Activity of feline interferon-omega after ocular or oral administration in cats as indicated by Mx protein expression in conjunctival and white blood cells

Thorsten Bracklein, Med Vet; Sandra Theise, Dr Med Vet; Alfred Metzler, PD; Bernhard M. Spiess, PD; Marianne Richter, Dr Med Vet

Objectives—To assess the biological response to recombinant feline interferon-omega (rFeIFN-ω) following ocular or oral administration in cats via estimation of Mx protein expression in conjunctival cells (CCs) and WBCs.

Animals—10 specific pathogen–free cats.

Procedures—In multiple single-dose drug experiments, each cat received various concentrations of rFeIFN-ω administered topically into both eyes (50 to 10,000 U/eye) and orally (200 to 20,000 units). The same cats received saline (0.9% NaCl) solution topically and orally as control treatments. The CCs and WBCs were collected prior to treatment (day 0), on day 1, and every third or seventh day thereafter until samples yielded negative results for Mx protein. Samples were examined for Mx protein expression via immunohistochemistry and immunoblotting procedures involving murine anti-Mx protein monoclonal antibody M143.

Results—After topical application of 10,000 U of rFeIFN-ω/eye, CCs stained for Mx protein for a minimum of 7 days, whereas WBCs were positive for Mx protein for a minimum of 31 days. After topical application of lower concentrations, CCs did not express Mx protein, in contrast to WBCs, which stained for Mx protein at 1,000 units for at least 1 day. Following oral administration, Mx protein was expressed in WBCs at rFeIFN-ω concentrations as low as 200 units, whereas CCs did not stain for Mx protein at any concentration.

Conclusions and Clinical Relevance—Results indicate that Mx protein expression (a marker of the biological response to rFeIFN-ω) in CCs and WBCs of rFeIFN-ω-treated cats depends on the dose of rFeIFN-ω, site of administration, and cell type. (Am J Vet Res 2006;67:1025–1032)

Interferons are produced in response to viral infections and contribute to host defense by establishing an antiviral state in target cells, wherein the replication of virus is blocked or impaired as a result of the synthesis of a number of enzymes that interfere with cellular and virus processes. The IFN-induced proteins that are important in antiviral actions include RNA-dependent protein kinase, 2′-5′ OAS (in conjunction with RNase L), and the Mx protein GTPases. Interferons can also slow the growth of target cells or make them more susceptible to apoptosis, thereby limiting the extent of virus spread. Furthermore, they have profound immunomodulatory effects and play an important role in the functioning of the innate immune response prior to the production of specific antiviral antibodies, which is also stimulated by IFNs.

Because of the short half-life of IFNs, several bioassays for IFN activity have been developed that are based on the capacity of IFNs to induce expression of IFN-responsive genes in peripheral blood mononuclear cells. Increased amounts of an IFN-induced gene product indicate that the cells have been exposed to IFN and are responding to it. Assays that measure the induction of 2′-5′ OAS, RNA-dependent protein kinase, indoleamine 2,3-dioxygenase, or guanylate-binding proteins in peripheral blood mononuclear cells or secreted products such as β2-microglobulin in serum or neopterin in urine determine activities of not only type I IFNs (IFN-α, -β, or -ω) but also of type II IFN (IFN-γ). An exception is the Mx protein, which is a specific biochemical marker for the action of type I IFNs. Mx gene expression is tightly and specifically regulated by type I IFNs and is induced in humans and various other mammalian species undergoing type I IFN treatment as well as in patients with viral or autoimmune diseases. Even minute quantities of endogenous IFN induced after live virus vaccination are detectable via determination of Mx gene expression. Furthermore, in contrast to parenterally administered type I IFN, oral administration does not result in detectable quantities of IFN or its biological markers.

ABBREVIATIONS

IFN Interferon
2′5′OAS 2′,5′-oligoadenylate synthetase
GTPase Guanosine triphosphatase
rHuIFN Recombinant human IFN
HSV Herpes simplex virus
rFeIFN-ω Recombinant feline IFN-omega
CC Conjunctival cell
SPF Specific pathogen free
MAb Monoclonal antibody

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From the Ophthalmology Unit, Department of Small Animals (Bracklein, Theise, Spiess, Richter), and the Institute of Virology (Metzler), Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zurich, Switzerland.
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Address correspondence to Dr. Richter.
β₂-microglobulin, neopterin, or 2'-5' OAS but does induce Mx mRNA. Therefore, at this time, Mx gene products are the only known markers for biologically active IFN after oral administration.

The Mx protein is a stable cytoplasmic protein, has GTPase and antiviral activities, and is promptly induced (within 1 to 2 hours) in cells stimulated with type I IFN, reaching maximum amounts in a relatively short period of time (36 hours). The biological half-life of Mx protein is relatively long (2.5 days). Moreover, cellular induction of Mx protein is not subject to feedback inhibition even at high doses of IFN. Thus, Mx protein is a rapidly inducible, sensitive, and reliable indicator of IFN action over a wide range of IFN doses. Horisberger et al. have described a dose-dependent induction of feline Mx protein after SC administration of an rHuIFN-α hybrid (rHuIFN-α B/D) in cats and stated that Mx protein is a sensitive, quantitative, and stable marker with which to monitor IFN activity in vivo in cats.

Interferons are approved therapeutics and have moved from application in the research laboratory to the clinic. There have been some studies of the effect of exogenous human type I IFN (HuIFN-α) in viral diseases in domestic animals. In addition to studies involving high doses of IFN administered parenterally, there also have been investigations in several species involving low doses of IFN administered orally to prevent or reduce clinical disease. Oral administration of low doses (0.5 or 5 units) of HuIFN-α prevented the experimental development of fatal FeLV-related disease in cats. Furthermore, low doses (0.05 to 0.5 units) of HuIFN-ω administered orally significantly reduced the clinical effects of infectious bovine rhinotracheitis virus infection in feedlot cattle in a dose-dependent fashion. In young pigs given a low dose (1.0, 10.0, or 20.0 units) of natural HuIFN-ω, survival rate was affected in a dose-dependent manner and was significantly greater than survival rate among placebo-treated pigs. Examination of ranges of doses of murine IFN-α/β (1 to 1,000 units) revealed that 10 units administered orally prior to virus infection was optimal for inhibition of murine cytomegalovirus replication.

In human medicine, oral administration of low to medium doses (10⁵ to 10⁶ units) has been evaluated because treatment with parenterally administered high doses of type I IFN is limited by clinical and chemical toxic effects and the induction of antibodies that abrogate its activity in vivo. Likewise, the duration of efficacy of long-term parenteral administration of HuIFN-α in virally infected cats may be shortened because of the generation of neutralizing antibodies against the IFN. In contrast to results of parenteral administration, type I IFNs do not induce antibody formation when administered via the oromucosal route. This phenomenon, called mucosal tolerance, is mainly attributable to interleukin-10 production by dendritic cells and to subsets of anergic regulatory or suppressor T cells in mucosal tissues.

Results of several studies indicate that orally administered IFN has many advantages over parenterally administered IFN: the oral route is convenient and may allow the use of lower doses, minimizes adverse effects, eliminates potential treatment difficulties associated with neutralizing antibodies, and provides enhanced efficacy via unique and potent immunoregulatory circuits. In mice and humans, inductible quantities of Mx mRNA after ingestion of IFN-α have been detected. Ingested IFN-α acts via established pathways of type 1 IFN signalling.

Ophthalmic applications of IFN have been investigated in animal models and humans in the context of viral infection, mainly HSV. Topical application of IFN has been shown to be effective in rabbits infected with HSV-1 and in humans with dendritic herpetic keratitis as a result of HSV-1 infection. In a study in monkeys, topical application of IFN resulted in inhibition of HSV keratitis when administered prior and simultaneous to HSV inoculation but had only minor effects when administered after inoculation with the virus. In humans, combined topical treatment of acyclovir and IFN-α has been shown to be effective in the treatment of herpetic keratitis. Because FHV-1 in cats and HSV-1 in humans are both Alphaherpesvirinae and induce very similar ocular signs, it seems to be of interest to ascertain whether topical application of IFN may have therapeutic effects in cats with herpetic keratitis as it does in humans and other animals.

Because mass production of stable rFeIFN-ω has been made possible, rFeIFN-ω is becoming of interest in the treatment of various viral diseases in cats. Recombinant FeIFN-ω has an antiviral action in vitro against feline herpesvirus, feline calcivirus, and feline infectious peritonitis virus. In vivo, rFeIFN-ω had good clinical efficacy against canine parvovirus infection in an experimental trial and in field trials with dogs.

To our knowledge, the biological activity of rFeIFN-ω in cats after topical (ocular) and oral application has not been evaluated. The purpose of the study reported here was to evaluate the activity of different concentrations of rFeIFN-ω after oral and oral administration in cats via assessment of Mx protein expression in CCs and WBCs.

Materials and Methods

Animals—Ten SPF cats (8 castrated males and 2 spayed females) used in this study were from a breeding colony of SPF cats. These cats were age matched (5 months of age) at the time of inoculation. Cats were treated with multiple single doses of different concentrations of rFeIFN-ω or only diluent orally and topically in both eyes, respectively. The study was approved by the Veterinary Office of the canton of Zurich, Switzerland, according to the Swiss Animal Protection Ordinance.

IFN—Lyophilized rFeIFN-ω was mixed with 1 mL of diluent (saline [0.9% NaCl] solution) provided by the manufacturer and was further diluted with saline solution to the final concentrations used in this study.

Procedure—First, single doses of 20 µL of saline solution containing 50, 100, 1,000, or 10,000 units of rFeIFN-ω were applied topically in both eyes (lower conjunctival sac) of the 10 SPF cats. In a second experiment, the cats received single oral doses of 20 µL of saline solution containing 200, 2,000, or 20,000 units of rFeIFN-ω. The same cats received 20 µL of saline solution without rFeIFN-ω topically in both eyes and orally (crossover design study) as control treatments. There was a 2-week washout period (when samples were negative for Mx protein at 3 consecutive sampling times) between each administration of rFeIFN-ω or saline solution.
Prior to administration of rFeIFN-ω or saline solution (day 0), CCs and WBCs were collected to ascertain the Mx protein-negative status of these cells in each cat. Further samples were collected 24 hours after a single application of each concentration of rFeIFN-ω or saline solution and every third or seventh day thereafter until all samples yielded negative results for Mx protein. Conjunctival smears were prepared by use of sterile brushlike devices for collection of exfoliative cells. Blood was collected in evacuated tubes containing EDTA and processed immediately. Samples of CCs and WBCs were examined for Mx protein expression via immunostaining and immunoblotting, respectively, by use of murine anti-Mx protein MAb M143, which is known to react with Mx proteins of a broad host range.1

**Immunostaining**—In a preliminary experiment, we investigated whether Mx protein expression was detectable in CCs via immunohistochemistry involving murine anti-Mx protein MAb M143. In this experiment, Mx protein was detected in CCs from cats with naturally occurring herpes virus–induced keratitis. In the present study, CCs were collected from the lower conjunctival sac of SPF cats by use of sterile brushlike devices and transferred to slides.2 Air-dried slides were fixed with an ice-cold (–20°C) acetone-methanol (50:50) solution for 10 minutes and divided into 2 fields by use of a water-repellent delimiting pen.3 One field was used as a negative control specimen. The positive control specimen consisted of Crandell-Rees feline kidney cells stimulated with 10,000 units of rFeIFN-ω and fixed onto slides. Immunohistochemical staining was accomplished by use of a staining kit4 and the murine anti-Mx protein MAb M143. First, 3 drops of peroxidase-blocking agent were applied on each field of the slides and rinsed off with PBS solution 5 minutes after incubation at room temperature (approx 25°C). Second, each field of the slides was treated with a protein-blocking agent in the same manner. Afterwards, the pretreated slides were incubated with 50 μL of MAb M143 on the right section and with 50 μL of PBS solution on the left section (negative control sample) for 1 hour in a humid chamber. After the slides were rinsed with PBS solution for 5 minutes, they were consecutively incubated for 30 minutes with a biotinylated rabbit anti-mouse antibody5 and a biotinylated horseradish peroxidase complex.6 Following a final wash step with distilled water, the preparations were incubated for 10 minutes with an appropriate substrate.7 For better identification of the CCs, slides were counterstained with hematoxylin for 10 seconds. Immunostaining of cells was evaluated qualitatively by use of a light microscope; cells were categorized as positive for Mx protein (uptake of staining) or negative for Mx protein (no uptake of stain).

**Immunoblotting procedure**—White blood cells from the SPF cats were obtained via ammonium chloride–induced RBC lysis of blood samples that had been collected into EDTA-containing tubes. Briefly, blood was collected in evacuated tubes containing EDTA and processed immediately. Two milliliters of blood was added to 8 mL of lysis buffer (0.15M NH4Cl, 10mM KHCO3, and 0.1M EDTA [pH, 7.2]) and incubated for 10 minutes at room temperature. After centrifugation (1,000 × g for 10 minutes at 4°C), the supernatant was discarded and the pellet WBCs were resuspended in PBS solution and recentrifuged (2,000 × g for 5 minutes) twice. After this washing step, the WBCs were used for an immunoblotting procedure according to the method of Müller-Doblies et al.8 For immunoblotting, the pellet was resuspended in 150 μL of SDS-PAGE sample buffer (250mM Tris-HCl [pH, 6.8], 5% SDS, 10% β-mercaptoethanol, 40% glycerol, and 0.03% bromphenol blue), boiled (at 96°C) for 3 minutes, and separated on 10% SDS-PAGE.1 Following electrophoretic separation, the proteins were electrotransferred in blotting buffer (25mM Tris base, 192mM glycine, and 20% methanol) to a nitrocellulose membrane for 1 hour at 100 V. Blots were blocked overnight (approx 16 hours) at 4°C in high-salt Tris-buffered saline solution consisting of 50mM Tris-HCl (pH, 7.0), 500mM NaCl, 0.2% (wt/vol) Tween 20, and 5% (wt/vol) skim milk. The blots were then washed 3 times (5 min/wash) with PBS, followed by incubation with murine anti-Mx protein MAb M143 for 1 hour at room temperature. After 3 further washing steps (5 min/wash), blots were incubated for 1 hour at room temperature with appropriately diluted peroxidase-labeled rabbit anti-mouse immunoglobulin G.9 After another 4 washing steps, blots were developed with enhanced chemiluminescence and exposed to radiographic films.9 For semiquantitative analysis of the blots, Mx protein signals were scored; the scores assigned ranged from − (ie, no signal) to +++ (strong signal). To follow the course of Mx protein expression in WBCs, the mean Mx protein signal score for each sampling period was evaluated.

**Results**

**Ocular application**—In smears of CCs collected from cats inoculated with a single dose of 10,000 units of rFeIFN-ω into each eye (total dose, 2 × 10,000 units), uptake of stain for Mx protein was evident in cells for 7 days in all 10 cats and as long as 21 days in 3 of them (Figure 1). Similarly, WBCs expressed Mx protein after topical application of 10,000 units of rFeIFN-ω into each eye, as indicated by an Mx protein signal in the blots (Figure 2). The WBC samples were positive for Mx protein for 31 days in all cats and as long as 45 days in 6 of the 10 cats; samples yielded negative results thereafter (Figure 3). Mean signals for Mx protein (via immunoblotting) decreased over time beginning 2 weeks after IFN administration (Figure 4). In smears from all cats inoculated with 1,000 units of rFeIFN-ω into each eye, CCs did not take up Mx protein stain; however, WBCs were positive for Mx protein for at least 1 day in 10 cats and for 3 days in 6 of 10 cats and were negative for Mx protein in all cats at the following sampling (8 days after IFN administration). Mean
signals for Mx protein were weaker after administration of 1,000 U of rFeIFN/eye, compared with those obtained after administration of 10,000 U of rFeIFN/eye. Inoculation of 50 units and 100 units of rFeIFN-ω into each eye did not induce Mx protein expression in CCs or WBCs in any cat at any time. Examination of smears of CCs yielded negative results in all cats when saline solution without IFN was applied (Figure 5).

Oral administration—Following oral administration of 200 units of rFeIFN-ω, Mx protein was detected in WBCs from 9 of the 10 cats for at least 1 day, but was not detectable in any cat 3 days after administration (Figure 3). Following oral administration of 2,000 units of rFeIFN-ω, Mx protein was detected in WBCs from all 10 cats for at least 1 day and from 7 of 10 cats for 3 days after administration; 7 days after administration of this dose, Mx protein was not detected in WBCs from any of the cats. After oral administration of 20,000 units of rFeIFN-ω, Mx protein was detected in WBCs from 9 cats (1 cat died because of an unrelated reason prior to oral administration of 20,000 units) for at least 28 days, from 8 of the 9 cats for 35 days, and from 6 of the 9 cats for 42 days; Mx protein was not detectable in WBCs from any cat thereafter. The course of mean Mx signal score after oral administration was similar to that associated with ocular application of the same IFN concentration (Figure 4). Mean signals for Mx protein were weaker after administration of 2,000 or 200 units, compared with mean signals obtained after administration of 20,000 units. In all 10 cats, CCs did not take up stain for Mx protein after oral administration of rFeIFN-ω at any concentration nor at any time. After oral administration of saline solution without IFN, WBCs and CCs were negative for Mx protein in all cats.

Discussion

Various routes of administration (including IV, SC, IM, topical, and intralesional) have been used to deliver IFN to the site of pathologic conditions. In general, the oral route has not been used because neither IFN nor its biological markers (2′-5′OAS, neopterin, or β2-microglobulin) have been detected in blood following oral administration.

In the present study, we identified the dose-dependent biologic activity of orally administered rFeIFN-ω in cats via evaluation of Mx protein expression in WBCs. Duration and intensity of Mx protein expression were dependent on the IFN concentration adminis-
tered. Following oral administration of a single dose of 200 units of rFeIFN-ω, 9 of 10 cats expressed Mx protein in their WBCs for only 1 day, whereas after oral administration of a single dose of 20,000 units, all 10 cats had Mx protein-positive WBCs for at least 28 days.

The therapeutic effect of IFNs may not require transit of intact IFN across the intestinal wall. Proteins that might not survive transit through the alimentary canal may still have immunomodulatory activity via lymphoid tissues, such as the oropharyngeal-associated lymphoid tissues, and via paracrine activity.48-50 Interferons, like other biologic response modifiers, are drugs that act outside the realm of classic pharmacologic parameters. Oropharyngeal delivery of IFN seems to achieve a far better drug-immune cell interaction than parenteral administration, having long-lasting biological effects in the absence of detectable serum IFN concentrations.49

At doses ranging from 2,000 to 4,000 units of IFN, high-affinity type I IFN receptors are expressed on all lymphoid cells. Interaction with specific high-affinity receptors on the surface of target cells is the initial step in IFN action and is necessary for subsequent biologic effects.51 Once activated, monocytes and lymphocytes, by virtue of their ability to circulate throughout the body, potentially can transfer their biological activities in the absence of circulating cytokines after contacting IFN or IFN-induced cells in the oropharyngeal-associated lymphoid tissues.51 At their destination, type I IFN-activated cells may release various cytokines that are able to influence neighboring cells.51 Thus, ingested type I IFNs may act outside the realm of classical pharmacology by activating a unique, natural, large regional immune system that originates in the lymphoid tissues. Ingestion of an immunoactive protein, such as type I IFN, potentially provides a continuous means of immunoregulation that is convenient and active at low doses with minimal adverse effects and may provide

Figure 4—Plots of mean ± SD Mx protein signal score (detected by use of an immunoblotting assay) in WBCs obtained from 10 cats* before and after administration (day 0) of single doses of different rFeIFN-ω concentrations either topically in both eyes (10,000 U/eye [A] and 1,000 U/eye [B]) or orally (20,000 units [C], 2,000 units [D], and 200 units [E]). *Prior to oral administration of 20,000 units, 1 cat died because of an unrelated reason.

Figure 5—Photomicrograph of a smear of CCs from the lower conjunctival sac of a cat 24 hours after single topical application of saline (0.9% NaCl) solution without rFeIFN-ω. Cytoplasm of a CC that does not contain Mx protein does not stain with murine anti-Mx protein MAb M143. Immunohistochemical staining and hematoxylin contrast staining; bar = 20 mm.
enhanced efficacy via unique and potent immunoregulatory circuits in the lymphoid tissue. The lymphoid tissue (ie, gastrointestinal- and oropharyngeal-associated lymphoid tissues) has multiple types of constituent immune cells, including Peyer's patches, tonsils, Waldeyer's ring, intraepithelial lymphocytes, T and B lymphocytes, macrophages, and dendritic cells, that may become immunoregulators via ingestion of type I IFN.48,49

Because of the short half-life of IFN and the facts that Mx gene expression is tightly and specifically regulated by type I IFNs and is induced in humans and various mammalian species undergoing type I IFN treatment, detection of Mx protein in blood cells has been shown to be a reliable marker for biological activity of type I IFNs.49-51 It has been determined that ingested type I IFN induces Mx mRNA in peripheral blood mononuclear cells of humans and in splenocytes of mice.50 In the present study, we could likewise identify dose-dependent Mx protein expression in WBCs of cats after oral administration of various concentrations of rFeIFN-ω. Furthermore, we also determined that topical application of IFN into the conjunctival sac resulted in Mx protein expression in feline WBCs. Possible explanations for this finding are that IFN may have been absorbed by the highly vascularized conjunctival tissue, that IFN may have passed the nasolacrimal duct system to reach the mucosa of the nasal and oropharyngeal cavities, or that a combination of both processes occurred. According to the aforementioned mechanism, oropharyngeal delivery (oral and nasolacrimal routes) of IFN activate or prime lymphoid tissues (ie, gastrointestinal- and oropharyngeal-associated lymphoid tissues) has multiple types of connections with surrounding lymphoid and nonlymphoid organs. Similarly, following topical application of IFN into the conjunctival sac, immune cells of the conjunctiva-associated lymphoid tissue and the immunoreactive compound IFN come into contact. Eventually, IFN-activated lymphocytes and monocytes may enter the vascular system and activate other cells via cell-to-cell contact and release of cytokines in a paracrine fashion.52

In vitro, HSV-1 induces only low amounts of IFN in rabbit corneal epithelial cells and no detectable quantities of IFN in stromal cells, in contrast to the effects of UV-inactivated bluetongue virus.53 In mice, type I IFNs play a major role in limiting mutant and wild-type HSV-1 replication in the cornea and nervous system.54 Because HSV-1 (and possibly the closely related feline herpesvirus-1) is itself a weak IFN inducer in corneal epithelial cells, exogenous topical application of IFN may have beneficial effects in controlling virus replication at the ocular surface. In cultured human corneal fibroblasts, Mx protein was induced at a concentration of IFN-α as low as 1 U/mL and maximal Mx protein expression was detected at 1 × 10^5 U/mL.55

In the present study, biological activity of IFN in CCl was identified for as long as 21 days after ocular application at a concentration of 10,000 U/20 µL (ie, 5 × 10^5 U/mL). In response to ocular application of lower IFN concentrations in cats, Mx protein was not detectable in conjunctival smears. Following ocular and oral administration of 2 × 1,000 units of rFeIFN-ω, respectively, Mx protein was expressed in feline WBCs for a short period (maximum duration, 3 days) and was not detectable in CCs. In WBCs, Mx protein was detectable at least for a short period (1 day), even after oral administration of only 200 units of rFeIFN-ω. The differences in Mx protein expression in CCs and WBCs following administration of different IFN concentrations in cats may be attributable to unequal responsiveness of these cell types to IFN or different methods of detection. Unequal responsiveness to IFNs has been identified in various rabbit cell types.55 In response to several HuIFNs, corneal epithelial cells were somewhat more sensitive than stromal cells or rabbit kidney cells; however, corneal epithelial cells were somewhat less sensitive to rabbit IFN than rabbit kidney cells.55 Moreover, another study56 revealed differential responses to IFN-α subtypes in human T cells and dendritic cells. Overall, sensitivity of cells to IFNs seems to vary with type or subtype of IFN and depends on cell type. In the present study, the immunoblotting procedure for detection of Mx protein in IFN-stimulated feline CCs and WBCs was performed according to the method of Müller-Doblies et al56, by use of this method, those investigators detected Mx protein in bovine kidney cells that were stimulated with as little as 1 U of IFN/mL. Thus, detection of Mx protein by immunoblotting techniques depends more on the IFN sensitivity of various cells than on the sensitivity of the examination technique itself.

Human IFN-α has been used effectively in the treatment of herpetic keratitis in rabbits and nonhuman primates when administered locally early in the course of infection.57-59 Results of studies57,58 have also indicated that IFN treatment can be effective in conjunction with administration of other antiviral agents or debride ment in the treatment of established HSV-associated ocular disease in humans. To date, FeIFN-ω has not been evaluated as a possible treatment option for herpes virus infection in cats; however, our data may be a basis for future research in this field.

Induction of Mx protein (as a marker of the biological activity of rFeIFN-ω) in CCs and WBCs of cats depends on the dose of IFN and site of application. Our findings indicate that the biological effect of rFeIFN-ω can be detected via evaluation of Mx protein expression in CCs and WBCs after a single topical application of 10,000 U of rFeIFN-ω/20 µL (ie, 5 × 10^5 U of rFeIFN-ω/mL) into each eye, whereas oral administration of the same dose of rFeIFN-ω induced Mx protein expression in WBCs but not in CCs. Our findings may encourage further investigations of the effectiveness of ocular and oral administration of exogenous IFN in the treatment of viral infections in cats.

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