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Short communication

Divergence between hepatic insulin-like growth factor (IGF)-I mRNA expression and IGF-I serum levels in *Leishmania* (*Leishmania*) *infantum chagasi*-infected dogs

Flaviane A. Pinho[a], Nilton A. Magalhães[a], Kleverton R. Silva[a], Aline A. Carvalho[a], Fernando L.L. Oliveira[a], Eduardo M. Ramos-Sanchez[b], Hiro Goto[b,c], Francisco A.L. Costa[a,*]

[a] *Setor de Patologia Animal, Centro de Ciências Agrárias, Universidade Federal do Piauí, 64049-550, Campus Socopo, S/N, Teresina, PI, Brazil*
[b] *Laboratório de Soroepidemiologia e Imunobiologia, Instituto de Medicina Tropical, USP, 05403-000, Av. Dr. Enéas de Carvalho Aguiar, 470, prédio II, 4° andar, São Paulo, SP, Brazil*
[c] *Departamento de Medicina Preventiva, Faculdade de Medicina, Universidade de São Paulo, São Paulo, SP, Brazil*

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ABSTRACT

Visceral leishmaniasis (VL) caused in the New World by *Leishmania* (*Leishmania*) *infantum chagasi* has dog as important peridomestic reservoir in its transmission cycle. Since VL in infected animals is similar to human VL, its study is interesting for human pathology. During *Leishmania* infection, insulin-like growth factor-I (IGF-I) plays a role in the host-parasite interaction, favoring parasite growth, particularly acting directly on *Leishmania*. We evaluated IGF-I mRNA expression in different organs/tissues, which was differently modulated in dogs naturally infected by *L.* (*L.*) *infantum chagasi*. We also evaluated the hepatic IGF-I mRNA and serum IGF-I levels in infected dogs. Hepatic mRNA IGF-I expression was higher in the infected dogs than in control animals. However, the serum levels of IGF-I, which are related to the production of this factor in the liver, were reduced in the infected dogs compared with the non-infected controls. Thus, we suggest interference in post-transcriptional processing in IGF-I production in active visceral leishmaniasis.

1. Introduction

Visceral leishmaniasis (VL) is a systemic disease caused by *Leishmania* (*Leishmania*) *infantum chagasi* (Shaw, 2006) in the American continent that proliferates within cells of the mononuclear phagocyte system (Hommel, 1999). In humans, symptomatic VL is lethal in some cases (Badaro et al., 1986). Dogs are the most important reservoir in the peridomestic environment, and they play an important role in the transmission to humans due to their susceptibility to infection, intense cutaneous parasitism and their close contact with humans (Ashford, 1996). Furthermore, the disease in dogs is similar to human VL, making its study interesting for human pathology (Costa et al., 2003).

The control or progression by *Leishmania* infection depends on the specific and non-specific host immune response and the ability of the parasite to evade the host response (Mougneau et al., 2011). Insulin-like growth factor-I (IGF-I), which is present in circulation (Cohick and Clemmons, 1993) and in macrophages (Arkins et al., 1993), is one of the early factors that interact with *Leishmania* promastigotes in the skin of the host and possibly with amastigotes after internalization by macrophages. Previous

* Corresponding author at: Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Clínica e Cirurgia Veterinária, Setor de Patologia Animal. Campus da Socopo, S/N, 64049-550, Teresina, Piauí, Brazil. Tel.: +55 86 3215 5760; fax: +55 86 3215 5753.
E-mail address: fassisle@gmail.com (F.A.L. Costa).

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studies have shown that IGF-I induces *in vitro* an increase in the proliferation of promastigotes and axenic amastigotes of *Leishmania* (Goto et al., 1998). In addition, it was also observed *in vivo* a significant increase in the lesion size and in the number of viable parasites in the skin of mice infected with *Leishmania amazonensis* promastigotes pre-incubated with IGF-I (Gomes et al., 2000).

Because there are still no studies on the expression of IGF-I in VL, the local expression of IGF-I was initially evaluated in preferential sites for parasitism in the dog, *i.e.*, the skin (ear and periungual region), popliteal lymph node, spleen and liver. Furthermore, we evaluated the serum levels of IGF-I, and because it is primarily produced in the liver (Schimpff et al., 1976; Han et al., 1988), we compared these data with hepatic IGF-I mRNA expression levels. The results showed that the serum levels were not consistent with the hepatic mRNA expression levels in dogs naturally infected with *L.* (*L.*) *infantum chagasi*.

2. Materials and methods

Adult male and female stray dogs from a Brazilian endemic area (Teresina, state of Piaui) with VL ($N = 22$) and controls ($N = 6$), with estimated age range of 2–8 years old and body weight range of 8–30 kg were included in this study. All of the experimental protocols were previously approved by the Research Ethics Committee of Federal University of Piauí (Protocol No. 004/09), and the dogs were anesthetized using 2.5% sodium thiopental (10 mg/kg) before of the procedures. Infection was confirmed through the identification of *Leishmania* amastigotes in the sternal bone marrow and/or popliteal lymph node aspirates. After the clinical examination, whole blood was collected to obtain serum. Subsequently, the dogs were sacrificed with an overdose of sodium thiopental (33%, 5 ml/kg, IV). Samples of the skin, spleen, liver and popliteal lymph node were obtained for touch imprint cytology and stained with Giemsa for parasite count in 50 random fields under the light microscope using oil immersion at a magnification of 100×. Then we collected 30 mg of each organ to extract total RNA using Trizol reagent (Invitrogen, California, USA). The RNA was reverse transcribed into cDNA using 200 U/ml reverse transcriptase (Invitrogen, California, USA) according to the manufacturer's protocol (Invitrogen, California, USA).

IGF-I mRNA expression was assessed in six infected and six non-infected control dogs with real-time PCR using Sybr Green (2×) (Life Technologies, California, USA) and an Applied Biosystems StepOne Real-time PCR System thermal cycler. The IGF-I primer sequences (NM:L08254) were forward, 5′GGG TTC TAC TTC AAC AAG CCC ACA3′, and reverse, 5′GGG CAC AGT GTG ACA CTA TCT GCC3′. The primers of the housekeeping β-actin gene (NM: AF021873) were forward, 5′CGT GAC ATC AAG GAA GAA GCT CTG3′, and reverse, 5′CGG GTT GTA CGT AGC TCT TCT CCA3′. The PCR conditions were as follows: an initial 10 min incubation step at 95 °C and 45 cycles of 15 s at 94 °C, 1 min at 61 °C, and 1 min at 70 °C. The expression levels were analyzed with the relative expression method and presented as $2^{-\Delta\Delta ct}$ relative to β-actin gene expression (Pfaffl, 2001).

Serum IGF-I concentrations were determined with a chemiluminescent assay (Immulite – Siemens) with an Immulite 2000 automated immunoassay (Diagnostic Products Corporation – DPC, CA, USA) according to the protocol provided by the manufacturer. The assay is based on the detection of the emmited ligh proportional to the amount of the analyte by chemiluminescent dioxetane substrate when it reacts with alkaline phosphatase. In the kit this enzyme is bound to the polystyrene beads coated with either anti-IGF-I or anti-IGFBP3 antibodies. For evaluation, 20 μL of the sample is mixed with the provided reagents and processed in the automated system.

For the data analysis, nonparametric statistics (Mann–Whitney and Kruskal–Wallis tests) were used.

3. Results and discussion

The frequent clinical manifestations in dogs with VL were skin lesions (86.4%), local or generalized lymphadenopathy (77.3%), weight loss (68.2%), onychogryphosis (54.5%), conjunctivitis (36.4%), apathy (31.8%), coryza (27.3%), fever (18.2%), anemia (18.2%), diarrhea (18.2%), subcutaneous edema (4.5%) and dyspnea (4.5%).

On cytologic analysis of six infected dogs, the presence of amastigote was observed free and inside the cytoplasm of mononuclear cells in all organs. The frequency was higher in ear (4/6) and lymph nodes (3/6) when compared to liver (1/6) and spleen (1/6).

We analyzed the mRNA expression of IGF-I in the skin from various areas and the spleen and we observed a higher tendency of IGF-I mRNA expression in infected dogs compared with the controls. The IGF-I mRNA expression in the liver was significantly higher in the infected dogs compared with the control animals ($P = 0.0325$, Mann–Whitney test). The IGF-I mRNA expression levels in the popliteal lymph node of infected animals tended to be lower than the control animals (Fig. 1). Interestingly, the lymph node is not a preferential organ for parasite growth (Reis et al., 2006). Thus, the data may suggest the association of local IGF-I mRNA expression and parasite growth in the tissue.

When we evaluated the IGF-I mRNA expression in the infected animals in relation to the number of clinical signs, we observed that the IGF-I mRNA expression was higher in animals with less clinical signs, except in liver (Table 1). These data may suggest different roles for IGF-I, *in situ*, in parasite growth during the infectious process, primarily in the earlier phase of infection. Moreover, we also evaluated the serum IGF-I concentration and did not observe any correlation between serum IGF-I concentration and parasite load. In addition, there was a significant decrease in IGF-I mRNA expression levels in the various organs (except for the liver) in animals with a more severe clinical presentation.

Analysis the serum IGF-I levels showed a higher proportion of dogs presenting lower levels of serum IGF-I among infected dogs compared with control animals. However, comparing serum IGF-I concentration with clinical manifestation of infected dogs we did not observe any correlation between them (Table 1). In addition, we observed that the infected dogs with lower serum IGF-I levels had an increase of mRNA IGF-I expression in the liver (Fig. 2A