TEST DI FUNZIONALITÀ TIROIDEA IN CANI CON LEISHMANIOSI: STUDIO PRELIMINARE

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Scopo del lavoro. La riduzione dei livelli sierici di tiroxina totale (tT4) e tiroxina libera (fT4) associata a concentrazioni normali di TSH in cani con malattie sistemiche in assenza di una malattia della tiroide si definisce *non-thyroidal illness syndrome* (NTIS). Nei cani con leishmaniosi l'ipotiroidismo viene spesso inserito nella lista delle diagnosi differenziali, ma ad oggi non è noto quale sia l'effetto della malattia sui test di funzionalità tiroidea. L'obiettivo di questo studio è valutare se la leishmaniosi può causare NTIS nel cane.
Materiali e metodi. Nove cani malati di leishmaniosi, con alterazioni clinico-patologiche attribuibili alla malattia, elevato titolo anticorpale (ELISA) e presenza del parassita riscontrata su un campione di midollo emopoietico o linfonodale attraverso la metodica real-time PCR specifica per *Leishmania infantum*, sono stati inclusi in maniera prospettica nello studio dopo aver ottenuto il consenso informato dei proprietari. I cani non avevano una precedente diagnosi di ipotiroidismo né alterazioni clinico-patologiche riconducibili a malattie diverse dalla leishmaniosi e non erano stati trattati nelle precedenti 12 settimane con farmaci in grado di causare alterazioni delle concentrazione sieriche degli ormoni tiroidei. Tutti i cani sono stati sottoposti a terapia con antimoniato di N-metilglucammina o miltefosina per 4 settimane e allopurinolo per 6 mesi. Al momento dell'inclusione e ogni 4 settimane nei successivi 8 mesi i cani sono stati controllati tramite esame fisico, esame emocromocitometrico, profilo biochimico, elettroforesi delle proteine sieriche, esame delle urine con rapporto proteinuria/creatininuria e test di funzionalità tiroidea. Le concentrazioni sieriche di tiroxina totale (tT4), tiroxina libera (fT4) e tireotropina (TSH) sono state misurate mediante chemiluminescenza (Immulite® 2000 Canine, Siemens Medical Solution Diagnostics Los Angeles CA, USA). Al termine della terapia con allopurinolo sono state ripetute la ricerca di anticorpi specifici anti-*Leishmania* spp. tramite ELISA e del parassita mediante real-time PCR su un campione di midollo emopoietico.
Risultati. In 6/9 cani, al termine della terapia con allopurinolo, è stata osservata la remissione dei segni clinico-patologici associata a riduzione dei titoli anticorpali specifici (5/5), riduzione della carica parassitaria indagata mediante real-time PCR (2/6) o negativizzazione della real-time PCR (3/6). Dei 3 cani restanti, in uno non sono stati osservati né miglioramento del quadro clinico-patologico né riduzione dei titoli anticorpali specifici né della carica parassitaria, uno è stato escluso dallo studio in seguito al peggioramento delle condizioni cliniche ed uno è morto poche settimane dopo l'inizio della terapia. Al momento dell'inclusione 5/9 cani avevano concentrazioni sieriche di tT4 (2/9), fT4 (2/9) o di entrambi gli ormoni (1/9) inferiori all'intervallo di riferimento per cani sani. Nessun cane aveva concentrazioni sieriche di TSH superiori all'intervallo di riferimento. In concomitanza con la progressiva remissione dei segni clinico-patologici le concentrazioni sieriche di tT4 e fT4 sono aumentate e tornate nell'intervallo di riferimento entro 2 mesi dall'inizio della terapia in 4/5 cani. Nel cane con scarsa risposta alla terapia le concentrazioni sieriche di tT4 e fT4 misurate 2 mesi dopo la sospensione della terapia erano inferiori a quelle misurate al momento dell'inclusione.
Conclusioni. I dati raccolti mostrano che la leishmaniosi, al pari di altre malattie sistemiche, è in grado di causare NTIS nel cane. Inoltre, nei cani con buona risposta alla terapia le concentrazioni di tT4 e/o fT4 tornano negli intervalli di riferimento parallelamente al miglioramento delle alterazioni clinico-patologiche. Sulla base di questi risultati i test di funzionalità tiroidea devono essere valutati con cautela in cani con leishmaniosi considerato anche che gli effetti dell'integrazione di ormoni tiroidei sul decorso della malattia protozoaria in caso di NTIS sono ad oggi sconosciuti.

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CLINICAL EFFICACY OF A LEISGUARD®-BASED PROGRAM STRATEGICALLY ESTABLISHED FOR THE PREVENTION OF CANINE LEISHMANIOSIS IN ENDEMIC AREAS WITH LOW PREVALENCE

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Work type: ***Original Research***
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Purpose of the work. *In leishmaniosis, the innate immune response has been claimed not only to be the first barrier against the parasite but also to play a pivotal role in the establishment of a cell-mediated (Th1) adaptive immune response protective against the disease (Bonilla-Escobar, 2005). In this respect, the adequate activation of the phagocytic cell populations involved in antigen processing and presentation to T-lymphocytes is of paramount importance.*
Leisguard® is a dopamine based oral suspension recently marketed in several European countries for both the treatment and prevention of canine leishmaniosis. Its repeated administration to dogs induces activation of phagocytic cells leading to an increase in their anti-Leishmania potential (Gomez-Ochoa et al. 2012), being this the rationale of its clinical indications.
In accordance to the manufacturer instructions, when administered for preventive use, repeated treatments with Leisguard® have to be strategically scheduled during the year according to both the parasite's transmission season and prevalence of the disease in a given geographical area.
The aim of the present controlled, randomized clinical trial was to evaluate the clinical efficacy of a Leisguard®-based program specifically established for the prevention of canine leishmaniosis in endemic areas with low prevalence.
Materials and used methods. *A total of 240 clinically healthy dogs, sero-negative to Leishmania (Direct Agglutination Test, DAT<1/400), of different age, breed and sex, were included in the study. All dogs were housed in open-air premises in a dog kennel located in Valladolid (Spain), with a previously known seroprevalence around 7%. The study was performed with the authorization of the Spanish Medicines Agency.*
All animals were included simultaneously in the study and randomly assigned either to a Treated or to a Non-Treated group with 120 dogs each. The Leisguard®-based program implemented in the Treated group consisted in two treatments with Leisguard® (1ml/10kg/day, during 30 consecutive days), one at the beginning of the estimated vector's activity period (May-June) and another one at the end of this period (September-October). The 120 animals in the Non-Treated group did not receive any product. No insect repellents were applied at all to any animal in both groups.
During the study, all animals underwent periodic blinded clinical examinations and two blood samplings determination of anti-Leishmania antibody titters: before the initiation of the first treatment and 3 months after the end of the second treatment (December-January). When, at a given examination, an animal was showing any clinical sign compatible with the disease, it underwent complementary serological analyses for anti-Leishmania antibody titters' determination. In case of positive results (DAT = 1/400) the animal was withdrawn from the study and treated according to the decision of the kennel's veterinary staff.
Outcomes. *All animals under the Leisguard®-based program remained healthy and seronegative to Leishmania right up to the end of the 9-month follow-up period. In contrast, seven dogs out of 120 in the Non-Treated group developed clinical signs compatible with canine leishmaniosis (peripheral lymphadenomegaly and alopecia) and anti-Leishmania antibody titters (DAT>1/400) during the last month of the study, thus indicating active infection and disease progression. In all seropositive dogs the presence of the parasite was confirmed by means of direct visualization in lymph node or bone marrow aspirate. Differences between groups in terms of incidence of the disease were statistically significant (0% vs 5.83% in the Treated and Non-Treated groups, respectively; p<0.001). Finally, no side effects were observed during the administration of the drug in the treated group.*
Conclusions. *The results of this study confirm that the implementation of a Leisguard®-based program consisting in two treatments with Leisguard® (1ml/10kg/24h, during 30 consecutive days), at the beginning and at the end of the estimated vector's activity period is highly efficacious in the prevention of canine leishmaniosis in dogs living in an endemic region with low prevalence.*

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EFFICACY OF A COMBINED THERAPY WHITH MEGLUMINE ANTIMONIATE AND DOMPERIDONE FOR TREATMENT OF CANINE LEISHMANIOSIS

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Introduction. *Meglumine antimoniate is a leishmanicidal compound that has been largely used for treating canine leishmaniosis (Oliva et al., 2010, Solano-Gallego et al., 2011). Domperidone is a dopamine D2 receptor antagonist that has been demonstrated to be effective for the control and reduction of clinical signs and antibody titers in dogs naturally infected by Leishmania infantum (Gómez-Ochoa et al., 2009). Meglumine antimoniate and domperdione have different mechanisms of action potentially leading to a complementary therapeutic effect against canine leishmaniosis, particularly in moderate or severe cases. However, combined therapy with both drugs as a therapeutic approach to this disease has not been described until now.*

Cases description. *The combination of meglumine antimoniate and domperidone for the treatment of dogs with canine leishmaniosis has been implemented in our hospital during the last four years with very good results. For this work we have selected fifty-three cases belonging to stages C (n=33), D (n=12) and E (n=8) according to the G.S.L.C. guidelines (Oliva et al. 2008). Dogs from stages C (moderately sick) and D (severely sick) had not been previously treated. Among animals from stage E (refractory/relapse), some dogs had not responded to an initial therapy with meglumine antimoniate (75-100 mg/kg sid, 90 days) or miltefosine (2 mg/kg sid, 28 days) + allopurinol (10mg/kg bid, 6 month) and others had showed early relapse after treatment within the following 30 days (stages Ea and Eb, respectively). All dogs were seropositive with high anti-Leishmania antibody titers and showed abnormal serum protein electrophoresis (SPE) patterns, with hyperbeta- hypergammaglobulinemia and hypoalbuminemia. In addition, they presented generalized lymphadenomegaly (53 cases), poor body condition and muscular hypotrophy (41 cases), pale mucous membranes (14 cases), hepatosplenomegaly (53 cases), epistaxis (3 cases), generalized exfoliative dermatitis (44 cases), ulcerative dermatitis (30 cases), nasal lesions (15 cases), ocular lesions (7 cases), and other clinicopathological alterations as anemia, thrombocytopenia, biochemical alterations of hepatorenal parameters, proteinuria and changes of PU/CU ratio. All animals were treated with meglumine antimoniate at a lower dose (50 mg/kg sid, 30 days) and domperidone (1 mg/kg bid, 90 days). During/after treatment animals underwent periodic clinical examinations for more than one year. At each examination, clinical evolution as well as biochemical/hematological and SPE parameters were evaluated. All dogs showed a significant improvement of both the clinical status and biochemical/hematological and SPE parameters. This was observed even in the more severe cases. In addition, all these parameters improved much faster that would have been expected after a conventional treatment. Some relapses were reported after one year among the most severe cases and in some dogs not responding to the initial conventional treatment. To control these recurrences, cyclic treatments with domperidone alone were administered with very good results (fast improvement of clinical status and biochemical/hematological and SPE values).*

Conclusions. *During treatment with meglumine antimoniate + allopurinol, clinical amelioration together with an improvement in hematologic and serum biochemical values is usually observed after a period of 1 or more weeks. However, restoration of SPE abnormalities back to reference limits is usually slower (Oliva et al., 2010). Our cumulate experience on combined use of meglumine antimoniate and domperidone has lead us to the conclusion that this therapeutic approach significantly accelerates the improvement of both the clinical status and the as biochemical/hematological and SPE values, compared to other treatments. In addition, when meglumine antimoniate is administered in association with domperidone its dose can be lower (50 mg/kg sid) and be administered for a shorter period (30 days) than normally used (75 - 100 mg/kg sid, 90 days).*

On the other hand, the results described in this work show that, after a first treatment with meglumine antimoniate at low dose in combination with domperidone, cyclic treatments with domperidone alone can control the disease progression.

According to all the above mentioned, in our opinion this is an efficacious therapeutic approach for treatment of dogs with moderate to severe canine leishmaniosis, even in cases not responding to conventional treatments or showing early relapse.

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