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Efficacy of passively transferred antibodies in cats with acute viral upper respiratory tract infection

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ABSTRACT

A commercial hyperimmune serum, containing antibodies against feline calicivirus (FCV), feline herpesvirus 1 (FHV-1), and feline panleukopenia virus, is available for treatment of cats with feline upper respiratory tract disease (FURTD), but its efficacy has not been rigorously evaluated in scientific studies. The aim of this randomised, placebo-controlled, double-blind clinical trial was to evaluate the efficacy of passive immunisation in cats with acute viral FURTD caused by FCV and/or FHV-1 infection. All cats received symptomatic treatment during the study period. Hyperimmune serum was administered to one group ($n = 22$) and an equivalent amount of saline was administered to the control group ($n = 20$) as placebo, for 3 consecutive days. In the treatment group, cats ≤12 weeks old received 2 mL, cats >12 weeks old received 4 mL, subcutaneously once daily and topically into eyes, nostrils, and mouth every 8 h. Clinical signs, including a 'FURTD score' and general health status, were recorded daily for 8 days and again on day 21. FCV shedding was determined by quantitative PCR on days 0 and 21.

Clinical signs and health status in both groups improved significantly over time ($P < 0.001$). Cats receiving hyperimmune serum significantly improved in terms of 'FURTD score' ($P = 0.046$) and general health status ($P = 0.032$) by day 3, while cats in the placebo group only improved significantly by day 7. There was no significant difference in the number of cats shedding FCV between the two groups. Thus, administration of hyperimmune serum led to a more rapid improvement of clinical signs in cats with acute viral FURTD, but by day 7, clinical signs had improved equally in both groups.

Introduction

Feline upper respiratory tract disease (FURTD) can be caused by several pathogens, leading to similar clinical signs of disease. Two viruses in particular, feline calicivirus (FCV) and feline herpesvirus 1 (FHV-1), are responsible for at least 80% of FURTD cases (Helps et al., 2005; Di Martino et al., 2007; Gould, 2011). In addition, bacteria, including *Chlamydophila felis*, *Bordetella bronchiseptica*, and *Mycoplasma* spp., can act as primary infectious agents (Jacobs et al., 1993; Hartmann and Hartmann, 2010; Hartmann et al., 2010). After initial infection, FHV-1 develops a state of latency, residing mainly in the trigeminal and vestibular ganglions, and cats become carriers, shedding virus during period of recrudescence (Townsend et al., 2004; Gaskell et al., 2007; Parzefall et al., 2010). FCV-infected cats can also become asymptomatic carriers, but shed virus more persistently (Wardley and Povey, 1977; Radford et al., 2007, 2009).

Several antiviral drugs are effective in vitro against FHV-1 (Nasisse et al., 1989; Maggs et al., 2000; Maggs and Clarke, 2004; Williams et al., 2004; Siebeck et al., 2006; Van der Meulen et al., 2006) and FCV (Povey, 1978a). Some treatment options are available clinically for treating cats affected with FHV-1, with most of these drugs being administered topically, e.g. cidofovir, idoxuridine, vidarabine, and trifluridine (Stiles, 1995; Fontenelle et al., 2008). Adverse effects can occur when anti-viral drugs are used systemically (Weiss et al., 1993; Nasisse et al., 1997), although oral administration of famciclovir (Famvir, Novartis) has recently been shown to improve clinical signs in FHV-1-infected cats, without causing adverse effects (Malik et al., 2009). In contrast, there is no specific treatment option available for FCV that has proven efficacy and tolerable adverse effects (Povey, 1978b; Hennet et al., 2011).

The commercially available product, Feliserin (IDT Biologika), is reported to contain antibodies against FCV, FHV-1, and feline panleukopenia virus, and is marketed in Germany (Product License number 35a/92) for the treatment of acute viral FURTD and feline panleukopenia. Its efficacy, however, has not been rigorously assessed in scientific studies. Therefore, the aim of the present study

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was to evaluate whether administration of Feliserin was beneficial in cats affected with acute viral FURTD.

Materials and methods

Study design

The study was performed as a randomised, placebo-controlled, double-blind clinical trial in cats affected with acute viral FURTD. Thirty-two cats (22 receiving Feliserin and 10 placebo) were prospectively recruited into the study and randomised to either treatment or placebo group. Data from a previous study including an additional 10 cats that had received the same symptomatic treatment protocol were also included as controls. When a cat was recruited, medications were drawn up and injected by a veterinarian not involved in the study to ensure that neither owner nor clinician was aware which group the cat had been assigned to. Decoding occurred after the study was completed and data entered for statistical evaluation.

The study fulfilled the general German guidelines for prospective studies with informed owner consent and was carried out with permission from the responsible German veterinary authority (Government of Upper Bavaria, Maximilianstrasse 39, 80538 Munich, reference number 55.2-1-54-2532-05-12).

Study population

Included in the study were 42 cats that presented with clinical signs of FURTD of less than 7 days duration and in which FCV and/or FHV-1 had been detected by quantitative polymerase chain reaction (qPCR) from oropharyngeal and/or conjunctival swabs. Cats were excluded if demonstrated to be infected with feline immunodeficiency virus (FIV) or feline leukaemia virus (FeLV), using a commercial immunoassay (FeLV Antigen/FIV Antibody Test Kit, IDEXX Laboratories). Cats that were affected with corneal ulceration, requiring surgical treatment, and those that were pregnant or lactating were excluded. Cats were also excluded if they showed evidence of systemic disease, as determined by clinical examination, complete blood count (Cell-Dyn 3500, Abbott) and serum biochemistry (Hitachi 911, Roche). Cats with a prior history of FURTD episodes, those that had received antimicrobial drugs within the previous 3 days, or those that had been vaccinated or received any type of passive immunisation, paramunity inducer, antiviral treatment, or glucocorticoid within the previous 4 weeks were also excluded.

The study population consisted of 38 European Shorthair cats, three longhair crossbreeds, and one Persian cat. Of the 22 females, one was neutered; all 20 males were intact. The youngest kitten was 3 weeks old, the oldest cat was 13 years (median, 0.15 years; 35 out of 42 cats (83%) were 12 weeks old or younger).

Treatment protocol

Twenty-two cats received Feliserin and 20 cats received placebo (physiological saline) subcutaneously once daily for 3 consecutive days. Cats younger than 12 weeks received 2 mL per injection; older cats received 4 mL per injection. This protocol was recommended by the manufacturer and has been used in Europe since 1992. Additionally, two drops (~0.1 mL) of antiserum were administered into eyes, nostrils, and on the oral mucosa every 8 h for 3 consecutive days. All cats received symptomatic treatment with 12.5 mg/kg amoxicillin-clavulanate (Synulox, Zoetis) orally twice daily for 10 days; bromhexine (Bisolvon, Boehringer Ingelheim) 0.5 mg/kg orally every 8 h for 8 days; inhalation with physiological saline and camomile (Kamillosan, MEDA) once daily for 8 days; cleaning of eyes and nostrils; nasal flushing with physiological saline once daily, as well as fluid and nutritional support where necessary.

Clinical examination

A clinical examination was performed each day from days 0 to 7, as well as on day 21. A 'FURTD score' was calculated, based on a bespoke scoring system that includes 13 parameters, graded from 0 to 3, according to their severity (see Appendix A: Supplementary Table S1). These clinical signs and the total 'FURTD score' were judged at each examination. In addition, quality of life and well-being of each cat were evaluated daily using the modified Karnofsky's score, ranging from 100% (no signs of disease) to 0% (death) (Hartmann and Kuffer, 1998).

Nucleic acid preparation

Oropharyngeal and/or conjunctival swabs were stored at –80 °C immediately after sampling until analysis. Total nucleic acid (DNA and RNA) was extracted using the Nucleospin Blood Kit (Macherey Nagel). Cotton swabs were placed in a solution consisting of 200 μL phosphate-buffered saline (PBS), 200 μL of buffer BQ1, and 20 μL of proteinase K. Swabs were incubated at 70 °C for 15 min with shaking at 700 rpm, then the manufacturer's protocol was followed. Total nucleic acid was eluted with 100 μL of buffer BE and stored at –80 °C.

Quantitative PCR

On day 0, qPCR for FCV and FHV-1 was performed for inclusion purposes and repeated for FCV on day 21 to detect viral shedding. Primers used in the study are detailed in Appendix A: Supplementary Table S2. Quantitative PCR was used to detect FHV-1 and feline 28S rDNA (endogenous internal control) as described by Helps et al. (2005), using an Agilent MX3005P thermocycler. Each reaction contained 5 μL of genomic DNA, 12.5 μL of 2 × GoTaq PCR Master mix (Promega), 200 nM each of 28S rDNA primers, 100 nM each of FHV-1 primers, 50 nM 28S rDNA Texas Red-BHQ2 probe, 50 nM FHV-1 CY5-BHQ3 probe, 4.5 mM $MgCl_2$ (final concentration), and water, to a final volume of 25 μL. Reactions were incubated at 95 °C for 2 min followed by 45 cycles of 15 s at 95 °C and 30 s at 60 °C. Fluorescence was measured at 610 nm and 665 nm after each annealing/elongation step.

Two separate real-time quantitative reverse-transcription (qRT) PCR assays were performed for FCV, due to genetic variability. PCR primers for the two FCV assays were designed to anneal to conserved regions of the FCV genome, which were determined by multiple sequence alignment. Ten microlitres of total nucleic acid were reverse transcribed by adding 4 μL of 5 × RT buffer, 2.4 μL of 25 mM $MgCl_2$, 1 μL of 10 mM dNTP, 1 μL of random hexamer primer (0.5 μg/μL), 0.6 μL of water, and 1 μL of Improm II reverse transcriptase (Promega), and incubated at 20 °C for 5 min, 42 °C for 30 min then 70 °C for 15 min in an MJ PTC 200 thermocycler. Thirty microlitres of RNase-free water were added to each 20 μL cDNA sample and stored at –20 °C prior to analysis.

For qPCR, an Agilent MX3005P thermocycler was used with reactions consisting of 5 μL cDNA, 12.5 μL GoTaq 2 × PCR Master mix, 200 nM primers (either FCV1, FCV2, or 28S rDNA; see Appendix A: Supplementary Table S2), 0.5 μL 1:2000 SYBR green I (Sigma-Aldrich) and water to a final volume of 25 μL. Samples were incubated at 95 °C for 2 min, followed by 40 cycles of 15 s at 95 °C and 30 s at either 60 °C (28S rDNA and FCV1) or 64 °C (FCV2). Fluorescence was measured at 516 nm after each annealing/elongation step. Following completion of the PCR, melting curve analysis was performed by incubating reactions at 70 °C for 10 s and taking fluorescence readings as the temperature increased incrementally by 1 °C for 10 s. Melting temperatures of FCV1 amplicons were between 82.5 and 86.0 °C, and those of FCV2 amplicons were between 83.5 and 86.0 °C.

The 28S rDNA cycle threshold (Ct) value of each sample was used to normalise the FCV Ct values, to take into account different swabbing efficiencies on days 0 and 21. The normalised viral Ct values were converted into relative copy numbers by assuming that one copy had a Ct value of 40, with an assay efficiency approximating 100%.

Detection of antibodies

Antibody titres were determined in Feliserin (Batch number: 0170612) and in sera from 13 cats at days 0, 3, 7, and 21, using a protocol modified from Dawson et al. (1998). Clotted blood samples were centrifuged at 1500 *g* for 10 min and serum heat-inactivated at 56 °C for 30 min. Serial dilutions (1:4) of Feliserin or serum were prepared with PBS in sterile 96-well culture plates (Dynatech Laboratories), with each well containing a final volume of 60 μL. For detection of FCV antibodies, 100 $TCID_{50}$ (tissue culture infective dose) of FCV 255 was added, and for detection of FHV-1 antibodies, 100 $TCID_{50}$ of FHV-1 605 was added (both isolates used by the manufacturer to produce Feliserin). Plates were incubated at 37 °C for 1–1.5 h, then 100 μL transferred onto monolayers of Crandell feline kidney cells (CrFK, CCL-94, ATCC) that had been maintained in complete medium consisting of Minimum Essential Medium (MEM, Biochrom) supplemented with 10% fetal calf serum (FCS, Biochrom). The neutralisation test was evaluated by direct microscopy for evidence of viral cytopathic effect, with the greatest dilution of serum capable of completely neutralising the virus (i.e. no cytopathic effect) indicating the antibody titre.

Statistical methods

The software program Graphpad Prism[1] was used for statistical analysis of data. The number of animals required was calculated by the Power Analysis and Sample Size Software (PASS, NCSS Statistical Software).[2] It was assumed clinically relevant if cats treated with Feliserin improved in terms of their 'FURTD score' by three points or more and their general health status by 15% or more, compared to cats in the placebo group, within the first 3 days. Assuming a power of 80% and a confidence interval of 95%, 17 cats per group were required to detect a difference between groups.

Fisher's exact test was used for the inter-group comparison of virus distribution on day 0. One-way analysis of variance (ANOVA) with Dunn's post test (Kruskal–Wallis test for not normally distributed data) was used to analyse mean values of the two scores of each group and between the groups at days 0, 3, 7, and 21, and relative FCV copy numbers on days 0 and 21. Changes over time (days 3, 7, and 21 vs. day 0) of the groups were compared to each other using the Mann–Whitney *U* test (not normally distributed data) or unpaired Student's *t* test (normally distributed data). FCV shedding between beginning and end of the study was analysed using

[1] See: http://www.graphpad.com/scientific-software/prism/
[2] See: http://www.statistical-solutions-software.com/ncss-home.