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Treatment of canine leishmaniosis with aminosidine at an optimized dosage regimen: A pilot open clinical trial

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ABSTRACT

Leishmaniosis due to *Leishmania infantum* (Syn: *L. chagasi*) is one of the most common diseases of dogs in Mediterranean countries and also has zoonotic potential. The aim of this study was to evaluate the efficacy of an optimized dosage regimen of aminosidine for the treatment of canine leishmaniosis (CanL) in terms of clinical remission, restoration of clinicopathological abnormalities, evolution of antibody titer, lymph node and bone marrow parasitic density and of PCR-based parasitological cure. Twelve non-uremic dogs without proteinuria, presenting clinical signs of CanL were included in the study. The diagnosis was confirmed by serology, microscopy and PCR of lymph node and bone marrow samples. Aminosidine was administered subcutaneously at the dose of 15 mg/kg body weight, once daily, for 21 consecutive days. A partial remission of the clinical signs, amelioration of clinicopathological abnormalities such as anemia, lymphopenia, hyperproteinemia, hyperglobulinemia, and reduced albumin/globulin ratio and reduced lymph node and bone marrow parasitic density were witnessed, although parasitological cure was not achieved. Since data are not supportive enough for the use of aminosidine as an alternative treatment, a large-scale controlled clinical trial using this optimized dosage regimen of aminosidine is warranted to compare efficacy against currently used drugs.

1. Introduction

Canine leishmaniosis (CanL) is endemic in many parts of the world, including the Mediterranean basin, Portugal, West Africa, Southern Asia and Latin America (Miró et al., 2008). Domestic dog is the primary reservoir of the parasite and phlebotomine sand flies are the biological vectors that transmit *Leishmania infantum* to other animals and humans (Dantas-Torres, 2007).

Pentavalent antimonials remain the drugs of choice for the treatment of CanL, as they usually induce clinical remission, although they do not prevent relapses; for this reason they are normally combined with allopurinol (Solano-Gallego et al., 2009). Many other drugs, such miltefosine, amphotericin B, pentamidine, metronidazole, spiramycin, enrofloxacin, and ketoconazole, have been also used, either alone or in combinations, with variable results (Manna et al., 2009; Miró et al., 2008).

Aminosidine, an aminocyclitol aminoglycoside antibiotic, is known to have anti-leishmanial activity both in vitro and in experimental animal models (Croft et al., 1997). Various open and controlled trials have shown that aminosidine is clinically effective for the treatment of CanL but at the same time toxic for the host (Oliva et al., 1998; Persechino et al., 1994; Poli et al., 1997; Vexenat

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et al., 1998). For this reason, an evidence-based systematic review concluded that there is only fair evidence for recommending aminosidine at the dose of 5 mg/kg body weight, twice daily (Noli and Auxilia, 2005).

Based on pharmacokinetic, pharmacodynamic and toxicologic data, a single daily administration of aminoglycosides significantly increased efficacy against susceptible organisms and at the same time reduces toxicity, compared to two or three divided daily doses. Increasing concentration above the therapeutic optimum, increases the bactericidal activity which persists for a variable period after the plasma concentrations fall below the MIC known as the post-antibiotic effect (PAE) (Isaksson et al., 1988). Consequently, it appears that once-daily dosage can be as effective as two- or three-times daily doses and may be less dangerous. However, the data supporting this conclusion were obtained in immunocompetent animals, whose defenses were able to restrain bacterial growth during the PAE. Although studies using once-daily administration of aminoglycoside antibiotics in immunocompetent animals have produced promising results (Belloli et al., 1996), more clinical trials are needed to confirm the efficacy of this dosage schedule in animals with compromised immune defense systems.

For this reason administration of aminosidine sulfate at the dosage regimen of 15 mg/kg body weight, once daily for 21 days has been proposed (Belloli et al., 1996), but to the best of our knowledge has never been evaluated for the treatment of CanL. Interestingly, this is the same dosage regiment that is currently recommended for the treatment of human visceral leishmaniasis (Sundar et al., 2007).

The aim of this pilot open clinical trial was to evaluate the efficacy of aminosidine sulfate administered at the dosage regimen of 15 mg/kg body weight, once daily for 21 days, in dogs with naturally occuring CanL in terms of clinical remission, restoration of clinicopathological abnormalities, evolution of antibody titers, lymph node and bone marrow parasitic density and of PCR-based parasitological cure.

2. Materials and methods

2.1. Animals

Twelve dogs with CanL were used. Most of them were mongrels (8/12), 4 were males and 8 females, their body weight ranged from 12.3–32 kg (median: 23.5 kg) and their age from 13 months to 4 years (median: 3 years). The diagnosis of CanL was based on the positive serology and the detection of *Leishmania* amastigotes and DNA in lymph node and bone marrow aspiration samples, using microscopy and PCR, respectively. Additional inclusion criteria were the presence of at least on clinical sign compatible with CanL, the absence of azotemia, proteinuria and concurrent systemic diseases, such as monocytic ehrlichiosis and dirofilariosis, and owners' informed consent.

2.2. Physical examination

Complete physical examinations were conducted before, once daily during the 21-day treatment period and 6 months later. Clinical signs of CanL were scored from 0 to 3 (0: absent or normal, 1: mild, 2: moderate, 3: severe) independently by the first two authors, before (time 0), at the end of the treatment period (time 1) and after 3 months (time 2) and the mean score for each sign at each time point was calculated (Koutinas et al., 2001). In addition all mean scores were added to calculate the total clinical score for each time point.

2.3. Clinicopathologic testing

Complete blood counts, serum biochemistry (total proteins, albumins, albumin/globulin ratio, urea nitrogen, creatinine, cholesterol, triglycerides, total bilirubin, alkaline phosphatase, alanino-aminotransferase, creatine kanase, calcium, inorganic phosphorus, potassium and sodium) and complete urinalysis including protein/creatinine ratio, were done at times 0, 1 and 2.

2.4. Serology for Leishmania spp

Anti-*Leishmania* spp antibodies were detected by the immunofluorescent antibody test (IFAT), using commercially available slides and conjugate (Fluoleish®, Bvt, Biovetotest Diagnostic Veterinaire, France). Serum samples obtained at times 0, 1 and 2 were tested at twofold dilutions in phosphate buffered saline (PBS), starting from 1/100 until reaching the end point titer. Sera reactive at a dilution of 1/200 or higher were considered positive.

2.5. Lymph node and bone marrow microscopy

The density of *Leishmania* amastigotes in Giemsa-stained lymph node and bone marrow aspiration smears, obtained at times 0, 1 and 2, was graded using a 0 (no amastigotes per 1000 oil immersion fields) to 6 (more than 100 amastigotes per oil immersion field) scale (Chulay and Bryceson, 1983; Koutinas et al., 2001).

2.6. PCR

DNA extraction and amplification was performed in lymph node and bone marrow aspirates obtained at times 0, 1 and 2, as previously described (Spanakos et al., 2002). Briefly, after digestion with 500 μg proteinase K in PCR buffer (50 mM KCl, 20 mM Tris–Cl pH 8.3, 2.5 mM $MgCl_2$, 0.5% Tween 20) and incubation at 56 °C, the enzyme was inactivated by heating at 95 °C for 10 min, the solution was centrifuged for 1 min at 100,000 × *g* and the supernatant was extracted with phenol–chloroform–isoamylalcochol 25:24:1 (Gibco-BRL) and chloroform. The primers Lei70L 5′-CGCAACCTCGGTTCGGTGTG-3′ and Lei70R 5′-CGCGGTGCTGGACACAGGGTA-3′ (MWG, Bioteck, Germany) were used in a final volume of 100 μl which contained 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 μM of each deoxyribonucleotide, 3 U Taq-DNA polymerase (Gibco-BRL) and 100 pM of each primer. The samples, including a positive and a negative control, were placed in a thermal cycler (PTC-100 MJ Research) and 40 cycles at 94 °C for 1 min, 65 °C for 1 min and 72 °C for 1.5 min were performed. PCR products were