

Cardiac markers: profile in rats experimentally infected with *Toxocara canis*

Marcadores cardíacos: perfil em ratos infectados experimentalmente com *Toxocara canis*

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Abstract

The aim of this study was to evaluate the profile of the enzymes creatine kinase (CK), creatine kinase MB (CK-MB) and lactate dehydrogenase (LDH) in Wistar rats infected with 250 (GI, $n = 24$) or 1000 (GII, $n = 24$) *Toxocara canis* eggs. Animals were evaluated on days 7, 15, 30, 60, 120 and 180 post-infection (DPI). Only the GI rats showed an increase in CK and CK-MB, at 15 and 30 DPI, respectively. Anti-*Toxocara* spp. antibodies were detected by ELISA in infected animals. Despite of the presence of eosinophilic infiltrate in the heart of three infected animals, none larva was recovered from the organ neither by acid digestion nor by Baermann procedure. Eosinophilia was observed in both groups but there was no significant difference in the eosinophil counts between GI and GII ($p = 0.2239$). It is possible to consider that cardiac lesion is an eventual finding in murine model for toxocariasis.

Keywords: Toxocariasis, cardiac lesions, cardiac biomarkers.

Resumo

O objetivo do presente estudo foi avaliar o perfil das enzimas creatinoquinase (CK), creatinoquinase-MB (CK-MB) e lactato desidrogenase (LDH) em ratos Wistar infectados com 250 (GI, $n = 24$) ou 1000 (GII, $n = 24$) ovos de *Toxocara canis*. Os animais foram avaliados nos dias 7, 15, 30, 60, 120 e 180 pós-infecção (DPI). Observou-se que apenas os animais do GI apresentaram aumento da atividade de CK e CK-MB aos 15 e 30 DPI, respectivamente. Anticorpos anti-*T. canis* foram detectados por ELISA nos animais infectados. Apesar da presença de infiltrado eosinofílico em três animais infectados, nenhuma larva foi recuperada do coração pela digestão ácida ou pela técnica de Baermann. Eosinofilia foi observada em todos os momentos em GI e GII, sem diferença significativa entre os grupos ($p = 0,2239$). Pode-se considerar que as lesões cardíacas foram um achado eventual no modelo murino para toxocaríase.

Palavras-chave: Toxocaríase, lesões cardíacas, biomarcadores cardíacos.

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Toxocariasis is an important zoonosis worldwide, mostly caused by *Toxocara canis*. Various organs may be affected, including liver, lungs, central nervous system and eyes. Ocular, cerebral and cardiac involvement tend to be more serious and may be fatal (HARALAMBIDOU et al., 2005). However, data regard to cardiopathy due to toxocariasis is presented in report cases.

Murine are considered an excellent model for toxocariasis due to their tolerance to heavy infections for long periods without suffering notable alterations and the larvae follow a cycle with signs and symptoms similar to those produced in humans (CHIEFFI et al., 2009). However, experimental studies concerning heart lesions caused by *Toxocara* spp. as well as the activity of isoenzymes creatine kinase (CK), CK-MB fraction and lactate dehydrogenase (LDH), commonly used as biomarkers to detect cardiac injury (RAJAPPA; SHARMA, 2005), are scarce in the literature. Therefore, the aim of this study was to evaluate the use of CK, CK-MB and LDH as markers of cardiac injury in Wistar rats (*Rattus norvegicus*) experimentally infected with *T. canis*.

A total of 60 rats, 30 males and 30 females, aged 8 months, free of parasites, were randomly distributed into two experimentally infected groups (GI and GII n = 24 in each group; 12 males and 12 females) and a control group (n = 12; 6 male and 6 female). On day 0, each rat in Group I and II (GI and GII) was orally infected with 250 or 1000 infective *T. canis* eggs, respectively. Animals were kept in individual cages under controlled temperature ($25 \pm 3^\circ\text{C}$) with a light-dark cycle of 12:12 PM. This study was approved by the Ethical Research Committee of Unoeste (protocol 12/OL/2009).

Blood samples and heart were collected from two males and two females of GI and GII as well as one male and one female from the control group on days 7, 15, 30, 60, 120 and 180 post-infection, after euthanasia by intraperitoneal administration of 40.0 mg/kg sodium thiopental (Tiopentax 2.5%, Cristália®). Blood was obtained from the caudal cava vein to perform haematological and biochemical analysis, and detection of IgG anti-*Toxocara* spp. Total leukocyte counting was obtained using an automated cell counter, while eosinophil differentiation was based on the microscopic observation of a blood smear stained with Diff-Quick (JAIN, 1993). Biochemical was performed

using kinetic and semi-automatic analysis with commercial kits (Bioclin®). Antibodies anti-*T. canis* were evaluated by indirect ELISA, using *T. canis* excretory-secretory larval antigen (TES) according to Savigny (1975), modified by Elefant et al. (2006). A commercial conjugated anti-rat IgG (Sigma®, A9417, USA) was employed. Reaction was stopped with sulphuric acid, and absorbance readings at 492 nm. Cut-off absorbance was defined by taking the mean absorbance for 30 negative control sera plus two standard deviations. Antibody levels are expressed as the reactivity index (RI), calculated as the following: absorbance of tested sample/cut-off (OD = 0.380).

For larvae recovery, fragments of the heart (1.0 cm²) were submitted to digestion in 0.5% HCl (XI; JIN, 1998) and to the Baermann procedure. Histopathology study was performed as described previously by Cookston et al. (1990), with some modifications.

Pearson's coefficient was calculated to assess the correlation between the serum CK, CK-MB and LDH activity, and the eosinophil count. The analyses were carried out using SPSS software v. 14 (Chicago, IL), considering statistically significant the *p* less than 0.05.

Some isoenzymes, including CK, CK-MB and LDH, are widely used markers for cardiac injury. CK has been considered the main enzyme for the determination of neuromuscular diseases, while CK-MB has been used either to confirm or exclude acute myocardial infarction in humans due to its high specificity for the cardiac muscle (RAJAPPA; SHARMA, 2005). CK-MB has also been described in animals presenting cardiac injuries (SCHOBER, 2005). In our investigation, the increase in CK and CK-MB activity was dose-dependent. The enzymatic elevation was observed only in GI animals, respectively, on 15 and 30 DPI (Table 1). According to Cardinet (1997), antigens secreted by the larvae may provoke continued tissue damage or trigger a delayed hypersensitivity reaction, resulting in a transitory increase in serum isoenzyme activity. Elevation in LDH activity has been observed in patients diagnosed with toxocariasis-associated myocarditis. The activity ranged from less than 279 U/L (HOFFMEISTER et al., 2007) to 2,126 U/L (ENKO et al., 2009). In our study, the LDH activity was within the normal range at all examined time points in both GI and GII.

Table 1. Serum activities (mean value±standard deviation in U/L) of creatine kinase (CK), creatine kinase MB fraction (CK-MB) and lactate dehydrogenase (LDH) in Wistar rats experimentally infected with 250 (GI) or 1000 (GII) *Toxocara canis* eggs, from seven to 180 days pos-infection (DPI).

Enzymes	DPI					
	7	15	30	60	120	180
CK						
GI	2277 ± 1309	2880 ± 847	2400 ± 325	2432 ± 136	2069 ± 113	1650 ± 369
GII	2415 ± 1183	2305 ± 142	1650 ± 392	1915 ± 763	2505 ± 106	1790 ± 764
CK-MB						
GI	272 ± 86	410 ± 35	674 ± 95	561 ± 80	295 ± 77	338 ± 115
GII	325 ± 105	383 ± 131	356 ± 171	566 ± 184	359 ± 90	228 ± 111
LDH						
GI	1577 ± 420	2270 ± 88	1795 ± 423	1715 ± 78	1862 ± 169	1594 ± 173
GII	1680 ± 394	1940 ± 268	2127 ± 177	1361 ± 485	1863 ± 169	1664 ± 319

Reference values: Data obtained from the Unoeste Biotery – 30 Wistar rats (Data not published). CK: 1965±703 (1262-2670); CK-MB: 494 ± 109 (385-605); LDH: 1937 ± 464 (1473-2401).

Eosinophilia is commonly found in human toxocariasis, including cases involving myocarditis (KWON et al., 2006). For instance, in Bahia, Brazil, eosinophilia was observed in 38.0% of the 268 blood donors, and associated to the ELISA positive test for anti-*Toxocara* spp. antibodies (DATTOLO et al., 2011). In our study, eosinophilia was verified in all the tested time points in GI and GII rats, with no significant difference ($p = 0.2239$). In addition, the presence of inflammatory clusters, consisting particularly of eosinophils in the myocardium of one animal in GI 180 DPI, and in two animals in GII (days 7 and 30 DPI), was observed. Nevertheless, no larvae were recovered from the cardiac tissue of the studied animals, corroborating the findings described by Alba-Hurtado et al. (2009) studying gerbils experimentally infected by *T. canis*. Cookston et al. (1990) observed myocarditis with eosinophilic infiltrate in infected mice, but a low number of larvae was observed in the heart. These authors stated the heart may be just a route of migration and cardiac injuries may be considered to be an eventual finding in toxocariasis. Due to the absence or the low number of *T. canis* larvae in the heart muscle, it is also plausible to consider that further studies are necessary to confirm whether murine are a good model for the study of cardiac injuries caused by *Toxocara* spp.

In our model, ELISA was run to detect the presence of antibodies, since laboratorial diagnosis of toxocariasis in humans is commonly based on this technique. In this study, the frequency of positive ELISA tests in infected animals ranged from 37.5% on day 7 to 100% on day 180. In addition, production of antibodies in infected animals was not dose-dependent, corroborating the findings observed by Fenoy et al. (2008), studying the antibody avidity in BALB/c mice infected with single and multiple doses of *T. canis* embryonated eggs.

In considering our results, some findings were intriguing. Firstly, the absence of larvae versus the eosinophilic infiltrate in the heart. A second point regards the dose dependence and delayed increase in enzymatic activity. Thus, it is possible to consider that cardiac lesion is an eventual, but not less important finding in toxocariasis, caused probably by the indirect action of antigens secreted by the larvae.

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