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9-[2-(Phosphonomethoxy)Propyl]Adenine Therapy of Established Simian Immunodeficiency Virus Infection in Infant Rhesus Macaques

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The long-term therapeutic and toxic effects of 9-[2-(phosphonomethoxy)propyl]adenine (PMPA) were evaluated in simian immunodeficiency virus (SIV)-infected newborn rhesus macaques. Four untreated SIV-infected newborn macaques developed persistently high levels of viremia, and three of the four animals had rapidly fatal disease within 3 months. In contrast, long-term PMPA treatment of four newborn macaques starting 3 weeks after virus inoculation resulted in a rapid, pronounced, and persistent reduction of viremia in three of the four animals. Emergence of virus with fivefold-decreased susceptibility to PMPA occurred in all four PMPA-treated animals and was associated with the development of a lysine-to-arginine substitution at amino acid 65 (K65R mutation) and additional mutations in the reverse transcriptase; however, the clinical implications of this low-level drug resistance are unclear. No toxic side effects have been seen, and all PMPA-treated animals have remained disease-free for more than 13 months. Our data suggest that PMPA holds much promise for the treatment of human immunodeficiency virus-infected human infants and adults.

More-effective and safer antiretroviral drugs are urgently needed to combat human immunodeficiency virus (HIV) infection and AIDS in humans. It has previously been shown that simian immunodeficiency virus (SIV)-infected newborn macaques are an excellent animal model for evaluating the therapeutic potential of antiviral drugs (29, 30). Newborn macaques infected with uncloned SIVmac251 develop persistently high levels of viremia, weak antiviral immune responses, and rapid disease progression, and most animals die within 3 months (17, 21, 31). In contrast, early 3′-azido-3′-deoxythymidine (zidovudine) (AZT) treatment resulted in decreased virus levels, enhanced antiviral immune responses, and delayed disease progression, with mild bone marrow toxicity and hematological changes (30). However, the emergence of AZT-resistant SIVmac mutants (with >100-fold resistance) ultimately limited the benefits of AZT therapy (27, 30). Although AZT proved to be far from ideal, these earlier studies established the usefulness of the SIV-infected newborn macaque model for evaluating antiviral drugs for efficacy, safety, and drug resistance. Information obtained with this pediatric animal model of AIDS should apply directly to HIV infection of human infants as well as adults (1).

A class of promising compounds which inhibit viral reverse transcription are the acyclic nucleoside phosphonate analogs, such as 9-[2-(phosphonomethoxy)ethyl]adenine (PMEA) (reviewed in reference 6). Chemoprophylaxis studies with adult macaques have recently demonstrated that a related compound, 9-[2-(phosphonomethoxy)propyl]adenine (PMPA), was able to prevent SIV infection, even when started 24 h after intravenous virus inoculation (25). However, the efficacy of PMPA against established infections is unknown, as are the potential for chronic toxicity and the emergence of PMPA resistance. In the current study, we investigated the antiviral and toxic effects of chronic PMPA treatment in SIV-infected newborn rhesus macaques.

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MATERIALS AND METHODS

Animals, virus, and PMPA administration. All newborn rhesus macaques (*Macaca mulatta*) were from the type D retrovirus-free and SIV-free colony at the California Regional Primate Research Center and were hand-reared in a primate nursery in accordance with American Association for Accreditation of Laboratory Animal Care Standards. We strictly adhered to the "Guide for the Care and Use of Laboratory Animals" prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council. When necessary, animals were immobilized with ketamine HCl (Parke-Davis, Morris Plains, N.J.) (10 mg/kg of body weight injected intramuscularly).

Between 1 and 3 days after birth, newborn macaques were inoculated orally with uncloned SIVmac. For each inoculation, 1 ml of an uncloned SIVmac251 stock was administered atraumatically by dispensing the virus slowly into the mouth. The animals were monitored to ensure swallowing of the oral inoculum. The virus stock used in this study consisted of uncloned SIVmac251, propagated on rhesus peripheral blood mononuclear cells (PBMC) with a titer of 10^5 50% tissue culture infective doses ($TCID_{50}$)/ml (18); this virus stock gives consistent infection after genital mucosal inoculation and has previously been shown to infect newborn macaques by the oral-conjunctival route (18, 31).

PMPA (provided by Gilead Sciences, Foster City, Calif.) was suspended in distilled water with NaOH added to a final pH of 7.0 at 40 or 60 mg/ml and filter sterilized (0.2-μm pore size; Nalgene). PMPA was administered subcutaneously once per day at a dose of 30 mg/kg of body weight (25). PMPA dosages were adjusted weekly according to body weight. The untreated control animals did not receive daily sham inoculations.

Determination of PMPA toxicity. Complete blood counts were performed on EDTA-anticoagulated blood samples from all animals. Samples were analyzed with an automated electronic cell counter (Baker 9000; Serono Baker Diagnostics); differential cell counts were determined manually. A standard serum chemistry profile (including liver enzymes) was done with the Dacos system (Coulter Electronics, Hialeah, Fla.). Bone marrow aspirates were obtained regularly, and smears were stained with Wright-Giemsa stain (with a Giemsa overlay) for morphologic evaluation.

Quantitative virus isolation (cell-associated and cell-free virus). Levels of infectious virus in cells and plasma of peripheral blood were determined regu-

TABLE 1. Summary and outcome of PMPA study with SIV-infected newborn macaques

Animal group and no.	Description	SIV administration[a]	PMPA administration[b]	Clinical status[c]
A				
28689 28690 28948 28972	SIV infected and untreated	At birth	None	Persistently high viremia; 3/4 animals dead at 3 months
B				
29003 29008 29045 29055	SIV infected and PMPA treated	At birth	Starting at 3 weeks	3/4 animals with very low viremia at 13 months; all healthy
C				
29046 29049	Given PMPA only (toxicity)	None	Starting at birth	Healthy at 13 months; no side effects

[a] One milliliter (10^5 $TCID_{50}$) of uncloned SIVmac251 (grown in rhesus PBMC) was given orally within 72 h after birth to groups A and B.
[b] PMPA was administered subcutaneously at 30 mg/kg once daily to groups B and C.
[c] Statistical analysis of survival and viremia levels showed significant differences between groups A and B ($P < 0.05$).

larly by a limiting-dilution culture assay (four replicates per dilution) of PBMC and plasma, respectively, with CEMx174 cells in 24-well plates and subsequent p27 core antigen measurement, according to methods previously described (28, 30, 31). In addition, for animals with a low or undetectable virus load, 1×10^6 to 5×10^6 PBMC were cocultivated for 8 weeks with CEMx174 cells in tissue culture flasks, as described previously (28).

Anti-SIV isotype-specific-antibody determination. For the SIV-specific immunoglobulin G (IgG) antibody enzyme-linked immunosorbent assay (ELISA), microtiter ELISA plates (Falcon 3912; Becton Dickinson, San Jose, Calif.) were coated with SIVmac251 (Advanced Biotechnologies Inc., Columbia, Md.) at 500 ng of total protein per well. The plates were incubated with test or control plasma samples (fourfold dilutions starting at 1:100), washed, next incubated with 1:2,000-diluted enzyme-conjugated goat anti-monkey IgG (Nordic, Capistrano Beach, Calif.), washed, and incubated with *O*-phenylenediamine (Sigma, St. Louis, Mo.) as a substrate, and the results were read spectrophotometrically as described previously (21). The SIV-specific IgM antibody ELISA was performed with plates coated with a 21-amino-acid synthetic peptide derived from the SIVmac transmembrane glycoprotein and is described elsewhere (21). Immunoblotting was performed as described previously (24).

T-lymphocyte phenotyping. T-lymphocyte antigens were detected by direct labeling of whole blood with fluorescein-conjugated anti-human CD8 (Leu-2a; Becton Dickinson Immunocytometry Inc., San Jose, Calif.) and phycoerythrin-conjugated anti-human CD4 (OKT4; Ortho Diagnostic Systems Inc., Raritan, N.J.). Erythrocytes were lysed, and the samples were fixed in paraformaldehyde by using the Coulter Q-prep system. Lymphocytes were gated by forward and side light scatter and were then analyzed with a FACSCAN flow cytometer (Becton Dickinson).

Sequencing of the viral RT region. CEMx174 cells infected with virus isolated from the SIV-infected animals were harvested as soon as culture supernatants were positive by antigen capture ELISA (15). The genomic-DNA preparation, PCR, and sequencing of the reverse transcriptase (RT) region were done according to previously described methods (10), with the only difference that PCR products in this study were directly sequenced (instead of being first cloned and then sequenced). This method can detect the presence of a 20% subpopulation in the PCR mixture. The RT DNA sequence was compared with the SIVmac251 molecular clone sequence obtained from GenBank and analyzed with the DNA STAR software program.

Drug susceptibility assay. Phenotypic drug susceptibility was characterized by an assay which is based on a dose-dependent reduction of viral infectivity and which has been described previously (30). In brief, titration of viral infectivity of tissue culture supernatants (by making fivefold dilutions with eight replicates per dilution, adding CEMx174 cells in 96-well tissue culture plates, and then measuring p27 antigen after 5 days [15]) was performed at various drug concentrations. Infectious titers were calculated and reduction of infectivity was determined for each drug concentration, as described previously (30).

Necropsy specimen collection and preparation of tissue samples. Euthanasia due to simian AIDS was indicated by three or more of the following clinical observations: (i) weight loss of >10% in 2 weeks or >30% in 2 months; (ii) chronic diarrhea unresponsive to treatment; (iii) infections unresponsive to treatment; (iv) inability to maintain body heat or fluids without supplementation; (v) persistent, marked hematological abnormalities, including lymphopenia, anemia, thrombocytopenia, or neutropenia; and (vi) persistent, marked splenomegaly or hepatomegaly (17, 31).

A complete necropsy was performed on all animals that died during the course of the study. Tissues collected at necropsy were fixed in 10% buffered formalin, embedded in paraffin, cut into 6-μm-thick sections, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical analysis. Statistical analysis was used to compare PMPA-treated versus PMPA-untreated SIV-infected animals with regard to survival and virus levels. Survival was compared by the generalized Wilcoxon test. The virus levels of the two animal groups were compared by calculation of the area under the curve for each animal, followed by analysis according to the Wilcoxon rank-sum test. It has previously been shown that these analyses can distinguish biologically relevant differences in survival and virus load (17, 30).

RESULTS

Study design. The toxic and antiviral effects of chronic PMPA treatment were evaluated in newborn rhesus macaques. To detect any long-term side effects, two uninfected newborn macaques were started on chronic PMPA treatment (30 mg/kg of body weight, given subcutaneously once per day [25]) within 3 days after birth (Table 1, group C). To study the therapeutic effects of PMPA, eight newborn macaques were inoculated orally, within 72 h after birth, with 1 ml (10^5 $TCID_{50}$) of an uncloned SIVmac251 virus stock. Four of these eight SIV-inoculated animals were used as untreated controls, while the other four infants were treated with PMPA (30 mg/kg subcutaneously, once daily) beginning at 3 weeks of age (Table 1, groups A and B). PMPA treatment was withheld for this period because previous studies demonstrated that newborn macaques infected with uncloned SIVmac251 had high-level viremia and widespread systemic virus dissemination and demonstrated signs of immunosuppression within 3 weeks (17, 21, 30, 31). An established infection is a more rigorous test of drug efficacy and is likely to increase the chance that drug-resistant viral variants would rapidly emerge (5). In addition, this situation would mimic directly that of human infants who become infected by HIV perinatally and who would start on PMPA therapy several weeks after birth.

The animals were monitored regularly for levels of PBMC-associated and plasma-associated virus, antibody responses, $CD4^+$ and $CD8^+$ T-cell counts, drug resistance, bone marrow morphology, serum biochemistry profiles, weight gain, and clinical signs, etc., according to methods extensively used in our previous studies with newborn macaques (17, 21, 30, 31).

Toxicology studies. Although the numbers of animals are small, no signs of toxic side effects have been observed over a 13-month period in any of the PMPA-treated animals, includ-

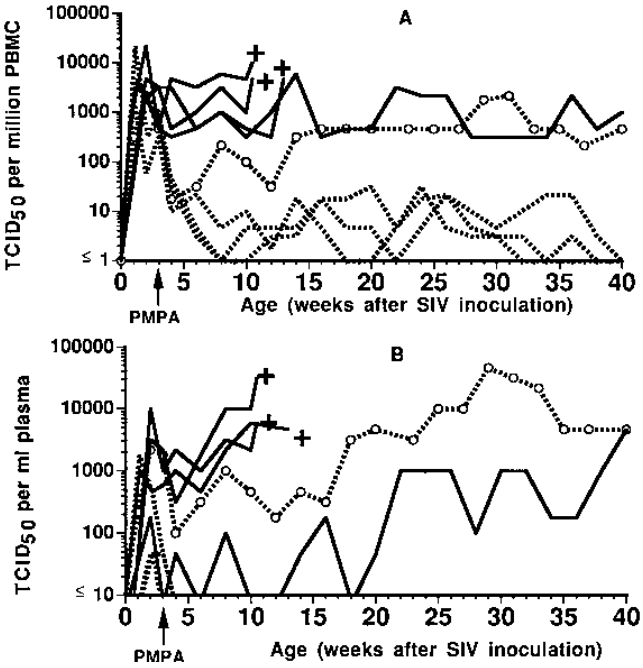

FIG. 1. Time course of oral SIVmac infection of newborn macaques and the therapeutic effects of PMPA. Levels of PBMC-associated virus (A) and virus in plasma (B) were determined by limiting-dilution culture of PBMC and plasma, respectively. All animals were inoculated orally with uncloned SIVmac at birth. Results are shown for the four SIV-infected untreated control infants (solid lines) and the four SIV-infected infants that were started on PMPA treatment (30 mg/kg subcutaneously, once daily) 3 weeks after virus inoculation (broken lines); results for animal 29055 (open circles) and euthanasia because of simian AIDS (+) are also indicated. Statistical analysis showed a significant difference in virus levels between the two groups ($P < 0.05$).

ing the two uninfected control animals that were started on PMPA treatment at birth (Table 1, group C). This absence of toxicity is based on regular, normal complete blood counts, serum biochemistry profiles (including liver enzymes), bone marrow morphology, $CD4^+$ and $CD8^+$ T-cell counts, weight gains, and clinical appearance for the duration of the study (data not shown).

Evaluation of efficacy. The four untreated SIV-infected control infants developed persistently high-level viremia (Fig. 1). All four animals showed an anti-SIV IgM response within 4 weeks after virus inoculation (data not shown), but three mounted no SIV-specific IgG response as determined by ELISA (Table 2) or immunoblotting (data not shown), indicating rapid immunosuppression (21). These three untreated control infants had persistently high plasma viremia and died between 10 and 13 weeks of age (Fig. 1B and Table 1), with clinical signs and gross and microscopic pathological changes consistent with immunodeficiency and terminal SIV infection (data not shown). The fourth untreated control animal, which made a strong antiviral IgG response (Table 2, animal 28972), had persistently high PBMC-associated virus levels and intermittent plasma viremia (Fig. 1) and a slower disease course. The fulminant course of SIV infection in the first three control infants was consistent with previous observations for newborn macaques inoculated intravenously ($n = 6$) or mucosally ($n = 3$) with uncloned SIVmac251 (17, 21, 30, 31). Therefore, the behavior of neonatal SIV infection in these three animals was representative of that observed in a larger number of historical control animals.

The four PMPA-treated animals (Table 1, group B) showed a course of infection similar to that of the untreated control animals up to 3 weeks, when PMPA therapy was started. One of the four PMPA-treated animals (animal 29008) already had a clear anti-SIV IgG response at 3 weeks; the remaining three animals showed a strong increase in anti-SIV IgG titer only after PMPA treatment was started (Table 2). PMPA treatment induced a rapid and strong reduction in viremia in three of the four animals (animals 29003, 29008, and 29045; Fig. 1). No virus has been isolated from the plasma of these three macaques during more than 12 months of PMPA treatment; the levels of PBMC-associated virus in these three animals were reduced 100- to 1,000-fold within 3 weeks of PMPA treatment and remained low (approximately 1 infected cell per 1×10^5 to 5×10^6 PBMC) for more than 12 months (Fig. 1). The fourth PMPA-treated infant (animal 29055), which was slower in making an anti-SIV IgG response following PMPA treatment than the other three animals in the PMPA-treated group (Table 2), maintained a persistent plasma- and PBMC-associated viremia (Fig. 1). While animals 29003, 29008, and 29045 have no clinical symptoms at all, animal 29055 has splenomegaly and lymphadenopathy at 13 months of age. All four PMPA-treated SIV-infected infants have normal weight gain and normal $CD4^+$ and $CD8^+$ T-cell counts and $CD4^+/CD8^+$ T-cell ratios. All PMPA-treated animals continue to receive daily PMPA treatment and will be monitored indefinitely.

TABLE 2. Anti-SIV IgG antibody response in untreated and PMPA-treated infant rhesus macaques after oral inoculation with uncloned SIVmac at birth

Treatment group	Animal no.	Anti-SIV IgG titer at the indicated time after virus inoculation (wk)[a]							
		0	2	3	4	6	8	10–13	20
Control	28689	<100	<100	100	<100	<100	<100	<100*	
	28690	<100	<100	<100	<100	<100	<100	<100*	
	28948	<100	100	100	100	<100	<100	<100*	
	28972	<100	<100	100	**1,600**	**6,400**	**25,600**	**102,400**	**102,400**
PMPA	29003	<100	<100	100	**1,600**	**1,600**	**6,400**	**6,400**	**25,600**
	29008	<100	**1,600**	**6,400**	**6,400**	**6,400**	**6,400**	**25,600**	**25,600**
	29045	<100	<100	100	**1,600**	**1,600**	**6,400**	**25,600**	**25,600**
	29055	<100	<100	<100	<100	**6,400**	**6,400**	**6,400**	**25,600**

[a] Anti-SIV IgG titers were determined by ELISA and are expressed as the reciprocal of the highest of fourfold dilutions (starting from a 1/100 dilution, with two replicates per dilution) which gave a positive optical density above cutoff value. Strong antibody responses (titer ≥ 400) are indicated in boldface. Antibody titers at time of death are indicated with an asterisk. Results of the ELISA were confirmed by immunoblotting: animals 28689, 28690, and 28948 showed no detectable anti-SIV antibody response, while the other five SIV-infected infants mounted a strong antibody response with reactivity to all structural proteins of SIV (data not shown).

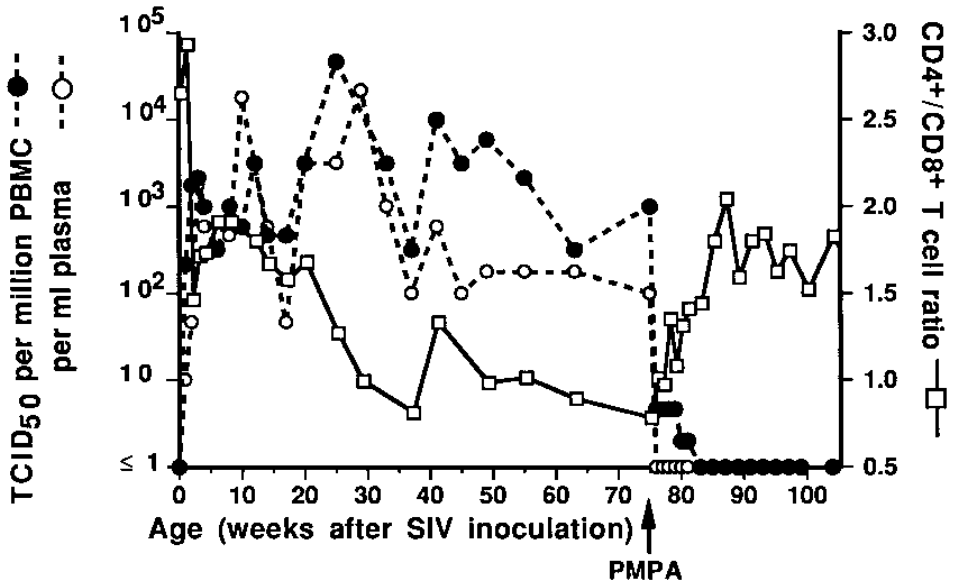

FIG. 2. Therapeutic effects of PMPA on chronic SIV infection. Animal 28214 was inoculated intravenously at birth with SIVmac239 (as described in reference 17) and started on PMPA treatment (30 mg/kg subcutaneously, once daily) at 75 weeks of age. Levels of PBMC-associated virus and virus in plasma (left *y* axis) were measured by limiting-dilution culture. $CD4^+/CD8^+$ T-cell ratios (right *y* axis) were determined by flow cytometry.

To further investigate and confirm the effect of PMPA treatment on established SIV infection, we also started PMPA treatment of a juvenile macaque (animal 28214) that had persistently high-level PBMC-associated and plasma viremia for 75 weeks since birth and had symptoms of simian AIDS (chronic diarrhea and dehydration and poor weight gain). Within 1 week after the start of PMPA treatment, plasma viremia became undetectable; PBMC-associated-virus levels dropped from 1 infected cell in 1,000 PBMC to <1 infected cell per 10^6 PBMC (Fig. 2). The $CD4^+/CD8^+$ T-cell ratio, which had been declining steadily prior to PMPA treatment, suddenly increased (Fig. 2), and clinical signs of disease reversed; the absolute $CD4^+$ T-cell counts were normal (>1,000/μl) during PMPA treatment. The strong suppression of viremia has persisted for more than 9 months of PMPA therapy. In our experience, untreated macaques with long-term SIV infection have never shown sudden, dramatic, and persistent changes of this magnitude (16, 17, 28).

Development of PMPA resistance. Virus isolated from the four PMPA-treated SIV-infected infants was tested in a drug susceptibility assay that was previously used to detect AZT-resistant SIVmac mutants (30). Virus isolated at the time of initiation of PMPA therapy (i.e., 3 weeks after virus inoculation) had susceptibility to PMPA similar to that of the virus inoculum (95% inhibitory concentration [IC_{95}] of 30 to 50 μM); for all four animals, an approximately fivefold-reduced in vitro susceptibility to PMPA was observed for virus isolated after 5 to 15 weeks of therapy (IC_{95} = 150 to 250 μM). This low-level drug resistance was temporally associated with the development of a lysine-to-arginine substitution at amino acid 65 of the RT (K65R) in all four animals (Table 3). Additional mutations emerged, and two patterns were observed: virus isolates from animals 29003, 29008, and 29045 all developed an I118V mutation at about the same time as or shortly after the K65R mutation and, most recently, an N69S mutation. In addition to the K65R mutation, virus from animal 29055 temporally developed R82K, S211N, N69S, and A158S mutations; the N69S mutation eventually became N69T at 6 months (Table 3). Development of these additional mutations did not result in a detectable further increase of resistance to PMPA.

DISCUSSION

PMPA appears superior to AZT for SIV-infected macaques because of its stronger virus suppression and absence of side effects (2, 28, 30). The most surprising antiviral effect of PMPA in SIV-infected rhesus infants was the strong reduction of PBMC-associated-virus levels (Fig. 1A). AZT had little or no effect on the levels of PBMC-associated virus in infant macaques once SIV infection was already established (28, 30). Strong reduction of PBMC-associated viremia was previously reported for SIV-infected adult macaques treated with PMEA, but toxic side effects were seen (26).

Virus with an approximately fivefold-reduced susceptibility to PMPA emerged in all four PMPA-treated SIV-infected infants after 5 to 15 weeks of PMPA therapy. The development of the K65R mutation in the RT was consistently observed and plays a central role in this low-level resistance to PMPA. The K65R mutation has previously been described for HIV type 1 selected for resistance to zalcitabine (dideoxycytosine) or PMEA in vitro and confers significant cross-resistance to lamivudine and didanosine but minor resistance to stavudine, (2*R*,5*R*)-9-[2,5-dihydro-5-(phosphonylmethoxy)-2-furanyl]adenine (D4API), and PMPA and is susceptible to AZT (4, 8, 9, 11, 34). Our PMPA-resistant SIVmac was found to have a very similar pattern of cross-resistance (data not shown). The present study is the first one to demonstrate the in vivo development of the K65R mutation under selection pressure of PMPA treatment. Thus, our data suggest that the K65R mutation (which confers an approximately fivefold-reduced susceptibility to PMPA for HIV and SIV) may arise in HIV-infected patients receiving prolonged PMPA therapy.

In addition to the K65R mutation, SIV isolates from the four PMPA-treated infants also showed progressive accumulation of other mutations in the RT gene (Table 3). Development of these additional mutations did not result in further increased

TABLE 3. Development of drug resistance in PMPA-treated SIVmac-infected infant rhesus macaques[a]

Animal no.	Mutation(s) in RT sequence at the indicated time after SIVmac inoculation								
	3 wk	6 wk	8 wk	12–14 wk	18 wk	6 mo	7 mo	8 mo	9 mo
29003	None (WT)	NA[b]	None (WT)	None (WT)	K65R I118V	K65R I118V	K65R N69S I118V	NA	K65R N69S I118V
29008	None (WT)	NA	None (WT)	K65R	K65R 1/2I118V[c]	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V
29045	None (WT)	NA	None (WT)	K65R I118V	NA	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V	K65R N69S I118V
29055	None (WT)	None (WT)	K65R R82K S211N	K65R N69S R82K S211N	NA	K65R N69T R82K A158S S211N	K65R N69T R82K A158S S211N	K65R N69T R82K A158S S211N	K65R N69T R82K A158S S211N

[a] Animals were inoculated at birth orally with uncloned SIVmac251 and started on PMPA treatment at 3 weeks of age. For study of genotypic resistance, DNA from CEMx174 cells infected with SIV isolated from PBMC of SIV-infected infant macaques was used for sequence analysis of RT. The RT amino acid sequence was compared with that of the virus inoculum of uncloned SIVmac251 (wild type [WT] is RT sequence identical to that of the virus inoculum). The RT sequence of the inoculum was found to be identical to that reported for the molecular clone SIVmac251 with the exceptions of three amino acid substitutions: alanine instead of threonine was found at amino acid positions 11 and 492, and at amino acid position 371 a mixture of isoleucine and valine was found in the virus inoculum, and either one of these two amino acids or a mixture was detected in virus isolates from these four animals at the different time points. Amino acid substitutions in RT are given by their respective single-letter designations. Analysis of virus isolated from plasma of animal 29055 at multiple time points revealed an RT sequence identical to that of virus derived from PBMC. For study of phenotypic resistance, all SIVmac isolates listed in this table were tested for PMPA susceptibility. All virus isolates with the wild-type RT sequence were PMPA susceptible (IC_{95} range of 30 to 50 μM); in contrast, all SIVmac isolates with mutations in the RT had approximately fivefold-reduced susceptibility to PMPA (IC_{95} range of 150 to 250 μM). Virus isolated from the untreated control animal 28972 after 9 months of SIV infection had maintained the wild-type RT sequence and the PMPA-susceptible phenotype (data not shown).

[b] NA, not available.

[c] 1/2, approximately equal mixture of wild-type and mutant amino acids.

resistance to PMPA; several of these mutations have been described for other wild-type SIV or HIV strains (19). Thus, a possible role of these additional mutations may be to compensate for impaired replicative capacity induced by the K65R mutation, rather than to contribute directly to the drug-resistant phenotype; this would be similar to observations with other mutations in HIV type 1 RT or protease (12, 13).

Despite the development of low-level PMPA resistance within a few months of PMPA treatment, viremia levels remained low for three of the four SIV-infected infants during more than 12 months of PMPA therapy (Fig. 1); these three animals are also healthy at 13 months of age. Thus, in contrast to observations with many other antiretroviral drugs which strongly suppress virus replication (3, 14, 22, 23, 33), emergence of PMPA-resistant virus did not result in a detectable rebound in viremia in these three animals. Virus isolated from the one PMPA-treated animal in this study which had persistently higher virus levels (animal 29055) had, in addition to the K65R mutation, a set of mutations different from those of the other three PMPA-treated animals (Table 3). Further investigation is needed to determine whether the different RT genotype of the PMPA-resistant virus versus some unidentified host or viral factor is responsible for this persistently higher-level viremia in animal 29055. Although PMPA therapy was clearly less efficacious in reducing viremia for animal 29055 than for the other SIV-infected infants in this study, animal 29055 has survived longer than other SIVmac251-infected infant rhesus macaques with similar persistently high-level viremia (17, 30, 31); thus, there is not enough evidence to conclude that PMPA therapy failed for animal 29055. Because PMEA has been found to have beneficial immunomodulatory effects in murine models (7, 20, 32), it is possible that PMPA also has a direct action on the immune system.

In summary, our preliminary data suggest that PMPA holds much promise for the treatment of HIV-infected human infants and adults. The current study demonstrates how preclinical evaluation of novel antiviral compounds in an appropriate animal model can provide valuable information about multiple aspects of drug therapy, including drug resistance. Future studies will focus on determining the clinical implications of PMPA resistance by inoculating newborn macaques with the different genotypes of PMPA-resistant SIVmac and on identifying possible immunomodulatory effects of PMPA.

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ADDENDUM IN PROOF

Persistently low-level viremia was maintained for animal 28214 (Fig. 2) after 1 year of PMPA therapy (<1 infected cell per 10 million PBMC; no detectable plasma viremia); 10 days after PMPA treatment was stopped, viremia levels have increased >100-fold.

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