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Research paper

# Use of recombinant interferon omega in feline retrovirosis: From theory to practice

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ABSTRACT

Type-I interferons (IFNs) are cytokines that have non-specific antiviral activity, participating mostly in innate defense mechanisms. Their administration has been proposed to treat several viral and immunomediated diseases as an immunomodulatory therapy. Due to its availability, recombinant human interferon-alpha (rHuIFN-α) has been studied in relation to feline retrovirosis, both in vitro and in vivo. However, IFNs are species-specific and antibodies have been shown to develop in response to the high rHuIFN-α doses necessary for an effective therapy. A recombinant feline IFN has been developed, which has been characterized as interferon-omega (rFeIFN-ω), designed to overcome these problems. Nonetheless, very few studies have been undertaken to evaluate its efficacy in cats naturally infected with FIV or FeLV. In an initial study, we here demonstrated that rFeIFN-ω can dramatically improve the clinical condition of infected cats, and induce improvement of hematologic parameters. Minor changes or no change was observed for hypergammaglobulinemia, CD4/CD8 ratio, proviral load, viremia and RT activity, suggesting that the overall effect of IFN was on innate immunity. More studies are needed in order to better understand its in vivo mechanisms.



## 1. Introduction

Feline retroviruses, notably feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV), induce chronic infections which eventually lead to the progressive weakening of cats and the presence of various clinical signs. In advanced stages of the disease, the immune suppression established may contribute to the death of the animal (review in Hartmann, 2006; Sellon and Hartmann, 2006). Treatment of animal retroviral diseases is usually based on supportive and symptomatic therapy, such as rehydration when needed, control of secondary infections with antibiotics, antiparasitic and antifungal drugs, among others, while the administration of antiviral drugs is uncommon (Hartmann, 2006; Sellon and Hartmann, 2006; Dunham and Graham, 2008). Most of the antivirals used for feline retrovirosis are the same as used in human medicine, including AZT (zidovudine), ribavirin, zalcitabine and foscarnet, singularly or in combination. The use of these drugs in cats has several disadvantages: doses and protocols are not well established, and they can be toxic for animals as well as producing secondary effects (Caney, 2005; Hartmann, 2006; Sellon and Hartmann, 2006; Dunham and Graham, 2008). As these infections are accompanied by a wide array of clinical signs (anemia, gingivitis, anorexia, secondary respiratory infections, tumors, etc.), there is no preferred curative treatment. Thus, a reasonable

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alternative for treatment is the use of immunomodulators, particularly type-I interferons that have an additional antiviral effect. The aim of the present work was both to review the literature on the possibility of using interferon-omega (IFN-ω) for treating feline retrovirosis, and to describe the results of a preliminary study conducted on 11 cats infected either by FeLV or FIV, observing clinical, biopathological and virological effects.

Innate immunity plays a role in protection against retroviral infections (Lehner et al., 2008), and includes both intracellular innate antiviral factors, and extracellular factors, particularly interferon. Interferons (IFNs) are cytokines with important multiple biological functions. Interferons are classified into type-I and type-II IFNs (Pestka et al., 2004). Type-I IFNs are produced by virus-infected cells and have non-specific antiviral activity on adjacent non-infected cells. Thus, they are known as "viral IFNs" and are associated with innate immunity. These interferons include IFN-α, IFN-β, and IFN-ω, among others. Each them has a general mechanism of action based on interaction with specific cell surface receptors and the subsequent induction of expression of interferon-stimulated genes (Sen, 2001; Pestka et al., 2004). These cytokines also induce anti-proliferative and anti-inflammatory responses, and therefore, can also participate in adaptive immune responses (Gerlach et al., 2006, 2009). Thus the administration of type-I IFN has been proposed as a treatment for several viral diseases as immunomodulatory therapy (Truyen et al., 2002; Collado et al., 2006). These products have been used empirically and quite successfully in feline medicine, without a profound knowledge of their molecular mechanisms.

The efficacy of human IFN-α (HuIFN-α) in the feline clinic was the first to be evaluated, as it was the first one commercially available (recombinant HuIFN-α(2a), rHuIFN-α(2a), Roferon®), as well as being the one with the highest in vitro antiviral effect. Even though clinical improvement and lengthening of the life expectancy of infected cats was observed, several disadvantages soon became apparent, such as: its activity in vivo may be lower than expected as cytokine activity is often species-restricted (it would have less effect on feline cells than on human cells); when injected, the higher doses (100,000 U/kg/day) that are able to induce adequate serum levels may lead to the development of specific neutralizing antibodies that block the active ingredient (Zeidner et al., 1990; Müller, 2002) and may have adverse effects (Caney, 2005).

These disadvantages could be overcome with the administration of a species-specific feline IFN (FeIFN). Several subtypes of recombinant FeIFN-α (rFeIFN-α) have been described that could have potential benefits for treating chronic viral infections in cats, given their in vitro antiviral activity (Wonderling et al., 2002). However, to date no rFeIFN-α is available for clinical use, although a recombinant feline interferon omega (rFeIFN-ω) is commercially available and used with relative success in feline viral infections of various etiologies.

IFN-ω, a type-I IFN secreted by virus infected leukocytes, was identified by Hauptmann and Swetly (1985) and is one of the more recently characterized interferons (Adolf, 1995). It was initially described in humans and is encoded by multiple IFN-ω or IFN-ω-like genes, which are present across mammalian groups, including cats (Roberts et al., 1998). Like other type-I IFNs, IFN-ω has species-restricted biological activity in vitro. It is able to bind to the same type-I IFN receptor complex as other type-I IFNs and therefore exerts similar antiviral, antiproliferative and immunomodulatory effects (Adolf, 1995). However, its antigenic structure is distantly related to IFN-α, -β and -γ, as it does not cross-react with antibodies against them (Adolf, 1995).

The rFeIFN-ω that has been developed (Nakamura et al., 1992; Ueda et al., 1993a) has a 60–65% homology to human IFN-α1. Its amino acid sequence consists of 170 amino acid residues and an N-glycosylation site at amino acid position 79. This recombinant IFN has been characterized as omega on the basis of its amino acid identity and the processing pattern of the N-terminal sequence (Ueda et al., 1993a; Adolf, 1995). It has a proven antiviral effect, both in vitro (Mochizuki et al., 1994; Truyen et al., 2002; Ohe et al., 2008) and in vivo against canine and feline parvovirus, herpesvirus, calicivirus, coronavirus and rotavirus (Truyen et al., 2002; De Mari et al., 2004; Ishida et al., 2004; Paltrinieri et al., 2007). In addition, the pharmacokinetic properties of rFeIFN-ω are comparable to those of human interferons in that it does not have a residual accumulation in the body (Ueda et al., 1993b). It has been licensed for use in veterinary medicine (Virbagen®, Virbac) in Europe, Japan, Australia, New Zealand and Mexico, although there are few clinical studies that support its use, and its in vivo molecular mechanisms are not well understood. In general, an improvement in clinical signs has been described, justifying its use in infections in which other treatments are not fully effective, such as retroviral diseases.

The in vitro effects of IFN-I on human retroviruses have been extensively studied. Generally, results from most studies have shown that retroviral protein synthesis is not affected, and suggest that IFN affects the latter stages of the viral cycle, preventing correct assembly or the release of viral particles (review in Gómez-Lucía et al., 2009). Unfortunately, few studies have focused on the effect of this cytokine on feline retroviruses (Rogers et al., 1972; Jameson and Essex, 1983; Yamamoto et al., 1986; Tanabe and Yamamoto, 2001; Collado et al., 2007).

In reviewing the literature, only Collado et al. (2007) have compared the effect of rHuIFN-α(2a) (Roferon®) and rFeIFN-ω (Virbagen®) on persistently FeLV-infected feline cells. Their results have indicated that, as with other retroviruses, rFeIFN-ω affects the FeLV cycle at the post-transcriptional level, as protein synthesis was not altered, while RT activity (used to estimate the number of infectious particles) decreased in a dose-dependent manner. Given its $IC_{50}$, rFeIFN-ω appeared to be around 50–90 times more potent than reHuIFN-α(2a) in inhibiting RT. In addition, the study revealed that IFNs induced a decrease in the viability of FeLV-infected cells, enhancing apoptosis in infected cells that were treated. The significance of this effect on cell viability and its in vivo impact is, at present, unknown.

In the case of FIV, similar experiments with rHuIFN-α(2a) and rFeIFN-ω in infected feline cells have shown

comparable results to that of FeLV (Tanabe and Yamamoto, 2001; Collado, unpublished data). The $IC_{50}$ of rFeIFN-ω suggests that it is *ca*. 25 times more effective than rHuIFN-α(2a) in inhibiting FIV RT (Collado, unpublished data). Collectively, results from treatment with rHuIFN-α(2a) and rFeIFN-ω of FeLV- or FIV-infected cells have demonstrated that these IFNs apparently do not inhibit feline retrovirus gene expression, but suppress the processing or assembly of viral proteins and/or the release of virions in the late stages of maturation.

In summary, a more profound antiviral effect of rFeIFN-ω over rHuIFN-α(2a) was observed in vitro against FeLV and FIV, which, in part, may be due to the species-specificity of type-I interferon and the homologous nature of rFeIFN-ω and feline cell lines.

It is difficult to predict the possible clinical applications of interferons based on in vitro studies due to the variety of physiological conditions present in vivo and the myriad of individual responses by treated animals. Nevertheless, the results of the in vitro studies seem to suggest that, although both rFeIFN-ω or rHuIFN-α(2a) may be of prophylactic and therapeutic clinical value, rFeIFN-ω would likely be more effective in vivo. Additionally, type-I IFNs have immune modulating properties concurrent with antiviral activity (which are not measured in vitro) as proposed by other researchers (Gerlach et al., 2009), and thus an enhanced effect of rFeIFN-ω may be expected in vivo.

Due to the therapeutic possibilities of interferon against feline retrovirosis, several studies have been undertaken with different type-I IFNs and protocols, although results, to date, are inconclusive. In initial experiments, rHuIFN-α was injected into FeLV-infected cats, singularly or in combination with AZT (Hoover et al., 1990; Zeidner et al., 1990), and viral antigenic load (antigenemia) was observed to decrease. However, this beneficial response had a limited duration as cats developed antibodies against human IFN (Zeidner et al., 1990). To reduce the development of antibodies, lower doses of IFN were administered orally (1–10 IU/kg body weight), resulting in longer survival rates during some studies, with clinical and analytical improvement noted (Steed, 1987; Cummins et al., 1988; Weiss et al., 1991); in others although there was no improvement (McCaw et al., 2001). Similarly, oral treatment of clinically sick FIV-infected cats with low doses of natural HuIFN-α resulted in an extended survival period and improved clinical signs (Pedretti et al., 2006). In spite of the clinical benefits of HuIFN-α treatment, FeLV- and FIV-infected cats became persistently viremic or had a reduced viral replication only during the treatment period (McCaw et al., 2001; Pedretti et al., 2006).

In addition, low oral doses of IFN-α were ineffective with regard to lymphocyte depletion, with no relevant variations in the CD4/CD8 ratio during treatment in naturally-infected FeLV cats (Riondato et al., 2003). These low doses even failed to maintain the balance between CD4+ and CD8+ cells in FIV-infected cats (Riondato et al., 2003; Pedretti et al., 2006). Moreover, the altered CD4/CD8 ratio had no evident correlation with clinical condition (Pedretti et al., 2006). However, a correlation was found between clinical condition and leukocyte counts, which suggested that HuIFN-α treated cats undergo a strengthened innate immune response with better control against opportunistic infections (Pedretti et al., 2006).

After the synthesis and commercialization of the recombinant feline IFN-ω molecule, most studies in feline medicine have focused on its application. Presently, it is the only interferon that the European Medicine Agency (http://emea.europa.eu) has registered in Europe for feline medicine. It has been approved for the treatment of parvovirosis in dogs from one month of age, and FeLV and/or FIV infections in non-terminal cats from the age of 9 weeks (http://emea.europa.eu). Yet very few studies have analyzed the effect of rFeIFN-ω in naturally-infected cats with FeLV and/or FIV. One of the most extensive studies undertaken evaluated this interferon in a multicentric, double-blind, placebo-controlled trial in FeLV-infected or FeLV/FIV co-infected cats with associated clinical signs (De Mari et al., 2004). Cats were treated subcutaneously with rFeIFN-ω ($10^6$ U/kg/day) daily for five consecutive days in three series (day 0, 14, 60). An improvement in clinical signs and decrease in the mortality rate was observed after treatment as compared to control animals. There was also an increase in the leukogram and in the RBC count in anemic cats (De Mari et al., 2004). However, as no virological (viremia or proviral loads) or immunological (such as CD4/CD8 ratio) parameters were measured throughout the study, it is difficult to conclude whether or not these effects were due to its immunomodulatory or antiviral activities. Since IFN-ω has biological properties similar to IFN-α, it is possible that the clinical benefits observed in treated cats corresponded to the activation of their innate immune system for an improved control of environmental pathogens, as compared with an improvement in their viremic state. Further studies are necessary in order to discover answers to these questions.

Only one controlled clinical trial has been performed in asymptomatic FIV-infected cats, using both low ($10^4$ U/cat/day orally for six weeks) and high ($10^6$ U/Kg/day by subcutaneous injection for five days) doses of rFeIFN-ω. No improvements in laboratory parameters such as proviral load, CD4+ T-cell counts and total white blood cell counts (WBCs) were observed (Caney et al., 2003). To our knowledge, no clinical studies have been performed on the effect of this interferon on symptomatic FIV-infected cats.

## 2. From theory to practice: administration of rFeIFN-ω in naturally-infected cats

Veterinary practitioners who treat FeLV- and/or FIV-infected cats usually do not know when the cat was infected, or the stage of the disease. Cats may have one or more clinical signs, or may be totally asymptomatic. Practitioners may not know the immune status of the animal, its CD4/CD8 ratio or the proviral load. Thus, treatment with rFeIFN-ω may produce different results than expected, ranging from no visible effects to an apparent clinical improvement. A big concern for the clinical veterinarian is whether this improvement correlates with a true improvement of the infection, i.e., a decrease in viremia or the proviral load of the cat, or whether the infection may revert when treatment of rFeIFN-ω is suspended. Treatment is also hampered by the cost of the product. The

company recommends three cycles of treatment for five consecutive days, on days 0, 14 and 60. As there is usually clinical improvement, oftentimes, owners consider that the cat has been cured, and suspend treatment before its completion. Another issue for practitioners is deciding when treatment should be repeated, when no analytical values are available. For these reasons, we undertook a preliminary study on the use of rFeIFN-ω in randomly selected household cats that were brought to the Complutense University Veterinary Clinic Hospital of Madrid, Spain.

## 3. Materials and methods

Eleven naturally-infected cats were treated (four infected by FeLV, FeLV$^+$; seven infected by FIV, FIV$^+$), with ages ranging between 6 months and 10 years, including both male (7/11) and female (4/11), most of them (10/11) of mixed breed, and with different regimes of roaming. They were treated for eight weeks with commercial rFeIFN-ω (Virbagen®, Virbac, France) following the protocol suggested by De Mari et al. (2004). Ten other cats (five FeLV$^+$ and five FIV$^+$), eight of them female, and housed in a cattery, were left untreated and used as controls. The date of infection was unknown in all cases; presumably, the cats were in different stages of the disease, since they included three asymptomatic FIV$^+$ cats, and ten cats (4 FeLV$^+$ and 6 FIV$^+$) with six or more different retrovirus-associated clinical signs (RACS) of various degrees of severity (clinical score ≥6, severe disease). The most frequent disorders were anorexia, apathy, pallor and oral lesions. Blood samples were taken before the beginning of treatment (V1), and two weeks after completion (V2), with equivalent sampling dates in untreated cats. Three treated cats were brought for follow-up examination until month 12 (V3). Blood sampling, hemogram, leukogram, biochemical profile, electrophoretogram, and CD4/CD8 determination were conducted as described previously (Miro et al., 2007). FeLV p27 and FIV p24 proteins were evaluated using commercial Petchek kits developed by Idexx (Westbrook, ME, USA), and RT activity was measured with kits provided by Cavidi Tech (Uppsala, Sweden). Quantitative PCR (qPCR) was conducted using the procedures described by Pinches et al. (2007) and Leutenegger et al. (1999). Results were statistically analyzed using Statgraphics. Differences in the ANOVA and Chi-square values of $p < 0.05$ were considered significant.

Animal handling, treatment, reagent manipulations and data collection were all carried out in compliance with guidelines for Good Clinical Practice, and Good Laboratory Practice of the Animal Welfare Committee of the Veterinary Clinical Hospital and the Complutense University (Madrid), and the experimental procedures were approved by the Institutional Animal Care and Use Committee of the Complutense University.

## 4. Results and discussion

After treatment with rFeIFN-ω (at V2 and for the subsequent 6 months), an evident clinical improvement was observed in all sick treated cats, especially those with a clinical score (CS) of ≥6 (Table 1). Such an improvement was not observed in untreated cats ($p < 0.05$). Asymptomatic cats or those with mild disease remained stable. This clinical improvement agrees with data from De Mari et al. (2004) and from studies with rHuIFN-α (Pedretti et al., 2006). One treated and one untreated-FeLV$^+$ cat, each with

**Table 1**
Changes in the clinical score (CS) and analytical parameters between V1 (immediately before treatment) and V2 (two weeks after treatment).

| Code | Virus | CS-V1 | CS-V2 | Erythrogram | | | Leukogram | | | Electroph. | | | Viral parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | PCV | Hgb | RBC | Leuko | Ntr | Lymp | γ–s | A/G | CD4/CD8 | p27 | RT | qPCR |
| C-1 | FeLV | 8 | 3 | n–n | n–n | n–n | n–n | n–n | n–n | ↑–↑ (U) | ↓–↓ (U) | n–nd | ↓ (F) | ↓ (F) | = (U) |
| C-3 | FeLV | 7 | 4 | n–n | n–n | n–n | n–n | n–n | n–n | ↑–n (F) | ↓–n (F) | ↓–↓ (U) | ↑ (U) | ↑ (U) | ↑ (U) |
| C-4 | FeLV | 5 | 0 | ↓–n (F) | ↓–n (F) | ↓–n (F) | n–n | n–n | ↓–n (F) | ↑–↑ (U) | ↓–↓ (U) | n–n | ↓ (F) | ↓ (F) | = (U) |
| C-6 | FIV | 9 | 5 | n–n | n–n | n–n | n–n | n–n | n–↓ (U) | ↑–n (F) | n–n | nd–nd | Neg. | Neg. | = (U) |
| C-7 | FIV | 2 | 2 | n–n | n–n | n–n | n–n | n–n | n–n | ↑–↑ (U) | ↓–↓ (U) | ↓–n (F) | Neg. | Neg. | ↓ (F) |
| C-8 | FIV | 8 | 1 | n–n | n–n | n–n | n–n | n–n | n–n | ↑–n (F) | n–n | ↓–↓ (U) | Neg. | Neg. | = (U) |
| C-9 | FIV | 0 | 0 | n–n | n–n | n–n | ↓–n (F) | ↓–n (F) | n–n | ↑–↑ (U) | n–↓ (U) | ↓–n (F) | Neg. | Neg. | = (U) |
| C-10 | FIV | 0 | 0 | n–n | n–n | n–n | n–n | n–n | n–n | ↑–n (F) | n–n | n–n | Neg. | Neg. | ↑ (U) |
| C-11 | FIV | 0 | 0 | n–n | n–n | n–n | n–n | n–n | n–↑ (U) | ↑–n (F) | ↓–n (F) | n–↓ (U) | Neg. | Neg. | = (U) |
| C-12 | FIV | 7 | 2 | n–n | n–n | ↓–n (F) | n–n | ↓–n (F) | n–n | ↑–↑ (U) | n–n | n–n | Neg. | Neg. | = (U) |

The CS was rated as described previously (Collado et al., submitted for publication). For the erythrogram, leukogram, electrophoretogram, and CD4/CD8 ratio the situation in V1 and V2 are provided as follows: n, within normal limits; ↓, below normal limits; ↑, above normal limits. For the viral parameters the change from V1 to V2 is provided as follows: ↓, ≥20% decrease; ↑, ≥20% increase; =, stable. (F), favorable change; (U) unfavorable change. PCV, packed cell volume; Hgb, hemoglobin concentration; RBC, erythrocyte counts; Leuko, leukocyte counts; Ntr, neutrophil counts; Lymph, lymphocyte counts; Electroph., electrophoretogram; γ–s, gammaglobulin concentration; nd, not determined; Neg., below the detection limit of the kit.

very severe initial disease (CS = 10 and CS = 8, respectively) died between V1 and V2.

Treated cats that initially presented erythrogram and leukogram values outside the norm showed improvement by V2. This agrees with previous reports that IFN was effective on anemic cats (De Mari et al., 2004). However, one treated FIV+ cat had developed lymphocytosis, and another, lymphopenia by V2 (Table 1). The three cats that were examined at V3 did not have a worse clinical or hematologic condition (data not shown). Most untreated cats initially had normal erythrogram and leukogram values. However, in three non-treated cats there was an unfavorable change in several hematologic parameters, although the values of neutrophil counts improved sporadically in two other non-treated cats.

Initially, cats had hypergammaglobulinemia, with a low A/G ratio in all FeLV+ cats and in two FIV+ cats. A significant decrease in gammaglobulin concentrations ($p < 0.001$) was observed at V2 in five treated cats (4 FIV+ and 1 FeLV+), but it was not sustained as cats followed till V3 were again hypergammaglobulinemic shortly after the V2 period (data not shown). Gammaglobulin levels in untreated cats in which this parameter had initially been abnormally high always remained above normal limits.

In the rFeIFN-ω treated cats, the CD4/CD8 ratio decreased in one FeLV+ cat and in two FIV+ cats. An improvement in the ratio was observed in two of them, but in another three, the CD4/CD8 ratio did not increase or decrease (Table 1). Riondato et al. (2003) observed no correlation between CD4/CD8 ratio variations and clinical signs, as sick and healthy cats had very similar ratios, which agrees with our results.

Proviral load was detected in all animals. Only one of the treated FIV+ cats decreased its initial proviral load by V2, but was still within the detection limits. No treated or untreated cat became negative. In one treated-FeLV+ and one treated-FIV+ cat the proviral load increased, though they did not display worsened clinical condition. As regards to viremia, only FeLV+ cats were positive for RT and capsid protein. In these cats, the response to interferon was variable (Table 1). One of the treated FeLV+ cats worsened in all the viral parameters, but its CS decreased. Caney et al. (2003) did not observe an effect of rFeIFN-ω on the proviral load.

To date, the in vivo mechanism of type-I IFNs on retroviral infections is unknown. In a murine model with the Friend murine leukemia retrovirus, the immunomodulatory effect of IFN-α seemed to be more important than the antiviral effect through the induction of antiviral enzymes (Gerlach et al., 2009). In our preliminary study, rFeIFN-ω induced an evident improvement of the clinical condition of treated cats, as well as of the hemogram parameters. The CD4/CD8 ratio improvement was inconsistent, and the virological parameters, such as proviral load and viremia were not seen to be affected by treatment. This suggests that rFeIFN-ω possibly lacks antiviral effects in vivo, but rather may have an immunomodulatory effect. Taking these results into consideration, it would seem advisable to treat retrovirus-infected cats with interferon when they have clinical signs, as they would benefit from its effects in improving their clinical condition.

## Conflict of interest statement

All authors declare no financial or commercial conflict of interest.

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