Imaging-guided gene therapy of experimental gliomas

Jacobs, A H; Rueger, M A; Winkeler, A; Li, H; Vollmar, S; Waerzeggers, Y; Rueckriem, B; Kummer, C; Dittmar, C; Klein, M; Heneka, M T; Herrlinger, U; Fraefel, C; Graf, R; Wienhard, K; Heiss, W-D

Jacobs, A H; Rueger, M A; Winkeler, A; Li, H; Vollmar, S; Waerzeggers, Y; Rueckriem, B; Kummer, C; Dittmar, C; Klein, M; Heneka, M T; Herrlinger, U; Fraefel, C; Graf, R; Wienhard, K; Heiss, W-D (2007). Imaging-guided gene therapy of experimental gliomas. Cancer Research, 67(4):1706-1715.

Postprint available at:
http://www.zora.uzh.ch

Posted at the Zurich Open Repository and Archive, University of Zurich.
http://www.zora.uzh.ch

Originally published at:
Imaging-guided gene therapy of experimental gliomas

Abstract

To further develop gene therapy for patients with glioblastomas, an experimental gene therapy protocol was established comprising a series of imaging parameters for (i) noninvasive assessment of viable target tissue followed by (ii) targeted application of herpes simplex virus type 1 (HSV-1) amplicon vectors and (iii) quantification of treatment effects by imaging. We show that viable target tissue amenable for application of gene therapy vectors can be identified by multitracer positron emission tomography (PET) using 2-18F-fluoro-2-deoxy-D-glucose, methyl-11C-L-methionine, or 3'-deoxy-3'-18F-fluoro-L-thymidine ([18F]FLT). Targeted application of HSV-1 amplicon vectors containing two therapeutic genes with synergistic antitumor activity (Escherichia coli cytosine deaminase, cd, and mutated HSV-1 thymidine kinase, tk39, fused to green fluorescent protein gene, gfp) leads to an overall response rate of 68%, with 18% complete responses and 50% partial responses. Most importantly, we show that the “tissue dose” of HSV-1 amplicon vector-mediated gene expression can be noninvasively assessed by [4-18F-fluoro-3-(hydroxymethyl)butyl]guanine ([18F]FHBG) PET. Therapeutic effects could be monitored by PET with significant differences in [18F]FLT accumulation in all positive control tumors and 72% in vivo transduced tumors (P = 0.01) as early as 4 days after prodruk therapy. For all stably and in vivo transduced tumors, cdIREStk39gfp gene expression as measured by [18F]FHBG-PET correlated with therapeutic efficiency as measured by [18F]FLT-PET. These data indicate that imaging-guided vector application with determination of tissue dose of vector-mediated gene expression and correlation to induced therapeutic effect using multimodal imaging is feasible. This strategy will help in the development of safe and efficient gene therapy protocols for clinical application.
Pretreatment with feline interferon omega and the course of subsequent infection with feline herpesvirus in cats

Clemens Haid,*¶ Simone Kaps,*,** Enikö Gönczi,† Michael Hässig,‡ Alfred Metzler,§ Bernhard M. Spiess* and Marianne Richter*

*Department of Small Animals, Ophthalmology Unit, †the Clinical Laboratory, the Department of Farm Animals, ‡Section of Population Medicine, §the Institute of Virology (Metzler), Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, 8057 Zürich, Switzerland

Abstract

Objective Recombinant feline interferon omega (rFeIFN-ω), a type I IFN, may have the potential to limit virus replication and associated clinical signs when administered early on in the course of feline herpesvirus type 1 (FHV-1) infection and reactivation, respectively. The effect of rFeIFN-ω pretreatment on the course of subsequent FHV-1 infection in cats was investigated.

Animals studied Nine SPF cats were divided into an IFN group (n = 5) and a control-group (n = 4).

Procedures The IFN group was pretreated for 2 days with 10 000 units rFeIFN-ω twice a day topically into both eyes and 20 000 units rFeIFN-ω once a day orally, whereas the control group was mock-treated. Subsequently all cats were infected with FHV-1. Samples for FHV-1 DNA detection and quantitation, virus isolation, and titration of FHV-1 antibodies were collected. Clinical and ocular signs were recorded and scored.

Results Courses of median individual clinical and ocular scores and virus load did not differ significantly between both groups using ANOVA for repeated measurements. Analysis (ANOVA) of each individual ocular parameter revealed significantly high scores for epithelial keratitis (P = 0.016) in the IFN group compared to the control group. Periods of virus shedding did not differ significantly between both groups using the Wilcoxon rank sum test.

Conclusions Results indicated a lack of beneficial effects of rFeIFN-ω pretreatment in the course of primary FHV-1 infection in cats.

Key words: cytokine, eye, feline, FHV-1, herpes, interferon, rhinotracheitis

INTRODUCTION

Feline herpesvirus-1 (FHV-1) is one of the most important ocular and upper respiratory pathogens of domestic cats worldwide. Clinical signs of acute infection include depression, fever, sneezing, serous nasal and/or ocular discharge, conjunctivitis and epithelial keratitis.1,2 Regular vaccination of FHV-1 in unexposed cats does not prevent infection, viral shedding and establishment of a FHV-1 carrier state when exposed to FHV-1.3 Furthermore, primary FHV-1 infection may occur in unvaccinated weanlings in close contact to a viral shedding dam.4 After productive infection in epithelial cells the virus develops neuronal latency. During periodic reactivation FHV-1 is transported back to surface sites where it may replicate.5,5 Innate and adaptive immunity are important in modulating herpesvirus infections of the epithelium and of the dorsal root ganglia.6

It has been shown in mice diluted for IFN receptors that type I IFNs play a major role in limiting replication of herpes simplex type 1 (HSV-1), an alpha-herpesvirus-like FHV-1, in the cornea and in the nervous system.7 Type I IFNs (IFN-α/β-ω) are rapidly inducible components of the innate immune response to infection.8 The potent activity of type I IFNs against viral infections is mediated both by the immune system and by intracellular antiviral pathways in vivo.7 IfNs do not act directly on the virus; rather, they trigger expression of various antiviral proteins and thus induce an antiviral state within the host cell to limit replication and spread of viruses.8 Furthermore, type I IFNs have been shown to potently enhance innate and adaptive immune responses in vivo through various immunomodulatory effects, such as activation of dendritic cells (DCs), amplification of antibody responses8 and enhancement of T-cell and natural killer cell cytotoxicity.11

© 2007 American College of Veterinary Ophthalmologists
In vitro studies using different cell types have shown antiviral activities of type I IFNs against FHV-1.\textsuperscript{12-15} In a preliminary study, cats experimentally infected with FHV-1 and treated orally with 25 U of human IFN alpha (HuIFN-\textalpha) once a day, beginning early on in the course of infection, had less severe clinical signs than control cats.\textsuperscript{16} As recombinant feline IFN omega (rFeIFN-\textomega) has greater anti-FHV-1 effects than does rHuIFN-\textomega \textalpha b \textit{in vitro},\textsuperscript{12} it is of interest to investigate anti-FHV-1 efficiency of rFeIFN-\textomega \textit{in vivo}. Antiviral activities of type I IFNs seem to be greater when used before rather than after viral infection, both \textit{in vitro}\textsuperscript{13,14} and \textit{in vivo}.\textsuperscript{17-19}

Usually, primary FHV-1 infection occurs early in life\textsuperscript{20} and, in the majority of cats presented to veterinarians with signs of herpesvirus infection, virus reactivation has occurred before possible IFN treatment could have been initiated. However, because of a time lag of approximately 1 week between exposure to stress or glucocorticoid administration and reactivation of FHV-1 in carrier cats,\textsuperscript{20} treatment with type I IFN prior to predicted periods of stress (pregnancy, lactation, cat shows, animal shelters, change of residence) or glucocorticoid administration may limit virus replication and shedding following reactivation in FHV-1 latently infected cats. Furthermore, type I IFNs are multifunctional proteins not only inducing an antiviral state at the cellular level but also enhancing the host's immune response.\textsuperscript{9} Thus, type I IFNs may have the potential to limit virus replication during primary FHV-1 infection as well as during reactivation in FHV-1 carrier cats.

In a previous study we have shown dose-dependent biologic activity of topically and orally applied rFeIFN-\textomega on conjunctival and white blood cells in cats.\textsuperscript{21} The present study aimed to investigate effects of rFeIFN-\textomega pretreatment on the course of primary FHV-1 infection in cats.

Materials and methods

Animals

Nine (two spayed female and seven castrated male) 30-month-old, specific pathogen-free (SPF) and FHV-1 seronegative cats (Harlan Sprague Dawley Inc., Madison, WI, USA) were randomly allocated into two treatment groups: five cats into the IFN group and four cats into the control group. All cats were housed as a single group in one large room. Experimental design was approved by the veterinary office of the canton Zürich, Switzerland according to the Swiss Animal Protection Ordinance. Cats had been used previously to investigate biologic activity of rFeIFN-\textomega.\textsuperscript{21}

Interferon

Lyophilized rFeIFN-\textomega (Virbagen® Omega 5 mU, Virbac, Switzerland) was mixed with 1 mL diluent (0.9% NaCl) provided by the manufacturer and was further diluted with 9 mL of 0.9% NaCl solution to a final concentration of 500 000 U/mL.

In vivo pretreatment protocol

Cats of the IFN group were pretreated with 1 drop (20 \textmu L, measured by a micropipette) of 500 000 U/mL (10 000 U/20 \textmu L) rFeIFN-\textomega topically into the conjunctival sac of both eyes twice a day as well as 2 drops of 500 000 U/mL rFeIFN-\textomega orally once a day for 48 h. Cats of the control group were similarly mock-treated with the diluent alone (0.9% NaCl solution).

Virus, cells and media

The FHV-1 field isolate UT 88 was used for infection, which has been proven previously to induce ocular and clinical signs in SPF cats at a concentration of 10^{-1} TCID\textsubscript{50}/mL.\textsuperscript{22} Crandell feline kidney (CRFK) cells, grown in RPMI-medium (RPMI 1640-L-Gluthamin, Gibco Invitrogen Corporation, Grand Island, NY, USA) including 2.5% fetal calf serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.5 mg/mL gentamicin, were used to grow the virus stock and for virus isolations and titrations, as described previously.\textsuperscript{23}

Virus inoculation

Sixteen hours after final pretreatment all cats were infected with FHV-1. For inoculation, 25 \textmu L of virus suspension (10^{-1} TCID\textsubscript{50}/mL) were dropped into the lower conjunctival sac of each eye.

Clinical and ophthalmic examination

Prior to the study, all cats were examined clinically and ophthalmologically. Following virus inoculation, all cats were examined daily for clinical symptoms (behavior, body temperature, appetite, nasal discharge, sneezing and dyspnea) and every other day for ocular signs (blepharospasm, epiphora, conjunctival hyperemia, chemosis, epithelial keratitis, corneal vascularization, and fluorescein and rose Bengal staining). The examiner (K.S.) was unaware of the assignment of the cats to treatment groups. Clinical and ocular symptoms were evaluated prior to sampling. Corneal parameters were assessed using a portable hand-held binocular biomicroscope (KOWA SL-14, Kowa, Tokyo, Japan) and fluorescein (Fluorescein, Haag Streit Int., Koniz, Switzerland) and rose bengal dye (Minims™, Chauvin, Kingston-Upon-Thames, UK). Fluorescein staining indicates epithelial defects. Rose bengal staining indicates disruption of epithelial tight junctions and tear film abnormalities and is used in the diagnosis of FHV-1 keratitis, as punctate and dendritic lesions may not retain fluorescein, but stain with rose bengal.\textsuperscript{24} Each ocular sign was given a score on a scale of 0 (absent) to 3 (severe). Clinical parameters were scored as ‘normal’ (0) or ‘abnormal’ (1). Body temperature (in °C) was scored as follows: 38.0–39.3 (0); 39.4–39.8 (1); 39.9–40.3 (2); ≥40.4 (3).

Sampling

Tear samples were obtained by placing a Schirmer tear test (STT) strip (Schirmer-Tränen-Test, Essex Tierarznei, München, Germany) into the nasal lower conjunctival sac of each eye. The paper strip was cut off at the 5-mm mark and
stored in a sterile tube at ~20 °C until further processing. For virus isolation, lower conjunctival sacs of both eyes were swabbed separately with sterile rayon tipped applicators (Transwab® ENT, Medical Wire & Equipment Co. Ltd. Corsham, Wilts, UK). Swabs from both eyes were pooled in a sterile tube containing 3 mL RPMI-medium and processed immediately for virus isolation. Blood samples were taken from the jugular vein to determine FHV-1 antibodies in a serum neutralization test. Sixteen hours after final pretreatment and prior to experimental infection ocular and blood samples from all cats were taken to ascertain FHV-1 free status. Ocular samples were collected 24 h following FHV-1 infection and twice a week thereafter until FHV-1 DNA and infectious virus were not detectable. Blood samples were taken 2 and 10 weeks after virus inoculation to determine FHV-1 antibody response.

Extraction of DNA
Extraction of DNA was performed using the DNeasy™ Tissue Kit (Qiagen AG, Hombrechtikon, Switzerland). Each STT strip was processed separately. First, strips were mixed with 22 μL proteinase and 220 μL ATL-buffer each, vortexed for 15 s and incubated at 56 °C for 1 h. Following a spin-down at 8000 g, 220 μL AL-buffer was added and the samples were incubated for 10 min at 70 °C. Following a second spin-down at 8000 g, 220 μL ethanol was added, mixed, and vortexed for 15 s. The samples were then transferred to a spin-column and processed according to the manufacturer’s instruction. Extracted samples were kept at ~20 °C until analysis by TaqMan PCR.

Detection and quantitation of FHV-1
Detection and quantitation of FHV-1 DNA was performed by Real-Time TaqMan PCR on an ABI PRISM 7700 sequence detection system (Applied Biosystems, Rotkreuz, Switzerland) as described elsewhere. The fluorescence threshold cycle (Ct), at which amplification of a PCR product is first detected, inversely correlates with the virus load, which is calculated by the following formula: log TCID₅₀ = −0.2611 Cₜ + 9.473. Ct-values ≥ 45, corresponding to calculated virus concentrations of ≤ 10⁻², were considered negative.

Virus isolation
Cell culture methods and techniques of viral isolation have been described previously. The cultures were examined daily for 5 days for a cytopathic effect (CPE) indicative of infectious virus. Supernatants of cultures negative for CPE at this time were incubated a second time on CRFK cells and examined for CPE for 5 days (blind passage).

Detection of FHV-1 antibodies
The constant virus-varying serum neutralization technique according to Povey and Johnson was used. Antibody titers were expressed as the reciprocal of the highest serum dilution at which CPE was inhibited in 50% of inoculated CRFK cell cultures.

Statistical analyses
Results were presented as median and minimum (min), maximum (max). Disease (clinical and ocular) scores and Ct-values were assigned as continuous variables and analyzed using ANOVA for repeated measurements if proven to be sufficient normally distributed for parametric analysis. As analysis of individual ocular parameters over the whole study period resulted in co-linearity and because of different onset and cessation of each individual ocular parameter, time frames of reactive differences between IFN and control group were extracted for analysis. Antibody titers, periods of virus isolation and median individual total disease scores were compared between both groups using Wilcoxon’s rank sum test. Values of P < 0.05 were considered significant and values of ≥ 0.05 P ≤ 0.2 were stated as tendency. For statistical analysis the software StatView 5.1 (SAS Systems, Cary, NC, USA) was used.

RESULTS
Clinical signs
Following infection with FHV-1 the course of clinical signs was similar (P = 0.79) in all cats of both groups (Fig. 1). Clinical signs started 2 days post infection (dpi) with reduced general condition, reached a peak 7 dpi with all clinical signs, and declined thereafter until cessation 2 weeks post infection in both groups. The median (min/max) individual total clinical score from 1 to 64 dpi was 26.0 (19/34) in the IFN group and 26.8 (22/33) in the control group and did not differ significantly between both groups (P = 0.73).

Ocular signs
Acute ocular signs (conjunctival hyperemia, chemosis, epithelial keratitis, epiphora, blepharospasm) developed within 6 dpi in all animals (Fig. 2). Onset of corneal vascularization was observed between 11 and 22 dpi in all animals. Peak of

© 2007 American College of Veterinary Ophthalmologists, Veterinary Ophthalmology, 10, 278–284
individual ocular scoring was reached on 8 dpi in both groups (median [min/max] = 8 [6.5/9.5] IFN group vs. 7.8 [6/10] control group) and was mainly due to conjunctivitis (hyperemia, chemosis), blepharospasm and epiphora. The median (min/max) individual total ocular score from 1 to 64 dpi was 57 (44.5/78.5) in the IFN group and 51 (49.5/54.5) in the control group and did not differ significantly between both groups (P = 0.32). Interaction of both groups for each ocular parameter was analyzed separately to evaluate contribution of each individual parameter to the course of ocular scoring: There was no statistically significant difference in conjunctival hyperemia, chemosis, epiphora and neovascularization between both groups throughout the observation period. However, blepharospasm, epithelial keratitis (dendritic and punctate lesions), and corneal staining properties were significantly different within defined time frames when comparing both groups (Table 1).

### Table 1. P-values, calculated for defined time frames (dpi = day post infection), and median (min/max) total scores of ophthalmic parameters from 1 to 64 dpi

<table>
<thead>
<tr>
<th>Ophthalmic parameter</th>
<th>P-value</th>
<th>Time frame (dpi)</th>
<th>IFN-group median (min/max) score</th>
<th>Control group median (min/max) score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epiphora</td>
<td>0.67</td>
<td>4–22</td>
<td>6 (5/8.5)</td>
<td>7.5 (4/15)</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>0.018*</td>
<td>1–64</td>
<td>4 (3/5.5)</td>
<td>5.8 (5/7)</td>
</tr>
<tr>
<td>Conjunctival hyperemia</td>
<td>0.66</td>
<td>1–18</td>
<td>12.5 (10/23.5)</td>
<td>12.8 (10/16.5)</td>
</tr>
<tr>
<td>Chemosis</td>
<td>0.14</td>
<td>4–13</td>
<td>2 (1/4)</td>
<td>3.8 (2.5/5.5)</td>
</tr>
<tr>
<td>Epithelial keratitis</td>
<td>0.016*</td>
<td>11–35</td>
<td>18.5 (14/24.5)</td>
<td>12.5 (8.5/17)</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>0.75</td>
<td>15–43</td>
<td>3.5 (1.5/7.5)</td>
<td>5.3 (0.5/6)</td>
</tr>
<tr>
<td>Fluorescein staining</td>
<td>0.045*</td>
<td>11–25</td>
<td>3 (2/5)</td>
<td>1.8 (0/3)</td>
</tr>
<tr>
<td>Rose bengal staining</td>
<td>0.047*</td>
<td>11–29</td>
<td>7 (3/9)</td>
<td>3.8 (2/5)</td>
</tr>
</tbody>
</table>

*Significant difference between both groups within given time frame. Note: time frames of reactive differences between both groups were extracted for analysis, as analysis over the whole study period resulted in co-linearity, and because of different onset and cessation of individual ocular parameters.

### Virus load

Samples of all cats taken immediately prior to FHV-1 infection were FHV-1 DNA negative by TaqMan PCR. Statistical analysis of the overall observation period (1–64 dpi) revealed no significant difference (P = 0.27) in the course of virus load between both groups (Fig. 3). Within the time frame of significant difference in epithelial keratitis (11–35 dpi), there was a weak tendency (P = 0.2) towards higher virus load in the IFN group compared to the control group.

### Virus shedding

No virus was isolated from samples of any cat taken immediately prior to FHV-1 infection. Post infection virus isolation was successful from day 1 for a median of 25 (18/35) days in IFN pretreated cats and for a median of 18 (18/29) days in control cats. The period of virus shedding did not differ significantly between both groups (P = 0.27). Virus was isolated...
from swab samples from the conjunctival sac of all cats whenever the Ct-value from the corresponding STT sample of the same cat was 27.4 or less. Virus isolation from swabs was not successful whenever the Ct-value of the corresponding STT was 28.6 and higher.

**FHV-1 antibodies**
The development of serum neutralizing (SN) antibody titers during the study is recorded in Fig. 4. Blood samples taken prior to infection were negative for SN antibodies against FHV-1 in all cats (data not shown). Comparing both groups, SN antibody titers of IFN-pretreated cats were lower ($P = 0.76$) at 2 weeks post infection, but were significantly higher ($P = 0.016$) at 10 weeks post infection than those of control cats.

**DISCUSSION**
The importance of early initiation of IFN administration in ocular herpesvirus infection has been described previously in rabbits, monkeys, and has been demonstrated in vitro. Early IFN administration is expected to induce greater antiviral activity because of induction of an antiviral state within the host cell prior to virus infection, and thus more efficiently limiting infection, replication and spread of virus than the induction of an antiviral state later in the course of infection.

In the present study we have shown dose-dependent biologic activity of topically and orally applied rFeIFN-ω on conjunctival and white blood cells in cats. Although type I IFNs are known for their antiviral and immunomodulating properties in numerous diseases, results of the present study did not demonstrate any beneficial effect of rFeIFN-ω pretreatment in preventing or limiting FHV-1 infection and associated clinical and ocular signs. Periods of virus shedding were longer in IFN-pretreated cats than in control cats, although differences were not statistically significant. Similarly, the course of virus load (FHV-1 DNA) did not differ significantly between both groups. The presence of FHV-1 DNA was demonstrated for longer periods than the prevalence of infectious virus in ocular samples, and the correlation between virus load and virus shedding was in agreement with that in a previous study. Antibody titers of IFN-pretreated cats were significantly higher at 10 weeks post infection than those of control cats, which may be explained by type I IFNs's ability to enhance antibody response by direct stimulation of B and T cells and via activation of DCs. There was no significant difference in the course and median individual scores of clinical and overall ocular symptoms and virus load between both groups. However, separate analysis of each individual ocular parameter revealed significantly higher scores for epithelial keratitis and corneal staining properties in the IFN group than in the control group.

In contrast to our results, in a preliminary study involving rats, cutaneous application of massive doses (10^8 U/kg) of HuIFN-ω resulted in low effectiveness, despite massive doses of HuIFN-ω. IFNs are cytokines from stimulated cells that are produced locally to act locally, thus inducing an antiviral state in surrounding cells. With respect to epitheliotropism of FHV-1, parenteral application of FeIFN-ω at a distant
site of viral replication may not be effective.\textsuperscript{35} FeIFN-\(\omega\) activity in conjunctival cells was seen after topical but not after systemic administration in cats.\textsuperscript{21} Similarly, HuIFN was effective in limiting herpetic keratitis in rabbits when applied topically but not so when applied intramuscularly.\textsuperscript{36} Furthermore, it was shown in a preliminary experimental study\textsuperscript{16} in cats that once-daily application of as little as 25 U of HuIFN-\(\alpha\) early on in the course of FHV-1 infection limited clinical signs, although there was no effect on virus shedding. Comparing results of this previous study with our present results, it seems that HuIFN-\(\alpha\) might be more effective at least in limiting clinical signs following FHV-1 infection compared to rFeIFN-\(\omega\).

Interestingly, type I IFNs possess some anti-FHV-1 activity \textit{in vitro}, with the rFeIFN-\(\omega\) being more effective than rHuIFN-\(\alpha\)2b in CRFK cell cultures.\textsuperscript{15} In contrast, results of our study and those of others\textsuperscript{16,14} have shown a lack of anti-FHV-1 activity of type I IFNs \textit{in vivo}. As biologic activity of type I IFNs in cats following topical and oral\textsuperscript{21} or subcutaneous\textsuperscript{37} administration has been demonstrated previously, lack of anti-FHV-1 effects of type I IFNs \textit{in vivo} appear to depend on cellular sensitivity to IFN-mediated antiviral effects and on relative resistance of FHV-1 to type I IFNs \textit{in vivo}.

Indeed, various studies demonstrate that the magnitude of antiviral effects strongly depends on type of target cell (host cell), infecting virus and (sub)type of IFN used.\textsuperscript{12,38–40} Therefore, \textit{in vitro} studies using cells other than cells naturally infected by a specific virus are inappropriate to predict antiviral effects of IFNs \textit{in vivo}.

Moreover, viruses have developed evolutionary countermeasures to facilitate productive infection and overcome host immune responses.\textsuperscript{31} HSV-1, an alpha-herpesvirus intensively investigated, is known to encode diverse functions to inhibit the IFN response and thus to be able to combat the IFN-mediated host defense, which has an important influence on virus pathogenesis.\textsuperscript{42–45} Similar to antiviral effects of IFN, virus countermeasures against the cellular IFN system vary with cell type.\textsuperscript{46} In fact, efficacy of type I IFNs has been shown to vary with the species investigated. While topical application of HuIFN-\(\alpha\) in monkeys\textsuperscript{48} and rabbits\textsuperscript{37} is efficient in limiting herpes virus-induced keratitis, topical HuIFN-\(\alpha\) has inconsistent efficacy in humans with herpetic keratitis when used as a single treatment.\textsuperscript{47–49} With regard to the lack of anti-FHV-1 effects of type I IFNs, we may speculate that FHV-1, similar to HSV-1, possibly possesses anti-IFN strategies responsible for relative resistance of FHV-1 to type I IFNs in cats.

In summary, considering the results of referenced studies, efficacy of exogenously applied IFN as a prophylactic or therapeutic option depends on complex and specific virus–host cell interactions. Many factors have an influence on antiviral efficiency of IFNs, such as time-point of IFN application in relation to virus infection, route of IFN administration in relation to site of virus infection (sub)type of IFN, IFN concentration, type of virus and host cell or species investigated.

**ACKNOWLEDGMENTS**

Virbagen\textsuperscript{®} Omega was kindly provided by Virbac, Switzerland. The FHV-1 field isolate UT 88 was kindly provided by David Lowery (Pfizer AG, Zürich, Switzerland). This study was funded by a research grant from the Vetsuisse faculty, University of Zürich, Switzerland.

**REFERENCES**

1. Povey RC. A review of feline viral rhinotracheitis (feline herpesvirus 1 infection). \textit{Comparative Immunology, Microbiology and Infectious Disease} 1979; 2: 373–387.


45. Cheng G, Brett ME, He B Val193 and Phe195 of the gamma 1 34.5 protein of herpes simplex virus 1 are required for viral resistance to interferon-alpha/beta. *Virology* 2001; 290: 115–120.


