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Limited efficacy of topical recombinant feline interferon-omega for treatment of cats with acute upper respiratory viral disease

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ABSTRACT

Despite a lack of controlled studies confirming its efficacy, recombinant feline interferon-omega (rfeIFN-ω) is used in the treatment of feline upper respiratory tract disease (FURTD), which is usually caused by feline calicivirus (FCV) or feline herpesvirus-1 (FHV-1). The aims of the present study were to investigate whether administration of rfeIFN-ω improves clinical signs in cats with acute FURTD and whether this treatment reduces shedding of FCV. Thirty-seven cats affected with acute FURTD were recruited into a prospective, randomised, placebo-controlled, double-blinded clinical trial. The presence of FCV and/or FHV-1 was determined by performing quantitative polymerase chain reaction (qPCR) on oropharyngeal and conjunctival swabs.

Cats were randomly assigned to treatment groups, receiving either placebo or rfeIFN-ω (2.5 MU/kg) subcutaneously, followed by 0.5 MU topically at 8-h intervals via the conjunctiva, intranasally, and orally for 21 days. All cats received additional treatment with antibiotics, expectorants, and inhalation of nebulised physiological saline with camomile. Clinical signs and FCV shedding were evaluated over 42 days. All cats demonstrated improvement in clinical signs during the course of the study, with no significant difference in any of the assessed variables when comparing the two groups. FCV copy numbers decreased more rapidly in cats receiving rfeIFN-ω. Treatment with rfeIFN-ω was not effective in ameliorating clinical signs of acute viral FURTD compared to placebo, but might accelerate a reduction in FCV load in infected cats.

Introduction

The major causative agents of feline upper respiratory tract disease (FURTD) are feline calicivirus (FCV) and feline herpesvirus-1 (FHV-1) (Binns et al., 2000; Helps et al., 2005). FHV-1 is usually shed for 3 weeks following primary infection (Weigler et al., 1997), with clinically recovered cats subsequently developing a state of latency and shedding virus episodically as a result of recrudescence (Gaskell and Povey, 1977). Cats infected with FCV shed virus for a variable duration of time, usually from 3 weeks to several months, but with some individuals becoming long-term carriers (Povey and Hale, 1974; Wardley, 1976; Wardley and Povey, 1977).

Type 1 interferons have been considered as a treatment option for acute viral FURTD, due to their antiviral effects. Such interferons are secreted by most nucleated cells during viral infection and replication (Tompkins, 1999), provoking a response in neighbouring cells in a paracrine fashion. Exposure of cells to type 1 interferons induces expression of viral-resistance genes, which encode proteins capable of inhibiting viral replication, degrading viral components, and down-regulating cell proliferation (Oritani et al., 2001; Randall and Goodbourn, 2008). Furthermore, these cytokines demonstrate immunomodulatory functions by upregulating antigen presentation to CD8+ cytotoxic T lymphocytes, promoting maturation of natural killer and dendritic cells, and activating macrophages and neutrophils (Le Bon and Tough, 2002; Randall and Goodbourn, 2008).

Recombinant feline interferon-ω (rfeIFN-ω) is licensed (Virbagen Omega, Virbac) in Europe, Japan, and Australia for the systemic treatment of canine parvovirus, feline leukaemia virus (FeLV) and feline immunodeficiency virus (FIV) infections.[1] Although not licensed for use in other virus infections, it has been shown to be efficacious against FHV-1 and FCV in vitro (Siebeck et al., 2006; Ohe et al., 2008).

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[1] See: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/veterinary/medicines/000061/vet_med_000195.jsp&mid=WC0b01ac058001fa1c.

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No adverse effects have been reported following mucosal administration of rfeIFN-ω to cats (Hennet et al., 2011; Slack et al., 2013), whereas subcutaneous (SC) administration can occasionally be associated with mild adverse effects, such as fever, lethargy, vomiting, and diarrhoea (de Mari et al., 2004). Topical administration of interferon is achievable for most pet owners, and would therefore be a convenient treatment option in veterinary clinical practice.

The aim of the present study was to evaluate whether topical administration of rfeIFN-ω improves clinical signs in cats with acute viral FURTD. In addition, it was hypothesised that administration of rfeIFN-ω would reduce shedding of FCV post-infection.

Materials and methods

Study population

Forty-two client-owned cats, presented to the Clinic of Small Animal Medicine, LMU University of Munich, Germany, with clinical signs of acute-onset FURTD, were enrolled into the study, with informed owner consent. Five of these cats were subsequently excluded, because they failed to test positive for FHV-1 or FCV. The study fulfilled the general German guidelines for clinical research and was approved by the Ethical Committee of the Centre for Clinical Veterinary Medicine, LMU University of Munich (Approval number: 10-20-06-13).

For inclusion into the study, cats were required to demonstrate clinical signs of acute FURTD of <7 days duration, such as ocular and nasal discharge, conjunctivitis, and sneezing. Exclusion criteria were concurrent infection with FeLV or FIV, presence of corneal ulcers, pregnancy or weaning, treatment with antimicrobial drugs within the previous 3 days, treatment with immunomodulatory drugs or glucocorticoids, vaccination, or passive immunisation within the previous 4 weeks. Cats with a prior history of FURTD were also excluded.

The final study population ($n = 37$) consisted of 31 domestic shorthair cats, two Siamese crossbreed cats and one each of British shorthair, Maine coon, Persian, and Birman crossbreed. The youngest cat was 3 weeks old, and the oldest 15 years (median of 1.8 months). Fourteen cats were male (all intact), and 23 female (four neutered).

Study design

The study was designed as a prospective, randomised, placebo-controlled, double-blinded trial. Cats were randomly assigned to one of two study groups. Cats in the rfeIFN-ω treatment group ($n = 18$) received one subcutaneous injection of 2.5 MU/kg (0.25 mL/kg) rfeIFN-ω (Virbagen Omega, Virbac) on day 0 of the study. In addition, starting on day 0, each of the cats was treated topically with 1 mL of a 0.5 MU/mL rfeIFN-ω dilution (10 MU rfeIFN-ω + 19 mL water for injection) at 8-h intervals for 21 consecutive days. Two drops (approximately 100 μL) of the solution were administered into both eyes and into both nostrils; the residual amount was administered orally. The approximate dose of rfeIFN-ω was 0.05 MU conjunctivally and nasally on each side, and 0.3 MU orally. Cats in the placebo group ($n = 19$) received 0.25 mL/kg of sterile physiological saline subcutaneously on day 0, followed by topical treatment with 1 mL of a placebo dilution (1 mL sterile physiological saline + 19 mL water for injection) according to the scheme described previously. Investigators and owners were blinded as to which treatment group each cat had been allocated, with the code subsequently broken at the end of the study.

Additional medical treatment was identical for both study groups and included amoxicillin-clavulanate (Synulox, Zoetis; 12.5 mg/kg orally twice daily) for 10 days, bromhexine (Bisolvon, Boehringer Ingelheim; 0.5 mg/kg orally three times daily) for 8 days, inhalation of nebulised physiological saline with camomile (Kamillosan, MEDA) daily for 8 days, cleaning of eyes and nostrils, nasal flushing with physiological saline daily for 8 days and fluid therapy/nutritional support if necessary.

Examination schedule

All cats were hospitalised during the first 7 days after initiation of treatment. Physical examination was performed daily from days 0–7, and on days 21 and 42. General health status and quality of life were evaluated by the modified Karnofsky's score (Hartmann and Kuffer, 1998), with quality of life expressed on a scale ranging from 100% (normal behaviour) to 0% (death of the cat).

A clinical scoring system with a scale of 0 (absent) to 3 (severe) was used to assess respiratory effort, sneezing, nasal and ocular discharge, conjunctivitis, gingivostomatitis, and oral ulceration (see Appendix: Supplementary Table S1). Body temperature was documented on each examination day. Complete blood counts (CellDyn 3500, Abbott Laboratories) were performed on days 0–3. On day 0, oropharyngeal and conjunctival swabs were obtained for FCV and FHV-1 testing. Additional oropharyngeal swabs for quantification of FCV load were taken on days 0, 7, 21, and 42. All swabs were immediately frozen at –80 °C until laboratory analysis was performed.

FHV-1 and FCV PCR

Nucleic acid (DNA and RNA) was isolated using the Nucleospin blood kit (Macherey Nagel). Cotton swabs were placed in a solution of 200 μL phosphate-buffered saline, 200 μL buffer BQ, and 20 μL proteinase K. Swabs were then incubated at 70 °C for 15 min with shaking at 700 rpm, after which the manufacturer's protocol was followed. Nucleic acid was eluted with 100 μL buffer BE and stored at –80 °C.

Real-time quantitative polymerase chain reaction (qRT-PCR) was used to detect FHV-1, multiplexed with an assay to detect feline 28S rDNA as an endogenous control (Helps and Harbour, 2003; Helps et al., 2003, 2005). A MX3005P (Agilent) thermocycler was used. Each reaction contained 12.5 μL GoTaq 2 × PCR Master mix (Promega), 200 nM each of 28S rDNA forward and reverse primers, 100 nM each of FHV-1 forward and reverse primers, 50 nM 28S rDNA Texas Red-BHQ2 probe (Metabion), 50 nM FHV-1 CY5-BHQ3 probe (Metabion), 4.5 mM $MgCl_2$; 5 μL nucleic acid and water to a final volume of 25 μL. Reactions were incubated at 95 °C for 2 min to activate the DNA polymerase, followed by thermal cycling for 45 cycles at 95 °C for 15 s and 60 °C for 30 s. Fluorescence was detected at 610 nm and 665 nm wavelengths at each annealing step (60 °C).

For detection of FCV, two separate real-time reverse transcription qPCR assays were performed, due to genetic variability of this virus, alongside detection of feline 28S rDNA to determine the amount of feline cellular material present on each swab (Helps et al., 2005). Initially, 10 μL nucleic acid were combined with 4 μL 5 × RT buffer (Promega), 2.4 μL 25 mM $MgCl_2$, 1 μL 10 mM dNTP (Promega), 1 μL random hexamer (0.5 μg/μL), 0.6 μL water and 1 μL Improm II reverse transcriptase (Promega). Reverse transcription reactions were then incubated at 20 °C for 5 min, 42 °C for 30 min and 70 °C for 15 min in a MJ PTC 200 thermal cycler (Bio-Rad). Thirty microlitres RNase-free water were added to each 20 μL cDNA sample and stored at –20 °C.

For the qRT-PCRs an Agilent MX3005P thermocycler was used. Reactions consisted of 12.5 μL GoTaq 2 × PCR Master mix, 200 nM FCV1 primers (FCV1 sense: 5′-GTTGGATGAACTACCCGCCAATC-3′, FCV1 antisense: 5′-CATATGCGGC YCTGATIGCTTGAAACTG-3′) or FCV2 primers (FCV2 sense: 5′-GAACTACCC GCCAATCAACATGTGGTAAC-3′, FCV2 antisense: 5′-GGGRCAGTTAGCACATCATATGCGGC-3′), 0.5 μL 1:2000 SYBR green I (Sigma–Aldrich), 5 μL cDNA and water to a final volume of 25 μL. Reactions were incubated at 95 °C for 2 min to activate the DNA polymerase, followed by thermal cycling for 40 cycles at 95 °C for 15 s and 60 °C for 30 s (FCV1) or 64 °C for 30 s (FCV2). Fluorescence was detected at 516 nm wavelength at each annealing step (60 or 64 °C). Following completion of the PCR, melting curve analysis was performed to assess the specificity of each reaction. The reaction efficiencies were 95% for FCV1 and 96% for FCV2.

PCR data were analysed using the MX3005P software. For FHV-1, a positive sample was defined as having a cycle threshold (Ct) value ≤45. For FCV, positive samples had Ct values ≤40 with appropriate amplicon melting temperatures (FCV1: 82.5–86.0 °C; FCV2: 83.5–86 °C). Positive samples with amplicon melting temperatures close to the cut off were repeated. For the calculation of relative viral copy numbers, samples were first normalised against the feline 28S rDNA Ct values to account for differences in swabbing efficiency. According to methods published previously (Peters et al., 2005), Ct values were converted to relative copy number using the equation $2^{(45-Ct)}$ (FHV-1) or $2^{(40-Ct)}$ (FCV).

Statistical methods

A clinically relevant difference in FURTD-associated signs between cats treated with rfeIFN-ω and those receiving placebo was assumed to be in the order of 40 vs. 80% of cats having clinical signs. Using these values, a power of at least 80% and a significance level of 5% could be achieved with a minimum of 17 animals per group (PASS, NCSS LLC).

Clinical signs, Karnofsky's score, body temperature, white blood cells (WBC), lymphocyte count, neutrophil count, packed cell volume, and relative FCV copy numbers were compared between the two treatment groups using a Kruskal–Wallis test with a Dunn post test (Prism 5.0, Graphpad). The distribution of pathogens was analysed by use of Fisher's exact test. The course of all variables from day 0 to 42 was examined by mixed model analysis (ProcMIXED, SAS). The change over time, referring to the number of cats shedding FCV, was determined by comparison of data obtained on days 0 and 7, 21, 42, respectively using chi-square test or Fisher's exact test (Prism 5.0, Graphpad). A P value <0.05 was considered significant for all analyses.

Results

Comparison between groups on day 0

In the rfeIFN-ω treatment group, nine cats were infected with FCV, two cats with FHV-1, and seven cats were co-infected. In the placebo group, 10 cats were infected with FCV, eight cats with FHV-1, and one cat was co-infected. There was no significant difference in the number of FCV-infected cats ($P = 1.000$), nor in the number of FHV-1-infected cats ($P = 0.062$) when comparing groups, but the