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Antiviral treatment of feline immunodeficiency virus-infected cats with (*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine

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Abstract

Feline immunodeficiency virus (FIV), the causative agent of an acquired immunodeficiency syndrome in cats (feline AIDS), is a ubiquitous health threat to the domestic and feral cat population, also triggering disease in wild animals. No registered antiviral compounds are currently available to treat FIV-infected cats. Several human antiviral drugs have been used experimentally in cats, but not without the development of serious adverse effects. Here we report on the treatment of six naturally FIV-infected cats, suffering from moderate to severe disease, with the antiretroviral compound (*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine ([*R*]-PMPDAP), a close analogue of tenofovir, a widely prescribed anti-HIV drug in human medicine. An improvement in the average Karnofsky score (pretreatment 33.2 ± 9.4%, post-treatment 65±12.3%), some laboratory parameters (ie, serum amyloid A and gammaglobulins) and a decrease of FIV viral load in plasma were noted in most cats. The role of concurrent medication in ameliorating the Karnofsky score, as well as the possible development of haematological side effects, are discussed. Side effects, when noted, appeared mild and reversible upon cessation of treatment. Although strong conclusions cannot be drawn owing to the small number of patients and lack of a placebo-treated control group, the activity of (*R*)-PMPDAP, as observed here, warrants further investigation.

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Introduction

Feline immunodeficiency virus (FIV) was first isolated in 1986 as 'a T-lymphotropic virus, causing an immunodeficiency-like syndrome'.[1] This retroviral lentivirus is an important pathogen of both wild and domestic cats worldwide, triggering a broad range of progressive diseases.[2–4] Currently, no species-specific antiviral drugs are available for the treatment of FIV-infected cats; hence, treatment is, at best, supportive and symptomatic, sometimes combined with immunomodulatory therapy.[5]

As FIV and human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT) enzymes are closely related, a number of nucleoside (NRTI) and/or nucleotide RT inhibitors (NtRTIs) of HIV also exert in vitro anti-FIV activity.[6–8] In vivo treatment of FIV-infected cats with these drugs has raised major concerns regarding toxicity and clinical efficacy.[9–13] The most extensively studied NRTI in cats is 3′-azido-3′deoxythymidine (AZT; zidovudine, Retrovir), as a single agent or combined with 2′,3′-dideoxy-3′-thiacytidine (3TC; lamivudine, Epivir). Despite an improvement in general health scores and recovery from opportunistic diseases, long-term use of AZT and the AZT/3TC combination

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treatment is jeopardised in cats owing to the development of non-regenerative anaemia.[9,10,14] Although not reaching statistical significance, a thioether lipid–AZT conjugate, fozivudine, appears to exhibit antiviral activity by decreasing viraemia during acute experimental FIV infection, without the development of adverse effects.[15]

A specific structural class of NtRTI—acyclic nucleoside phosphonates (ANPs)—exert broad-spectrum antiviral activity. Treatment of FIV-infected cats with the ANP 9-2-(phosphonylmethoxyethyl)adenine (PMEA; adefovir) showed greater potency and efficacy than AZT in improving clinical health parameters, but at the cost of a decline in packed cell volume (PCV) and haemoglobin levels.[10,12] However, (*S*)-9-(3-fluoro-2-phosphonylmethoxypropyl)-adenine ([*S*]-FPMPA), an analogue of the HIV-drug tenofovir (ie, PMPA or [*R*]-9-[2-phosphonylmethoxypropyl]adenine) was better tolerated than PMEA in cats, but proved somewhat less antivirally and clinically effective for the treatment of FIV in cats.[11]

Another more closely-related PMPA analogue, (*R*)-9-(2-phosphonylmethoxypropyl)-2,6-diaminopurine ([*R*]-PMPDAP), was reported to be not only a more potent inhibitor of FIV replication than PMEA, but also better tolerated in cats.[12,16] Moreover, clinically healthy, experimentally infected cats showed a significant decrease in FIV viral load when treated with (*R*)-PMPDAP.[12] Although only a tendency towards improvement in clinical parameters was reported in naturally FIV-infected cats treated with (*R*)-PMPDAP, most cats included had mild general health problems, with a mean Karnofsky score (KS) >70% at the beginning of the treatment.[16,17] Hence, the margin for amelioration was narrow. A quantitative monitoring of the viral load during (*R*)-PMPDAP treatment of naturally FIV-infected cats has not been reported.

Here, we report on the initial presentation of naturally FIV-infected cats with moderate-to-severe clinical disease and their clinical, as well as viral, response to treatment with the antiretroviral compound (*R*)-PMPDAP.

Materials and methods

Patient medical records

Medical records of client-owned FIV-positive cats treated with (*R*)-PMPDAP between September 2010 and August 2011 were reviewed. For all cats, owners had given their written informed consent for the treatment of their pets with (*R*)-PMPDAP. The compound had been manufactured in accordance with good manufacturing practices and administered as a formula magistralis.

FIV infection status was determined using a commercial kit (Witness FeLV–FIV; Synbiotics Europe) and confirmed by RT quantitative polymerase chain reaction (qPCR) at a commercial laboratory (Okapi Sciences NV, Heverlee, Belgium).

Records were included if physical examination, complete blood count (CBC) and serum biochemistry findings before and during treatment were available, and if cats had an initial KS ⩽50%. Records were excluded if cats had received other antiviral therapy prior to treatment with (*R*)-PMPDAP.

The KS represents a scoring system to assess a cat's wellbeing and quality of life. The index is based on a questionnaire for the owner or caretaker, considering various aspects of the cat's behaviour, together with a clinician score assessing the general condition of the cat.[17]

Treatment

(*R*)-PMPDAP was administered by subcutaneous injection twice a week at a dose rate of 25 mg/kg. The KSs were used to determine the duration of treatment. If the cat had reached a KS ⩾60% after 3 weeks, treatment was stopped; otherwise, treatment was continued for another 3 weeks. (*R*)-PMPDAP was provided by Okapi Sciences NV, Heverlee, Belgium. Concurrent, supportive medication was administered when necessary. During treatment, all cats were hospitalised at the Small Animal Clinic of Ghent University.

Physical examination and KS

Weekly detailed physical examinations and KSs were available. A follow-up visit was advised 1 month after cessation of treatment. This information was also included if available.

Blood and urine analysis

Blood samples were taken from the jugular vein before the first administration of (*R*)-PMPDAP, every 3 weeks during treatment and at follow-up 1 month after last treatment. Urine samples were taken before and/or during treatment if clinically indicated. CBC, serum biochemistry profile and urinalysis were performed at a private commercial laboratory (Medic Lab Laboratories, Aalst, Belgium).

Viral load quantification

FIV viral load quantification in plasma was determined by Okapi Sciences NV, Heverlee, Belgium. Viral RNA was extracted out of 140 µl cat plasma using the QIAgen Viral RNA mini kit according to the manufacturer's instructions. The extracted viral RNA was reverse transcribed to complementary DNA (cDNA) using 2 µl of the extract in an RT reaction containing 1 × RT reaction mix, 7 mM $MgCl_2$, 2 µl deoxynucleotide triphosphates (2.5 mM each), 2.5 µM random hexamers, 0.2 µl RNase Inhibitor (20 U/µl; Life Technologies) and 0.25 µl Multiscribe RT (50 U/µl; Life Technologies) in a

total volume of 10 µl. The RT programme consisted of following steps: 10 mins at 25°C, 30 mins at 45°C and 5 mins at 95°C. An FIV 1416p real-time PCR targeting the FIV *gag* gene was performed[14] using 2 µl of cDNA added to 20 nM probe; and 100 nM forward and reverse primers, and Fast Real Time Mastermix (Life Technologies) in a total volume of 20 µl. Amplification was done with a StepOnePlus thermocycler (Life Technologies) using the standard Fast programme.

Incorporation of a standard synthetic FIV RNA dilution series (using the RiboMax Large Scale RNA kit [Promega] following the manufacturer's instructions) allowed for absolute FIV viral load quantification. The synthetic FIV RNA was synthesised from a 300-base pair PCR-amplified fragment of the *gag* gene of the FIV Glasgow-8 isolate, targeted by the FIV 1416p real-time PCR. The synthetic FIV RNA obtained was treated with DNase I (Life Technologies) and purified on a MicroSpin G-25 column (GE Healthcare). The synthetic RNA was separated and extracted from a formaldehyde agarose gel to remove remaining FIV DNA contaminants from the FIV RNA template. To generate a standard curve for viral load quantification, 2 µl of a 10-fold dilution series of the synthetic FIV RNA was used in each RT reaction ranging from 10^6 to 10^0 FIV RNA copies per reaction. The limit for quantification for this RT-qPCR is 10^3 virions per ml plasma.

Results

The records of 10 client-owned FIV-positive cats treated with (*R*)-PMPDAP were available. Three cats were excluded as they had previously been treated with antiviral drugs. One additional patient was not considered, as the initial KS was >50%.

Signalment and clinical signs

The age of the included cats ranged from 3 to 11 years; the majority (4/6) over 8 years of age. Male cats were overrepresented (4/6); all cats were neutered. The cats suffered from a variety of clinical diseases: oral disease (4/6), dermatological conditions (2/6) and conjunctivitis (2/6). All cats but one (cat K) were underweight, with a body condition score of 3 on a 9-point scale.[18] Cat M presented with a mandibular phlegmon, cat P concurrently suffered from hypertrophic cardiomyopathy, and cat F had a history of polydipsia and polyuria. Cat K was known to be feline leukaemia virus (FeLV)-positive and presented with vague symptoms—inappetence, occasional vomiting and lethargy—without abnormal findings on physical examination.

Treatment schedule and concurrent medication

Two cats (cats F and J) received the antiviral treatment for 3 weeks, three cats (cats P, N and K) for 6 weeks and cat M received the treatment for only 1 week (as described below). Cat K was treated four times a week instead of twice a week from the second week of treatment onwards. All cats but one (cat F) received concurrent medication, including antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs).

General health

During treatment The main clinical signs (ie, stomatogingivitis, dermatitis, polyuria and polydipsia, conjunctivitis) remained stable throughout the treatment period in four cats (cats F, J, P and N), but the condition of two cats (cats M and K) deteriorated markedly after 1 week of treatment to a point requiring intensive care therapy.

Cat M had to be humanely euthanased owing to recurrent hypotension not responding to treatment. Autopsy revealed acute diffuse interstitial pneumonia, liver necrosis, immune complex-mediated glomerulonephritis and non-suppurative encephalitis.

Cat K showed abnormalities on abdominal ultrasonography suggestive of cholangitis/cholangiohepatitis, together with hyperbilirubinemia and a moderate elevation of serum bile acids. The cat recovered following intensive treatment. Further supportive care for cat K consisted of corticosteroid administration for recurrent episodes of fever despite antibiotic therapy and the daily addition of insulin owing to the development of diabetes mellitus. Consequently, the frequency of antiviral treatment was doubled from a twice a week to a four-times weekly dosing schedule from the second week of (*R*)-PMPDAP treatment onward.

One month after cessation of antiviral therapy Of the five cats completing (*R*)-PMPDAP treatment, four cats (cats F, P, N and K) underwent a follow-up visit 1 month after the last administration of the antiviral compound. The remaining cat (cat J) was euthanased 2 weeks after discharge because of persisting anorexia.

In three cats (cats F, P and K), clinical presentation seemed similar to their condition at the end of (*R*)-PMPDAP treatment. Cat N had received supportive treatment with an antibiotic and an NSAID for an episode of pyrexia that occurred after discharge and showed, still on this treatment, an ameliorated clinical condition (ie, ameliorated dermatitis and gingivitis) at follow-up.

KS

The evolution of KS is shown in Table 1. The average KS was 33.2 ± 9.4% at initial presentation (D0). Compared with D0, all cats (n = 5) showed an improvement in the KS at discharge (Tend), with an average increase of 28.6%.

At follow-up (Tend + 1 m), all four cats showed a higher KS when compared with their initial score (D0). However, compared with the end of the treatment period

Table 1 Evolution of Karnofsky scores expressed in percentages before and during treatment of six FIV-positive cats with (*R*)-PMPDAP

Cat	D0	D0→ Tend	D0→ Tend + 1 m
F	31	+30	+16
J	36	+24	–
P	31	+27	+5
N	40	+19	+50
K	44	+43	+38
M	17	–	–

D = day of treatment; D0 = first day of (*R*)-PMPDAP treatment; Tend = end of treatment; D0→Tend = evolution from onset until end of treatment (ie, 3 or 6 weeks); D0→ Tend + 1 m = evolution from onset until one month following last treatment

(Tend), 3/4 cats (cats F, P and K) presented with a lower score at follow-up (Tend + 1 m).

Laboratory results

Haematology The main haematological parameters are summarised in Table 2.

Four cats (cats F, J, P and M) had anaemia at presentation (PCV <24%), while at follow-up only one (cat F) was anaemic.

Before treatment, two cats (cats K and M) were leukopenic as a result of a lymphopenia. For only one of these cats (cat K) were follow-up data available. One month after the cessation of (*R*)-PMPDAP treatment, the leukocyte count had normalised, but the cat remained lymphopenic.

Three cats (cats F, P and N) initially presented with a leukocytosis, which was still present on follow-up. In one of these cats (cat P), an increased lymphocyte count was noted at initiation, which had returned to within normal limit values at follow-up examination.

Cat M showed a pancytopenia at the start of antiviral treatment, with an aggravated anaemia following 1 week of treatment.

Biochemical profile Two cats (cats F and J) were mildly azotaemic; two others (cats N and M) showed elevated urea levels without an increased creatinine concentration. Mild-to-moderate hyperphosphataemia was seen in three cats (cats F, N and K). Four cats (cats F, J, P and N) presented with hyperproteinaemia and a persistent hypergammaglobulinaemia. At the end of treatment, total protein concentration had normalised in two cats (cats P and N). Electrophoresis showed a decreased gamma globulin fraction in one of them (cat N). Cat K showed persistent hypoproteinaemia with hypogammaglobulinaemia. The acute phase protein serum amyloid A (SAA) was elevated in all samples and decreased during treatment in all but one cat (cat F).

As previously mentioned, cat K developed diabetes mellitus. Blood glucose and serum fructosamine concentrations had normalised upon follow-up analysis while under daily insulin treatment. At the end of the (*R*)-PMPDAP treatment period, an elevated alanine aminotransferase (ALT) activity (172 U/l, reference interval [RI] <122 U/l) was noted for cat K. Subsequently, abdominal ultrasound showed a focal echoic structure within the gallbladder lumen without signs of cholangitis. During follow-up, several liver values increased, namely aspartate aminotransferase activity (AST) (80 U/l, RI <65 U/l) and ALT (164 U/l), total bilirubin (25.7 µmol/l, RI <17.1 µmol/l) and serum bile acids (96 µmol/l, RI <20 µmol/l). Abdominal ultrasound was not repeated.

Urinalysis Urinalysis results of only four cats (cats F, J, N and K) were available. One cat (cat K) had a normal urinary protein:creatinine (UPC) ratio (<0.4) on repeated urinalysis from day 28 of (*R*)-PMPDAP treatment (D28) onwards.

The other three cats showed an increased UPC without active sediment. One of these (cat N) had a UPC ratio of 0.9, together with a urinary specific gravity >1.035 on D0. Unfortunately, no repeated measurements were available in this cat.

The remaining two cats (cats F and J), who were also azotaemic at the onset of antiviral treatment, showed isosthenuria and a substantial increase in the UPC ratio (average UPCs of 3.3 and 15.7, first measured on D0 and D7, respectively), which was persistent. An abdominal ultrasound was performed in the cat (cat F) with the highest UPC, which showed significant renomegaly. This cat had a systolic blood pressure (SBP) of 110 mmHg, measured at a single occasion during treatment.

Viral load

Figure 1 displays the FIV viral load in four cats before, during and after treatment. Cat M survived for only 1 week after the initiation of treatment with (*R*)-PMPDAP; therefore, only the initial viral load is shown. Viral load results below a reliable quantification limit (<10^3 virions/ml plasma) are not shown in Figure 1, namely at all time points for cat J and at D21 for cat F. Viral load decreased during antiretroviral treatment in 3/4 cats (cats F, P and N), while an increase was noted in the remaining cat (cat K). At follow-up, FIV viral load had decreased in two cats (cats N and K) compared with their levels at the end of treatment with (*R*)-PMPDAP. In the other two cats (cats F and P), FIV viral load had increased again.

Discussion

Herein, we report on the efficacy and safety of (*R*)-PMPDAP treatment administered for 3–6 weeks to a

Table 2 Haematological parameters before and during treatment with (*R*)-PMPDAP of six FIV-positive cats

Haematological variable	D0 (n = 6)		D21 (n = 5)		D42 (n = 3)		D0→Tend		Tend + 1 m (n = 4)		D0→ Tend + 1 m	
	Mean ± SD	(Range)	Mean ± SD	(Range)	Mean ± SD	(Range)	↓	↑	Mean ± SD	(Range)	↓	↑
PCV (%)	23.7 ± 4.1	(20–31)	23.0 ± 6.4	(18–33)	29.0 ± 5.6	(23–34)	3/5	2/5	28.3 ± 4.6	(22–33)	-	4/4
Leukocyte count (10^3/µl)	10.9 ± 11.2	(1.2–28.7)	14.7 ± 7.8	(4.8–23.7)	17.5 ± 5.1	(11.9–21.9)	2/5	3/5	18.2 ± 8.3	(6.3–25.2)	2/4	2/4
Lymphocyte count (10^3/µl)	3.7 ± 3.8	(0.2–10.6)	3.4 ± 2.4	(0.9–6.7)	5.5 ± 3.9	(1.0–8.3)	2/5	3/5	4.3 ± 2.9	(0.8–6.8)	2/4	2/4

D = day of treatment; D0 = first day of (*R*)-PMPDAP treatment; Tend = end of treatment; D0→Tend = evolution from onset until end of treatment (ie, 3 or 6 weeks); D0→ Tend + 1 m = evolution from onset until 1 month following last treatment; PCV = packed cell volume

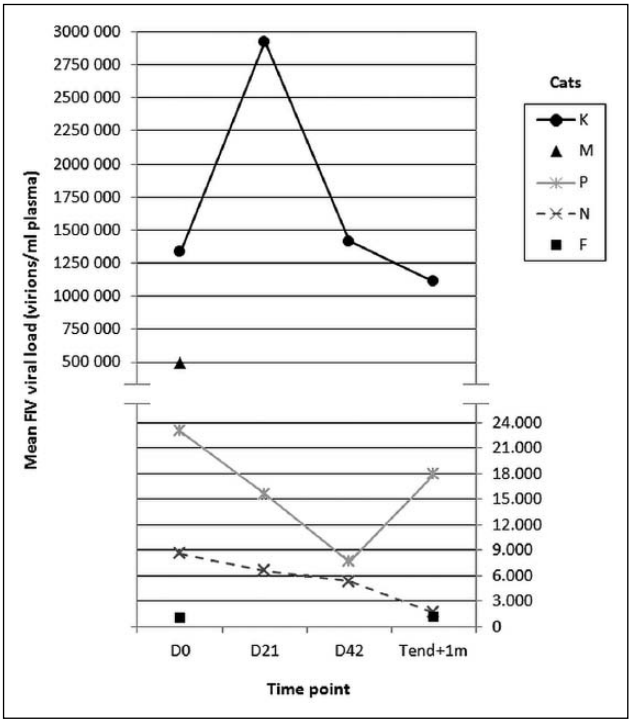

Figure 1 Viral load before and during treatment of five FIV-positive cats with (*R*)-PMPDAP. Cat M survived for only 1 week after the initiation of antiviral treatment. Viral load values $<10^3$ virions/ml plasma were considered unreliable for quantification and are not shown. Viral load results were below a quantification limit at all time points for cat J and at D21 for cat F. D = day of treatment, D0 = first day of (*R*)-PMPDAP treatment; Tend + 1 m = 1 month after last treatment

small number of naturally FIV-infected cats. The following data were available: physical examinations, including a KS (modified for cats),[17] blood and urine examinations, and viral load quantification. Where available, follow-up data were reported to assess longer-term treatment effects and the possible reversibility of any adverse events.

Signalment and clinical presentation of the six included FIV-positive cats were consistent with the scientific literature.[3,5,19,20]

During (*R*)-PMPDAP treatment, the KS of all cats improved, suggesting an improved general health status and quality of life. An increase in the KS of FIV-positive cats following (*R*)-PMPDAP treatment has been described previously, although it was not found to be statistically significant compared with a placebo control arm.[16] However, the initial average KS in the aforementioned placebo-controlled study was about 40% higher than the scores observed in our study. Hence, cats included in our study presented with more severe clinical conditions at the start of treatment and thus had markedly lower KSs. The average KS increased from 33.2 ± 9.4% to 65.0 ± 12.3% at the end of antiviral treatment.

However, the potential beneficial effect of concurrent medication has to be considered as it is likely to have contributed to the improvement in the KSs. Indeed, FIV-infected cats may fully recover, even from a moribund condition, with adequate and proper care.[21]

Historically, an objective assessment of the wellbeing and quality of life of cats has been challenging for veterinary clinicians. The modified KS for cats was proposed by Hartmann and Kuffer[17] as an attempt to facilitate this task. To the best of our knowledge, validation of this quantitative scoring system has not been performed. Moreover, the influence of confounding factors on the KS determination, for example, the acclimatisation of cats during hospitalisation and the unblinded aspect of the antiviral treatment, cannot be excluded.

The relapsing hypotension leading to euthanasia during treatment for one cat is, to the best of our knowledge, not a reported side effect of ANP antivirals. In contrast, antiviral treatment is rather associated with

hypertension in HIV-infected humans.[22] The presence of a mandibular phlegmon, neutropenia, anaemia and hypoglycaemia in this cat could reflect sepsis. No blood culture was performed; hence, the diagnosis remains unclear.

Apart from the situation with the pancytopenic cat, haematological anomalies were common in the treated cats, both before and during treatment. Several haematological deficits in FIV-positive cats, such as the observation of anaemia, leukopenia, lymphopenia and neutropenia akin to the presence of these cytopenias in HIV-infected humans, have been reported in numerous papers.[2,23–25] However, a clear association of these abnormalities with FIV infection appears inconsistent. When compared with FIV-negative control cats, matched by age and clinical condition, most haematological deficits were not associated with FIV infection, except for neutro- and monocytopenia.[26–28] Consequently, and in contrast with the cytopenias traditionally assigned to FIV-infection, it is interesting to note that three cats showed a leucocytosis throughout the whole treatment period.

During treatment of FIV-infected cats, ANPs are known to cause a mild-to-severe anaemia.[10–12,29] For (*R*)-PMPDAP specifically, a mild decrease in PCV is reported in naturally infected FIV cats,[16] whereas another study reported no toxic side effects in experimentally FIV-infected cats.[12] Therefore, two cats showing an improvement in PCV values at the end of treatment is noteworthy. Moreover, all four cats seen at follow-up examination had an increased PCV. Hence, as some cats developed a lower PCV during treatment, a negative haematological effect cannot be excluded, but reversibility of this potential adverse event seems likely.

Notable on serum biochemistry were elevated phosphorus, urea and creatinine levels in several cats. In humans, an HIV-associated nephropathy has been described, with proteinuria and progressive deterioration of renal function.[30,31] Previous studies have also suggested an FIV-associated nephropathy in cats.[32–36] However, recent studies could not assign an absolute association with renal azotaemia,[37,38] but proteinuria did appear to be associated with FIV infection.[38] Two of the azotaemic cats in this study had persistent substantial proteinuria indicative of glomerular proteinuria. As hypertension can have an influence on proteinuria, and a previous study described a significantly higher SBP in FIV-positive cats,[39] further studies to evaluate proteinuria and SBP in naturally FIV-infected cats are recommended. In our cats, SBP was recorded in only one azotaemic cat, and appeared normal.

The hyperproteinaemia with high gamma globulin fractions in all but one cat is in agreement with previous observations in naturally FIV-infected cats.[24,26,35,40–42] The only cat with hypogammaglobulinaemia was co-infected with FeLV, which can be the cause of hypogammaglobulinaemia through a B-lymphocyte function deficit.[43]

At the end of antiviral treatment, two cats showed total protein concentrations within the normal RI, with decreased gamma globulin levels in one of them. As FIV infection can cause a polyclonal B-cell activation,[44] this decrease may be considered favourable.

SAA is one of the major and most rapidly responsive acute phase proteins in cats. It appears to be a useful marker for the status of inflammatory diseases and for evaluating the response to treatment in cats.[45–47] Therefore, it is interesting to note that several cats showed a decline in their overall increased SAA level at the end of treatment.

Viral load declined during treatment in all but one cat, confirming previous studies describing significant decreases in viral load in experimentally FIV-infected, (*R*)-PMPDAP-treated cats.[12,48] Plasma viraemia appears to be, akin to HIV-1 infected humans,[49] a possible indicator of the disease stage in FIV-infected cats, and quantitative analysis might act as a predictive value of disease progression.[50,51] In accordance, the two cats that developed a clinical deterioration also had the highest viral loads. Again, a potential beneficial influence of concurrent medication on the viral load levels cannot be excluded,[21] as viral load decreased when concurrent diseases were better regulated (eg, diabetes mellitus in one cat).

Conclusions

This study suggests that treatment with (*R*)-PMPDAP of six naturally FIV-infected cats, suffering from moderate to severe clinical disease might lead to an improvement of the cats' quality of life and wellbeing, as measured by the modified KS, as well as a decrease in FIV viral load in plasma. In addition, some biochemical parameters appear to improve. Concurrent medication likely contributed to this amelioration.

A mild decrease in PCV was observed during treatment in three cats, but these changes seemed reversible within 1 month of stopping the antiviral therapy.

Owing to the small number of patients and lack of a control group, no definite conclusions can be drawn. Nevertheless, the data suggest that the potential use of (*R*)-PMPDAP should be further explored for the treatment of FIV infections in cats.

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Conflict of Interest Nesya Goris, Joeri Auwerx and Johan Neyts have a financial interest in Okapi Sciences NV.

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