy. The long term success of PMEA in this patient population will likely depend on the availability of an oral formulation containing PMEA itself, or a prodrug of PMEA.

Existing pharmacokinetic data for PMEA in a number of species suggest that it will have limited oral bioavailability in humans. Studies in the rhesus monkey (3,4) have compared intravenous and oral administration at relatively high doses. Oral administration of PMEA at 250 mg/kg resulted in a bioavailability of less than 1%. Oral bioavailability of PMEA determined in the rat at a 30 mg/kg dose was 7.8%, based on ^{14}C-PMEA concentrations in plasma (5).

In a further study, the oral bioavailability of ^{14}C-PMEA was assessed in cynomolgus monkeys at a dose of 10 mg/kg, as part of a comparison of four routes of administration (6). Although bioavailabilities for the intramuscular and subcutaneous routes were essentially identical to intravenous injection, oral bioavailability of PMEA in this study was only 4.0 ± 1.0%.

The low oral bioavailability of PMEA observed in monkeys and rats appears to be a consequence of limited intestinal permeability of the phosphonate, which exists as a dianion at physiological pH (pKa values are 2 and 6.8) (7). A bis(pivaloyloxymethyl) ester prodrug of the phosphonate, bis(POM)-PMEA (Fig. 1) was prepared, with the aim of increasing the lipophilicity of the molecule and hence its ability to cross cell membranes (8). The *in vitro* activity of bis(POM)-PMEA against HSV-2 was 150 fold greater than that of PMEA itself. This enhanced activity relative to PMEA is due to increased cellular uptake and metabolism of the prodrug, resulting in elevated intracellular concentrations of PMEA (9). In addition, bis(POM)-PMEA shows 8 fold greater epithelial permeability across cultured Caco-2 intestinal cell monolayers (10), suggesting that the prodrug may have improved oral absorption *in vivo*.

Unfortunately, the comparatively low aqueous solubility of bis(POM)-PMEA limits the choice of potential oral formulations of the prodrug for clinical use. In addition to potential dissolution limitations, *in vitro* chemical and metabolic stability data suggest that this prodrug may be labile in the gastro-intestinal tract (10).

The present study was designed to compare three potential oral formulations of bis(POM)-PMEA: 1) an aqueous complex of bis(POM)-PMEA with hydroxypropyl-β-cyclodextrin (HPBCD); 2) a polyethylene glycol (PEG 400) cosolvent solution; 3) an aqueous suspension of particulate bis(POM)-PMEA. The formulations selected each represent prototypes designed to overcome specific physicochemical limitations of bis(POM)-PMEA, and each reflects a significantly different formulation strategy. Formulations were evaluated in the cynomolgus monkey, since extensive pharmacokinetic (6) and toxicological data were already available for PMEA in this species.

MATERIALS AND METHODS

Materials

PMEA (lot #1965-BL-3) was synthesized by Raylo Chemicals (Edmonton, Canada). Bis(POM)-PMEA (lot #477-24-33) was synthesized by Gilead Sciences using published procedures (8). [adenine-2,8-^{3}H]-PMEA (lot #104-083-011) (specific activity 11 Ci/mmol, radiochemical purity 99.6%) and [8-^{3}H]-Bis(POM)-PMEA (lot #111-102-020) (specific activity 20 Ci/mmol, radiochemical purity 98.3%) were prepared by catalytic hydrogen exchange by Moravek (Brea, CA). For the suspension formulation, [8-^{3}H]-Bis(POM)-

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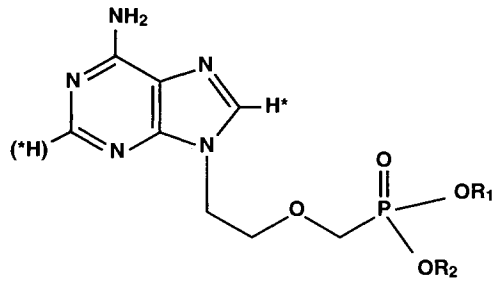

Fig. 1. Structure of bis(POM)-PMEA (R_1 = R_2 = $CH_2OCOC(CH_3)_3$), mono(POM)-PMEA (R_1 = $CH_2OCOC(CH_3)_3$; R_2 = H) and PMEA (R_1 = R_2 = H).

PMEA was recrystallized in the presence of unlabelled bis-(POM)-PMEA to a specific activity 2 mCi/g (lot #112-034-002, radiochemical purity 95.3%) by Moravek. Hydroxypropyl-β-cyclodextrin (HPBCD) (MW 1500) and trifluoroacetic acid were obtained from Aldrich (Milwaukee, WI). Polyethylene glycol 400 (PEG 400), benzyl alcohol, propylene glycol and polysorbate 20 were obtained from Spectrum (Gardena, CA). Glycerin (99.5%) was obtained from Humco (Texarkana, TX). Sodium carboxymethylcellulose was obtained from Aquolon (Wilmington, DE). Tetrabutylammonium hydrogen phosphate (TBAHP) was obtained from Eastman Kodak (Rochester, NY). Acetonitrile was obtained from Baxter (Muskegon, MI). Dibasic potassium phosphate, monobasic potassium phosphate and sodium chloride were obtained from Mallinkrodt (St. Louis, MO).

Formulations

PMEA Intravenous Formulation

Tritiated PMEA (0.5 mCi/ml, specific activity 11 Ci/mmol) was evaporated under reduced pressure to remove ethanol and reconstituted in 2 ml of water for injection to a final concentration of 2 mCi/ml. Unlabelled PMEA (1.52 g) was dissolved in 20 ml of water for injection by adjusting pH to 6.5 with 6 N sodium hydroxide, and diluted to a PMEA concentration of 60 mg/ml with water for injection. Unlabelled PMEA solution (16.12 ml) and ^{3}H-PMEA solution (1.88 ml) were then mixed and filtered into sterile vials using a sterile 0.2 μm Acrodisc 13 filter (Gelman, Ann Arbor, MI). The resulting formulation comprised an isotonic solution of PMEA for intravenous administration, containing 54.5 mg/ml PMEA and 200 μCi/ml ^{3}H-PMEA. The final dose was 0.2 ml/kg (10.9 mg/kg PMEA, 40 μCi/kg ^{3}H-PMEA).

Oral Bis(POM)-PMEA Hydroxypropyl-β-cyclodextrin Complex

Hydroxypropyl-β-cyclodextrin (103.5 g) was dissolved in 103.5 ml of water for injection and filtered using a 0.45 μm Nalgene filter unit (Nalgene Co., Rochester, NY). Benzyl alcohol (1.8 g) was added, and an aliquot (58 ml) of the resulting solution was mixed with 1.5 g of unlabelled bis-(POM)-PMEA and 2 ml of ^{3}H-bis(POM)-PMEA (1.5 mCi/ml, specific activity 20 Ci/mmol) until all prodrug was dissolved. The final formulation comprised 25 mg/ml bis(POM)-PMEA and 50 μCi/ml ^{3}H-bis(POM)-PMEA in 50% w/w cyclodextrin, with 0.9% benzyl alcohol as preservative. The dose was 0.8 ml/kg (20 mg/kg bis(POM)-PMEA, 40 μCi/kg ^{3}H-bis(POM)-PMEA) by oral gavage.

Oral Bis(POM)-PMEA PEG Cosolvent Solution

Polysorbate 20 (1 g), glycerin (4 g), propylene glycol (5 g) and benzyl alcohol (0.9 g) were mixed and diluted to 100 g with polyethylene glycol 400 (PEG 400). An aliquot of the resulting cosolvent mixture (58 ml) was mixed with 1.5 g of unlabelled bis(POM)-PMEA and 2 ml of ^{3}H-bis(POM)-PMEA (1.5 mCi/ml, specific activity 20 Ci/mmol) until all prodrug was dissolved. The final formulation comprised a solution of 25 mg/ml bis(POM)-PMEA and 50 μCi/ml ^{3}H-bis(POM)-PMEA in a PEG 400 cosolvent mixture. The dose was 0.8 ml/kg (20 mg/kg bis(POM)-PMEA, 40 μCi/kg ^{3}H-bis(POM)-PMEA) by oral gavage.

Oral Bis(POM)-PMEA Aqueous Suspension

Sodium carboxymethylcellulose (1 g) was dissolved in 120 ml of water for injection by heating to 40–50°C for 2 hours. To this solution was added sodium chloride (1.8 g), benzyl alcohol (1.8 g) and polysorbate 20 (1.0 g), and the mixture diluted to 200 ml with water for injection. An aliquot of this suspension vehicle (76 ml) was mixed with 1.9 g of recrystallized ^{3}H-bis(POM)-PMEA (lot #112-034-002, specific activity 2 mCi/g). The mixture was triturated in a mortar and pestle until the prodrug was uniformly suspended in the vehicle. Any remaining agglomeration was removed by sonicating the suspension for 20 minutes in a sonicator bath (Baxter, Muskegon, MI). The resulting formulation comprised 25 mg/ml bis(POM)-PMEA and 50 μCi/ml ^{3}H-bis(POM)-PMEA in a carboxymethylcellulose based aqueous suspension. The final dose was 0.8 ml/kg (20 mg/kg bis-(POM)-PMEA, 40 μCi/kg ^{3}H-bis(POM)-PMEA) by oral gavage.

Animals

Four adult male cynomolgus monkeys (*Macaca fascicularis*) were used for the study. The mean body weight at the time of the first dose was 3.4 ± 0.6 kg. Animals were fasted for 12 hours prior to dosing. All animals were sedated with intramuscular ketamine hydrochloride (20 mg/kg) prior to dosing, and as needed throughout the initial 4 hours of sampling. Food (Purina Monkey Chow) and water were provided *ad lib*. Following recovery from sedation.

Study Design

This study was a four-way crossover comparison of one intravenous formulation of PMEA and three oral formulations of bis(POM)-PMEA in four monkeys. Each formulation was administered as a single dose to one monkey at each of four dosing periods. The required volume of dosing solution was calculated based on the body weight determined prior to each administration. Individual vials of formulation were provided for each animal. The intravenous formulation was administered via a saphenous vein, followed by 1.0 ml sterile phosphate buffered saline. Oral formulations were administered by gavage, followed by 30 ml water. A one week washout period was allowed between administrations.

Sample Collection

Following oral dosing, blood samples (1.0 ml) were collected from the saphenous vein of each animal into heparinized tubes at 0 (pre-dose), 15 and 30 minutes and 1, 2, 3, 4, 6, 8, 12 and 24 hours post-dose. Following intravenous dosing, blood samples (1.0 ml) were collected from the opposing saphenous vein of each animal into heparinized tubes at 0 (pre-dose), 5, 15, 30 and 45 minutes and 1, 2, 4, 6, 8, 12 and 24 hours post-dose. Blood was processed immediately for plasma by centrifugation at 2000 rpm for 10 minutes. The RBC/plasma ratios at 37°C for PMEA and bis(POM)-PMEA were estimated as 0.49 and 1.07, respectively, using the corresponding radiolabelled materials. Plasma samples were frozen and maintained at −20°C until analyzed.

Determination of Radioactivity in Plasma

Total Radioactivity

Total radioactivity in plasma samples was determined by scintillation counting. Aliquots (200 µl) of each plasma sample were mixed with 20 ml ReadiSafe scintillation cocktail (Beckman, Fullerton, CA). Samples were counted for five minutes on a Packard TriCarb Model 2500 TR scintillation counter (Packard, Meriden, CT) and corrected for counting efficiency using appropriate standards.

Radiochromatographic Analysis

The radiochemical composition of selected plasma samples was determined by extraction and reverse phase HPLC using radioactive flow detection. For samples obtained following intravenous administration of PMEA, plasma (100 µl) was centrifuged though a 10,000 MW cutoff filter (Millipore, Bedford, MA). The supernatant was directly injected into the HPLC. For samples obtained following oral administration of bis(POM)-PMEA formulations, plasma (350 µl) was mixed with 700 µl 0.1% trifluoroacetic acid in acetonitrile to denature protein. Samples were then evaporated to dryness under reduced pressure at room temperature (Savant Speed-Vac). Dried samples were reconstituted in 100 µl Mobile Phase A (see below), vortexed, and centrifuged for 5 minutes at 14,000 RPM in an Model 5402 centrifuge (Eppendorf, Fremont, CA). Supernatant was then transferred to autoinjector vials for HPLC analysis. The HPLC system comprised a Model P4000 solvent delivery system with a Model AS3000 autoinjector and a Model UV1000 UV detector (Spectra Physics, San Jose, CA). The column was a Vydac C18 (5 µm, 250 × 4.6 mm) (The Separation Group, Hesperia, CA) equipped with a Brownlee RP-18 Newguard guard column (7 µm, 15 × 3.2 mm) (Alltech, Deerfield, IL). The mobile phases used were: A, 2% acetonitrile in 25 mM potassium phosphate buffer, pH 6.0; B, 65% acetronitrile in 25 mM potassium phosphate buffer, pH 6.0. The flow rate was 1.0 ml/min and the column temperature was maintained at 35°C by a column oven. The gradient profile was 100% A until 2.0 min, then a linear gradient to 100% by 13.0 minutes, returning immediately to 100% A. Detection was by UV absorbance at 262 nm and tritium using a Model Flo-one Beta radioactive flow detection system (Packard, Meriden, CT). The injection volume was 50 µl. Data was acquired and stored by a PeakPro data acquisition system (Beckman, Palo Alto, CA).

PMEA concentrations determined by tritium counting were further confirmed by analysis of selected samples using a published HPLC method involving fluorescence derivatization of the drug (5).

Formulation Analysis

All formulations were analyzed for final concentrations of PMEA or bis(POM)-PMEA using the analytical methods described above. Radiochemical purity and content was confirmed by HPLC using radioactive flow detection.

Pharmacokinetic Calculations

The pharmacokinetic parameters for intravenous PMEA and the three oral formulations of bis(POM)-PMEA were calculated using noncompartmental methods. The area under the serum concentration time curve up to the 12 hour time point, AUC_{0-12}, was calculated by the linear trapezoidal method. Where possible, the slope of the terminal elimination phase (ke) was estimated by linear regression. The total plasma clearance (CL_{tot}) was calculated as Dose/AUC_{0-12}. The half-life of the terminal elimination phase ($t_{1/2}$) was calculated were possible as $0.693/k_e$. The maximum plasma concentration (C_{max}) and time to C_{max} (T_{max}) were determined by inspection.

RESULTS AND DISCUSSION

Radioactivity in Plasma

Mean plasma ^{3}H-PMEA concentration vs. time curves are presented in Fig. 2 for the intravenous ^{3}H-PMEA formulation and the three oral formulations of ^{3}H-bis(POM)-PMEA. Concentrations are expressed in µg-equivalents/ml, calculated using the relevant specific activity of each radiolabelled material administered and the tritium counting effi-

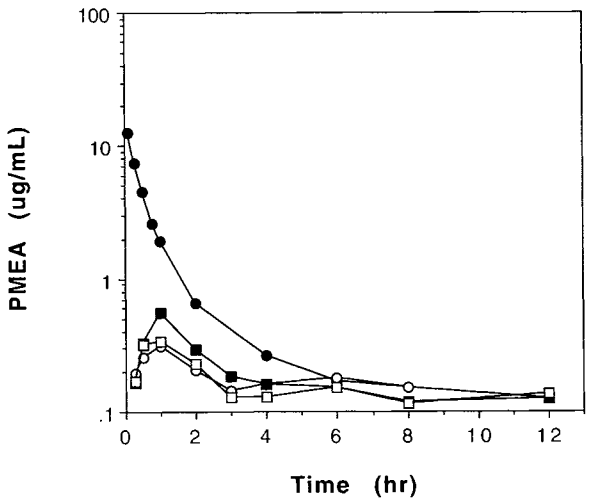

Fig. 2. Mean PMEA concentrations in plasma following administration of intravenous ^{3}H-PMEA and three oral formulations of ^{3}H-bis(POM)-PMEA to cynomolgus monkeys (n = 4). (●, intravenous PMEA; ○, oral HPBCD complex; ■, oral PEG solution; □ aqueous suspension).

ciency of the liquid scintillation counter under the conditions used (63%).

The data for intravenous PMEA suggest the possible presence of a prolonged elimination phase. However, such a phase would be very difficult to verify in a region where the plasma concentrations approach the limit of quantitation. Representative plasma samples obtained at the peak of the plasma concentration time curves after administration of each formulation were extracted and analyzed by HPLC with radioactive flow detection. The extraction conditions were designed to allow determination of bis(POM)-PMEA, the corresponding monoester (mono(POM)-PMEA) (Figure 1) and PMEA itself. Despite the relatively low levels of radioactivity present, radiochromatography was able to demonstrate that all counts present were attributable to ^{3}H-PMEA (retention time 4.3 min). Bis(POM)-PMEA (retention time 15.2 min) and mono(POM)-PMEA (retention time 9.8 min) were not detected in any of the plasma samples examined, suggesting that the absorbed prodrug is essentially subject to complete first pass metabolic cleavage in the intestine or liver. PMEA itself is not subject to first pass metabolism and is recovered intact in the urine of mice (12) and rats (13). The small proportion of PMEA that penetrates cells is phosphorylated to the active form (14). No phosphorylated metabolites of PMEA have been observed in the systemic circulation. This is probably a result of low membrane permeability and the presence of extracellular phosphatases.

Initial plasma radioactivity at the pre-dose time point for each dosing period showed a measurable increase over the four weeks of the study. In addition, all three oral formulations gave rise to increasing plasma radioactivity levels during the final 12 hours of sampling. Radiochromatography of the pre-dose plasma samples and those obtained in the terminal phase of the plasma concentration profile (12–24 hr) revealed the presence of radioactivity not associated with PMEA. Analysis of the same samples using a fluorescence derivatization method confirmed that the observed increase in background radioactivity over the course of the study was not due to PMEA accumulation. It is likely that the source of this radioactivity was low level tritium exchange with water, leading to prolonged tritium elimination. The decomposition rates reported by the manufacturer for the tritiated PMEA and bis(POM)PMEA materials employed in this study were approximately 1% per month at −20°C. To avoid any contribution of exchanged tritium to the calculated AUC values, zero time radioactive counts were subtracted from all subsequent samples for each time course obtained, and AUC values were restricted to the first 12 hours.

Pharmacokinetics

Pharmacokinetic parameters from non-compartmental analysis of plasma concentration data obtained following intravenous administration of PMEA are summarized in Table I. The observed mean AUC(0–12 hr) value was 8.66 ± 2.12 μg-eq.hr/ml. The total plasma clearance of PMEA was 1.4 ± 0.4 L/hr/kg, which is similar to that observed previously (0.9 ± 0.3 L/hr/kg) for ^{14}C-PMEA in the same species (6).

Noncompartmental pharmacokinetic data for the three oral formulations of bis(POM)-PMEA are also summarized in Table I. The observed Cmax values for HPBCD complex, PEG solution and suspension were not significantly different, based on one way ANOVA ($P > 0.05$). The longer mean Tmax for the HPBCD complex was due to an unusual delay in the absorption of prodrug from this formulation in only one animal, and was not significantly different from the other formulations. The mean bioavailabilities of PMEA from bis(POM)PMEA for the HPBCD complex, PEG solution and suspension were not significantly different ($P > 0.05$).

HPBCD forms inclusion complexes with hydrophobic molecules to effectively achieve a molecular dispersion of the drug (11). As such, prodrug within the HPBCD complex does not need to undergo dissolution before it becomes available for absorption. In the absence of a dissolution barrier, the less than complete absorption of bis(POM)-PMEA seen for the HPBCD complex is probably a result of intestinal instability and limited intestinal permeability. However, our data cannot rule out a high first pass extraction of the prodrug by the liver.

The present study has established that bis(POM)-PMEA can be formulated to deliver an acceptable dose of PMEA by the oral route. The PEG cosolvent solution formulation is compatible with commercial soft gelatin capsule technology, while data for the suspension formulation suggest that an optimized tablet/capsule with a controlled drug particle size and carefully selected excipients may offer a viable alternative. The choice of formulation is apparently not limited by the relatively low solubility of the prodrug.

Bis(POM)-PMEA does not reach the systemic circulation intact. This is probably a consequence of cleavage of the prodrug during transit across the intestinal epithelium, or during passage through the liver, as suggested by the apparent enzymatic cleavage of bis(POM)-PMEA in vitro in human intestinal and liver homogenates (10). Oral bioavailability of PMEA from bis(POM)-PMEA is more likely limited by hydrolytic stability of the prodrug in the intestinal environment.

Table I. Mean (SD) Non-compartmental Pharmacokinetic Parameters For Intravenous ^{3}H-PMEA and Oral ^{3}H-Bis(POM)PMEA Formulations at 10.9 mg-eq/kg.

Parameter	Intravenous PMEA	Bis(POM)PMEA Oral Formulations		
		HPBCD Complex	PEG Cosolvent Solution	Aqueous Suspension
AUC_{0-12} (μg-eq.hr/ml)	8.7 (2.1)	2.0 (0.1)	2.2 (0.6)	1.7 (0.7)
C_{max} (μg-eq/ml)	12.58 (2.23)	0.33 (0.10)	0.61 (0.21)	0.35 (0.15)
T_{max} (hr)	0.08	2.1 (2.6)	0.9 (0.3)	0.8 (0.3)
Bioavailability (%)	100	24.7 (6.5)	27.3 (12.3)	22.2 (15.6)

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REFERENCES

1. J. Balzarini, L. Naesens, P. Herdewijn, I. Rosenberg, A. Holy, R. Pauwels, M. Baba, D. G. Johns and E. De Clercq. Marked in vivo antiretrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine, a selective anti-human immunodeficiency virus agent. *Proc. Natl. Acad. Sci. U.S.A.* 86:332–336 (1989).
2. M. R. Blum, S. H. T. Liao, S. S. Good and P. de Miranda. Pharmacokinetics and bioavailability of zidovudine in humans. *Am. J. Med.* 85(Suppl. 2A):189–194 (1988).
3. J. Balzarini, L. Naesens, J. Slachmuylders, H. Niphius, I. Rosenberg, A. Holy, H. Schellekens and E. De Clercq. Potent anti-simian immunodeficiency virus (SIV) activity and pharmacokinetics of 9-(2-phosphonyl-methoxyethyl)adenine (PMEA) in rhesus monkeys. In H. Schellekens, and M. C. Horzinek (eds.), *Animal Models in AIDS,* Elsevier Science Publications, Amsterdam, 1990, pp. 131–138.
4. J. Balzarini, L. Naesens, J. Slachmuylders, H. Niphius, I. Rosenberg, A. Holy, H. Schellenkens and E. De Clercq. 9-(2-phosphonylmethoxyethyl)adenine (PMEA) effectively inhibits retrovirus replication in vitro and simian immunodeficiency virus (SIV) infection in rhesus monkeys, *AIDS* 5:21–28 (1991).
5. J. E. Starrett, M. M. Mansuri, J. C. Martin, D. R. Tortolani and J. J. Bronson. April 1992. European Patent 481,214,A1.
6. K. C. Cundy, and W. A. Lee. Oral, subcutaneous and intramuscular bioavailability of the antiviral nucleotide analog 9-(2-phosphonylmethoxy-ethyl)adenine (PMEA) in cynomolgus monkeys. *Antimicrob. Agents Chemother.* 38:365–368.
7. G. Palu, S. Stefanelli, M. Rassu, C. Parolin, J. Balzarini and E. De Clercq. Cellular Uptake of Phosphonylmethoxyalkylpurine Derivatives, *Antiviral Res.* 16:115–119 (1991).
8. J. E. Starrett, D. R. Tortolani, M. J. M. Hitchcock, J. C. Martin and M. M. Mansuri. Synthesis and *in vitro* evaluation of a phosphonate prodrug: bis(pivaloyloxymethyl)-9-(2-phosphonylmethoxyethyl)adenine, *Antiviral Res.* 19:267–273 (1992).
9. J. Balzarini, Z. Hao, P. Herdewijn, D. G. Johns and E. De Clercq. Intracellular metabolism and antiretrovirus action of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) a potent anti-HIV compound. *Proc. Nat. Acad. Sci. U.S.A.* 88:1499–1503 (1991).
10. J. P. Shaw and K. C. Cundy. Biological screens of PMEA prodrugs. *Pharm Res.* 10(Suppl.):S294 (1993).
11. K. Uekama and M. Otagiri. Cyclodextrins in drug carrier systems. *Crit. Rev. Ther. Drug Carrier Syst.* 3:1–40 (1987).
12. L. Naesens, J. Balzarini and E. De Clercq. Pharmacokinetics in mice of the antiretrovirus agent 9-(2-phosphonylmethoxyethyl)adenine. *Drug Metab. and Disposit.* 20:747–752 (1992).
13. J. W. Russell, D. Marrero, V. J. Whiterock, L. J. Klunk and J. E. Starrett. Determination of 9-[(2-phosphonylmethoxy)ethyl]adenine in rat urine by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. (Netherlands)* 572:321–326 (1991).