Comparative Immunology, Microbiology and Infectious Diseases 48 (2016) 41–47

Contents lists available at ScienceDirect

Comparative Immunology, Microbiology and Infectious Diseases

journal homepage: www.elsevier.com/locate/cimid

Clinical improvement in feline herpesvirus 1 infected cats by oral low dose of interleukin-12 plus interferon-gamma

Filomena Fiorito[a,b,*], Antonietta Cantiello[a], Giovanna Elvira Granato[a], Luigi Navas[a], Carmine Diffidenti[c], Luisa De Martino[a,*], Veeramani Maharajan[d], Fabio Olivieri[e], Ugo Pagnini[a], Giuseppe Iovane[a]

[a] Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy
[b] Department of Chemistry, Istituto Zooprofilattico del Mezzogiorno, Portici, Naples, Italy
[c] Veterinary Clinic Noè, Telese Terme, Benevento, Italy
[d] CNR, Institute of Cybernetics, Pozzuoli, Naples, Italy
[e] Clinical Research Unit, GUNA S.p.a., Milan, Italy

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 27 June 2016
Accepted 20 July 2016

Keywords:
Feline herpesvirus-1
Cats
Clinical trial
Interleukin-12 plus interferon-gamma
Low dose therapy
Sequential Kinetic ActivationSequential kinetic activation

ABSTRACT

Feline herpesvirus 1 (FHV-1) is a widespread cat pathogen inducing rhinitis, conjunctivitis and corneal ulcers. To alleviate acute FHV-1-induced disease, antiviral agents are used often with antibiotics. But sometimes, these treatments, as well as conventional doses of cytokines have moderate efficacy and/or collateral effects. Herein we have investigated the effects of low dose interleukin (IL)-12 plus interferon (IFN)-gamma, prepared by Sequential Kinetic Activated (SKA), on the treatment of FHV-1 infection. Twenty-five, unvaccinated FHV-1-positive cats were recruited into a prospective, randomized, placebo-controlled, double-blinded clinical trial. Fifteen cats were treated for 6 months with oral low doses of SKA IL-12 plus IFN-gamma and 10 cats were treated with placebo. At 1, 6 and 12 months (follow-up) after the beginning of treatment, clinical assessment, PCR assay and blood count were carried out. At follow-up, in treated group, we observed significant ($p < 0.05$) improvements in clinical signs and PCR became negative in 12/15 cats (80%). In placebo, 10/10 cats were PCR-positive, with improvements (30%) or worsening (70%) in clinical signs. Blood values were normal in both groups.

Our results show that the low dose therapy, based on activated solutions of IL-12 plus IFN-gamma, represents a novel approach to treat FHV-1 infection in cats.

1. Introduction

Feline herpesvirus 1 (FHV-1), a widespread cat pathogen, is a member of Herpesvirales order. It is involved in the feline upper respiratory tract syndrome, often along with other pathogens, such as feline calicivirus (FCV) and/or *Chlamydophila felis*, *Bordetella bronchiseptica*, *Mycoplasma* species, *Staphylococcus* species or *Escherichia coli* [1]. Typical clinical signs of FHV-1 infection include fever, depression, anorexia, serous or serosanguineous ocular and/or nasal discharge, conjunctival hyperaemia, sneezing and less frequently, salivation and coughing. Secondary bacterial infections are common and secretions become purulent. In susceptible kittens, primary pneumonia and viraemic states have been identified to produce severe generalized signs and eventual death [1–4]. Infectious virus can be detected in oropharyngeal and nasal swabs as early as 24 h post infection and generally persists for 1–3 weeks, although viral DNA may be detected by PCR assay for longer periods [1,2,5]. Like a typical alphaherpesvirus, FHV-1 establishes latency mainly in the trigeminal ganglia and the cats become latently infected carriers. Reactivation gives rise to intermittent viral shedding in oronasal and conjunctival secretions which can lead to keratitis, conjunctivitis, dermatitis and blindness. The use of antibiotic therapy is encouraged to control secondary bacterial infection in cases of acute upper respiratory tract disease (URTD), for instance, doxycycline, since it has activity against *Mycoplasma felis*, *Bordetella bronchiseptica* and *Chlamydia felis* and has been shown to produce clinical improvements in treating cats with clinical signs of URTD [6]. Clinical signs of FHV-1 ocular dis-

* Correspondence authors at: Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Via Delpino 1, 80137 Naples, Italy. Tel.: +39 0812536180; fax: +39 0812536179; Istituto Zooprofilattico Sperimentale del Mezzogiorno, Via Salute 2,80055 Portici, Naples, Italy. Tel.: +39 0818675167; fax: +39 0818675164.
E-mail addresses: filomena.fiorito@unina.it (F. Fiorito), luisa.demartino@unina.it (L. De Martino).

http://dx.doi.org/10.1016/j.cimid.2016.07.006
0147-9571/

ease are generally treated with antiviral therapy administered as ophthalmic solutions, ointments or oral solutions. Though, many of these drugs show excellent results in the treatment of FHV-1 *in vitro*, sometimes they have poor efficacy and/or bioavailability *in vivo*. Moreover, some antiviral agents are toxic when administered systemically in cats [4,7–10]. In general, several antiviral agents developed for the treatment of herpetic disease in humans have also been tested in cats. For example, oral administration of famciclovir (1–3 times/d for more than), alone or in combination with topical mucinomimetic and antimicrobial treatments, improved clinic outcomes in cats experimentally infected with FHV-1 [11]. But the main barrier to the use of famciclovir in cats is the expense of treatment. Additionally, sometimes these therapeutic approaches require daily multiple dose administration, for prolonged periods, which can be associated with poor compliance mainly in less tractable cats [9].

Interferons (IFNs), described as cytokines since their discovery, are signaling molecules essential for regulating the activation of immune cells as an antiviral response [12,13]. Several pathologies with an important inflammatory component are characterized by the presence of a shift in the immunological balance which is mainly reflected in an imbalance between the cytokines expressed by the two major lymphocyte subpopulations, Th1 and Th2. Th1 cells secrete the cytokines IFN-g and tumor necrosis factor (TNF)-b, which allow these cells to be effective against intracellular infections by viruses and bacteria. Th2 cells secrete interleukin (IL)-4, -5, -10 and -13, which up-regulate antibody production and target parasitic organisms [14]. Examples have been growing recently and include members of different viral families including herpesviridae [15]. Thus, these cytokines have been used in several studies, in order to restore the balance between Th1/Th2 responses, lost due to infectious diseases [16]. It has been shown that recombinant feline interferon-omega (rfeIFN-omega) is efficacious against FHV-1 *in vitro* [17–20]. But, experimental trials with conventional doses of cytokines are found poorly effective against FHV-1 infection [10,21–24]. There are few studies on the susceptibility of FHV-1 to cytokines immune-modulatory action and most of these experiments were carried out in *in vitro* systems. Feline interferon or recombinant human leukocyte interferons, rFeIFN-omega and/or interferon-alpha were used with success in FHV-1 infection, in cells of feline lung [17], kidney [20], embryo [18] or cornea [19]. However, attempts to obtain these results *in vivo*, with conventional doses of cytokines, like rFeIFN-omega, have only yielded modest improvements of the clinical signs and reductions in concurrent viral excretion in naturally infected FHV-1 cats [22]. Topical ocular administration of rFeIFN-omega or human recombinant interferon alpha-2b did not improve clinical disease or virus shedding in cats with naturally acquired keratoconjunctivitis induced by FHV-1 [23]. Similar results were observed in cats which were pretreated with rFeIFN-omega before FHV-1 infection [21]. Recently, a placebo controlled clinical study evaluated the effect of rFeIFN-omega treatment in cats. A single subcutaneous dose of rFeIFN-omega followed by topical treatment for 21 days did not improve the outcome, compared to placebo, in acute upper respiratory viral disease caused by FHV-1. While, locally administered rFeIFN-omega might benefit those cats affected with oromucosal ulceration [24]. Interestingly, no adverse effects have been reported following mucosal administration of rfeIFN-omega to cats [23,24]. In addition, subcutaneous administration of feline cytokines to cats can occasionally be associated with mild adverse effects, such as fever, lethargy, vomiting and diarrhoea [25]. Whereas, increasing evidence suggests that oral administration of certain cytokines is not only safe and effective, but also avoids the collateral effects of systemic administration [26]. Low-dose oral administration of cytokines is effective both *in vivo* and *in vitro* [26]. Basic research studies and clinical trial showed that low dose cytokines could induce rebalancing activity on the Thl/Th2 response [27]. For instance, low-doses of IL-12 modulate human functional activities of T cell subpopulations from non-small cell lung cancer patients [28]. It has been shown that if cytokines are activated by the pharmaceutical preparation process known as "sequential kinetic activation" (SKA) (underwent a shaking process characterized by the following parameters: vertical shaking; 10 centimetres motion range; shaking speed corresponding to 100 oscillations in 10 s), the activated solutions, compared to non-activated solution, showed a good therapeutic activity in a murine model of allergic asthma [29]. Using low dose cytokines SKA is highly effective *in vitro*/*ex vivo* models [27]. Indeed, SKA cytokines displayed their functional activities even at physiologically low concentrations, with a complete recovering from the experimental asthmatic state [29]. Moreover, low-doses of SKA IFN-gamma enhanced the *ex vivo* cytotoxicity of peripheral blood natural killer cells from human patients with early-stage colorectal cancer [30]. And, low doses of SKA IL-4 and IL-12 induced an enhancement of the immunostimulatory functions of *ex vivo*-generated dendritic cells from early-stage human colon cancer patients [31]. Finally, in a murine model of allergic asthma, oral administration of an association of IL-12 plus IFN-gamma low dose activated solutions is able to solve the bronchial hyperresponsiveness condition of mice, by establishing normal cytokine levels [29]. Moreover, the high efficacy is not accompanied by the collateral effects in mice [29].

Therefore, the aim of this study was to test low doses of SKA IL-12 plus IFN-gamma in cats naturally infected with FHV-1. Fifteen cats were treated for 6 months with oral low doses of SKA IL-12 plus IFN-gamma and 10 cats were treated with placebo. Clinical assessment, PCR assay and blood count were carried out at 1 and 6 months after the beginning of treatment. Moreover, in order to evaluate the further variations in both clinical and biochemical signs when the low doses treatment with cytokines has been stopped [29], we also analyzed cats 6 months after the discontinuation of treatment (follow-up).

2. Materials and methods

2.1. Enrolment of animals

Cats were selected with informed consent and voluntary acceptance of the owners attending the veterinary clinics of the Department of Veterinary Medicine and Animal Production (University of Naples Federico II, Naples) and from a private clinic (Veterinary Clinic Noè, Telese Terme, Benevento). The study fulfilled the general Italian guidelines for clinical research and was approved by the local Institutional Ethical Animal Care and Use Committee, University of Naples Federico II, Naples (Italy) (Approval number: 2012/0022732, 03/01/2012). A detailed profile was maintained for each animal including information on sex, age, gender, lifestyle habits (indoor or outdoor), household variables (single or multiple cat households), and clinical history. For inclusion into the study, cats were required to demonstrate clinical signs of FHV-1 disease, such as ocular and nasal discharge and conjunctivitis. Exclusion criteria were concurrent infection with Feline Immunodeficiency Virus-FIV or Feline Leukemia Virus-FeLV, pregnancy or weaning, any vaccination and multiple cat households. From January 2013 to June 2014, 333 nasal, oropharyngeal or conjunctival swabs were collected from 35 cats with respiratory signs related to feline upper respiratory tract syndrome.

2.2. Study design

Out of the above 35 animals, only 25 were positive for FHV-1, as detected by PCR assay, and were enrolled into a prospective, randomized, placebo-controlled, double-blinded clinical trial.