Undetectable proviral DNA and viral RNA levels after raltegravir administration in two cats with natural feline leukemia virus infection

Valores indetectáveis de DNA Pró-viral e RNA viral após o uso de raltegravir em dois gatos com infecção pelo vírus da leucemia: relato de caso

Carla Regina Gomes Rodrigues Santos[1] ● Isabela Torres Ferreira[2] ● , Renata Beranger[3] ● , Julia Possebon Santi[4] ● , Mariana Palha de Brito Jardim[1] ● & Heloisa Justen Moreira de Souza[5] ●

[1] Veterinarian, MSc. Programa de Pós-Graduação em Medicina Veterinária (PPGMV), Departamento de Clínica e Cirurgia Veterinária (DMCV), Instituto de Veterinária (IV), Universidade Federal Rural do Rio de Janeiro (UFRRJ). *Campus* Seropédica, Seropédica, RJ, Brazil.
[2] Veterinarian, Rio de Janeiro, RJ, Brazil
[3] Infectologist, Rio de Janeiro, RJ, Brazil
[4] Veterinarian, Resident. Programa de Residência em Medicina Veterinária – Clínica Médica dos Gatos Domésticos, DMCV, IV, UFRRJ. *Campus* Seropédica, Seropédica, RJ, Brazil.
[5] Veterinarian, DSc. DMCV, IV. *Campus* Seropédica, Seropédica, RJ, Brazil.

Abstract

Feline leukemia virus (FeLV) infection was discovered over 50 years ago; however, the serious clinical changes associated with FeLV infection still have great importance in the diagnosis, prevention, and clinical management of symptomatic patients. Progressive infection with FeLV leads to a reduction in the patient's life expectancy and quality of life. This report describes the use of an antiretroviral integrase inhibitor, raltegravir, in two cats with natural FeLV infection. Raltegravir was administered orally at a dose of 40 mg/cat every 12 h in both cases. In case one, 13 weeks after starting raltegravir, RNA loads were undetectable, while proviral DNA loads were still detectable. In case two, proviral DNA loads were undetectable after 32 weeks of medication, while RNA loads were undetectable throughout the treatment. No adverse effects or laboratory test abnormalities were detected with the use of raltegravir in either patient. The patients are currently clinically healthy, still receiving the drug, and are under close observation. To our knowledge, this is the first report describing the use of raltegravir in naturally infected FeLV-positive cats and its effects on circulating viral load. Moreover, the patients described here were followed-up for a longer period than those in previously reported cases.

Keywords: antiretroviral, integrase inhibitor, cats, retroviruses.

Resumo

A infecção pelo vírus da leucemia felina foi descoberta há mais de 50 anos, mas as graves alterações clínicas associadas à infecção pelo FeLV, ainda denotam grande importância no diagnóstico, nas medidas de prevenção e no manejo clínico de pacientes sintomáticos. A infecção progressiva pelo FeLV acarreta na redução do tempo e qualidade de vida do paciente. Este relato descreve o uso de um antirretroviral inibidor da integrase, o raltegravir, em dois gatos com infecção natural pelo FeLV. O raltegravir foi utilizado na dose de 40 mg/gato por via oral a cada 12 horas em ambos os casos. No primeiro caso após treze semanas do uso do raltegravir, a quantificação de RNA foi indetectável e no segundo caso, após trinta e duas semanas o número de cópias de DNA pró-viral foi indetectável. A carga de RNA nunca foi detectada neste paciente. Nenhum efeito adverso, nem alterações laboratoriais foram detectadas com o uso do raltegravir em ambos os casos. Atualmente, os pacientes encontram-se clinicamente saudáveis, fazem uso do antirretroviral com monitorização contínua. Este é o primeiro relato que descreve o uso do raltegravir em gatos infectados naturalmente pelo FeLV e seus efeitos na carga viral circulante. Além disso, os pacientes descritos aqui foram acompanhados por um período maior que os trabalhos anteriores descritos.

Palavras-chave: antiretroviral, inibidor de integrase, gatos, retroviroses.

*Correspondence
Carla Regina Gomes Rodrigues Santos
Departamento de Clínica e Cirurgia Veterinária (DMCV), Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro – UFRRJ
Rodovia BR 465, Km 7, Campus Universitário, Bairro Zona Rural
CEP 23897-000 - Seropédica (RJ), Brasil
E-mail: carlavetuff@yahoo.com.br
Tel: +55 (21) 96469-2559

Introduction

Feline leukemia virus (FeLV) infection is highly prevalent in cats worldwide. High infection rates have been observed in developing countries and regions, such as South America and Africa (Al-Kappany et al., 2011; Muchaamba et al., 2014; Ortega-Pacheco et al., 2014; Azócar-Aedo & Montí, 2015; Ludwick & Clymer, 2019; Ortega et al., 2020). In Brazil, the actual number remains unknown, since few animals have been tested for this infection and published data are scarce. Nonetheless, previous studies have pointed out that it may be as high as 31% in antigenemic cats and up to 47.5% in PCR-positive cats (Da Costa et al., 2017; Coelho et al., 2011). FeLV is a typical retrovirus with a single-stranded RNA genome, which is transcribed by the reverse transcriptase (RT) enzyme in a DNA strand, named provirus.

The provirus is subsequently integrated into the host cell genome. Unlike other major feline retroviruses, such as feline immunodeficiency virus (FIV), FeLV is a gammaretrovirus and is much more pathogenic and fatal than FIV (Hartmann, 2012; Hartmann, 2015; Hartmann & Hofmann-Lehmann, 2020). Lymphoid neoplasms, hematologic diseases, immunosuppression, neuropathies, and reproductive disorders are possible consequences of FeLV infection. These clinical outcomes, besides their potential to lead to patient death, are motives for emotional and financial damage to owners (Hartmann, 2012; Little et al., 2020).

Antiretroviral drugs have been used for the treatment of human immunodeficiency virus infections for more than 40 years. Throughout this time, a lot of research has been performed and antiretroviral therapy was improved, allowing new drugs to be incorporated into the therapeutic arsenal , thus enhancing the treatment success, minimizing adverse effects, and maximizing viral suppression (Sepkowitz, 2001; Gulick et al., 1997; Ho, 1995). Cats experimentally and naturally infected with FIV and FeLV were used as experimental models for human antiretroviral therapy (Tavares et al., 1989; Hartmann et al., 1992; Bisset et al., 2002; Van Rompay, 2010). The RT inhibitor nucleoside analogs zidovudine (AZT), lamivudine, tenofovir, and integrase inhibitors, such as raltegravir, are potential drugs for the treatment of feline retroviruses (Bisset et al., 2002; Cattori et al., 2011; Medeiros et al., 2016). However, few studies have evaluated antiretroviral therapy in FeLV-infected cats. This report aimed to describe the decrease in RNA and proviral DNA loads to undetectable levels in two cats naturally regressively infected with FeLV and receiving antiretroviral therapy with raltegravir (40 mg/cat PO BID), along with their clinical improvement.

Case reports

Case one was an approximately 5-year-old intact male Persian cat that was rescued from the streets and immediately taken for veterinary care. On physical examination, the patient had 7% dehydration, weighed 2.7 kg, had a body condition score (BCS) of 2/9, dull and unkept coat, and had a normal conscious state. Oral examination revealed severe periodontal disease aggravated by the presence of myiasis, hard palate ulceration, and necrosis. Bilateral keratitis, conjunctivitis, and uveitis with secondary glaucoma were observed in the right eye. In the left eye, the pupillary light reflex was absent, and fundoscopy revealed retinal atrophy. No other abnormalities were detected during the physical examination. The patient remained hospitalized for treatment and laboratory and imaging examinations. Clindamycin (10 mg/kg every 12 h for 10 days), single-dose nitenpyram, and meloxicam 0.05 mg/kg once a day for 3 days were prescribed. Ophthalmic treatment consisted of 2% dorzolamide hydrochloride eye drops and ciprofloxacin based on chondroitin. Abdominal ultrasonography and echocardiography revealed no changes.

The complete blood count (CBC) and serum biochemistry revealed unremarkable findings. A point-of-care (POC) screening test (FIV Ac/FeLV Ag Test Kit, ALERE™) was performed with a whole blood sample, and the result was negative for both agents. PCR assay for chemotropic *Mycoplasma sp.* and serology for *Toxoplasma gondii* IgM and IgG were negative. However, when qPCR and qRT-PCR respectively, were performed for FeLV proviral DNA and viral RNA loads, both yielded positive results with 2.0×10^7 and 5.6×10^4 copies/mL of whole blood, respectively.

After the laboratory results were obtained, antiretroviral therapy was initiated using raltegravir (Isentress™ 400 mg, Merck Sharp & Dohme) re-compounded into 40 mg capsules and administered orally *bis en die* (BID) continuously. Surgical treatment for periodontal disease was performed 30 days after initiation of raltegravir. Hematological, biochemical, and qPCR follow-ups were performed sequentially until 22 weeks post-therapy (Tables 1 and 2). At 13 and 18 weeks, the

RNA loads were undetectable at both time points, whereas the proviral DNA loads were 1 x 10^6 and 4.7 x 10^4 copies/mL of whole blood, respectively. No clinical or laboratory abnormalities were observed during the 22 weeks when raltegravir was administered. During this period, the cat showed considerable weight gain and the BCS improved to 6/9. At the time this report was published, the patient was still receiving antiretroviral therapy and was being monitored.

Case two was a 2-year-old, non-neutered, male domestic shorthair cat that presented with dyspnea, cough, sneezing, diarrhea, and pruritus. It was adopted from the streets 2 weeks earlier, already presenting the aforementioned signs. On physical examination, it weighed 3.37 kg and had a BCS of 3/9. Thoracic auscultation revealed a discontinuous sternum. High airway stridor and serous nasal discharge were evident, and there were lice eggs, bilateral otitis, and extensive alopecic areas throughout the fur. Wood's lamp test was positive for dermatophytosis. Thoracic radiography revealed a broncho interstitial pattern, and abdominal ultrasonography demonstrated mild enteritis and hepatopathy. The CBC and biochemistry panel revealed moderate neutrophilic leukocytosis (32400 cells/mm^3, range 2500–12500 cells/mm^3) and increased alanine aminotransferase (508 U/L, range 5–60 UI/L).

Treatment was initiated with a combination of single-dose ivermectin and topical praziquantel, famciclovir 125 mg/cat every 12 h for 10 days, itraconazole 10 mg/kg every 12 h for 60 days, and marbofloxacin 2 mg/kg orally twice a day until clinical improvement. An FIV Ac/FeLV Ag POC test (Idexx Laboratories®) was performed, which was negative for both agents. However, when quantitative PCR was performed for both RNA and proviral DNA loads, the former was undetectable and the latter was positive (3.3 x10^4 proviral DNA copies/mL of whole blood). All PCR assays (from this case and the previous one) were performed at Vet Análises Diagnóstico Veterinário (Rio de Janeiro, Brazil), following the protocol described by Torres et al. (2005).

Raltegravir (Isentress™ 400 mg, Merck Sharp & Dohme) therapy was initiated 4 weeks after presentation. It was re-compounded into 40 mg/capsule and orally administered BID continuously.

The patient's condition improved satisfactorily. Follow-up CBC, biochemistry, and qPCR were performed 2 and 32 weeks after therapy initiation (Tables 1 and 2). At the 32nd week, the proviral DNA was undetectable. No adverse effects or laboratory test abnormalities were detected with raltegravir use. The patient is clinically healthy and is still receiving the drug under close monitoring.

Discussion

Current guidelines emphasize the need for more than one diagnostic test to confirm the individual real FeLV status. POC tests are useful screening tools and should be used to detect antigenemia; however, their association with PCR assays enables further characterization of the infection status and understanding of discordant results (Westman et al., 2019; Little et al., 2020). In this report, both patients exhibited negative POC test and positive PCR results for proviral DNA. The first patient tested positive for viral RNA. This can confuse the infection status. However, antigen testing is less sensitive than qPCR. The same applies to PCR for RNA compared with qPCR for DNA, with the former being much less sensitive (Torres et al., 2008). This could explain the second case, in which a fair DNA load was detected at two time points; however, no RNA was detected. Agreement between these two markers is expected (both positive and negative), with only 24 of 264 discordant results (Tandon et al., 2005; Torres et al., 2008; Helfer-Hungerbuehler et al., 2015).

Owing to the lack of antigenemia in both cases, the probable regressive status in these cats, and therefore, the clinical benefits of antiviral therapy may be argued. However, it is indisputable that reactivation and progression to a progressive status are possible (Hofmann-Lehmann et al., 2001; Helfer-Hungerbuehler et al., 2015). Moreover, for both patients, the owners were unwilling or unable to separate these cats from others at home. Therefore, antiretroviral therapy was initiated to halt viral replication and the possible development of a progressive infection, once this could lead to the dissemination of the virus among other cats in the household.

AZT is an antiretroviral with proven long-term efficacy in cats naturally infected with FIV (Medeiros et al., 2016); however, it is not effective against gammaretroviruses, such as FeLV, in comparison with integrase inhibitor drugs (Smith et al., 2010). Tavares et al. (1989) published the first *in vitro* and *in vivo* study using AZT at different dosages and times after experimental infection with FeLV, revealing that an early antiretroviral therapy, with Zidovudine within a

Table 1. Hematological and biochemical parameters of two cats with FeLV infection before and during raltegravir treatment.

Hematological analysis	Reference range	Case 1					Case 2		
		Week 0	Week 5	Week 13	Week 18	Week 22	Week 0	Week 2	Week 32
RBC (x10⁶ cells/µL)	5.0-10.0	4.9	9.1	7.4	6.8	8.1	7	7	9.8
Ht (%)	24.0-45.0	25.1	48.4	35	33.6	41.2	39	37	50
Hb (g/dL)	8.0-15.0	8.4	17.1	12.2	10.9	12.8	12.5	12	17
MCV (fL)	39.0-55.0	50.3	52.7	47.2	49.1	50.3	55.7	25.9	51
MCHC (g/dL)	30.0-36.0	33.5	35.3	34.9	32.44	31	32.1	32.4	34.6
PLT (cells/µL)	200000-600000	344000	394000	569000	225000	226000			266000
LEUC (cells/µL)	5500-19500	5600	5600	7430	18500	13000	32400	8600	11100
NEU (10³/µL)	2500-12500	4704	3360	5201	9250	7410	29808	7396	7548
LYMP (10³/µL)	15000-7000	784	1680	1635	8695	4290	1296	688	2220
MON (10³/µL)	0-850	56	168	74	185	260	0	172	1332
EON (10³/µL)	100-1500	56	392	520	370	1040	1296	344	0
TP (g/dL)	5.5-8.0	9	7	7.8	8	8.4	9.2	8.8	9.4
Biochemical analysis									
Urea (mg/dL)	30-60	30.4			58	63.9	36		
Creatinine (mg/dL)	0.5-1.9	1			1.2	1	0.8		
ALT (U/L)	5-60	48.8			50	122.4	508		
AP (U/L)	0-90	18.1			32.3	31.77			
Albumin (g/dL)	2.1-3.3								1.9
Globulin (g/dL)	2.6-5.1								5.6

RBC = red blood cells; Ht = hematocrit; Hb = hemoglobin; MCV = mean corpuscular volume; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets; LEUC = leukocytes; NEU = neutrophils; LYMP = lymphocytes; MON = monocytes; EOS = eosinophils; TP = total plasma proteins; ALT = alanine aminotransferase; AP = alanine phosphatase. Reference values (Willard & Twedt, 2004).

Table 2. Results of proviral load (DNA) and viral load (RNA) using qPCR and qRT-PCR, respectively in whole blood of two cats with FeLV infection before and during raltegravir treatment.

	Case 1				Case 2		
	Week 0	Week 5	Week 13	Week 18	Week 0	Week 2	Week 32
RNA viral (Copies/mL)	5.6×10^4	2.1×10^2	Undetectable	Undetectable	Undetectable	Undetectable	Undetectable
Proviral DNA (Copies/mL)	2.0×10^7	2.0×10^6	1.0×10^6	4.7×10^4	3.3×10^4	2.2×10^4	Undetectable

week post-infection could prevent the progressive infection (Hartmann et al., 1992). However, in naturally infected cats, short-term antiretroviral therapy with zidovudine failed to decrease antigenemia or induce clinical improvement (Stuetzer et al., 2013).

To our knowledge, this is the first report describing raltegravir therapy inducing RNA and proviral DNA load reduction to undetectable values in Brazil. The patients described herein were treated for longer periods than experimentally infected cats were (Boesch et al., 2015). Raltegravir inhibits provirus integration into the host DNA. Compared to RT inhibitors, it is more selective and shows better control over gammaretrovirus replication (Smith et al., 2010; Singh et al., 2010). It efficiently curbs FeLV replication *in vitro*, and its safety and efficacy have been observed *in vivo* in experimentally infected animals treated for short durations (Catorri et al., 2011; Boesch et al., 2015). Antiretroviral-associated adverse effects are mostly described in cats receiving AZT, such as emesis and anemia; however, they are responsive to symptomatic therapy and reversible after treatment discontinuation (Gómez et al., 2012). Raltegravir is metabolized via glucuronidation, a metabolic pathway that is deficient in cats (Smith et al., 2010; Singh et al., 2010). However, no clinical, hematological, or biochemical abnormalities were observed during the treatment period in the cats described herein. Nonetheless, in a study by Boesch et al. (2015), after the discontinuation of therapy, the viral loads exhibited a rebound effect, with the elevation surpassing the initial levels in all but one cat (1/18). This implies that raltegravir therapy should be administered continuously.

Four and three qPCR and RT-PCR assays were performed for the first and second cases, respectively. In the first case, in which both proviral DNA and RNA were positive, the RNA load was undetectable by the 13th week, whereas the DNA load was reduced by 10^3 copies. According to Beall et al. (2021), cats in which the viral load is $<4 \times 10^4$ copies/mL are considered low, and the infection course is most likely regressive. In the second case, the patient was positive only for proviral DNA, which was undetectable in the 32nd week. No controlled studies with naturally infected cats undergoing antiretroviral therapy with raltegravir have been reported; therefore, the viral load reductions cannot be compared. Nonetheless, continuous reduction was observed in both patients, as already described in the study with experimentally infected cats subjected to treatment with the drug. The decrease in proviral DNA load was a surprising finding since experimentally infected cats receiving this medication suffered a 5x RNA load reduction; nonetheless, the proviral DNA loads were not altered (Boesch et al., 2015).

Another point of concern is the possibility of viral resistance associated with non-suppressive and low-potency monotherapy. This has been known in human medicine since 1995, when an iconic study showed the emergence of viral resistance through a mere base pair substitution. The term HAART (highly active antiretroviral therapy) then arose, enabling a therapeutic combination of different drug classes and prolonging viral suppression (Ho, 1995; Gulick et al., 1997; Sepkowitz, 2001). Despite the absence of adverse effects and the outcomes of both current patients, this report was limited due to the number of cats. Moreover, there is a concern over long-term monotherapy efficiency, which was not evaluated due to the short follow-up time. Thus, it is of great importance to developing controlled studies to assess the response to antiretrovirals and drug-induced resistance emergencies. These results may contribute to attaining a consensus on feline antiretroviral therapy and improve the quality and life expectancy of retrovirus-infected cats.

Conclusions

Raltegravir, when administered at a dose of 40 mg/cat PO BID, was successful in reducing viral loads to undetectable levels in two cats with natural FeLV infection. These findings suggest the need for more controlled studies to assess the efficacy of this drug in a larger population of FeLV-positive cats, and to evaluate antiretroviral therapy for improving patient prognoses.

Ethics statement

All procediments were consented by the animal owner (for case reports)

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Conflict of interests

No conflict of interest

Authors' contributions

CRGRS, ITF, JPS- Development of methodology; preparation and writing the initial draft CRGRS, RB, MPBJ, HJMS - Writing, Review and Editing manuscript.

Availability of complementary results

All information obtained as a result of the study is included in the manuscript.

The study was carried out at the Hospital Veterinário de Pequenos Animais – HVPA, from the Universidade Federal Rural do Rio de Janeiro, Campus Seropédica, RJ, Brasil.

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