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The role of selenium in intestinal motility and morphology in a murine model of *Trypanosoma cruzi* infection

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Abstract

Infection with *Trypanosoma cruzi* causes mega-syndromes of the gastrointestinal (GI) tract in humans and animals. In the present study, we employed magnetic resonance imaging to non-invasively monitor the effect of selenium supplementation on alterations in the GI tract of *T. cruzi*-infected mice. CD1 mice infected with *T. cruzi* (Brazil strain) exhibited dilatation of the intestines similar to that we recently reported in infected C57Bl/6 mice. The average intestine lumen diameter increased by 65% and the increase was reduced to 29% in mice supplemented with 2 ppm selenium in the drinking water. When supplemented with 3 ppm selenium in chow the lumen diameter was also significantly reduced although the difference between the infected and infected supplemented mice was smaller. Intestinal motility in infected mice fed with selenium-enriched chow was increased

compared with infected mice fed with normal unsupplemented chow and was not significantly different from intestinal motility in uninfected mice. We suggest that Se may be used to modulate the inflammatory, immunological, and/or antioxidant responses involved in intestinal disturbances caused by *T. cruzi* infection.

Introduction

Acute *Trypanosoma cruzi* infection is followed by a lifelong chronic phase during which 10% to 30% of infected patients develop clinical Chagas disease (Tanowitz et al. 2009). Chronic Chagas disease is associated with progressive inflammatory destruction of the heart, muscles, nerves, and gastrointestinal (GI) tract tissue. Seven to 10% of patients develop megasyndromes involving the GI tract, in particular, the esophagus and the colon (Tanowitz et al. 1992; Kirchoff 1996; da Silveira et al. 2007). These megasyndromes are coupled with gastrointestinal motility disturbances in patients with Chagas disease (Madrid et al. 2004; Madrid and Defilippi 2006). Mega-organ syndromes and associated symptoms are also exhibited in animal models of Chagas disease (Postan et al. 1986; 1987; Scremin et al. 1999; De Rossell et al. 2000; Boczko et al. 2005). Recently, in separate studies, we demonstrated decreased intestinal motility in *T. cruzi* (Y strain) infected Swiss Webster mice (de Oliveira et al. 2008) and intestinal dilation in *T. cruzi* (Brazil strain) infected C57Bl/6 mice (Ny et al. 2008).

The response to infection with *T. cruzi* includes upregulation of the inflammatory response including inducible nitric oxide synthase (iNOS or NOS2), tumor necrosis factor- α interferon (IFN)- γ , and other proinflammatory cytokines (Silva et al. 2003). This leads to the generation of nitric oxide (NO). Reactive oxygen species (ROS; such as superoxide, hydrogen peroxide, and hydroxyl radical) can combine with NO to form other reactive nitrogen species (RNS). ROS and RNS have damaging effects in various tissues including the GI tract. These reactive species play a role in several diseases of the GI tract including inflammatory bowel disease. In the GI tract selenium-dependent glutathione peroxidase is one of the enzymes involved in reducing hydrogen peroxide. In addition selenium (Se), an essential trace element, may have antioxidant properties distinct from those associated with glutathione peroxidase (Chu and Esworthy 2004).

Results from several studies suggest that Se deficiency may be an important factor in the pathogenesis of Chagas disease. A significant percentage of patients with chronic chagasic cardiomyopathy have low Se levels that are positively correlated with cardiac insufficiency (Rivera et al. 2002). In that study, low Se was found in six of ten chagasic patients with digestive megasyndromes and none of the patients in the study were living in Se-deficient geographic region. In animal models, we observed a higher susceptibility to the infection in *T. cruzi*-infected mice fed with Se-deficient chow (de Souza et al. 2002), while low-dose oral Se supplementation alleviated heart damage (de Souza et al. 2003). In addition, other authors reported increased severity of myopathy in Se depleted chronically infected mice (Gomez et al. 2002); and reduced parasitemia and mortality after Se supplementation (Davis et al. 1998). Based on our previous results and those of other researchers, we investigated the role of Se supplementation in mice infected with *T. cruzi* (Brazil strain) in development of megasyndromes of the GI tract and associated changes in intestinal motility and inflammation.

Materials and methods

Parasitology and pathology

The Brazil strain of *T. cruzi* was maintained by serial passages in C3H/He mice (Jackson Laboratories, Bar Harbor, ME, USA). Male CD1 mice (WT; Jackson Laboratories) were

studied in two separate cohorts. The first cohort of mice was supplemented with sodium selenate (Sigma Chemical Co., MO, USA) added at a concentration of 2 ppm to the drinking water and offered ad libitum from 6 weeks of age, as previously described (de Souza et al. 2003). These mice were fed with standard rodent chow (Pico Labs 5053). Supplementation with oral selenium was continued for 100 days. Groups in cohort 1 consisted of infected, infected+2 ppm Se, uninfected, uninfected+2 ppm Se ($n=14$ in each group at the final data point). Subsequently, a second cohort of mice was fed with a Se-enriched diet containing 3 ppm Se as Se-methylselenocysteine incorporated into purified AIN-93G diet (Research Diets, Inc.). Groups in Cohort 2 consisted of uninfected, infected, and infected+3 ppm Se ($n=8$ for uninfected, $n=9$ for infected, and $n=8$ for infected+3 ppm Se at the final data point). Two weeks after initiating Se supplementation (either in the drinking water or chow) the animals were infected by intra-peritoneal injection of 5×10^4 trypomastigotes (Brazil strain). Parasitemia was determined from 15 through 37 days post-infection (dpi). Mice were examined several times using non-invasive magnetic resonance imaging (MRI) during chronic infection, 150–280 dpi. After the final imaging session (at 280 dpi) mice were sacrificed and dissected intestinal tissue was fixed in 10% buffered formalin and stained with H&E. These studies were approved by The Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine which adheres to the guidelines established by the Association for the Assessment and Accreditation of Laboratory Animal Care and the United States Office of Laboratory Welfare.

Magnetic resonance imaging

Mice were anesthetized with 1–2% isoflurane administered with a nose cone and were positioned in a 40 mm birdcage RF coil (RF-Sensors LLC, New York, NY, USA) as previously described (Ny et al. 2008). Images were acquired using a vertical 9.4 T wide bore General Electric Omega spectrometer operating at a proton frequency of 400 MHz and equipped with 50-mm shielded gradients. The temperature within the gradient coils was maintained at 30°C with a Neslab water cooling/heating unit to maintain body temperature during the imaging experiment. Slice selection was achieved with a 90° sinc pulse. A 51.2-mm field of view was used. Each 128×256 pixel image was interpolated to 256×256 pixels. There was no separation between image slices. An echo time of 18 ms and repetition time of 400 ms was used. After locating the gastrointestinal tract a series of transverse, sagittal, and coronal images were acquired to delineate the entire intestine. Image data was transferred to a PC for offline processing using MRI analysis software running in MATLAB and intestinal lumen diameters were measured.

Charcoal motility test

Analysis of intestinal motility was performed during the chronic stage of infection (at 180 dpi) in the second cohort of mice (those fed with the Se-enriched chow). Briefly, 3 h after food deprivation, 0.2 ml of a liquid suspension of charcoal (Toxiban, Vetamix, IA, USA) was orally administered to each mouse by gavage. The mice were observed at 5 min intervals until feces blackened by charcoal were eliminated as previously described (Marona and Lucchesi 2004).

Statistics

The data are expressed as the standard error of the mean (SEM). When statistical differences between two means were determined, an unpaired Student's *t* test was performed and $P < 0.05$ was regarded as significant. When statistical analyses between means were performed, all values refer to different animals if not otherwise stated.

Results

Parasitemia, mortality, and histology

In cohort 1 all infected mice, supplemented or not, presented a peak of parasitemia between 20 and 24 dpi, with no statistically significant differences among the two groups ($11.8 \times 10^5 \pm 4.3/\text{ml}$ (control group) and $7.1 \times 10^5 \pm 3.6/\text{ml}$ (Se-supplemented group)). Cumulative mortality of the unsupplemented infected mice was 18.2% at 40 dpi, while all mice supplemented with 2 ppm Se in the drinking water survived beyond that day. In cohort 2 parasitemia was also not significantly different in infected mice regardless of the diet ($3.3 \times 10^5 \pm 1.5/\text{ml}$ (control group) and $5.2 \times 10^5 \pm 3.9/\text{ml}$ (Se-supplemented group)). By 37 dpi parasitemia had waned. Cumulative mortality of mice fed with unsupplemented chow was 20% and cumulative mortality of the mice fed with Se-supplemented chow was 26% (at 45 dpi). The remaining mice in cohort 2 survived to the chronic phase of infection and were studied by MRI and charcoal gavage. Histological examination of the colon and small intestine revealed inflammation in all layers of the GI tract. There were occasional amastigotes observed in the muscle layers. However, we could not detect any significant differences in the histological appearance in infected alone compared with infected and treated with Se.

MRI and charcoal defecation time evaluation

Mice in cohort 1 were studied only by MRI. Intestine lumen diameters were evaluated at 160 dpi. As we observed in our recent study of C57Bl/6 mice, there was significant intestinal dilation of the intestines (intestine lumen diameter >2.35 mm which is the largest diameter we measured for an uninfected control mouse) in seven out of 14 infected CD1 mice compared to uninfected controls (Fig. 1). There was no significant difference in the average lumen diameter of uninfected mice if they were supplemented with 2 ppm Se in the drinking water (1.36 ± 0.09 mm) or if they were provided with normal drinking water (1.47 ± 0.1 mm). Intestine lumen diameter was significantly increased in infected mice provided with unsupplemented drinking water (2.42 ± 0.2 mm) when compared to either uninfected mice or infected mice supplemented with 2 ppm Se in the drinking water (1.76 ± 0.1 mm; Fig. 1). Infected mice supplemented with Se in the drinking water exhibited significantly larger lumen diameters than uninfected mice although their percent increase (29%) in lumen diameter was less than that of the infected unsupplemented mice compared to their respective control (65%). Based on these findings, we proceeded to study mice fed with Se-supplemented chow.

Mice in cohort 2 fed with chow enriched with 3 ppm Se were examined at 160 and 260 dpi by MRI. Four out of the nine infected mice developed enlargement of the intestines, with average lumen diameters >2.35 mm. The range of values for all nine infected mice was 1.76–2.86 mm (mean \pm SEM, 2.18 ± 0.05). Figure 2 includes representative images with the 3D reconstruction of the GI tract of one of the infected mice with enlarged intestines at 160 dpi (B) in comparison with an uninfected age-matched control mouse (A) and a Se-supplemented infected mouse (C). Se-supplemented infected mice had lumen diameters ranging from 1.62 to 2.18 mm (mean \pm SEM, 1.93 ± 0.04). The average lumen diameter of the eight Se-supplemented mice was significantly smaller than that of the infected unsupplemented mice ($P < 0.005$; comparing all mice from each group). Charcoal defecation time was evaluated in these mice at 180 dpi and was significantly increased in unsupplemented infected mice. This demonstrates that motility was significantly reduced in the unsupplemented infected mice (charcoal transit time was 231 ± 31 min) compared to unsupplemented controls (89 ± 12 min), whereas infected mice fed with Se-enriched chow exhibited motility (109 ± 12 min) that was not significantly different from that of uninfected control mice (Fig. 3). Charcoal transit time tended to be longer in mice with the largest lumen although increased transit time did not always predict increased lumen diameter as shown in Fig. 4 (see point marked with *). This is not completely unexpected since not all infected mice develop megasyndromes of the GI tract. Intestine diameters of the same

mice were evaluated again by MRI at 260 dpi. At 260 dpi the intestine lumen diameter of unsupplemented infected mice was 2.11 ± 0.02 mm while Se-supplemented infected mice still exhibited significantly smaller lumen diameters (1.81 ± 0.02).

Discussion

In our previous study of intestinal motility, we examined mice during the acute stage of infection (de Oliveira et al. 2008). In that study charcoal defecation time was increased in all infected mice suggesting that neuronal damage compromising intestinal motility had already occurred. In our present study we examined mice infected with Brazil strain of *T. cruzi* that survived to the chronic stage. We observed intestinal dilation in infected mice that was reduced with Se-supplementation in either the drinking water or chow. Se has a variety of functions, but the mechanism by which Se restricts the intestinal enlargement in *T. cruzi*-infected mice is not understood. Se can behave as both an antioxidant and an anti-inflammatory agent (Rayman 2000; Mishra et al. 2007). It has been suggested that the GI-specific enzyme (GI-GPx or GPx2) has a specific role in protecting the GI tract against inflammation and cancer development (Esworthy et al. 2005). It is possible that infected mice supplemented with Se presented a rapid production of GPx2 which protected the intestine against the oxidative stress commonly caused by *T. cruzi* infection. We did not observe reduced inflammation in Se-supplemented infected mice; however, inflammation was not extensively investigated in our study.

Selenoproteins reduce oxidative stress and balance redox in tissues involved in innate and adaptive immune responses. We suggest that Se could participate in immunological and/or inflammatory responses involved in the development of intestinal dilation. Da Silveira et al. (da Silveira et al. 2007) reported an increase of CD-57 natural killer cells and TIA-1 (granule-associated protein of cytotoxic T-cells and NK cells) within the enteric ganglia in chagasic patients with megacolon. Recently, Oliveira et al. (Oliveira et al. 2009) demonstrated that patients with severe GI syndromes in the setting of chronic Chagas disease presented evidence of an immune response to myelin components, suggesting that myelin basic protein is a potential target on the peripheral nerve for autoimmune reactions in those patients. It is possible that Se could affect cytotoxic cells, based on findings in different diseases. Lymphocyte performance related to cytotoxic T lymphocyte-driven tumor lysis, mitogen-induced proliferation of lymphocytes, and mixed lymphocyte reaction proliferation of lymphocytes was increased in patients after receiving Se supplementation (Kiremidjian-Schumacher and Roy 2001). Se can also influence the T-helper phenotype. The experiments of Broome et al. (2004) demonstrated that Se supplementation in human subjects resulted in increased production of IFN γ and IL-10, and an increased percent of T-helper cells in response to the vaccine based on oral live attenuated poliomyelitis.

Intestinal motility was disturbed in unsupplemented infected mice while mice fed with Se-enriched chow exhibited charcoal defecation rates that were not different from uninfected mice. Delayed evacuation time was previously observed in infected mice at the chronic stage by Mori et al. (1995) using X-ray technology and only one out of 12 of those mice had document megacolon. Thus, our results and those of Mori et al. (1995) demonstrate that disturbances in intestinal motility are not necessarily accompanied by megacolon. The control of motility of the gastrointestinal tract depends on acetylcholine, noradrenaline, and non-adrenergic non-cholinergic neurotransmitters (Matsuda and Dantas 2008). Several substances identified in the enteric nervous system of mammals can be released and participate in the control of gastrointestinal motility by acting directly on the gastrointestinal smooth muscle, or indirectly by modulating the release of inhibitory or excitatory mediators (Matsuda and Dantas 2008). Since Se supplementation prevented the delayed evacuation time in infected mice, it would be worthy to explore the effect of Se on factors related to disturbances in intestinal motility, in

addition to those caused by *T. cruzi* infection, such as decreased levels of tachykinins and vasoactive intestinal peptide (Maifrino et al. 1999), decreased somatostatin (Maifrino et al. 2005), and reduced number of interstitial cells of Cajal (Hagger et al. 2000).

In this study we compared the effect of two forms of Se on the intestinal alteration caused by *T. cruzi* infection. We demonstrated that either an inorganic form (sodium selenate) or an organic form (Se-methylselenocysteine) were able to reduce the intestine lumen diameter and increase intestinal motility at the chronic phase. In addition, no adverse effects were observed after using 2 or 3 ppm, showing that those supranutritional concentrations (marginally high) can be used with no restrictions in those models. Further studies are required to demonstrate the protective effect of Se on pre-existing intestinal disturbances. Based on our previous observation of a beneficial effect of sodium selenate on cardiac damage (de Souza et al. 2003) and the present results showing beneficial effects on digestive alteration, we suggest that Se supplementation in combination with specific anti-*T. cruzi* agents may provide a superior therapeutic protocol to modulate the inflammatory, immunological, and antioxidant responses involved in intestinal and cardiac disturbances caused by *T. cruzi* infection.

Acknowledgments

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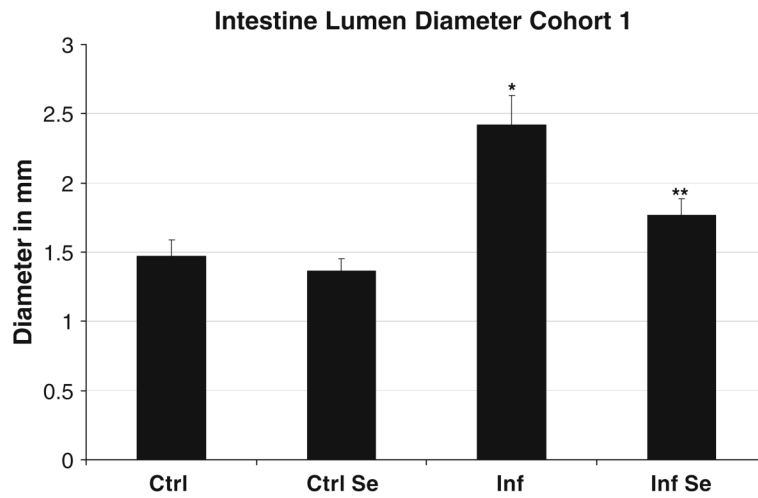


Fig. 1.

Average intestine lumen diameters in mice, Cohort 1. Mice were provided with either unsupplemented or 2-ppm Se-supplemented drinking water ad libitum. Intestine lumen diameters were evaluated during the chronic phase of infection by MRI. Average lumen diameter was increased to a greater extent in unsupplemented infected mice while Se-supplemented mice showed a smaller change in lumen diameter when infected. * $P < 0.005$, unsupplemented uninfected (*Ctrl*) versus unsupplemented infected (*Inf*); ** $P = 0.012$, unsupplemented infected (*Inf*) versus Se-supplemented infected (*Inf Se*) and supplemented infected (*Inf Se*) versus Se-supplemented uninfected (*Ctrl Se*)

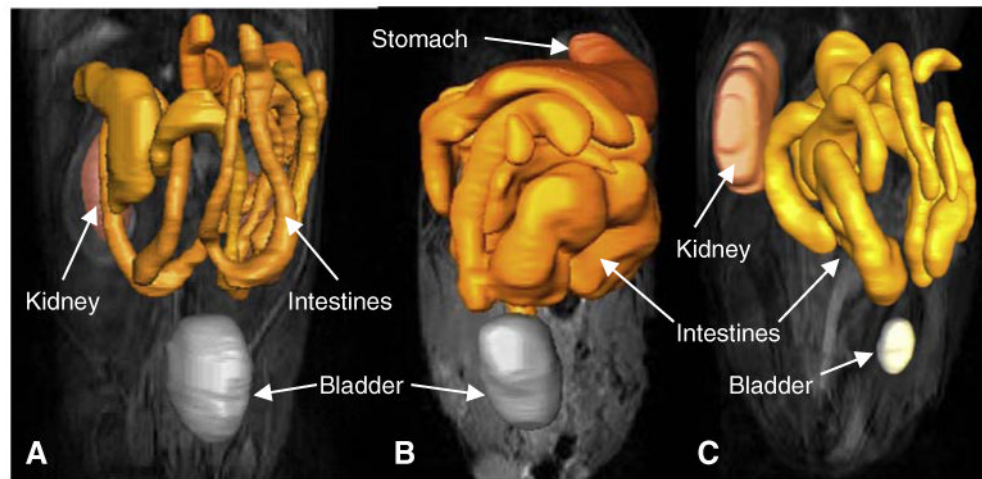


Fig. 2. Representative MRI of uninfected (a), unsupplemented infected (b), and Se-supplemented infected (c) mice. Images were acquired of the entire mouse GI tract. 3D reconstruction of the GI tract was created and is overlaid on one of the MRI images. Enlargement of the intestines is observed in the infected mouse (b)

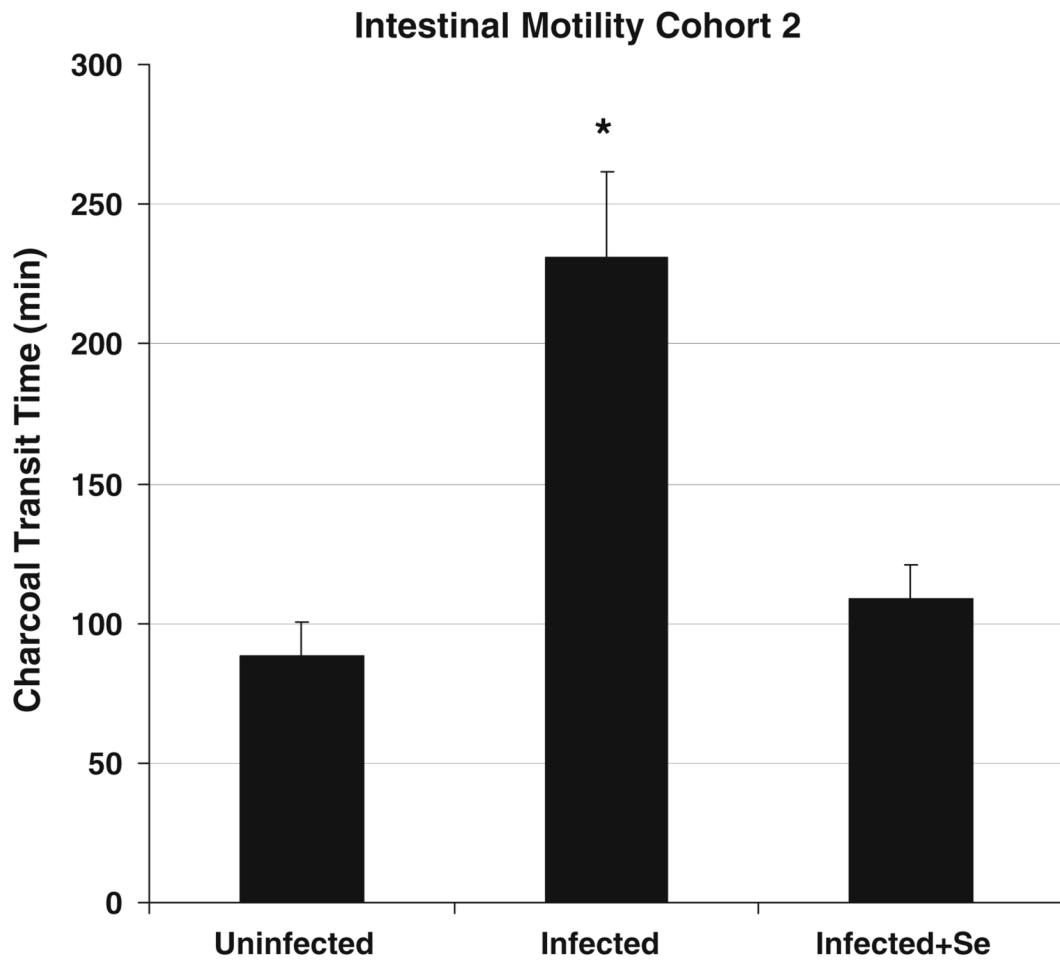


Fig. 3. Intestinal motility in mice. Mice were fed with control or 3 ppm Se-supplemented chow and intestinal motility was evaluated by charcoal transit time (in min). * $P < 0.005$, unsupplemented uninfected (*Uninfected*) versus unsupplemented infected (*Infected*) and unsupplemented infected (*Infected*) versus Se-supplemented infected (*Infected+Se*)

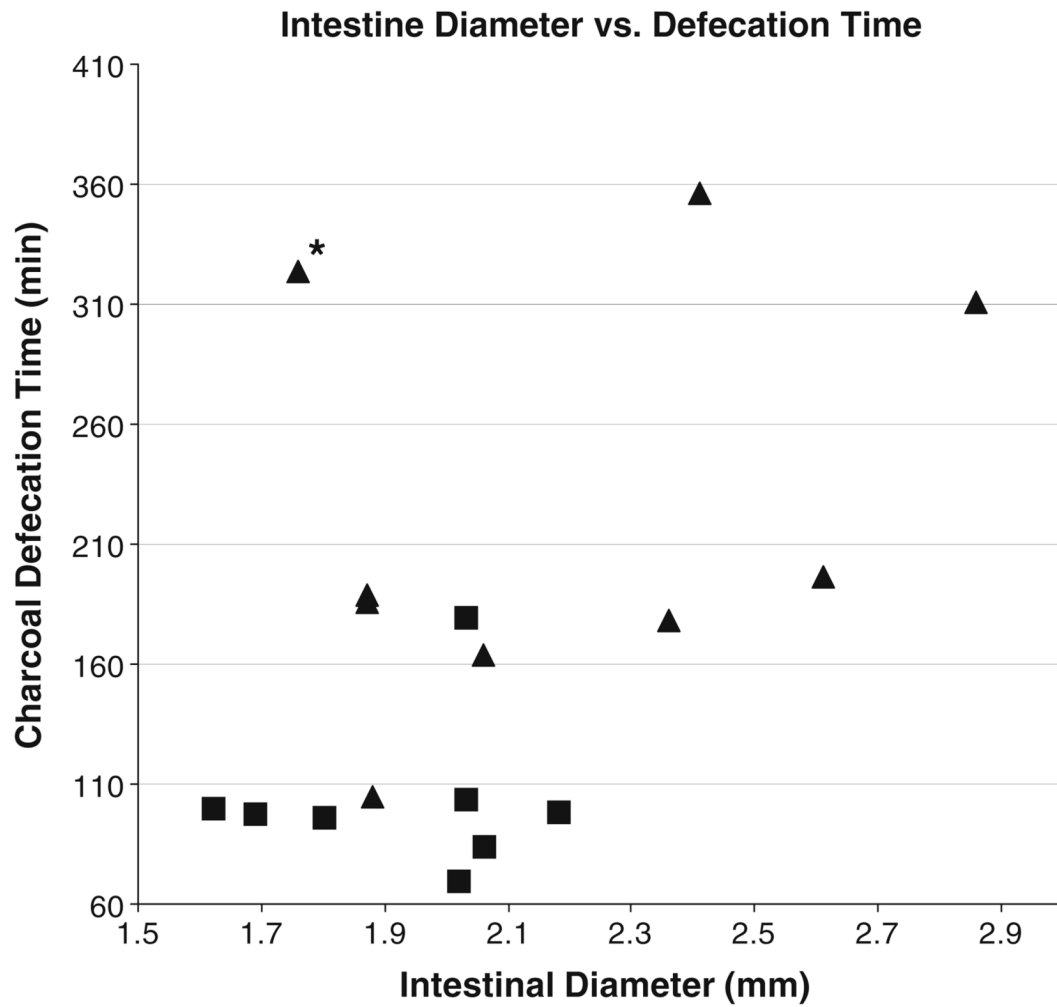


Fig. 4. Intestinal diameter vs. charcoal defecation time. Intestine diameter of infected (*filled triangles*) and Se-supplemented infected (*filled squares*) are plotted against their corresponding charcoal defecation time. Intestine diameters were measured at 160 dpi and charcoal defecation time was measured at 180 dpi. The point marked with * represents an infected mouse with normal lumen diameters but very long charcoal transit time