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Use of two virustatica (AZT, PMEA) in the treatment of FIV and of FeLV seropositive cats with clinical symptoms

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ABSTRACT

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In the present study the therapeutic efficacy and the side effects of two antiretroviral compounds used in human acquired immunodeficiency syndrome (AIDS) research, 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine, Retrovir) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA), were investigated in the treatment of cats naturally infected with feline immunodeficiency virus (FIV) and cats naturally infected with feline leukemia virus (FeLV). AZT was administered subcutaneously at a dose of 5 mg kg^{-1} body weight every 12 h and PMEA was administered subcutaneously at a dose of 2.5 mg kg^{-1} body weight every 12 h during a 3 week hospitalization. The therapeutic efficacy of both compounds was investigated. There was a stronger potency of PMEA than of AZT on the regression of stomatitis in FIV and in FeLV infected cats. In addition, in FIV infection PMEA had a stronger effect on the improvement of the general clinical status. Both antiretroviral compounds were potent agents to improve the immunologic status of FIV infected cats by raising the CD4/CD8 ratio. In FeLV infection PMEA and AZT appeared to reduce antigenemia. The hematological side effects caused by PMEA were severe and stronger than those of AZT. Therefore the advantage of PMEA in clinical and immunologic improvement was diminished by the hematologic disorders, which do not allow long term treatment with this drug in the dose used.

ABBREVIATIONS

AZT, 3′-azido-2′,3′-dideoxythymidine; AZTTP, 3′-azido-2′,3′-dideoxythymidine triphosphate; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; PMEA, 9-(2-phosphonylmethoxy-

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ethyl)adenine; PMEApp, 9-(2-phosphonylmethoxylethyl)adenine pyrophosphate; RT, reverse transcriptase.

INTRODUCTION

The compounds 3′-azido-2′,3′-dideoxythymidine (AZT, zidovudine, Retrovir) and 9-(2-phosphonylmethoxyethyl)adenine (PMEA) are potent and selective inhibitors of the in vitro replication of a number of retroviruses. At present, only AZT has been officially licensed for the treatment of human immunodeficiency virus infection (Balzarini et al., 1990b). The structure of AZT is shown in Fig. 1. The molecule is identical to endogenous thymidine in all aspects except for the modification at the 3′-position of the sugar. Mitsuya et al. (1985) demonstrated that AZT inhibits HIV-1 replication in vitro. AZT readily enters cells and is converted to nucleotide forms by the intracellular kinases which activate thymidine. There is no evidence of preferential activation of AZT within infected cells. The apparent retroviral selectivity of AZT is a result of preferential utilization of 3′-azido-2′,3′-dideoxythymidine triphosphate (AZTTP) as a substrate for the viral reverse transcriptase (RT) compared with the mammalian cellular DNA polymerases. AZTTP binds to the retroviral RT and the incorporation of azidothymidilate into the growing DNA strand leads to chain termination (Mitsuya et al., 1985; Collins and Unadkat, 1989). The antiviral activity of AZT against feline retroviruses in vitro has also been demonstrated. Tavares et al. (1987) found AZT to be a potent inhibitor of FeLV replication. North et al. (1989) demonstrated the antiretroviral efficacy against FIV.

Recently a new group of acyclic nucleosides, the phosphonate derivatives, first synthesized by Holy and Rosenberg (1987), was found to selectively inhibit HIV replication in vitro. Balzarini et al. (1990b) demonstrated that the basic substance, PMEA (Fig. 2), exerts a significant antiviral effect in lentivirus infected monkeys. Intracellularly PMEA may be directly converted to its diphosphorylated derivative 9-(2-phosphonylmethoxyethyl)adenine py-

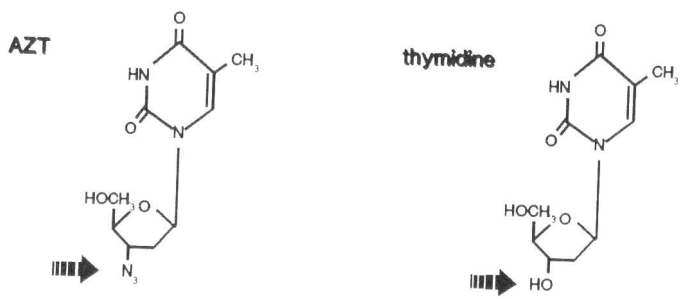


Fig. 1. Chemical formulae of AZT and thymidine.

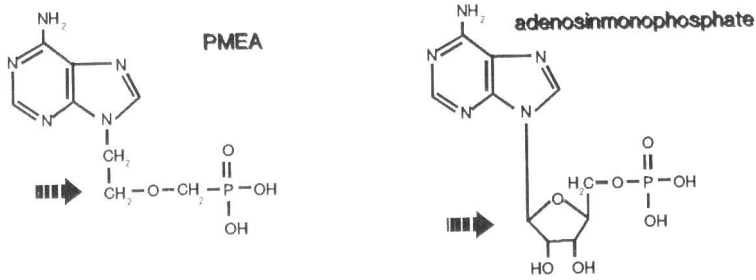


Fig. 2. Chemical formulae of PMEA and adenosine monophosphate.

rophosphate (PMEApp). This phosphorylation is accomplished by 5-phosphoribosyl-1-pyrophosphate synthetase. PMEApp is targeted at the retroviral RT and can act as a competitive inhibitor of the RT reaction. Being an alternative substrate to deoxyadenosine triphosphate, PMEApp also acts, if incorporated, as a potent DNA chain terminator. This double functioning may explain the potent antiretrovirus activity (Balzarini et al., 1991). PMEA is a potent and selective inhibitor of the replication of several human, simian and murine retroviruses in vitro and of the murine sarcoma virus in vivo. In the latter system PMEA has a stronger antiretroviral potency and selectivity than AZT. In vitro PMEA was found to efficiently block FeLV and FIV replication in feline cells (Balzarini et al., 1990a; Egberink et al., 1990; Hoover et al., 1991).

MATERIAL AND METHODS

Four thousand four hundred and thirteen field cats were tested for their FIV and FeLV status by enzyme linked immunosorbent assay (ELISA). FIV specific antibodies were assayed using the ELISA PetChek FIV (IDEXX, Portland, ME) and FeLV antigens were assayed using the ELISA PetChek FeLV (IDEXX). Positive FIV results were confirmed by Western blot and radioimmunoprecipitation assay and positive FeLV results by indirect immunofluorescence assay and virus isolation.

Thirty-three FIV and 32 FeLV infected cats were included in the study. The cats were eligible to enter the study if they showed chronic oral inflammations. The status of the oral cavity, classified in degrees 0–3, was the main criterion for therapy success.

The clinical trial was designed as a placebo-controlled double-blind study. The period of the treatment was standardized to a 3 week hospitalization. Based on preliminary pharmacokinetic studies, AZT was administered subcutaneously at a dose of 5 mg kg^{-1} body weight every 12 h and PMEA subcutaneously at a dose of 2.5 mg kg^{-1} body weight every 12 h. No other drugs were used. Eleven of the FIV infected cats were assigned to AZT, nine to

PMEA and 13 to placebo. Eleven of the FeLV infected cats were assigned to AZT, 12 to PMEA and nine to placebo. The therapeutic effect of the compounds was determined by monitoring the status of the oral cavity, the general clinical condition, hematologic and biochemical parameters, the counts of CD4+ and CD8+ peripheral blood cells differentiated by flow analysis cytometer system, and virological parameters throughout the 3 week treatment phase. Statistically significant differences in these parameters between the three groups of compounds were evaluated by multifactorial variance analysis. Statistical significance (P) was fixed as less than or equal to 0.05.

RESULTS

FIV specific antibodies were detected in 165 (3.7%) of the 4413 sera tested. Two hundred and eighty-four cats (6.4%) were seropositive for FeLV antigen. Eighteen (0.4%) were found to be infected with both viruses (Fig. 3).

Between the beginning and the end of the 3 week therapy statistically significant differences between the three compound groups given to FIV infected cats were demonstrated in the status of the oral cavity, the general clinical condition, the CD4/CD8 ratio and the hematocrit and hemoglobin values. In FeLV infected cats significant changes were detected in the status of the oral cavity, the hematocrit and hemoglobin values and the FeLV p27 antigen level.

Status of the oral cavity

The status of the oral cavity was evaluated at the beginning and the end of the therapy (Fig. 4). Changes in the degree of stomatitis were registered from -1 to 2. Significant differences ($P_{\text{FIV}}=0.029$, $P_{\text{FeLV}}=0.029$) in the degree of stomatitis were demonstrated in both retroviral infections. The degree of stomatitis improved by an average of 1.1 degrees with PMEA, 0.8 with AZT and

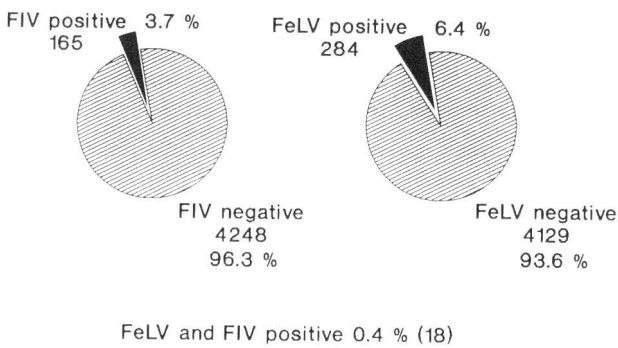
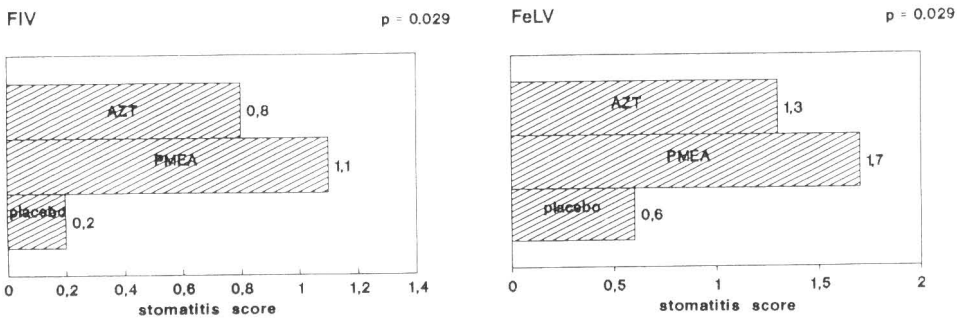


Fig. 3. Prevalence of FIV and of FeLV positive cats in 4413 serum samples.



compound	mean	std dev
total (33)	0.6	0.8
AZT (11)	0.8	0.9
PMEA (9)	1.1	0.6
placebo (13)	0.2	0.7

compound	mean	std dev
total (33)	1.3	1.0
AZT (11)	1.3	0.7
PMEA (9)	1.7	0.6
placebo (13)	0.6	1.4

Fig. 4. Mean changes in the stomatitis degree (−1, deterioration; 0, no difference; 1, slight improvement; 2, strong improvement) between the end and the beginning of treatment in FIV and in FeLV infected cats.

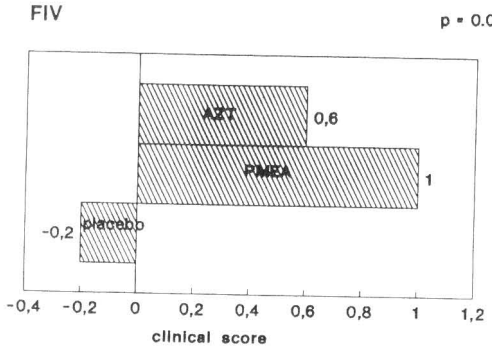
0.2 with placebo in the FIV infected cats. In the FeLV infected cats an average improvement in the degree of stomatitis of 1.7 with PMEA, 1.3 with AZT and 0.6 with placebo was found.

General clinical condition

The general clinical condition was evaluated by the number and the severity of the alterations in the affected organs, the weight gain and the well-being of the cats (Fig. 5). The FIV infected cats treated with antiretroviral drugs significantly ($P=0.007$) improved by an average degree of 0.6 when receiving AZT and by 1.0 when receiving PMEA. The placebo group deteriorated by an average degree of 0.2. The changes in the clinical condition of the FeLV infected cats were not statistically significant ($P=0.166$).

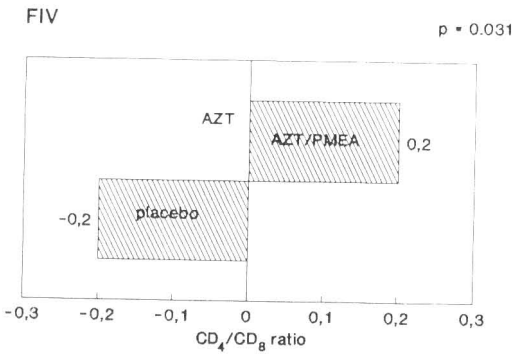
CD4/CD8 ratios

Upon investigating the CD4+ and CD8+ peripheral blood cells of the FIV infected cats, significant differences ($P=0.031$) in the CD4/CD8 ratios were detected in the course of the treatment (Fig. 6). By application of each of the two nucleoside analogues, the ratios increased by an average of 0.2 compared with the pretreatment values. No difference in the influence on the CD4/CD8 ratio was found between PMEA and AZT. Placebo treated cats showed a decrease in the CD4/CD8 ratio of 0.2. In the FeLV infected cats the changes in the CD4/CD8 ratio were not statistically significant ($P=0.226$).



compound	mean	std dev
total (33)	0.4	1.0
AZT (11)	0.6	1.1
PMEA (9)	1.0	0.9
placebo (13)	-0.2	0.6

Fig. 5. Mean changes in the general clinical status (-1, deterioration; 0, no difference; 1, slight improvement; 2, strong improvement) between the end and the beginning of treatment in FIV infected cats.

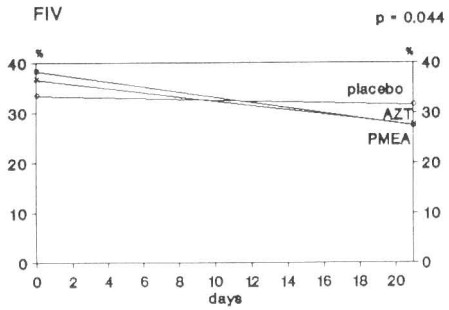


compound	mean	std dev
total (30)	0.0	0.4
AZT/PMEA (18)	0.2	0.3
placebo (13)	-0.2	0.6

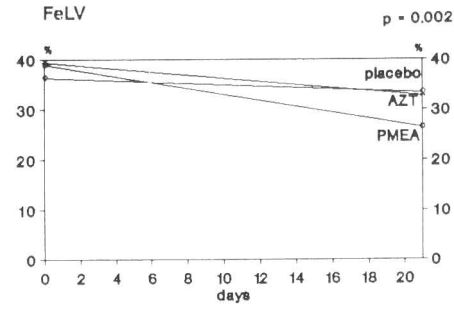
Fig. 6. Mean decrease/increase in the CD4/CD8 ratios between the end and the beginning of treatment in FIV infected cats.

Hematologic values

Significant changes were seen in the hematocrit (HCT) (Fig. 7) as well as in the hemoglobin (Hb) values (Fig. 8) in both infections ($P_{\mathrm{HCT/FIV}}=0.044$, $P_{\mathrm{HCT/FeLV}}=0.002$, $P_{\mathrm{Hb/FIV}}=0.007$, $P_{\mathrm{Hb/FeLV}}=0.001$). The decrease of the val-

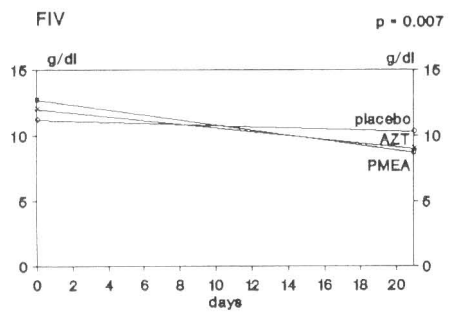


compound	mean	std dev
total (33)	-6.7	9.5
AZT (11)	-9.0	9.4
PMEA (9)	-11.0	9.0
placebo (13)	-1.8	8.2

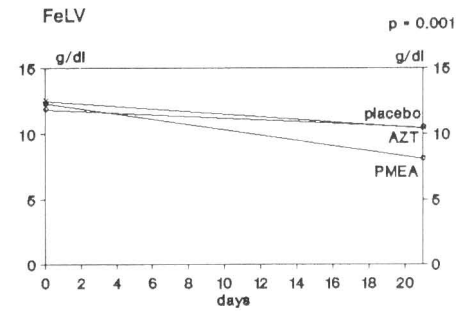


compound	mean	std dev
total (32)	-7.6	6.7
AZT (12)	-6.6	5.3
PMEA (11)	-12.5	5.1
placebo (9)	-2.8	6.3

Fig. 7. Mean hematocrit values of the FIV and FeLV infected cats.



compound	mean	std dev
total (33)	-2.4	2.5
AZT (11)	-3.0	2.2
PMEA (9)	-4.0	2.5
placebo (13)	-0.9	2.0



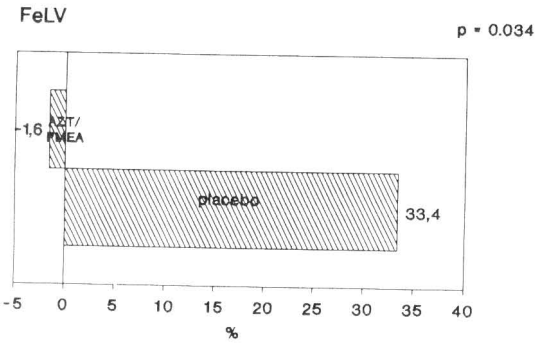
compound	mean	std dev
total (32)	-2.6	2.0
AZT (12)	-2.1	1.6
PMEA (11)	-4.2	1.7
placebo (9)	-1.3	1.5

Fig. 8. Mean hemoglobin values of the FIV and FeLV infected cats.

ues of the cats treated with PMEA was greater than the decrease in the AZT treated cats. The placebo treated cats showed a slight decrease.

FeLV p27 antigen level

Among the FeLV infected cats the average antigen levels declined significantly ($P=0.034$) in those that received antiretroviral drugs (percentage decrease -1.6). In contrast in those cats that received placebo (percentage increase 33.4), increasing levels of the p27 antigen were seen (Fig. 9).



compound	mean	std dev
total (14)	8.4	28.9
AZT/PMEA (10)	-1.6	26.4
placebo (4)	33.4	26.4

Fig. 9. Mean percentage decrease/increase in the FeLV p27 antigen levels between the end and the beginning of treatment in FeLV infected cats.

DISCUSSION

As several authors have demonstrated in vitro (North et al., 1989; Egberink et al., 1990), the therapeutic efficacy of the compounds AZT and PMEA against FIV infection was also demonstrated in naturally infected cats. Both compounds had a beneficial effect on the severity of oral inflammation in the FIV infected cats. PMEA showed a greater potency on the regression of stomatitis than AZT. Complete healing of the oral lesions could not be achieved in all cats because no additional systemic or local therapy was applied. In addition both drugs induced an improvement in the general clinical condition of the FIV infected cats. A better efficacy was reached by administering PMEA. In placebo treated cats, a deterioration of the general clinical status was noticed. Hospitalization or the progress of the disease could be regarded as possible reasons. Both drugs registered a beneficial effect on the CD4/CD8 ratio of the FIV infected cats. Between the two compounds there were no significant differences observed in this immunological parameter.

In FeLV infected cats both AZT and PMEA effected an improvement in several of the investigated parameters. Both compounds showed a therapeutic efficacy in the treatment of the oral cavity, with PMEA as the more effective substance. On the other hand no statistically significant influence was seen in the general clinical condition or in the CD4/CD8 ratio of the FeLV infected cats. The reason may be that, in FeLV, in contrast to FIV infected cats, the CD4+ cells are not selectively destroyed. Measurement of the FeLV p27 antigen levels showed that administration of the antiviral drugs effected a decrease of the FeLV p27 antigen. The steep increase of the antigen level by

administering placebo can be traced back to the stress of the 3 week hospitalization.

For both compounds hematologic side effects were observed. All FIV and FeLV infected cats showed a decrease of hematocrit and hemoglobin values during the 3 week therapy. A slight decrease was even found in the placebo group, probably caused by taking several blood samples. The decrease of the hematologic parameters was mild and tolerable in the AZT recipients. In some cats, which were assigned to AZT for longer than 3 weeks, the anemia even disappeared. A severe decline of hematocrit and hemoglobin levels was produced by therapy with PMEA. Some cats even developed a strong anemia, which disallowed continuation of the therapy. Therefore the advantage of PMEA in the clinical and the immunologic improvement is diminished by strong hematologic disorders, which do not allow long term treatment with this drug in the dose used in this study.

REFERENCES

Balzarini, J., Naesens, L. and de Clercq, E., 1990a. Anti-retrovirus activity of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) in vivo increases when it is less frequently administered. Int. J. Cancer, 46: 337–340.

Balzarini, J., Naesens, L., Slachmuylders, J., Niphuis, H., Rosenberg, I., Holy, A., Schellekens, H. and de Clercq, E., 1990b. Potent anti-simian immunodeficiency virus (SIV) activity and pharmacokinetics of 9-(2-phosphonylmethoxyethyl)adenine (PMEA) in rhesus monkeys. In: H. Schellekens and M.C. Horzinek (Editors), Animal Models in AIDS. Elsevier, Amsterdam, pp. 131–138.

Balzarini, J., Hao, Z., Herdewijn, P., Johns, D.G. and de Clercq, E., 1991. Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl)adenine, a potent anti-human immunodeficiency virus compound. Proc. Natl. Acad. Sci. USA, 88: 1499–1503.

Collins, J.M. and Unadkat, J.D., 1989. Clinical pharmacokinetics of Zidovudine. Clin. Pharmacokin., 17: 1–9.

Egberink, H., Borst, M., Niphuis, H., Balzarini, J., Neu, H., Schellekens, H., de Clercq, E., Horzinek, M.C. and Koolen, M., 1990. Suppression of feline immunodeficiency virus infection in vivo by 9-(2-phosphonomethoxyethyl)adenine. Proc. Natl. Acad. Sci. USA, 87: 1–5.

Holy, A. and Rosenberg, I., 1987. Synthesis of 9-(2-phosphonomethoxyethyl)adenine and related compounds. Collect. Czech. Chem. Commun., 52: 2801–2809.

Hoover, E.A., Ebner, J.P., Zeidner, N.S. and Mullins, J.I., 1991. Early therapy of feline leukemia virus infection (FeLV-FAIDS) with 9-(2-phosphonyl-methoxyethyl)adenine (PMEA). Antiviral Res., 16: 77–92.

Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Lehrman, S.N., Gallo, R.C., Bolognesi, D., Barry, D.W. and Broder, S., 1985. 3′-Azido-3′-desoxythymidine: an antiretroviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic lentivirus type III/lymphadenopathy associated virus in vitro. Proc. Natl. Acad. Sci. USA, 82: 7096–7100.

North, T.W., North, G.L. and Pedersen, N.C., 1989. Feline immunodeficiency virus, a model for reverse transcriptase-targeted chemotherapy for acquired immune deficiency syndrome. Antimicrob. Agents Chemother., 33: 915–919.

Tavares, L., Roneker, C., Johnston, K., Lehrman, S.N. and de Noronha, F., 1987. 3′-Azido-3′-deoxythymidine in feline leukemia virus-infected cats: a model for therapy and prophylaxis of AIDS. Cancer Res., 47: 3190–3194.