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Kinetics of acute infection with *Toxoplasma gondii* and histopathological changes in the duodenum of rats

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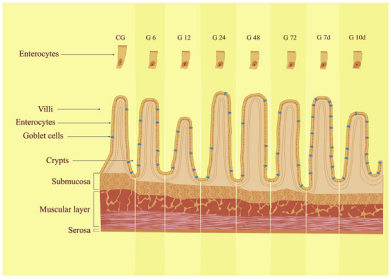
HIGHLIGHTS

- We analyzed the duodenum of rats infected with T. gondii during acute infection.
- Acute infection was tested in the durations of 6 h–10 days after infection.
- Morphometric and quantitative changes were found in epithelial cells.
- Morphological changes occurred in villi and crypts.
- There was atrophy of the submucosa, the muscular layer and the total wall.

GRAPHICAL ABSTRACT



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ABSTRACT

Toxoplasma gondii crosses the intestinal barrier to spread into the body. We investigate the intestinal wall and epithelial cells of the duodenum of rats infected with *T. gondii* during different time points of acute infection. Male Wistar rats, 60 days of age, were assigned into groups that were orally inoculated with 5000 sporulated oocysts *T. gondii* for 6 h (G6), 12 h (G12), 24 h (G24), 48 h (G48), 72 h (G72), 7 days (G7d), and 10 days (G10d). The control group (CG) received saline. The rats were killed and the duodenum was processed to obtain histological sections stained with hematoxylin and eosin, Periodic Acid Schiff, and Alcian blue (pH 2.5 and 1.0). Morphometry was performed on the layers of the intestinal wall and enterocytes, and the number of goblet cells and intraepithelial lymphocytes was counted. The data were compared by ANOVA considering 5% as level of significance. The infection provoked an increase in the width of villi and crypts; decrease in enterocyte height; increase in the smaller-diameter and reduction in the larger-diameter of the enterocytes nuclei, increased number of goblet cells secreting neutral (G6, G12 and G7d) and acidic (G7d and G10d) mucus, and increase in the number of intraepithelial lymphocytes (G48). The infected groups showed atrophy of the submucosa and muscular layers and the total wall. Acute infection with *T. gondii* caused morphological changes in the intestinal wall and epithelial cells of the duodenum in rats.

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1. Introduction

Toxoplasmosis is an infection that is caused by the parasite *Toxoplasma gondii*. Is highly prevalent in humans and animals. It is transmitted primarily through the ingestion of contaminated food and water that contain oocysts or undercooked meat that contains tissue cysts. In acute infection, tachyzoites multiply rapidly and reach different host cells (Dubey, 1998). During the course of infection, bradyzoites tend to form tissue cysts, especially in nervous and muscle tissue, thus leading to the chronic form of the infection (Hill and Dubey, 2002).

Felines are the definitive hosts, and the others warm-blooded animals are the intermediate hosts (Ferreira et al., 2003; Weiss and Kim, 2007). Infection is often asymptomatic, but immunocompromised individuals may present clinical manifestations, such as encephalitis, that result from the reactivation of latent infections (Dubey, 1998; Ettinger and Feldman, 1997). The transplacental transmission can be serious and can occur brain injury, physical deformities and abortion of the fetus (Bonametti et al., 1997).

T. gondii needs to cross the intestinal barrier to spread in the body, causing a state of alertness in the mucosa, marked mainly by the migration of lymphocytes (Buzoni-Gatel and Werts, 2006; Furness, 2006). Enterocytes are the most numerous cells in the intestinal epithelium and are responsible for the absorption of water and nutrients. Goblet cells secret mucins that comprise the mucus, forming a protective layer on the intestinal epithelium (Gartner and Hiatt, 2003). Sporozoites of *T. gondii* reach segments of the digestive system and enter enterocytes, goblet cells, and lymphocytes, reaching the intestinal wall and several other tissues (Speer and Dubey, 1998; Weiss and Kim, 2007). Such infection can result in disorders associated with intestinal inflammation and diarrhea (Wingstrand et al., 1997).

Studies of animals that are chronically infected with *T. gondii* have reported alterations in the structure of the intestinal wall, number of goblet cells, and size of enterocytes (Bonapaz et al., 2010; Braga et al., 2011; Da Silva et al., 2010; Shiraishi et al., 2009). These changes depend on the parasite's lifecycle and strain, the duration of infection, the host species, and the region of the intestine that is affected. However, changes in the histological structure of the intestine with acute *T. gondii* infection are unknown because there are no studies reporting the response of the intestinal wall and epithelial cells on different time of acute infection. Given the close relationship between the intestine and parasite at the time of infection, the present study analyzed the layers of the intestinal wall and dynamics of the epithelial cells in the duodenum of Wistar rats infected with *T. gondii* oocysts. This analysis was performed in different time points of acute infection to understand the role of the intestinal barrier in the kinetics of acute *T. gondii* infection in rats.

2. Materials and methods

2.1. Experimental design

Sixty-four Wistar rats (*Rattus norvegicus*) with 60 days of age weighing on average 272.77 ± 10.9 g were made available by the animal house of the State University of Maringa. The experimental protocol was approved by the Ethics Committee on Animal Experiments of the State University of Maringa (protocol number 079/2013). The animals received oral antiparasitic pretreatment at 28 days of age (500 mg/kg metronidazole suspension for 5 days and 50 mg/kg fenbendazole in a single dose suspension) and after seven days of pretreatment, a stool test was performed to verify the absence of parasites. These rats were randomly assigned into a control group (CG) and groups that were inoculated with *T. gondii* and assessed at 6 h (G6 group), 12 h (G12 group), 24 h (G24 group), 48 h (G48 group), 72 h (G72 group), 7 days (G7d group), and 10 days (G10d group), considering $n = 8$ per group.

Each infected rat received orally 5000 *T. gondii* sporulated oocysts (ME-49 strain, genotype II) that were resuspended in 1 mL of sterile saline. Rats from the CG group received only saline. The oocysts were obtained from the Parasitology Veterinary Laboratory, State University of Londrina. All animals were kept in cages with controlled temperature (24 ± 2 °C) and a 12 h/12 h photoperiod (lights on 6:00 AM-6:00 PM) and received standard rodent chow (Nuvilab, Colombo, PR, Brazil) and filtered water *ad libitum*.

At the end of each experimental period, tail blood was taken to verify the presence of IgG anti-*T. gondii* by a direct agglutination test, with positive titers above >25 (Desmonts and Remington, 1980; Dubey et al., 1988).

2.2. Euthanasia and tissue collection

After the experimental period, the rats were sacrificed by deep anesthesia with halothane vapor (Vivas et al., 2007). After vertical laparotomy, the duodenum was removed, having the duodenal-jejunal flexure as distal anatomical limit, and submitted to histological processing.

2.3. Histological processing

Two-inch-long rings of the duodenum were collected from all of the animals. The tissue was opened at the mesenteric border, set in Styrofoam with the aid of tacks, and placed in Bouin fixative solution for 6 h. Initially, the duodenum samples were embedded in paraffin, cut into semi-serial 4 μm cross-sections, and arranged on glass slides. These sections were subjected to a series of deparaffinization and hydration for staining with hematoxylin and eosin (HE), which was used to determine the morphometry of the villi, crypts, enterocytes, submucosa, muscular layer, and total wall and also quantify intraepithelial lymphocytes. Part of the sections was stained with Periodic Acid Schiff (PAS) in order to detect neutral mucins. Alcian blue (AB) staining was used to detect sialomucins (at pH 2.5) and sulphomucins (at pH 1.0). All of the analyses were performed in a blinded analyzer.

2.4. Morphometric analysis of enterocytes

We measured the width and height of 50 enterocytes and the smallest- and largest-diameters of the nucleus of these cells. Morphometry of enterocytes that had the complete morphology present in villi and crypts of sixteen microscopic images of the intestinal epithelium was performed up to a total 50 measurements each rat. To perform this, four images of each section were taken with a digital camera (Pro series 3CCD camera) coupled to an optical microscope (Olympus BX50) using the 40 × objective. The width and height measurements of enterocytes and their nuclei were made using ImagePro Plus software (Media Cybernetics).

2.5. Counting of goblet cells

Four sections from each rat were stained with PAS, AB pH 1.0, and AB pH 2.5. Six images from each section were taken with a digital camera (Pro series 3CCD camera) coupled to an optical microscope (Olympus BX50) with a 20 × objective. The number of goblet cells that were present in 0.96 mm^2 in the mucosa of each animal was quantified for each staining (Bonapaz et al., 2010) using ImagePro Plus software (Media Cybernetics).