http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 11 (12): 1630-1633, 2008
ISSN 1028-8880

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

[1]M. Sayari, [2]R. Avizeh and [2]F. Barati
[1]Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran
[2]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, Khozestan 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 antimoate (glucnime), *Mycobacterium vaccae* adjuvant (SRL 172), alone and in association with *L. major* promastigote and the latter compound in association to glucantime, in dog with visceral leishmaniasis. In this trial 18, mixed bred dogs with different ages, receiving amastigte 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} Glucantime for 30 days, IM. Group 2: Receiving 3 mg dog^{-1} (0.1 mL) of *Mycobacterium vaccae* adjuvant suspension intradermaly. Group 3: receiving *L. major* promastigote plus *M. vaccae* adjuvant each of them 0.1 mL intradermaly by one month intervals for 3 months. Group 4: receiving Glucantime in association *L. major* promastigote plus *M. vaccae* 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, sever glomerulosclerosis, membranoproliferative glomerulonephritis and also non suppurative nephritis were the lesions in 5 groups. The prescription of *Mycobacterium vaccae* adjurant 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 files 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 of 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 thoromboembolic lesions.

Under certain circumstances, particularly in peridomestic and domestic transmission foci, aynanthropic 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; Valladares *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, glomerunephritis was observed and it was concluded that this syndrome may be due lymphatic cells imbalance as well as their proliferation in the medulla (Hroudae *et al*., 1998).

At necropsy, dogs with visceral leishmaniosis are emaciated and have an enlarged liver and lymph nodes.

Corresponding Author: M. Sayari, Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Lymph node aspirates contain macrophages in which contain organisms (Weisinger *et al*., 1978; Workman and Hernau, 2003). Dogs with visceral leishmaniosis 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 same results were concluded (Deplazes *et al*., 1995). Membranoproliferative glomerulonephritis was the most common cause of chronic renal failure. Mesangioproliferative was tubulointerstitial 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 titters 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 intradermaly 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.

G**roup 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.
- Extraxcellular destruction by proteases and solvability 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 glomerularosclerosis is end stage of the renal glomerular lesions and is a sign of severe tissue destruction and is considered an irreversible phenomenon. Cecci *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 glomerularosclerosis in the end stage of glomerulonephritis. In a research done by Valli and Pary (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 a 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 leishmania 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 IgG_1a 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 membrano proliferative 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 factors 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 antimoant derivatives together with immunotherapy (S_2) 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 if 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

Cecci, L., V. Marrano, F. Petazzi, N. Kondaiah, A.S.R. Anjaneyulu, N. Keshava, N. Sharma and N. Joshi, 1986. Qualitative and quantitative determination of circulating immune complex in dog with leishmaniosis. J. Vet. Sci., 39: 335-339.

Dantas-Torres, F., 2007. The role of dogs as reservoirs of *Leishmania* parasites, with emphasis on *Leishmania* (*Leishmania*) *infantum* and *Leishmania* (*Viannia*) *braziliensis*. Vet. Parasitol., 10: 139-146.

Denerolle, P. and G. Bourdoiseau, 1999. Combination allopurinol and antimony treatment versus antimony alone and allopurinol alone in the treatment of canine leishmaniosis (96 cases). J. Vet. Intern. Med., 5: 413-415.

Deplazes, P., N. Smith, P. Arnold, H. Lutz and J. Eckert, 1995. Specific IgG_1 and IgG_2 antibody response of dogs to *Leishmania infantum* and other parasites. Parasite Immunol., 17: 451-458.

Font, A. and M. Closa, 1997. Ultrasonographic localization of caudal vena cava thrombus in dog with leishmaniasis. Vet. Radiol. Ultrasound, 38: 394-398.

Font, A., J. Mascort, J. Altimira, J.M. Closa and M. Vilafranca, 2004. Acute paraplegia association with vasculitis in dog with leishmaniasis. J. Small Anim. Practice, 45: 199-201.

Gradoni, L., 2000. An update on antileishmania vaccine candidates and prospects for canine *Leishmania* vaccine. Vet. Parasitol., 100: 87-103.

Grauer, G.F., 1992. Glomerulonephritis seminar of veterinary medicine surgery in small animals. Seminar Vet. Med. Surg. Small Anim., 7: 187-197.

Hofman, V., P. Brousset, E. Mougneau, P. Marty, L. Lamant, J.C. Antoine, N. Glaichenhaus, P. Hofman, 2003. Immunostaining of visceral *Leishmania* caused by *Leishmania infantum* using monoclonal antibody (19-11) to the *Leishmania* homologue of receptors for activated C-kinase. Am. J. Clin. Pathol., 120: 567-574.

Hroudae, D., B. Baban, W. Dunsmuir, R. Kirby and A. Dalgleish, 1998. Immunotherapy of advanced prostate cancer phase I/II trial using *Mycobacterium vaccae*. J. Urol., 82: 568-573.

Joao, A., M.A. Pereira, S. Cortes and G.M. Santos-Gomes, 2006. Canine leishmaniosis chemotherapy: Dog 's clinical condition and risk of *Leishmania* transmission. J. Vet. Med., 53: 540-545.

Lamoth, J., 2001. Activity of amphotericin B in lipid emulsion in the initial treatment of canine leishmaniosis. J. Small Anim. Practice, 42: 170-175.

Mcgavin, M.D. and J.F. Zachary, 2007. Pathological Basis of Veterinary Disease. 4th Edn. Mosby, New York, USA., pp: 622-630.

Mohebali, M., E. Hamzavi and Z. Zarei, 2001. Study of canine visceral leishmaniosis in some part of Iran and its health importance. J. Univ. Vet. Med., 56: 55-59.

Moritz, A., S. Steuber and M. Greiner, 1999. Clinical follow-up examination after treatment of canine leishmaniosis. Exp. Vet. Med., 23: 279-293.

Murray, H.W., 2001. Clinical and experimental advance in treatment in visceral leishmaniasis. Antimicrob. Agents Chemother., 47: 2513-2517.

Nieto, C., M. Navarrete, M. Habela, F. Serrano and E. Rodendo, 1992. Pathological changes in kidneys of dog with natural *Leishmania* infection. J. Parasitol., 45: 33-47.

Plevraki, K., A.F. Koutinas, H. Kaldrymidou, N. Roumpies, L.G. Papazoglou, M.N. Saridomichelakis, I. Savvas and L. Leondides, 2006. Effects of allopurinol treatment on the progression of chronic nephritis in canine leishmaniosis (*Leishmania infantum*). J. Vet. Intern. Med., 20: 228-233.

Riera, C., M. Valladares, M.J. Gallego, S. Alisa, R. Catille Jo, N. Fisa, J. Ribas, J. Carrio, M. Lerola and M. Arboix, 1999. Serological and parasitological follow-up in dogs experimentally infected with *Leishmania infantum* and treated with glucantime. Vet. Parasitol., 84: 33-47.

Silva, F.G., 2004. Chemical-induced nephropathy: A review of the renal tubulointerstitial lesions in humans. Toxicol. Pathol., 32: 71-84.

Slappendel, R.J. and E. Teks, 1997. The effect of intravenous or subcutaneous administration of meglumine antimonate (glucantime) in dogs with leishmaniasis. A randomized clinical trial. Vet. Q., 19: 10-13.

Valladares, J.E., C. Riera, M.J. Alberola, M. Gallego, C. Portus, C.F. Cristofol, C. Ranhuelo and M. Arboix, 2001. Long term improvement in the treatment of canine leishmaniosis using an antimoant liposomal formulation. Vet. Parasitol., 92: 15-21.

Valli, O.E.V. and W.B. Pary, 1993. Pathology of Domestic Animals. 4th Edn. Academic Press, Inc., pp: 249-250.

Weisinger, R., A. Pinto, A. Velazhuez, F. Bronstein and F. Tapanes, 1978. Clinical and histological kidney involvement in human kalazar. J. Trop. Med. Hyg., 27: 357-357.

Workman, H. and W. Hernau, 2003. Chronic lymphacytic leukemia in dogs and cats. J. Vet. Clin. Pathol., 33: 1379-1399.