Microscopic Evaluation of Renal Changes in Experimental Canine Visceral Leishmaniosis after Chemo- and Immunotherapy

M. Sayari, R. Avizheh and F. Barati
Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
Department of Clinical Sciences, College of Veterinary Medicine, University of Shahid Chamran, Ahvaz, Iran

Abstract: Visceral Leishmania (VL) with diverse clinical manifestation is prevalent and remains a major public health problem in Iran. This study was performed in Ahwaz, Khuzestan province southwest to increase immune system and to reduce of the renal lesions. Treatment of dogs with visceral leishmaniosis is basically the same as the treatment of human. However, cure is not usually achieved, leaving the sacrifice of animal as the only feasible choice. The goal of this work was to test the therapeutic efficacy of N-methyl glutamic antimote (glucamine), Mycobacterium vaccae adjuvant (SRL 172), alone and in association with L. major promastigote and the latter compound in association to glucamine, in dog with visceral leishmaniasis. In this trial 18, mixed bred dogs with different ages, receiving amastigote promastigote of L. infantum intravenously were used. They were monitored for 6 months. Serologic assays (ELISA, Dot and IFAT) were performed on blood samples of each animal. The animals were divided into six groups, each having 3 dogs: Group 1: receiving 100 mg kg⁻¹ day⁻¹ Glucamine for 30 days, IM. Group 2: Receiving 3 mg dog⁻¹ (01 mL) of Mycobacterium vaccae adjuvant suspension intradermal. Group 3: receiving L. major promastigote plus M. vaccae adjuvant each of them 0.1 mL intradermal by one month intervals for 3 months. Group 4: receiving Glucamine in association L. major promastigote plus M. vaccai adjuvant with previous doses. Group 5: Receiving no treatment. Group 6: was control group with no infection and treatment. In microscopic evaluation following lesions have been shown in kidney: Chronic, interstitial nephritis, severe glomerulosclerosis, membranoproliferative glomerulonephritis and also non supportive nephritis were the lesions in 5 groups. The prescription of Mycobacterium vaccae adjuvant was able to reduce the number of parasites in the macrophages of liver and spleen in this round of treatment.

Key words: Microscopic evaluation, renal changes, immunotherapy

INTRODUCTION

Leishmaniosis occurs in humans, domestic and wild animals. Visceral leishmaniosis is an infectious disease transmitted by sand flies and caused by various species of Leishmania parasites. These parasites (e.g., L. donovani, L. chagasi and L. infantum) cause a wide spectrum of clinical manifestations and it is estimated that annual occurrence of human visceral leishmaniosis is 500,000 (Mohebali et al., 2001) worldwide.

It is an important zoonosis throughout the world. In visceral leishmaniosis, the macrophages in the reticuloendothelial system are attacked by L. chagasi (Moritz et al., 1999). In one study performed by Font and Closa (1997), demonstrated that Leishmania infected people were highly susceptible to Pneumonic and Pneumococci infections because of loss of immunoglobulin and or complements components, as well nephrotic and thromboembolic lesions.

Under certain circumstances, particularly in peridomestic and domestic transmission foci, aphanthrop and domestic animals can as source of infection for phlebotomine sand fly vectors. Dogs have long been implicated as the main domestic reservoirs Leishmania (Leishmania) infantum (Dantas-Torres, 2007; Valladores et al., 2001).

Nieto et al. (1992) not only reported the interstitial nephritis and glomerulonephritis but also they observed the overall glomerulosclerosis together with acute tubular atrophy.

In a survey performed on biopsy samples from infected human with visceral leishmaniosis, glomerubnephritis was observed and it was concluded that this syndrome may be due lymphatic cells imbalance as well as their proliferation in the medulla (Hrouda et al., 1998).

At necropsy, dogs with visceral leishmaniosis are emaciated and have an enlarged liver and lymph nodes.
Lymph node aspirates contain macrophages in which contain organisms (Weisinger et al., 1978; Workman and Hernau, 2003). Dogs with visceral leishmaniosi developed hind limb edema and distension of caudal epigastric veins (Font and Closa, 1997).

Glomerular disease with nephritic syndrome and hypercoagulable state diagnosed. Sonographically there was massive thrombosis of the caudal vena cava (Font et al., 2004).

In various investigations renal syndromes including tubulo-interstitial nephritis were reported and some results were concluded (Deplazes et al., 1995). Membranoproliferative glomerulonephritis was the most common cause of chronic renal failure. Mesangiproliferative was tubulo-interstitial nephritis were detected (Plevraki et al., 2006).

Having regard to high importance of the kidneys in this disease as well mitigation of renal damages due to this infection, the objective of this survey is to demonstrate the pathological lesions in infected dogs with visceral leishmaniosis after chemotherapy (treatment with glucantime) and immunotherapy (adjuvant of Mycobacterium vaccae with Leishmania major promastigotes).

MATERIALS AND METHODS

Eighteen cross breed healthy dogs were provided, their health was checked by serological and blood smears every two weeks, after two weeks each dog received 5 mL of homogenised infected dog spleen with visceral leishmaniosis intravenously.

For detection of antibodies titers sera were collected, this sampling was performed till all sera were positive, when all dog sera were positive they were categorized to six groups of three dogs randomly. In the first group, 3 dogs were treated with glucantime (100 mg kg\(^{-1}\) day\(^{-1}\) for 30 days IM) this group was called chemotherapy group, in 2nd group three dogs were treated with killed suspension of Mycobacterium vaccae as adjuvant (0.1 mL kg\(^{-1}\) month plus Mycobacterium vaccae as adjuvant intradermal three times). In 3rd group (immunotherapy group) in this group 3 dogs were treated with Leishmania major antigen, 0.21 mg\(^{-1}\) dog\(^{-1}\) along with Mycobacterium vaccae adjuvant with the same dosage of 2nd group, three times with one month interval intradermally in 4th group, three dogs (chemotherapy+ immunotherapy group) were treated with glucantime and Leishmania major antigen along with adjuvant in same dosage of the 2nd and 3rd groups dosages. In 5th group: three dogs were as positive control group for 30 days with glucantime daily the quantity of the injection was the same as the 2nd group, distilled water was injected intramuscularly. Group 6 was negative control group with no inoculation of parasite. Each group was kept separately and investigated clinically. After 3 months which the duration of the treatment was terminated, the dogs were killed and autopsies were performed, the abdominal cavity of the dogs were investigated and for histopathological tests the kidneys were cut to provide the 5 cm. Pathological samples were fixed in 10% formalin, processed and embedded in paraffin, sectioned at 5 µm and stained with Haematoxylin and Eosin and special stain PAS were used where found necessary.

RESULTS

The main findings are:

Group 1: Chemotherapy group treated with glucantime, the renal lesions were mild interstitial nephritis in tow cases as well glomerulosclerosis together with acute protein deposition.

Group 2: Treated with Mycobacterium vaccae adjuvant the following finding were observed as: membranous glomerulonephritis, proliferation of parietal epithelium, an influx of monocytes.

Group 3: Membranoproliferative in three cases, hypercellularity and capillary basement membrane thickening are present.

Group 4: (Immunotherapy and chemotherapy group) in this group no lesion was observed.

In 5th group (positive control) which were infected without treatment, all three dogs serologically were positive after three months and renal lesions were non purulent multifocal acute interstitial nephritis (2 dogs) and proliferative membranous glomerulonephritis.

DISCUSSION

Group 1: Weisinger et al. (1978) showed the return of kidney’s activity to normal in histopathological section of kidney of a woman suffering from glomerulonephritis due to leishmaniosis after treatment.

Gradoni (2000) believes residues related to glomeruli especially glomerulonephritis to be recurring and regard the decrease in immunological sediments present in glomerular as natural. He believes the reasons are:

- Increase in antigen dissolve, phagocytosis by neutrophils, macrophages and mesangial cells.
- Passing from mesangial channels and penetration to vascular pole.
Transport of complexes by mesangial cells through epithelial cells and penetration to urinary space as well as destruction in mesangial matrix.

- Extracellular destruction by proteases and solubility under influence of complement system.

Hence omission of entering antigens in the blood in many diseases can be helpful in destroying sediment complexes. They emphasize that glomerularcrosis is end stage of the renal glomerular lesions and is a sign of severe tissue destruction and is considered an irreversible phenomenon. Ceci et al. (1986) showed that complex sediments returned in dog's kidney circulation infected with leishmaniosis under glucantime treatment. After treatment this event can be the reason for acute glomerulonephritis and result is glomerularclerosis in the end stage of glomerulonephritis. In a research done by Valli and Fary (1993) they demonstrated that glomerulonephritis could be due to various factors including idiopathic factors, so glomerulosclerosis occurrence may be due to glomerulonephritis factors simultaneously. Mcgavin and Zachary (2007) believe that since the factors responsible for interstitial nephritis are unknown, this phenomenon in treated dogs can be caused by weakening of the immune system due to parasites.

The low efficacy of glucantime in achieving parasitological cure despite marked clinical improvement is highlighted by number of studies. In a study of 15 naturally-infected dogs, parasites were cultivated from 79% of lymph node biopsies after glucantime therapy (Deplazes et al., 1995).

In further study of 41 naturally-infected dogs treated with glucantime, 35 dogs (85.4%) achieved partial or complete remission in clinical signs and negative cytological examination for parasites after 3-6 weeks of treatment (Slappendel and Teks, 1997). Twenty six (74.3%) of these dogs experienced clinical relapse with one year. In another study, 10 naturally-infected dogs were treated with two cycles of intravenous glucantime, starting with 50 mg kg⁻¹ day⁻¹ for 2 days and then 100 mg kg⁻¹ day⁻¹ for an additional 8 days. Nine (90) remained PCR positive for leishmaniasis DNA following therapy despite improvement of clinical signs (Riera et al., 1999; Moritz et al., 1999).

**Group 2:** In all the organs of the dogs in this group membranous glomerulonephritis was present with epithelium crescent. Yet with the omission of disease factors and side affects such as B lymphocyte proliferation and plasma cells in the white pulp the destruction of the cause of disease is expected (Lamoth, 2001). Membranous glomerulonephritis due to treatment with *Mycobacterium vaccae* adjuvant is as an antigen in blood circulation of these patients, even if the time necessary for recovery from glomerulonephritis has not passed.

**Group 3:** Membranoproliferative glomerulonephritis has been observed in all of these groups. This event is a cause of chronic antigenemia in cases. Deplazes et al. (1995) demonstrated that the IgG2a and IgG1 increased due to medication with *Leishmania infantum* under influence of Th1, Th2, cells. Hence the presence of these antigens in circulation with immunoglobulin is sufficient for propagated membranoproliferative glomerulonephritis occurrence. It should be noted that even though immune complexes are sufficient for creating sediments in glomeruli, the amount is not enough to consider the antibodies (Grauer, 1992). Anyhow, the presence of *Mycobacterium vaccae* adjuvant itself can be another factor in increasing the antibodies titers which causes the above mentioned complex residues (Silva, 2004). This finding in the second group called treated with *Mycobacterium vaccae* Adjuvant can be found with a less severity.

**Group 4:** Having regard to high titer of antibodies in serum of dog which could be a positive case the lack of any glomerular lesion in the above mentioned case. The exact reason behind this phenomenon has not been mentioned. Two mentioned points can be a cause in recrudescence of the disease. A short while after the recrudescence of the disease immunological sediments lead to kidney side affects (Murray, 2001).

A research done by Moritz (1999) for evaluation of the period of treatment by immunotherapy and chemotherapy against American visceral leishmaniosis shows that the prescription of antimon derivatives together with immunotherapy (S₄) demonstrated the reduction of the amount of chemical drug and as a result fewer side effects. The findings of the research performed 3 months after final treatment shows the presence of residues and macrophages containing parasites and is a sign of the disease's recrudescence. It sometimes difficult to diagnose leishmaniosis in tissue sections or in smears particularly in unusual sites or if few parasites are present in the lesion. *Leishmania* species must be differentiated morphologically from a variety of other microorganisms (Denerolle and Bourdoiseau, 1999; Hofman et al., 2003). The different treatments used in the other studies did not completely eliminate parasite (Joao et al., 2006).

Only the prescription of *Mycobacterium vaccae* adjuvant was able to reduce the number of parasites in the macrophages of liver and spleen in this round of treatment. But this mixture was not able to prevent renal lesions in this round. Anyhow, for the first time
prescription of this adjuvant in treatment of infected leishmaniosis due to high antibody titer and lack of infection load is important and could be considered in the future research.

REFERENCES


