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Oral Recombinant Feline Interferon-Omega as an alternative immune modulation therapy in FIV positive cats: Clinical and laboratory evaluation ☆

S. Gil [a,*,1], R.O. Leal [a,1], D. McGahie [b], N. Sepúlveda [c,d], A. Duarte [a], M.M.R.E. Niza [a], L. Tavares [a]

[a] Centro de Investigação Interdisciplinar em Sanidade Animal (CIISA), Faculdade de Medicina Veterinária, University of Lisbon (ULisboa), Av. Universidade Técnica, 1300 477 Lisbon, Portugal
[b] Virbac, 13[e] rue LID – BP 27, F 06511 Carros cedex, France
[c] London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E7HT, United Kingdom
[d] Centro de Estatística e Aplicações da Universidade de Lisboa, FCUL, Bloco C6-Piso 4 Campo Grande, 1749 016 Lisboa, Portugal

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ABSTRACT

Recombinant-Feline Interferon-Omega (rFeIFN-ω) is an immune-modulator licensed for use subcutaneously in Feline Immunodeficiency virus (FIV) therapy. Despite oral protocols have been suggested, little is known about such use in FIV-infected cats. This study aimed to evaluate the clinical improvement, laboratory findings, concurrent viral excretion and acute phase proteins (APPs) in naturally FIV-infected cats under oral rFeIFN-ω therapy (0.1 MU/cat rFeIFN-ω PO, SID, 90 days). 11 FIV-positive cats were treated with oral rFeIFN-ω (PO Group). Results were compared to previous data from 7 FIV-positive cats treated with the subcutaneous licensed protocol (SC Group). Initial clinical scores were similar in both groups. Independently of the protocol, rFeIFN-ω induced a significant clinical improvement of treated cats. Concurrent viral excretion and APP's variation were not significant in the PO Group. Oral rFeIFN-ω can be an effective alternative therapy for FIV-infected cats, being also an option for treatment follow-up in cats submitted to the licensed protocol.

1. Introduction

Recombinant Feline Interferon-Omega (rFeIFN-ω, Virbagen Omega®, Virbac) is an immune-modulator drug licensed for use in Europe, Australia and some Asian countries. Among its main therapeutic indications, it is frequently used in the management of Feline Immunodeficiency Virus (FIV) and Feline Leukemia Virus (Hosie et al., 2009; Lutz et al., 2009). rFeIFN-ω therapy induces clinical improvement of retroviral infected cats (de Mari et al., 2004; Domenech et al., 2011; Gil et al., 2013), and increases their survival time (de Mari et al., 2004). Slightly changes in some clinical parameters such as hypergammaglobulinemia, CD4/CD8 ratio, proviral load and viremia have been previously reported in retroviral infected cats during rFeIFN-ω therapy (Domenech et al., 2011). According to these authors, rFeIFN-ω is thought to act on innate immunity (Domenech et al., 2011). More recently, another study reported that the rFeIFN-ω licensed protocol improves the clinical presentation and reduces concurrent viral excretion in naturally retroviral-infected cats living in catteries, suggesting its usefulness in multi-cat environments where viral-related disorders are often a clinical problem (Gil et al., 2013). In that study, no significant abnormalities were observed in the hematology or biochemistry profiles during treatment (Gil et al., 2013).

The rFeIFN-ω licensed protocol consists of 3 therapeutic cycles of 5 daily subcutaneous injections (1 MU/kg/day), beginning respectively on days 0, 14 and 60. This protocol can be expensive and its cost may limit a more frequent use. To bypass this problem, alternative protocols such as oral ones have been suggested as an alternative use of rFeIFN-ω in certain situations (Addie, 2012; Bracklein et al., 2006; Hennet et al., 2011).

Some authors previously described the increased expression of Mx protein, a specific biomarker of an IFN-induced antiviral response, in specific-pathogen-free cats treated orally with various concentrations of rFeIFN-ω (Bracklein et al., 2006). In that study, it was shown that a higher oral dose of rFeIFN-ω induced higher levels of Mx protein expression, confirming its activity in oral protocols (Bracklein et al., 2006). Another recent study reported its successful use in a randomized double-blind study of calicivirus-positive, retrovirus negative cats with caudal stomatitis (Hennet et al., 2011). The protocol consisted of daily oro-mucosal rFeIFN-ω

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* Corresponding author. Tel.: +351 91 748 48 20; fax: +351 213652821.
E-mail addresses: 1solange@fmv.ulisboa.pt, solange@fmv.utl.pt (S. Gil).

[1] These authors contributed equally to the work.

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administration (0.1 MU/cat) for 90 days and was associated with a significant clinical improvement of lesions. Other authors describe its use in the non-effusive form of Feline Infectious Peritonitis (FIP) (50,000 U/cat PO daily) (Addie, 2012). Nevertheless, the beneficial effect of rFeIFN-ω for management of FIP is still not fully established (Addie et al., 2009; Ishida et al., 2004; Ritz et al., 2007).

To date, little is known about the clinical benefits of rFeIFN-ω via the oral route in cats with retroviral infections. Similarly to what is observed with Human Interferon-Alpha (HIFN-α) therapy, daily oral administration of rFeIFN-ω may provide effective immune modulation in FIV cats. To the authors' knowledge, only one trial has been performed, which used experimentally infected and asymptomatic FIV cats (Caney et al., 2003). It described the successful use of a daily oral rFeIFN-ω dose (0.1 MU/cat) for 6 weeks, but was not intended to assess any clinical benefit. Although no significant changes were obtained in provirus loads or CD4:CD8 ratio, the animals increased their bodyweight and the oral protocol was well tolerated (Caney et al., 2003). However, no further studies were performed in order to evaluate whether oral administration of rFeIFN-ω is efficient in cats with naturally occurring retroviral infections or its use over longer periods in symptomatic cats.

In daily practice, there are a few clinical parameters that permit a direct or indirect assessment of the immune-stimulation induced by rFeIFN-ω. Among them, Serum Protein Electrophoresis (SPE) and Acute Phase Proteins (APP) are potential complementary exams to evaluate the immune system of treated cats. SPE is a laboratory test that allows the separation of serum proteins based on size and electrical charge. Serum proteins are therefore divided into different fractions (alpha, beta and gamma-globulins) whose increase or decrease can be interrelated (Taylor et al., 2010). The gamma-globulin fraction has a special relevance in FIV cats. An increase in this fraction is associated with chronic antigenic stimulation and, according to previous studies, tends to occur in FIV positive cats (Gleich and Hartmann, 2009). This is mainly due to concurrent infections and a polyclonal B-cell activation which are a direct consequence of FIV infection and is seen even in apparently healthy FIV-positive cats (Ackley et al., 1990; Flynn et al., 1994; Gleich and Hartmann, 2009; Hartmann, 2011). In human medicine, especially in low resource areas, SPE has been used to monitor the response of anti-retroviral therapy in HIV patients (Sarro et al., 2010). Therefore, SPE could be a promising complementary exam in FIV infected cats.

APPs have been recently measured in various feline diseases (Paltrinieri, 2008). They seem to modulate the innate immune response, reinforcing the body defenses during inflammation (Ceron et al., 2005; Paltrinieri, 2008; Petersen et al., 2004). Hence, APP serum levels seem to be indirect indicators of innate immune system stimulation. A recent study described the use APP levels to monitor the effect of the licensed rFeIFN-ω protocol on innate immunity in FIV-positive cats. All the treated cats increased Alpha-1-glycoprotein (AGP), Serum Amyloid A (SAA) and C-Reactive Protein (CRP) suggesting that these parameters may be reliable in the individual monitoring of rFeIFN-ω immune-modulation therapy (Leal et al., 2012).

The present study aimed to evaluate the clinical improvement, laboratory findings (CBC, biochemistry and SPE), concurrent viral excretion and acute phase proteins (AGP, SAA and CRP) in naturally FIV-infected cats treated with an oral rFeIFN-ω protocol, in comparison to the licensed one.

2. Materials and methods employed

2.1. Animals and treatment protocols

11 FIV-positive cats were treated with oral rFeIFN-ω (PO Group) and the results were compared with data previously obtained from 7 FIV-positive cats treated with the licensed protocol (SC Group). The inclusion criteria used were based on previous publications (de Mari et al., 2004; Gil et al., 2013). No medications apart from rFeIFN-ω were allowed during the period of the study. Retroviral status was confirmed in all the animals by ELISA kits using serum or plasma samples from D0 (Viracheck/FIV and Viracheck/FeLV, Synbiotics). The results of the SC Group were previously published as a single-arm trial which evaluated clinical improvement and concurrent viral excretion (Gil et al., 2013). The data from this group were considered as a positive control for the current study. The PO Group consisted of 11 naturally FIV-infected cats referred to the Veterinary Teaching Hospital, which were treated, after obtaining the owner's consent, with 0.1 MU/cat rFeIFN-ω orally, once a day for 90 consecutive days. 6/11 cats were single-housed or lived indoor with no more than one other cat while 5/11 cats were outdoor animals or came from a multi-cat environment. To obtain the correct dose, a vial containing the rFeIFN-ω freeze-dried pellet (10 MU) was diluted in 25 ml of sterile physiological saline. Single-doses were prepared using 1 ml syringes containing 0.25 ml each by one of the members of the research group and given to owners who were instructed how to administer the therapy. The syringes were kept frozen (−18 to −20 °C) after preparation and owners defrosted each single dose shortly before administration. All animals were submitted to full clinical evaluations on days 0 (before therapy), 10, 30 and 65. Animals in the PO Group had an additional evaluation on day 90 (end of therapy).

2.2. Ethics

The study was approved by the Committee for Ethics and Animal Welfare of the Faculty of Veterinary Medicine – Technical University of Lisbon (CEBEA).

2.3. Clinical evaluation

Clinical improvement was evaluated using a score-scale (Gil et al., 2013) which included the most important parameters associated with retroviral infections namely: oral ulcers/gingivitis (score 0–2), caudal stomatitis/palatitis (score 0–2), ophthalmic abnormalities (score 0–2), lymphadenopathy (score 0–2), ocular and nasal discharge (score 0–2), mucous membrane color (score 0–2), coat appearance (score 0–1), body score (score 0–2), faecal appearance (score 0–1) and concurrent diseases/co-morbidities (score 0–2). At each time point, the total score for each cat was obtained by summing up all the corresponding clinical scores. These overall scores were then compared during the study period. Clinical improvement was classified as 'marked' (>50% improvement of the initial score), 'mild' (up to 50% improvement), 'stable' (same final and initial score) or 'worse' (final score more elevated than the initial).

2.4. Concurrent viral excretion assessment

Oral and Rectal swabs were collected at each time point to assess potential variations in concurrent excretion of herpesvirus (FHV-1), calicivirus (FCV), coronavirus (FCoV) and parvovirus (FPV). Swabs were processed for viral DNA/RNA extraction (QIAamp MinElute Virus Spin Kit, Qiagen, Portugal).

The determination and quantification of concurrent viral excretion was performed using the diagnostic procedures available in the Virology Laboratory of the Faculty of Veterinary Medicine – Technical University of Lisbon. In detail, screening and quantification of FHV-1 and FCoV was performed by Real-Time Polymerase Chain Reaction (PCR) and FCV presence was assessed by conventional reverse transcriptase PCR. The methodology used was the same as previously published (Gil et al., 2013). Due to a technical

update, FPV was also assessed by Real-Time PCR in the PO Group. FPV primers and TaqMan® probes were calculated using the Primer designing tool of NCBI (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), based on the nucleotide sequence of the vp1 gene (AN: AB437433.1). Screening and quantification of FPV was assessed by Real Time Polymerase Chain Reaction (PCR) amplification (Applied 7300 instrument, Applied Biosystems), in a 20 μl reaction, using TaqMan® Gene Expression 2× Master Mix (Applied Biosystems), 0.9 μM of forward primer (5′GGGCCTGGGAACAGTCTTGACC-3′), 0.9 μM of reverse primer (5′ACCAGAGCGAAGATAAGCAGCGT-3′) and 0.25 μM of TaqMan® probe (FAM 5′-CGCCGCTGCAAAAGAA-CACGACGAAGC-3′ TAMRA) and 10 ng of template. The cycling conditions comprised an initial denaturation step at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 1 min at 60 °C. To estimate FPV copy number serial tenfold dilutions (10^{-1}–10^{-6}) of recombinant plasmid DNA were used to generate a standard curve with a correlation efficiency of $r^2 = 0.997$, using the 7300 System SDS software.

2.5. Hematology and biochemistry

Blood samples were collected by jugular venipuncture during each clinical evaluation. Complete blood-cell count (CBC), hepatic enzymes (alanine-transaminase, aspartate-transaminase) and renal function (serum creatinine and urea) were assessed. CBC and biochemistry were performed, respectively, on Cell-Dyn 3700 (Abbott diagnostics division) and Kone Optima 4.2 (Kemia Cientifica).

2.6. Acute phase proteins

SAA and CRP were measured by ELISA using previously validated kits (Phase SAA Multispecies/Tridelta and Cat CRP ELISA/Kamiya Biomedical Company, respectively). AGP was assessed by single radial immunodiffusion (Feline AGP, SRID, Tridelta). All the measurements were performed according to the manufacturer's instructions.

2.7. Statistical analysis

For each clinical and immunological parameter of interest, the two groups were compared at the beginning and end of therapy using the Mann–Whitney–Wilcoxon test for independent samples. In each group, the comparison between measurements at the beginning and end of therapy was also carried out by the Mann–Whitney–Wilcoxon test but now for paired samples. In these tests we use their version for small sample sizes (e.g., with continuity correction). The significance level was fixed at 5%. In the PO Group, we presented a simple descriptive statistical analysis for indoor and outdoor cats, where appropriate, due to their small sizes. Also for concurrent viral excretion, where results were marginal due to low initial excretion rates, a descriptive statistical analysis was only presented. All calculations were undertaken in the R statistical software (version 3.0, www.r-project.org).

3. Results

3.1. Clinical improvement

Regarding clinical scores, groups were indistinguishable at the beginning and at the end of therapy ($p = 0.71$ and 0.74, respectively). Although not significant, the PO Group revealed overall higher clinical scores than the SC one. Regarding clinical improvement, in the SC Group, 5/7 (71%) cats improved their overall score ($p = 0.025$). In particular: 4/7 (57%) had a marked improvement, 1/7 (14%) a mild improvement and 2/7 (29%) remained stable. No worsening was observed. Oral lesions were the most common clinical sign at D0. The individual clinical scores for each cat on D0 and D65 (end of therapy) are presented in Fig. 1. In the PO Group, 9/11 (82%) treated cats significantly improved their overall scores with therapy ($p = 0{,}007$). Specifically: 3/11 (27%) of treated cats showed a marked improvement, 6/11 (55%) revealed a mild improvement and 2/11 (18%) remained stable. Similarly to the SC Group, no worsening was observed. The individual clinical scores for each cat on D0 and D90 (end of therapy) are presented in Fig. 1. Although some animals displayed only few clinical signs, an overall rapid improvement of cats was observed after the beginning of the study. 10 days after the onset of oral therapy, there was a significant reduction of the overall clinical score, which homogeneously dropped until the end of the study (data not shown). A very mild increase of the overall clinical score was observed from day 65 to day 90, due to specific worsening episodes of oral granulomas in one cat, and ocular and nasal discharge in another one (data not shown). At D0, the most significant clinical parameters affected were once again the oral lesions (oral ulcers and caudal stomatitis) and ocular discharge. With the exception of ocular and nasal discharge, which showed slight fluctuations during therapy, all parameters revealed a homogenous improvement (data not shown). Coat appearance, body condition score and ocular discharge were the clinical parameters which showed the most remarkable improvement.

There was no statistical difference between groups in the proportion of cats showing an improvement (Pearson's Chi-square test with Yates continuity correction for small sample sizes; $p = 0.95$). Moreover, there were also no differences in the grade of clinical improvement (mild or marked) between groups (Pearson's Chi-square test; $p = 0.23$). The overall results of both groups are presented in Table 1.

3.2. Concurrent viral excretion

In the PO Group, the concurrent viral excretion was very minor. None of the cats were positive for FHV-1 at the beginning of therapy. Only occasional excretion was detected and it was considered clinically irrelevant. At the end of therapy all the cats were FHV-1 negative. 5/11 (4 indoor and 1 outdoor) cats showed a very limited initial excretion of FPV which became negative. Also 5/11 cats (3 indoor and 2 outdoor) were residually excreting FCoV on D0. Despite some fluctuations, after the therapy two of them became negative, one increased the viral excretion and two reduced it. 7/11 (5/5 outdoors and 2/6 indoor cats) were positive for Calicivirus on D0, retaining this status throughout the therapy. One indoor cat that was negative on D0 became positive at the end of therapy. In opposition, and as previously published, the SC Group revealed a significant reduction of concurrent viral excretion (Gil et al., 2013).

3.3. Hematology and biochemistry

Concerning CBC results on D0, 3/11 cats (2 indoor and 1 outdoor cats) from the PO Group revealed a mild to moderate leucopenia; 2/11 outdoor cats were slightly anemic and 3/11 (2 indoor and 1 outdoor cat) showed a clinically unremarkable erythrocytosis, clinically compatible with mild subclinical dehydration. During therapy, 2 out of 3 leucopenic cats normalized their leucocyte counts. One cat still had a persistent mild leucopenia at the end of the therapy, despite an improvement of the leucocyte count. The red-blood cell counts of the 2 slightly anemic cats fluctuated during therapy. At the end of the protocol, the hematocrit of one of the cats normalized whilst the other worsened, revealing at D90 a moderate non-regenerative normocytic and normochromic anemia. The differential leukogram of the PO Group cats remained stable and unremarkable during therapy.

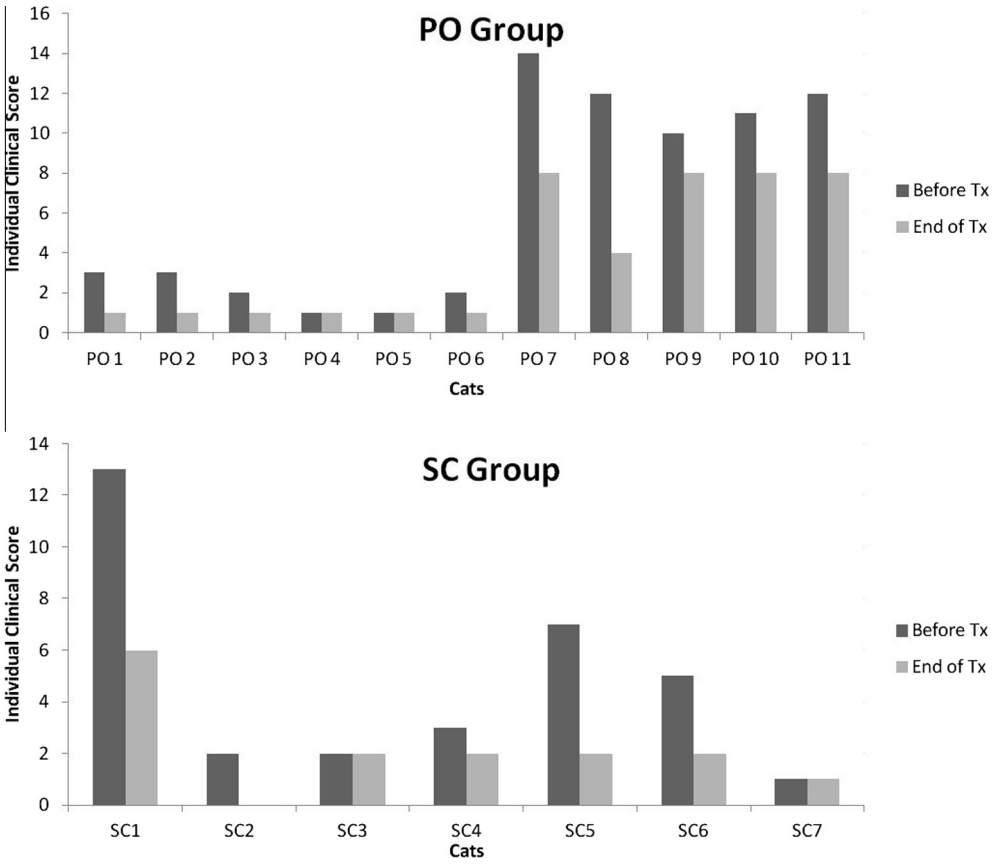

Fig. 1. Individual clinical scores for each cat of each group, before and after rFeIFN-ω therapy. SC Group refers to cats treated with the licensed sub-cutaneous protocol while the PO Group refers to cats treated with oral protocol.

Table 1
Overall clinical improvement of FIV positive cats treated with the rFeIFN-ω licensed protocol (SC Group) and rFeIFN-ω PO protocol.

Clinical improvement	SC Group		PO Group	
Improvement	5/7 (29.0–96.3)[a]	Marked: 4/7 Mild: 1/7	9/11 (48.2–97.7)[a]	Marked 3/11 Mild 6/11
No improvement	2/7	Stable: 2/11 Worsening: 0/7	2/11	Stable: 2/11 Worsening: 0/11

[a] Confidence intervals (shown in brackets) were calculated at 95% and refer to the percentage of cats showing clinical improvement overall.

Urea and Creatinine serum levels remained within the reference interval before, during and after therapy. Regarding hepatic transaminases, 2 outdoor cats had a mild to moderately increased ALT and AST on D0. For one, this normalized by D10 and remained within the reference interval until the end of study, whereas the other persisted for longer but normalized by D90.

3.4. Serum protein electrophoresis

No significant changes in SPE were detected in either group during therapy (Fig. 2). Both groups initially presented with the same pattern on D0 with an increase in total proteins and a hypergammaglobulinemia. Also in both groups, albumin was within the normal range before and after the study. Despite being non statistically significant, the PO Group experienced a slight decrease in total proteins while the SC Group increased this parameter. Conversely, hypergammaglobulinemia remained stable in the PO Group whilst in the SC Group it increased with therapy. When considering the environment of the cats (Indoor/Outdoor) submitted to the PO protocol, outdoor cats had overall higher values of gamma-globulins and total proteins than indoor ones both before and after therapy.

3.5. Acute phase proteins

Regarding the APP profile on D0, the PO Group showed significant higher serum levels of AGP, CRP and SAA than the SC one ($p = 0.018$, 0.02 and 0.00006 respectively). In detail, the baseline values (D0) of AGP, CRP and SAA in the PO Group were, respectively, three, nine and seven times higher than the SC one. Results are shown on Table 2. As previously published, the APP levels increased in animals treated with the SC protocol (Leal et al., 2012). In contrast, cats treated with the PO protocol did not demonstrate significant changes in APP serum levels after therapy ($p = 0.9$; 0.4 and 0.9 for CRP, SAA and AGP, respectively).

4. Discussion and conclusions

This study showed that, independently of the protocol applied, rFeIFN-ω induces a significant clinical improvement of treated FIV-infected cats. Unexpectedly and although not statistically significant, the PO Group had slightly higher overall clinical scores than the SC Group. This can be due to the fact that the five outdoor cats revealed higher clinical scores than any cat from the SC Group,

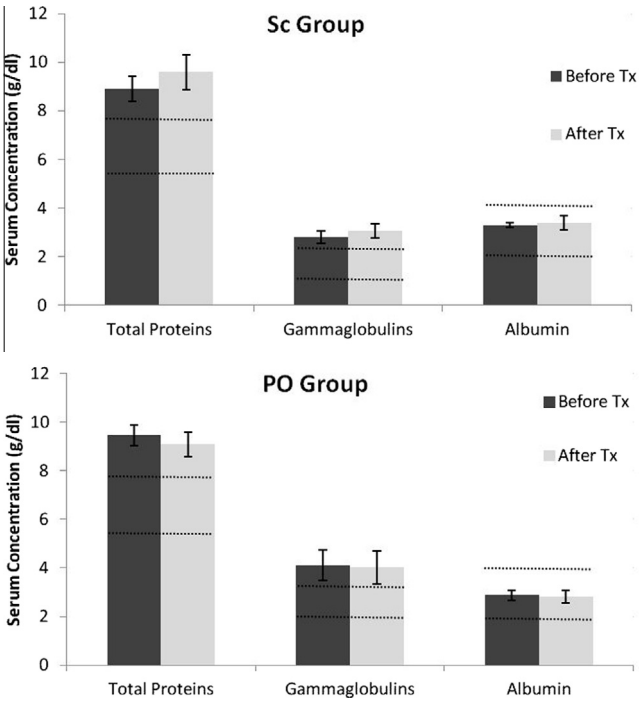

Fig. 2. Total Proteins, Gammaglobulins and Albumin serum levels of FIV positive cats treated with two different protocols of rFeIFN-ω (Mean values ± standard error). The SC Group refers to cats treated with the licensed protocol while the PO Group refers to cats treated with oral rFeIFN-ω. Horizontal lines (—) represent, respectively, the upper and lower limits of the normal range for each parameter.

counter-balancing the almost asymptomatic indoor cats. In fact, no significant differences were observed on clinical improvement between groups suggesting that, in a clinical setting where cost might be a limiting factor and subcutaneous administration might be problematic, rFeIFN-ω may be administered orally with success in FIV positive cats. However, despite the lack of statistical difference, the rFeIFN-ω licensed protocol (subcutaneous injections) appeared to induce a marked clinical improvement in a larger proportion of the cats (Gil et al., 2013). This suggests that the licensed protocol seems to be a better choice in more symptomatic cats when an effective and marked clinical improvement is desired. Being a feline recombinant product, it does not induce neutralizing antibodies meaning that the high-dose protocol may be used safely and efficiently even if repeat administration is required. This is an important factor to consider in a condition where management will be life-long.

As expected, the concurrent viral excretion was minimal in the PO Group mainly due to the fact that these animals were not living in a shelter condition, where opportunistic infections are more difficult to control. Despite the apparent overall reduction in the viral loads of FHV-1, FCoV and FPV during oral treatment, these findings were considered to be without clinical significance taking into account the low initial viral loads in this group. Further studies are required to fully clarify the role of oral rFeIFN-ω in the reduction of concurrent viral excretion, particularly in shelter medicine. Regarding the calicivirus status, no changes were observed in positive animals during oral treatment, and one of the negative cats became positive during therapy. This had no relationship with the clinical presentation, as oral rFeIFN-ω induced a useful clinical improvement in the animals in spite of the fact that they remained FCV positive, and agrees with previous studies which describe the long-term carrier state of many cats (Coyne et al., 2007). According to these authors, truly persistent infection is relatively rare and most of the FCV-positive cats undergo cyclical reinfections. These results in the oral group are also somewhat in contrast to the group receiving higher doses by subcutaneous injection, where reduction of viral excretion was more marked. Nevertheless, this study reveals that beneficial immune-modulation can be obtained even with low oral doses of rFeIFN-ω. In a similar fashion to HIFN-α(Tompkins, 1999), it may induce a local stimulation of lymphoid tissues which results in a systemic modulation of the immune response. Studies regarding rFeIFN-ω pharmacokinetics are scarce (Ueda et al., 1993). In contrast to HIFN-α, some authors report that rFeIFN-ω is acid resistant, which means that it may have a greater relative oral absorption or have activity on the gut-associated lymphoid tissue, thus better potentiating the overall immune system (Addie, 2012; Ueda et al., 1993). In contrast to the licensed protocol (Gil et al., 2013), when the PO protocol was used the improvement of oral lesions (ulcers and caudal stomatitis) was milder. This is in contrast to the effects seen in a previous study where the same oral administration protocol induced significant improvements in cats with refractory caudal stomatitis (Hennet et al., 2011). One major difference is that in that study all cats were retrovirus negative. This suggests that when treating retrovirus positive cats for caudal stomatitis, better results may be obtained with a protocol which, at least initially, uses higher injectable doses. Despite the more limited impact on caudal stomatitis and viral excretion, the oral protocol resulted in a significant improvement in other parameters such as body condition and coat appearance. Coat appearance is a non specific sign in cats but it is the authors' opinion that a good esthetical improvement in the animals might be a favorable point for improving the owners' compliance. Regarding the body condition, it was previously reported that rFeIFN-ω may be helpful in the initial resolution of anorexia in hyperthermic cats (Lutz et al., 2011). Although there was no anorexia reported in this study, animals increased their weight which is in agreement with the previously cited trial using oral rFeIFN-ω in FIV-positive asymptomatic cats (Caney et al., 2003). This is particularly important in thin and debilitated animals. As also described by some authors (Hennet et al., 2011), the clinical improvement observed may be related to the relief of oral lesions, even if only mild, which helps improve mastication and increase appetite. Animals with ocular discharge also showed a good improvement which is probably related to the control of opportunistic infections subsequent to immune modulation rather than a direct local antiviral effect.

Regarding hematology and biochemistry, despite mild fluctuations, no significant changes were observed in either group. The unremarkable erythrocytosis observed in 3 cats in the PO Group on D0 was clinically compatible with mild subclinical dehydration. The cat that developed a moderate non-regenerative normocitic

Table 2
Mean values ± standard error of Serum Amyloid A (SAA), Alpha-1-Glycoprotein (AGP) and C-Reactive Protein (CRP) serum levels in FIV positive cats before and after therapy with licensed rFeIFN-ω (SC Group) and oral (PO Group) protocols.

	SAA μg/ml		AGP μg/ml		CRP μg/ml	
	Before Tx	After Tx	Before Tx	After Tx	Before Tx	After Tx
Licensed protocol (SC Group)	2.2 ± 0.2	2.8 ± 0.2	341.4 ± 56.8	544.3 ± 123.8	116.6 ± 17.7	215.4 ± 16.5
Oral protocol (PO Group)	15.9 ± 6.5	8.5 ± 1.9	929.1 ± 126.9	945.5 ± 157.3	1048.4 ± 68.4	985.8 ± 93.8

and normochromic anemia on D90 was submitted to a clinical workup. The anemia was considered to be resulted of chronic inflammation and concurrent respiratory tract disease. After the end of the study, this animal was treated with antibiotics (Cefovecine) which improved the respective blood results. Considering the two outdoor cats in the PO Group that revealed transitory elevations of ALT and AST serum levels, a possible hepatic lipidosis secondary to an inappropriate food intake or a subclinical pancreatitis was considered. However, recognizing that both animals did not show any other clinical abnormalities, and that this increase was only analytical and values normalized within the period of study, the owners refused to perform the respective complementary exams suggested. Similarly to the licensed protocol, and as expected, oral rFeIFN-ω does not induce significant hematological or biochemical changes. The SPE results showed that all the animals of both groups presented an hypergammaglobulinemia and a concurrent hyperproteinemia at the beginning of the study. These findings corroborate the results of previous studies that describe an hypergammaglobulinemia in FIV positive cats, due to a concurrent opportunistic infections and polyclonal B-cell activation (Gleich and Hartmann, 2009; Hartmann, 2011). Considering that no significant changes were observed in either group, rFeIFN-ω does not seem to interfere with SPE, independently of the protocol administered. However, despite the lack of statistical significance, there appeared to be a tendency for the hypergammaglobulinemia to increase in the SC Group, while concurrent viral excretion reduced (Gil et al., 2013), suggesting that it could be related to a subtle but detectable immune stimulation increasing the activity of B-cells. In contrast, this was not observed in the PO Group where the hypergammaglobulinemia and total proteins remained stable during therapy. As expected, outdoor cats showed a more evident hypergammaglobulinemia and raised total protein levels than indoor ones, which could simply be related to a higher level of antigen-exposure in different environments.

Considering APP profile, on D0 the PO Group revealed higher AGP SAA and CRP serum levels when compared to the SC one. Previous results described an increase of APP levels in FIV-positive cats treated with the SC protocol (Leal et al., 2012). In contrast, administration of oral rFeIFN-ω did not induce significant changes in CRP, SAA and AGP serum levels. This is in agreement with a previous study performed in dogs where APPs were higher in dogs from private householders in comparison with cleaned kennels (Yamamoto et al., 1994). Thus, one possible explanation relies on a wide exposure to different environmental factors. Although they were living in a shelter, cats from the SC Group remained restricted to a particular area whilst cats from the PO Group, even indoor ones, were probably in contact with a larger variety of different daily stimuli. However, there are some arguments which do not support this theory. In fact, cats from the animal shelter were positive to other concurrent viruses on D0 (Gil et al., 2013), suggesting that environmental factors were less controlled in this group. Subsequently, as these cats improved their clinical conditions during rFeIFN-ω therapy, their APP levels increased and concurrent viral excretion decreased. Therefore, more than simply the environmental exposure to pathogens, these data support the hypothesis that the shelter cats had a more evident immune-suppressed basal health status. This can explain a poor innate response and subsequent lower levels of APP despite the clear evidence of opportunistic infections. Consequently, the shelter-housed SC Group showed lower initial levels of APP, which increased with therapy suggesting a restoration of the immune competency. Oral rFeIFN-ω did not significantly change the APP profile meaning that, despite the chronic oral therapy, the observed clinical benefits do not seem to be related with an increase on APP profile in these animals. A suggested explanation for this relies on the fact that, a chronic oral therapy may induce an overall clinical improvement due to a local action directly into the mucosa and localized lymphoid areas, potentiating a local immune response rather than a systemic one. Therefore, while the SC protocol, based on pulsate cycles of higher doses of rFeIFN-ω seems to have a relevant systemic role potentiating the innate immunity, the oral protocol suggests to act differently and directed in the local immune response. Considering the higher initial APP levels in the PO Group, this study alone is not sufficient to determine whether the licensed protocol is more potent in potentiating the innate-immune response or if this is simply the impact of the other factors such as living environment and better initial immune competence. Further studies namely the evaluation of cytokine profile expression namely pro-inflammatory ones (such as Interleukin-6, Interleukin-1 and Tumor Necrosis Factor) and its relation with the APP profile would clarify these major differences between groups.

A limitation of this study is the use of cats living in different environments. It seems reasonable to assume that animals from a shelter tend to be more exposed to conditions of higher morbidity than housed cats and, therefore, marked clinical improvement may be more likely when immune-modulation therapy is performed. However, recognizing that both protocols have different durations and routes of administration, a blinded study seemed unreasonable. More than simply comparing protocols, this study allowed us to assess the clinical improvement of cats individually with the baseline data of each cat also providing useful comparative information. Despite the heterogeneity of environments, initial clinical scores did not differ between groups, which made the SC Group a reliable positive control for clinical improvement assessment. Furthermore, having rFeIFN-ω treated FIV-infected cats that had been previously studied in a single-arm trial permitted a reduction of the number of animals used for this research. However, in other parameters namely APPs, differences in groups on D0 are significant and reflect different basal immune status. Rather than house-hold cats, it seems reasonable to assume that cats from an animal shelter and living in catteries have different extrinsic factors that can affect the immune response. Therefore, particularly in these parameters, the positive group control is less reliable. Even though, APPs are intrinsically variable even in healthy cats, reason why it is recommended that the animal should act as its own reference (Ceron et al., 2005). Then, more than comparing both groups, this study evaluated APP's tendency after the oral protocol, having the baseline values as the intrinsic own reference. This analysis minimized the initial discrepancy observed between groups.

This is the first study describing the successful application of an oral rFeIFN-ω protocol in symptomatic FIV-infected cats, opening new insights into more detailed immunological studies. It is highly probable that the licensed protocol provides sufficient levels of interferon systemically to induce a direct antiviral stimulus, in contrast to the oral protocol where systemic absorption is relatively limited and the doses used are also significantly lower. This may explain the apparently greater benefit of the injectable protocol in cats with an initially higher clinical score and in cats with high initial levels of virus shedding, and suggests that this should be taken into account when choosing the protocol for an individual cat in a clinical setting. Although the laboratory changes are subtler than those observed in the SC protocol, oral rFeIFN-ω nevertheless resulted in a useful improvement of the animals' condition. Considering the significantly reduced cost of the product, it could be an interesting alternative for immune-modulation therapy of FIV-infected cats with a mild to moderate clinical presentation if the current licensed protocol is difficult to perform. Additionally, this may be an interesting option for treatment follow-up after the licensed protocol once the condition of the cat is better stabilized and secondary viral infections are better controlled.

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