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The effects of prednisone on haemostasis in leishmaniotic dogs treated with meglumine antimoniate and allopurinol

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Abstract

Thirty dogs naturally infected with *Leishmania infantum* were studied in order to determine the effects of treatment on haemostatic function. The animals were divided randomly into two treatment groups: Group 1 received meglumine antimoniate and allopurinol; Group 2 dogs were given the same treatment plus prednisone. Ten healthy animals were used as untreated controls. Clinical examination and determination of platelet aggregation, coagulation factors and biochemical parameters were undertaken before treatment and after 15, 30 and 60 days. A significant improvement in platelet aggregation was detected after 60 days in Group 1, but only after 15 days in Group 2. In both treated groups, platelet aggregation was lower than in the control group at the end of the study. The results suggest that prednisone may be a useful tool in the treatment of haemostatic disorders during canine leishmaniosis. The potential benefits and risks due to the use of corticosteroids in the treatment of leishmaniosis are discussed.

Keywords: Canine Leishmaniasis; Platelet aggregation; Meglumine antimoniate; Allopurinol; Glucocorticoids

Introduction

Several clinical signs of bleeding such as epistaxis, haematuria, haemorrhagic diarrhoea and disseminated intravascular coagulation have been reported in canine leishmaniasis (CL) (Font et al., 1993; Ciaramella et al., 1997; Koutinas et al., 1999; Ciaramella and Corona, 2003). The pathogenesis of the bleeding is uncertain, but it may be caused by alterations in primary and/or secondary haemostasis (Ciaramella et al., 2005).

In our previous studies, thrombocytopenia has been reported in 29.3% of cases of canine leishmaniasis (Ciaramella et al., 1997) and a deficiency in platelet aggregation has been found in all infected dogs (Ciaramella et al., 2005). Thrombocytopenia and thrombocytopathy may result from changes in the vessel wall due to vasculitis, altered thrombocytopoiesis, or increase in platelet destruction following renal and/or hepatic failure (Ferrer, 1992; Slappendel and Ferrer, 1998; Ciaramella et al., 2005). An immunological component has also been suspected in CL associated with the presence of platelet-bound antibodies in kala-azar patients (Kharazmi et al., 1982).

Recently, a pathogenic association between thrombocytopenia and the presence of antibodies against the platelet membrane has been observed in dogs naturally infected with *Leishmania infantum* (Terrazzano et al., 2006). For these reasons, the authors hypothesised that glucocorticoids could be useful in addition to classic chemotherapy for the treatment of CL. The conventional antileishmania drugs used in human therapy (pentavalent antimonials, amphotericin B, pentamidine or miltefosine) have low clin-

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ical efficacy in dogs as they induce only a temporary remission of clinical signs and do not prevent relapses. Pentavalent antimonials alone or in combination with allopurinol remain the most commonly used drugs for the control of canine CL (Baneth and Shaw, 2002). It is reported that the combined treatment is more effective, because allopurinol blocks RNA synthesis of *Leishmania* spp. and the antimonials simultaneously inhibit leishmanial enzymes needed for glycolytic and fatty acid oxidation (Martinez et al., 1988; Denerolle and Bourdoiseau, 1999). In addition, higher parasitological cure rates (53.3–66.7%) have been achieved with the combined use of allopurinol and meglumine (*n*-methylglucamine) antimoniate (Roura et al., 1997).

To the authors' knowledge, no data were available concerning haemostatic abnormalities during the therapy of CL. The aim of the present study was to evaluate haemostatic and platelet function in dogs naturally infected by *Leishmania infantum* treated with a combination of meglumine antimoniate and allopurinol, alone or in combination with prednisone.

Materials and methods

Animals

Thirty dogs of different breeds and sex, aged between 5 and 9 years, and naturally infected with *L. infantum* were included in the clinical trial. A control group of 10 untreated healthy dogs was also studied. All infected animals showed typical clinical signs of leishmaniasis, but without haemorrhagic pathology. The main symptoms seen were systemic lymphadenomegaly, splenomegaly, diffuse dry exfoliative dermatitis and weight loss. The clinical diagnosis of leishmaniasis was confirmed by direct observation of the protozoa in Giemsa-stained fine needle aspirates of bone marrow and/or lymph nodes, and serologically by an immunofluorescent antibody test (IFAT). A threshold titre of 1/160 was considered indicative of infection.

All dogs were serologically negative for *Ehrlichia canis* (IFAT titre <1:100) and had received no previous treatment with specific anti-*Leishmania* drugs within the previous 8 months. The infected dogs were randomly divided in 2 groups of 15 animals each (Groups 1 and 2). Animals from Group 1 were treated with a combination of meglumine antimoniate (Glucantime, Merial) at the standard dose of 100 mg/kg, subdivided into two daily doses of 50 mg/kg subcutaneously for 30 days plus allopurinol (Zyloric, Wellcome) at the dose of 15 mg/kg bid orally for 30 days. The animals from Group 2 were treated with the same therapy as Group 1 with the addition of oral prednisone (Deltacortene, Lepetit) at a dose of 2 mg/kg/day for 7 days, then 1 mg/kg/day for 7 days and finally 0.5 mg/kg/day for a further 7 days.

Blood collection

Blood was collected by jugular venepuncture from all dogs at 0800 h and from dogs of Groups 1 and 2 before (time 0), and 15, 30 and 60 days after the beginning of the therapy. Blood samples were placed in plastic tubes to obtain serum (for clinical chemistry), or in anticoagulant tubes with sodium citrate 3.8% (to measure haemachrome, platelet aggregation and coagulation factors). Samples were stored at room temperature.

Full blood count, haematocrit

A full blood count was performed within 30 min of collection, using a semi-automated cell counter (Genius S; SEAC Radom Group).

Platelet aggregation

Adenosine 5′-diphosphate (ADP; 0.5–10 μM) and collagen type I calf skin (5–200 μg/mL) were used as agonists. Platelet-rich plasma (PRP) was obtained by centrifuging whole blood at 180 *g* for 20 min, at room temperature (20 ± 25 °C). Autologous platelet-poor plasma (PPP) was prepared from PRP by further centrifugation at 2000 *g* for 15 min. Platelets were counted in a haematocytometer chamber, with a phase-contrast microscope. The PRP counts were adjusted to a platelet count of 250,000/mL by dilution with autologous PPP. Aliquots of 225 μL PRP were incubated for 1 min at 37 °C, before 25 μL of agonist, at varying concentrations, was added. The aggregation profile was recorded for 5 min. All determinations were performed within 3 h of sampling. During this time, blood samples were kept at room temperature. PRP aggregation responses were measured with a Chronolog aggregometer (Haverton) coupled to a recorder.

Quantification of aggregation was determined by measuring the percentage difference in light transmission between PRP and autologous PPP at 5 min. Aggregation of 100% was considered to be equivalent to an 80 ± 85% increase in light transmission. The results represent the mean values of aggregation percentage ± SD, with each test being run in duplicate. The reversibility of platelet aggregation induced by each dose of agonist was assessed from the shape of the aggregation tracings, with a monophasic curve taken to indicate irreversible aggregation.

Coagulation factors

Prothrombin time (PT) (Thromborel S; Dade Behring), activated partial thromboplastin time (APTT) (Pathromtin SL; Dade Behring) and fibrinogen (Multifibren; Dade Behring; the Clauss method) were determined using a semi-automatic coagulometer (Labor Fibrintimer; Coa Data 1000).

Clinical chemistry

Serum total plasma protein (TP), serum protein electrophoresis (on cellulose acetate support), alanine transaminase (ALT), creatinine and urea levels were measured for each dog using commercial kits (Reactivos Spinreacter S.A.).

Statistics

The Student–Newman–Keuls multiple comparisons test was used for the statistical evaluation. $P < 0.01$ and $P < 0.05$ were considered significant.

Results

As shown in Table 1, platelet aggregation (medium% ± SD) in Group 1 was markedly reduced in the control group both for ADP and collagen. A significant improvement in platelet aggregation was observed after 60 days from the onset of treatment and these results were accompanied by a better clinical, haematological and biochemical profile (Table 2), although aggregation values were still significantly lower in respect of the control group ($P < 0.01$). Table 3 shows the results for animals in Group 2. The significant improvement ($P < 0.01$) in platelet aggregation, detected only after 15 days of treatment, remained stable up to day 30 and a further improvement was detected at day 60. Again, platelet aggregation after 60 days from the onset of treatment was lower than in healthy control dogs, but this difference was significant only for