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ORIGINAL PAPER

Applicability of the use of charcoal for the evaluation of intestinal motility in a murine model of *Trypanosoma cruzi* infection

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Abstract Chagas disease, caused by the protozoan *Trypanosoma cruzi,* remains a serious public health problem in Latin America. In relation to digestive problems, 4.5% of patients show mega syndromes (megacolon) in the chronic phase. In this article, we evaluated intestinal motility at the acute phase of *T. cruzi* infection through charcoal ingestion in adult mice. After infection, Swiss mice were administered an aqueous suspension of charcoal in water by gavage. Decrease in intestinal motility was determined by increased time of appearance of charcoal in the feces. The uninfected group showed a mean time of charcoal elimination of 109.0± 14.6 min throughout the assay. On the other hand, infected mice presented a significant increase in charcoal defecation time during infection. At 15 days postinfection, infected mice showed a significant increase in charcoal defecation time, 310.2±67.4 min when compared to the uninfected group, which presented 97.8±31.8 min, indicating that the *T. cruzi* infection interferes with intestinal motility. Our results demonstrate that the use of charcoal is an ethical and efficient procedure to evaluate the intestinal motility in the murine model of *T. cruzi* infection.

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Introduction

Trypanosoma cruzi infection remains a serious public health problem in Latin America. The acute phase is followed by a chronic phase, where approximately 30% of the individuals develop a chronic symptomatic form with cardiac, digestive, and neurological disturbances (WHO 2002). Heart insufficiency is related to the cause of death in 58% of patients, whereas arrhythmias have been associated with unexpected deaths in 36.5%. The remaining 4.5% of patients with chronic Chagas infection show mega syndromes (megaesophagus and megacolon; reviewed by Teixeira et al. 2006). Chagas disease with gastrointestinal association involves an inflammatory invasion of the enteric plexuses and degeneration of enteric neurons. It is known that glial cells can be involved in enteric inflammatory responses (Da Silveira et al. 2007). In megacolon, motility disturbances and constipation are associated with enlargement of this organ (Köberle 1968). The rectum and the sigmoid colon are the most compromised segments, exhibiting striking luminal enlargement and muscular hypertrophy (Köberle 1968). Inflammatory lesions in the enteric nervous system are related to a substantial reduction in the number of neurons, which has been thought to underlie the clinical findings in megasyndromes (Adad et al. 2001). Recently, the participation of the immune system in neuronal loss was reported in chagasic patients with megacolon (Da Silveira et al. 2007). Those patients presented increased CD-57 natural killer cells and TIA-1 cytotoxic lymphocytes within the enteric ganglia. In addition, the loss of S-100-IR glial cells, in the colon of those patients, was reported, suggesting that reduction might induce loss of colon homeostasis and contribute to the development of chronic megacolon (Da Silveira et al.

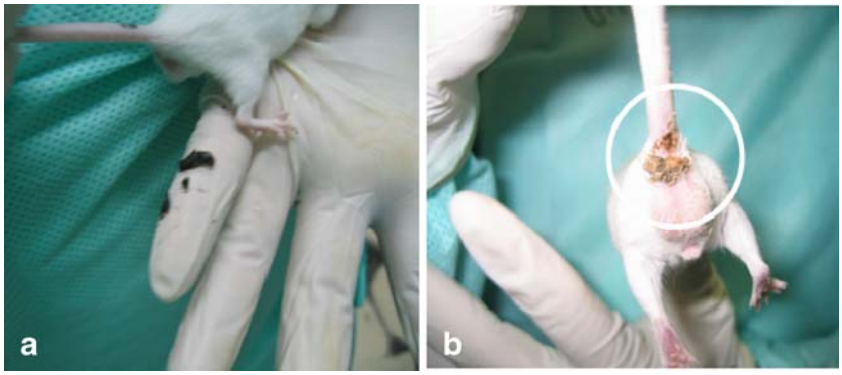

Fig. 1 Charcoal presence in feces (**a**) and constipation (**b**): Note feces presence in anal region with abnormal form (**b**), suggestive of alterations in intestinal transit

2007). Denervation in the myenteric plexus is evident and presumed to be the major contributory factor to the malfunction of motility and secretory mechanisms (Köberle 1968), which has been supported by results in experimental models (Gabella 1994). To study gastrointestinal motility, Marona and Lucchesi (2004) proposed a new protocol using charcoal evacuation time in animals deprived of food for a short time. As intestinal motility is altered in chagasic subjects, we evaluated intestinal motility using the charcoal ingestion protocol in mice during the acute phase of *T. cruzi* infection.

Materials and methods

Male Swiss Webster mice at 12 weeks of age (n=14) were intraperitoneally infected with 10^4 bloodstream forms of the Y strain of *T. cruzi* (Silva and Nussenszweig 1953). Uninfected mice were used as controls (n=12). Analyses of motility were performed before and during the acute stage of infection (2, 8, 15 and 22 days postinfection). Briefly, 3 h after food deprivation, 0.3 ml of aqueous suspension of 5% charcoal in 10% water was orally administered to each animal by gavage. The animals were observed at 5 min intervals until feces with charcoal were eliminated (maximum time of observation was 450 min, Fig. 1a). Charcoal was observed on the feces using normal light or using a microscope to help with the identification of the black spots of charcoal (Marona and Lucchesi 2004). Parasitemia, survival rates, and condition of the mice and their behavior were monitored during the experiment. After the procedure, all animals had free access to water and commercial rodent chow (Nuvital Nutrients, Paraná, Brazil). The results were calculated based on the time for charcoal evacuation, and are expressed as means±standard deviation (SD). Statistical significance (p<0. 05) was evaluated using Student's t test.

Results and discussion

Infected mice exhibited mean parasitemia of 341.8×10^4 trypomastigotes/ml (±77.1) peaking at dpi 8, displaying characteristic ascending and descending phases (data not shown). Infection with the *T. cruzi* Y strain in adult mice (12 weeks of age) resulted in lower cumulative mortality (50%, data not shown) at the end of the experiment (dpi 22), in contrast to Y strain infection of younger mice, which usually presents 100% mortality during the acute phase (De Souza et al. 2000; Olivieri et al. 2006). The choice of the present model of infection was based on results of De Rossel et al. (2000), who observed dilation of the

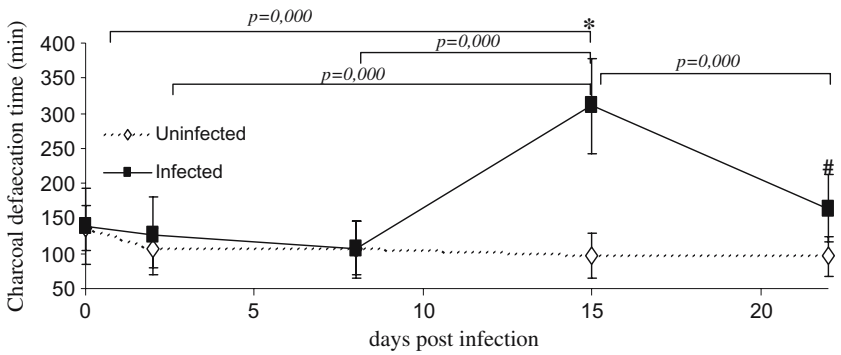

Fig. 2 Charcoal elimination time: (*empty diamond*) uninfected mice (n=12); (*filled square*) infected mice (n=14). The results are expressed in mean and SDs. *Asterisk* indicates significant differences (p<0.05) between uninfected and infected group at 15 dpi, while the *number sign* indicates significant differences among these groups at dpi 22. *Lines* indicate differences within infected mice at different days postinfection

colon in adult mice infected with *T. cruzi*. All uninfected mice survived during the experiment. Decrease in intestinal motility was based on increased time of appearance of charcoal in the feces (Marona and Lucchesi 2004; Fig 1a).

Before infection, no difference was observed between the control and infected groups, which presented similar charcoal elimination times (mean 137.9±11 min). The uninfected group exhibited a mean charcoal elimination time of 109.0±14.6 min throughout the assay (Fig. 2). At 15 dpi, infected mice showed a significant increase in charcoal evacuation time, 310.2±67.4 min (p=0,000) when compared to the uninfected group, which presented 97.8± 31.8 min (Fig. 2), indicating that *T. cruzi* infection is able to interfere with intestinal motility. At dpi 22, there was also a significant difference (p=0.001) between infected (mean 163.8±47.6 min) and uninfected mice (mean 96.2±28.1; Fig. 2). The significant increase in charcoal defecation time was observed throughout the course of infection (p= 0.0001: dpi 0×dpi 15; dpi 3×dpi 15; dpi 8×dpi 15). At the end of the experiment, the charcoal elimination time of the infected mice (dpi 22: mean 163.8±47.6 min) was lower than at dpi 15 (mean 310.2±67.4; p=0.0001), suggesting that the inflammatory infiltrate, frequently observed at 15 dpi in this model, was able to cause neuronal destruction and, consequently, altered the colon motility, being more intense than 20 dpi. There are important events that culminate in severe tissue damage observed at the second week after *T. cruzi* infection (Olivieri et al. 2006; De Souza et al. 2002).

Delay in evacuation time was observed by Mori et al. (1995), through means of X-rays in mice at the chronic stage of the *T. cruzi* infection. In addition, in 1 out of 12 mice, the opaque enema documented the existence of megacolon (Mori et al. 1995). Later, Maifrino et al. (1999) observed that experimental infection with *T. cruzi* resulted in denervation of the myenteric plexus, which could be responsible for the decreased tachykinin content (TK, which rapidly induces contraction of gut tissue) and vasoactive intestinal peptide (VIP). Such reduction in TK and VIP activity could be related to the disturbances in intestinal motility observed in the chronic phase of Chagas disease. Recently, the same group suggested that disturbances in intestinal motility observed in patients in the chronic phase of Chagas' disease could also be related to the decrease in somatostatin (SOM), which occurs in the neurons and nerve fibers of the myenteric plexus, where chronically infected mice presented less intensely stained neuron bodies and varicose SOM-positive nerve fibers (Maifrino et al. 2005). Studying the interstitial cells of Cajal (probably involved in the pathophysiology of gut disorders), Hagger et al. (2000) reported that the density of these cells in patients with Chagasic megacolon was much reduced in comparison to normal colonic tissue in the longitudinal muscle layer, intermuscular plane, and circular muscle layer, suggesting that interstitial cells of Cajal may play a role in the development of megacolon and symptoms of constipation.

Our results demonstrate that the use of charcoal is an ethical and efficient procedure to evaluate intestinal motility. This new protocol used here, for the first time in an animal model challenged with *T. cruzi* infection, is a suitable model for the intestinal motility test, taking into consideration the welfare of the animals used, as no alterations in physical activity and behavior were observed during the experiment. The new protocol uses reduced time of food deprivation and allows the reuse of the animals. The protocol was adapted in our laboratory to satisfy the 3 R's concept (Replacement, Reduction, and Refinement) by reducing animal discomfort according to Marona and Lucchesi (2004). Our findings indicate that charcoal is a useful approach, particularly for monitoring the efficacy of new agents designed to compensate for alterations in intestinal motility caused by *T. cruzi*.

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