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Use of domperidone in the treatment of canine visceral leishmaniasis: A clinical trial

P. Gómez-Ochoa [a,*], J.A. Castillo [a], M. Gascón [a], J.J. Zarate [a], F. Alvarez [b], C.G. Couto [b]

[a] *Department of Animal Pathology, Veterinary Faculty of Zaragoza, c/ Miguel Servet 177, CP 50013 Zaragoza, Spain*
[b] *Veterinary Teaching Hospital ,Department of Animal Pathology, College of Veterinary Medicine, The Ohio State University, 601 Vernon L. Tharp Street, Columbus, 43210 OH, United States*

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Abstract

The aim of this study was to evaluate the effects of domperidone, a dopamine D2 receptor antagonist, in dogs naturally infected by *Leishmania infantum*. Ninety-eight dogs were treated with single-agent domperidone at 1 mg/kg twice a day orally for 1 month. Clinical, serological, biochemical and immunological examinations were conducted for the following 12 months. Domperidone was effective in controlling and reducing clinical signs and antibody titre. Significant decreases in reciprocal serum antibodies were seen in 74.3% of the dogs with mild clinical signs and 40% of the dogs became seronegative. In dogs with several clinical signs and high antibody titres, clinical improvement occurred in 86% of animals and the reciprocal serum antibody titres decreased in 38% of these dogs. A significant increase was noted in the immune cellular status, as measured by the leishmanin skin test and a lymphocyte proliferation assay.

Keywords: Leishmaniasis; Immunomodulation; Domperidone

Introduction

Canine leishmaniasis is widespread in the Mediterranean basin. It is transmitted by the sandfly *Phlebotomus perniciosus*, and is a zoonosis considered by the WHO as an emergent disease; indeed, every year some 500,000 new cases arise in areas in which health services are poorly developed (WHO, 1990). The clinical signs of leishmaniasis in naturally infected dogs are variable, ranging from asymptomatic dogs with only mild lymphadenopathy or skin lesions, to dogs with ulceration, bleeding complications, anemia, cachexia, and renal failure (Valladares et al., 1997).

Although there are many protocols using several drugs such as aminosidine, amphotericin B, meglumine antimoniate and allopurinol (Baneth and Shaw, 2002; Noli and Auxilia, 2005), the most frequently used treatment regime, supported by much clinical evidence, is long-term administration of allopurinol after an initial course of meglumine antimoniate. One suggested protocol consists of an initial treatment with both drugs for a minimum of 3 weeks followed by long-term allopurinol treatment (Noli and Auxilia, 2005).

Virtually no treatment will completely eliminate parasites from the infected animal, and even if temporary clinical remission is achieved a relapse is to be expected weeks to years after drug withdrawal (Baneth and Shaw, 2002; Ciaramella and Corona, 2003b). However, currently available treatment options are limited (Pennisi et al., 2005). The susceptibility of the leishmania parasite to antimonials (Gramiccia et al., 1992), amphotericin B (Mbongo et al., 1998) and aminosidine (Fong et al., 1994) decrease progressively (Pennisi et al., 2005). The limitations of antimonial therapy in dogs (induction of parasite resistance, lack of parasitological cure, toxicity and expense) demand more

* Corresponding author. Tel.: +34 976761633; fax: +34 976761612.
E-mail address: pablogomezochoa@gmail.com (P. Gómez-Ochoa).

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affordable alternatives with greater efficacy (Baneth and Shaw, 2002). The adverse effects of existing drugs (antimonials, amphotericin B and aminosidine) provide an additional motivation (Davidson, 1998; Pennisi et al., 2005; Rodrigues et al., 2006). Moreover, the long-term requirement for parenteral administration and/or high cost may reduce the rate of compliance of the dogs' owners during treatment.

Leishmaniasis in humans and in dogs should be treated with drugs that act via separate mechanisms in order to minimise the danger of generating resistant parasite strains (Baneth and Shaw, 2002). The prognosis and clinical course of leishmaniasis are variable, and the host's immune system plays a pivotal role in the establishment of infection and in the outcome of therapy (Pinelli et al., 1994; Ciaramella and Corona, 2003a). Immunomodulation added to conventional treatment could be a therapeutic key to success in treating the disease.

We have previously proposed a protective role of lactation against leishmaniasis in the Syrian Hamster model (*Mesocricetus auratus*) (Gomez-Ochoa et al., 2003). This ability to kill the parasite seems to be related to the hyperprolactinaemic status during lactation. Prolactin has a central role in the immune reaction, but its mechanism of action is largely unknown. The hormone, whose main function is to stimulate milk production in mammals, is now classified as a proinflammatory lymphocyte-derived cytokine (Hinterberger-Fischer, 2000). An increase in serum prolactin concentration generates an increment in CD4+Th1 subsets, and in interleukin (IL)-2, IL-12, interferon (INF)-γ and tumour necrosis factor (TNF)-α release, leading to a natural killer (NK) cell and macrophage activation, followed by a decrease in CD4+Th2 subsets and TNF-β (Di Carlo et al., 1993; Richards et al., 1998; Majumder et al., 2002).

The main goal of the present study was to evaluate the effects of a hyperprolactinaemic drug in dogs with leishmaniasis. We tested domperidone (EV-4820 Esteve Veterinary, Dr. Esteve Labs. SA), a gastric prokinetic and antiemetic drug that is also a dopamine D2 receptor antagonist that results in the release of serotonin, which in turn stimulates prolactin production. It has been well documented that this drug significantly increases serum prolactin concentration (Berczi et al., 2000).

Materials and methods

Ninety-eight dogs (males and females of various breeds and ages ranging from 2–13 years) admitted to the Veterinary Faculty Hospital of the University of Zaragoza and naturally infected with leishmaniasis were included in the study. Sixty-two were housed outdoors and 36 indoors. Deltamethrin-impregnated collars were fitted on all dogs, which were kept in the same conditions throughout the study period.

Due to the broad spectrum of clinical signs, haematological and biochemical abnormalities that are found in dogs with leishmaniasis, we divided the affected dogs into two groups. Group A included 70 dogs with low antibody titres (1/800–1/1600 as measured with a Direct Agglutination Test [DAT]); of these 70 dogs, 34 (48.57%) had no obvious clinical signs, and the remaining 36 animals had clinically relevant lymphadenopathy and skin lesions. Twenty-eight dogs were included in Group B; the inclusion criteria were the presence of multisystemic clinical signs (exfoliative dermatitis, alopecia, lymphadenopathy, pale mucous membranes, weight loss, anterior uveitis, and onycogriphosis) and high antibody titres (>1/1600 using DAT).

At the time of diagnosis, each dog underwent popliteal lymph node and bone marrow aspiration from the costochondral junction for culture; specimens were inoculated into Novy–MacNeal–Nicolle (NNN) culture medium and incubated at 28 °C for 1 month. Culture tubes were examined every 10 days for the presence of motile promastigotes by ordinary light microscopy. Initially, 117 dogs positive for *Leishmania* were included in the study but 19 were later excluded (11/19 were serologically positive but *Leishmania* was not isolated in tissue culture; the other 8/19 showed concurrent diseases (six with ehrlichiosis and one with chronic otitis), or were being treated with other drugs (one with corticosteroids and the other with antibiotics).

All experiments were performed in accordance with the University of Zaragoza Ethics Committee guidelines and the owners gave prior informed consent. The dogs were fully monitored and treatment could be changed to antimoniate meglumine/allopurinol at the owner's request if clinical or biochemical impairment was noticed. After inclusion, domperidone (EV-4820 Esteve Veterinary, Dr. Esteve Labs. SA) was orally administered at a dosage of 1 mg/kg every 12 h for 1 month as the sole drug.

Animals were checked on days 0, 15, 30, 60, 90, 120, 150, 180, 270 and 360 after the treatment had started, and clinical signs and lesions associated with leishmaniasis were evaluated and monitored. A complete blood count, biochemistry profile (liver, kidney and electrolytes), and serum protein electrophoresis were performed on all dogs. DAT were also performed, with 1/800 used as the cut-off titre. To check the cellular immunity status the leishmanin skin test reaction (LST) was used on days 0, 90 and 360 and an in vitro lymphocyte proliferation assay (LPA) (cell proliferation detection kit III, Boehringer/Roche) on days 0, 30, 180 and 360 after the treatment commenced.

The LST was performed as described elsewhere (Solano-Gallego et al., 2001a). Briefly, 0.1 mL of a suspension of 3×10^8 inactivated *Leishmania infantum* (MHOM/FR/78/LEM75) promastigotes per millilitre diluted in 0.4% phenol–saline were injected intradermally (ID) in the skin of the right groin. The same amount of 0.4% phenol–saline was injected ID in the skin of the left groin as a negative control. An induration diameter of >5 mm after 72 h was considered positive.

LPA was performed using the whole blood microassay technique previously described for dogs (Shifrine et al., 1978). In short, 200 μL of heparinised blood were diluted in 3 mL of RPMI medium supplemented with 2 mM glutamine, 100 μg of streptomycin and 100 IU penicillin/mL. One hundred microlitres of diluted blood were placed in triplicate in 96 well flat-bottomed culture plates to which *Leishmania* antigen was added at concentrations of 1.0, 1.25 and 5 μg/mL (100 μL/well) to standardise the test. The results of the optimal concentration (1 μg/mL) were carried out in duplicate. The cells were incubated at 37 °C in 5% O_2. The proliferation was checked using 5-bromo-2′-deoxyuridine (BrdU) (Huong et al., 1991) added over 18 h on day 4 after incubation. Cell proliferation was determined using an ELISA technique according to the manufacturer's instructions (BrdU, cell proliferation detection kit III, Boehringer/Roche). The cells that had incorporated BrdU to the DNA were recognised using a monoclonal antibody against BrdU and measured by an enzyme conjugated second antibody. Cell proliferation was expressed as a stimulation index (SI), which represents differences in optical density (OD) between the stimulated and non-stimulated cultures; (SI) = (OD stimulated cells − OD background)/(OD non-stimulated cells − OD background). The absorbance was read using a plate reader at 405 nm, considering values >2 for SI positives (Fernandez-Bellon et al., 2005).

The data were submitted to statistical analysis using the Instat program (GraphPad). Statistical methods were chosen according to the data features. The Gaussian distribution of the data was assessed with the Kolmogorov–Smirnov test. A one-way analysis of variance for repeated-measures was used to assess differences between males and females, and to compare differences in all the variables between day 0 and the rest of the time points.

Results

No differences were found between males and females in any of the variables analysed so all the dogs in each group were analysed together. At the fifth check point (day 90) all animals from group A were normal in their physical examination and no lymphadenopathy or skin lesions were found. An increase in antibody titre was not seen in any dog. There was a decrease in titre in 52/70 (74.3%) of the dogs, and 28/70 (40%) of the dogs seroconverted (i.e. became negative) at the end of the study (Fig. 1). This decrease was very significant ($P < 0.001$) when comparing day 0 to days 90, 120, 150, 180, 270 and 360 after treatment had commenced. The results of clinicopathological testing were normal throughout the year.

A significant increase in diameter induration was found in group A for LST ($P < 0.001$) between days 0 and 90. However no differences were noted between days 90 and 360. The LPA showed a significant increase ($P < 0.001$) in the SI in this group comparing day 0 against days 30, 180 and 360 after treatment had started. This response remained stable during all the year and no differences were found comparing days 30, 180 and 360.

In animals from group B, two dogs suffering from chronic renal failure died, being euthanased at the owner's request 15 and 21 days, respectively, after entering the study. Clinical signs remitted in 24/28 (85.7%) dogs whose clinicopathological abnormalities improved during the study period. Antibody titre increases in 2/28 (7.2%) of the dogs remained stable in 16/28 (57.1%), and decreased in 10/28 (35.7%) by the end of the year (Fig. 1). This decrease was significant ($P < 0.05$) when comparing day 0 to days 90, 120, 150, 180, 270 and 360 after treatment had started.

Significant differences were found in the induration diameter in group B between days 0 and 360, but no differences were found when comparing day 0 to day 90, nor when comparing day 90 to day 360 (means ± SD are shown in Table 1). The SI index from LPA showed a significant increase in this group comparing day 0 to days 30, 180 and 360 after treatment had started ($P < 0.001$).

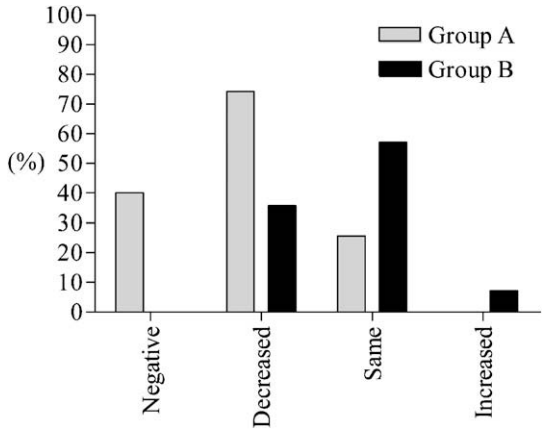

Fig. 1. Graph showing the percentage of antibodies titre tendency at the end of the year in both groups. Negative titres were considered <1/400 using the Direct Agglutination test.

Table 1
Induration diameter in millimetres (mean ± SD) 72 h after intradermal injection. A diameter >5 mm was considered positive

	Day 0	Day 90	Day 360
Group A	5.6 ± 2.7	9.1 ± 3.5	8.4 ± 2.9
Group B	3.8 ± 2.3	4.6 ± 2	5.3 ± 2.3

Discussion

Due to the current lack of an effective therapy for canine leishmaniasis, new drugs, delivery systems and treatment strategies are necessary to achieve a cure in infected dogs (Baneth and Shaw, 2002). This is the first clinical trial testing domperidone against canine leishmaniasis. Prior to this study, we evaluated the dosage scheme in an experimental dog population (20 Beagle dogs) and, using a commercial ELISA kit, demonstrated a significant increase in serum prolactin concentration (P. Gómez-Ochoa et al., unpublished data).

Dogs from group A showed better results than those in group B, probably because they were at an earlier stage of the disease. In addition, anti-leishmanial antibody levels did not increase in the majority of the dogs in the 3–5 month period post-treatment, indicating a regression of antigenic stimulation (Baneth and Shaw, 2002; Rodriguez-Cortes et al., 2007). High antibody levels have been associated with the dissemination of the parasite to different tissues (Martinez-Moreno et al., 1995; Solano-Gallego et al., 2001b), therefore it might be hypothesised that the absence of this rise in antibody levels implies there is no massive *Leishmania* dissemination.

An evident increase in cellular immunity status was found in both groups. This did not lead to a complete cure in all cases, implying that cellular stimulation is necessary but not sufficient alone to cause improvement (Freeman et al., 2000; Corrada et al., 2006). LST and LPA, the test used to check immune status, are more feasible in a clinical trial and, together with the IFN-γ cytopathic effect inhibition bioassay (IFNB), are considered to be the most suitable tests for checking cellular immunity in canine leishmaniasis (Fernandez-Bellon et al., 2005). The results found using LPA were in agreement with recent studies in which no significant correlation was found between specific response and clinical score (Rodriguez-Cortes et al., 2007). The LST, reported to be the most useful test to evaluate specific cellular immunity (Cardoso et al., 1998; Solano-Gallego et al., 2000; Fernandez-Bellon et al., 2005) may however cause interference. Moreover, the repeated use of LST could affect the humoral and cellular responses in *Leishmania*-infected dogs and studies assessing the effect of multiple doses of LST in dogs are necessary.

In the present study we did not include a positive control group. We felt that its inclusion in this kind of clinical assay

was not ethically admissible, since there are several references regarding to leishmaniasis disease progression (Rodriguez-Cortes et al., 2007). Field trials may also be influenced by factors such as the virulence and susceptibility to drugs of the strain parasite and the genetic susceptibility of individual dogs (Pennisi et al., 2005). A long-term study has revealed that 15% of all infected dogs were able to circumvent the establishment of disease or demonstrate spontaneous resolution (Fisa et al., 1999). In order to face this problem without a control group, it was decided to include in the study a large number of animals which were serologically (DAT) and parasitologically (NNN cultures) positive.

The advantages of domperidone are that it is an inexpensive drug that can be administered orally, and that no side effects were observed during the study, allowing us to treat *Leishmania*-affected dogs with renal failure. Central effects have been reported on rare occasions but are not generally expected since the blood-brain barrier is not crossed by domperidone (Matera and Mori, 2000). There are several aspects, however, that require investigation. Since there is an absence of references, other dosages must be tested. A preliminary clinical study such as this one was necessary to find out if domperidone worked as a single-agent. Taking into account that antimoniate meglumine/allopurinol protocols have proven efficacy (Noli and Auxilia, 2005), the next step could be the integration of a immunomodulatory drug such as domperidone in these treatment schedules. It will also be necessary to monitor prolactin serum concentrations. Due to the circadian rhythm, these are very difficult to examine in a clinical assay, which implies that there is a need to take all samples throughout the experiment at the same hour of the day and also to consider the reproductive status of female dogs (Freeman et al., 2000; Corrada et al., 2006).

Owing to the zoonotic nature of leishmaniasis the infective status in which dogs remain after domperidone treatment must be examined. It might be supposed that domperidone, as with other anti-leishmania drugs, does not achieve a complete parasitological cure (Noli and Auxilia, 2005), but the clinical improvement and the decrease in the antibody levels we found could indicate lower infectivity. This was demonstrated in a recent study in which symptomatic dogs showed the highest rate of infectivity compared to oligosymptomatic and asymptomatic animals (da Costa-Val et al., 2007). In a longitudinal study conducted in naturally infected dogs, infectivity was positively correlated with anti-*Leishmania* antibody concentrations, the presence of *Leishmania* DNA in bone marrow and clinical scores (Courtenay et al., 2002).

Conclusions

Domperidone was effective in controlling and reducing the clinical signs of leishmaniasis in dogs and the antibody titre. It is now necessary to obtain more data on oral treatment with domperidone using a large number of dogs and extending the follow-up period after treatment. Further clinical trials are needed to determine the ideal dose schedule and to investigate whether the dogs remain infectious to phlebotomine sand flies.

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