Acta Tropica 182 (2018) 27–33

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Metaperiodate deglycosylation of *Strongyloides venezuelensis* larvae: Immunochemical characterization and antigen production for human strongyloidiasis diagnosis

Henrique Tomaz Gonzaga[a], Daniela da Silva Nunes[a], Vanessa da Silva Ribeiro[b], Nágilla Daliane Feliciano[a], Jair Pereira da Cunha-Junior[a], Julia Maria Costa-Cruz[a,*]

[a] Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Av. Pará 1720, 38400-902, Uberlândia, MG, Brazil
[b] Unidade Acadêmica Especial de Biotecnologia, Área de Ciências Biológicas, Universidade Federal de Goiás, Regional Catalão, Av. Dr. Lamartine Pinto Avelar 1120, Bloco M, 75704-020, Catalão, GO, Brazil

ARTICLE INFO

Keywords:
Deglycosylation
Diagnosis
Strongyloidiasis
Strongyloides venezuelensis
Sodium metaperiodate

ABSTRACT

Strongyloidiasis is an important helminthiasis affecting million people worldwide. The aim of this study was to use sodium metaperiodate (MP) treatment to immunochemically characterize *Strongyloides venezuelensis* filariform larvae and use MP-treated heterologous antigen to detect IgG and subclasses in serum. Samples from individuals with definitive diagnosis of strongyloidiasis (n = 50), other parasitic diseases (n = 60) and negative endemic (n = 50) were tested. TG-ROC and two-way ANOVA were applied. MP-treatment resulted on differential localization of carbohydrates at larval structure and no carbohydrate content in saline extract (SE). Electrophoretic profiles were similar before and after treatment. ELISA sensitivity and specificity were: 90%; 88.2% for SE and 92.0%; 94.6% for MP, respectively. When using MP treated antigen we observed reduction in IgG1 and IgG3 detection in strongyloidiasis group and decrease of cross reactions in control groups. Our data demonstrate the role of carbohydrate residues in cross reactions and on the recognition of anti-*Strongyloides* IgG and its subclasses.

1. Introduction

Strongyloides stercoralis infection is considered the fourth most important intestinal nematode infection, after hookworm, *Ascaris lumbricoides* and *Trichuris trichiura* (Stephenson et al., 2000). Soil-transmitted helminths of the genus *Strongyloides* currently infect 30–370 million people worldwide (Bisoffi et al., 2013). The disease is common in many warm and humid areas where sanitary conditions are poor. However, the parasite is also endemic in several subtropical and temperate regions, e.g. southern areas of the USA, Europe and Japan (Olsen et al., 2009).

Due to the low parasitic load in most of the infected subjects, parasitological assays show low sensitivity because of the irregular larval output by intestinal adult females (Dreyer et al., 1996). One of the most important limitations in strongyloidiasis immunodiagnosis is the difficulty to obtain large amounts of *S. stercoralis* filariform larvae to produce antigens. Thus, studies have been conducted using heterologous antigenic sources such as *Strongyloides venezuelensis* (Costa-Cruz et al., 1997, 1998; Gonzaga et al., 2011, 2013; Machado et al., 2008). Serology, in combination with fecal-based methods, is recommended for the screening, diagnosis and strongyloidiasis follow up (Buonfrate et al., 2017).

Given the challenges for direct larval detection, serological tests for specific IgG by enzyme-linked immunosorbent assay (ELISA) have been developed, showing high sensitivity, but cross-reacting with other helminthic infections (Costa et al., 2003; Lindo et al., 1994; Sudré et al., 2007), maybe due to glycosylated antigens shared among different parasites.

To diminish false positive results, efforts have been made treating antigens with sodium metaperiodate (Alarcón de Noya et al., 2000; Kirinoki et al., 2011; Sudré et al., 2007). Metaperiodate (MP) oxidizes carbohydrates to aldehydes, disrupting carbohydrate domains, allowing to verify if cross-reactive responses target the carbohydrate or protein epitopes of an antigen (Eylar and Jaenloz, 1962; Woodward et al., 1985). Considering the challenges and the necessity to develop a better immunological test using heterologous antigens, the aim of this study was to characterize histochemically *S. venezuelensis* larvae after treatment with MP and evaluate the ability of MP-treated saline extract to

* Corresponding author at: Laboratório de Diagnóstico de Parasitoses, Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Avenida Pará 1720, 38400-902, Uberlândia, MG, Brazil.
E-mail address: costacruz@ufu.br (J.M. Costa-Cruz).

https://doi.org/10.1016/j.actatropica.2018.02.001
Received 24 August 2017; Received in revised form 24 January 2018; Accepted 10 February 2018
Available online 15 February 2018
0001-706X/

detect IgG and its subclasses in serum samples from patients with strongyloidiasis.

2. Methods

2.1. Serum samples

Serum samples were collected from 160 subjects divided into three groups: Group 1 (G1): 50 patients attending the outpatient departments or admitted to the Hospital de Clínicas de Uberlândia, Universidade Federal de Uberlândia (UFU), Minas Gerais State, Brazil, harboring *S. stercoralis* larvae, coprologically detected according to Baermann-Moraes (Baermann, 1917; Moraes, 1948) and Lutz (1919) methods. Group 2 (G2): 60 patients with other parasitic diseases including: *A. lumbricoides* (n = 8), *Entamoeba histolytica/dispar* (n = 2), *Enterobius vermicularis* (n = 5), *Giardia lamblia* (n = 4), hookworm (n = 10), *Hymenolepis nana* (n = 5), *Schistosoma mansoni* (n = 8), *Taenia* sp. [n = 8; taeniasis (n = 5) and cysticercosis (n = 3)], *T. trichiura* (n = 4), and co-infected patients (n = 6): hookworm + *A. lumbricoides* + *T. trichiura* (n = 1), hookworm + *A. lumbricoides* (n = 1), *A. lumbricoides* + *T. trichiura* (n = 1), hookworm + *E. histolytica/dispar* (n = 1), hookworm + *H. nana* (n = 1), and *E. histolytica/dispar* + *H. nana* (n = 1). Group 3 (G3): 50 endemic individuals parasitologically negative, based on clinical observation they were apparently healthy; without evidence of contact with *S. stercoralis*, no previous history of strongyloidiasis and three fecal samples tested negative after Baermann-Moraes (Baermann, 1917; Moraes, 1948) and Lutz (1919) methods. The study received approval from the Research Ethics Committee of UFU (protocol numbers 041/2008 and 188/2009).

2.2. Larvae obtention and antigen preparation

S. venezuelensis third-stage larvae (L3) were obtained from charcoal cultures of feces from experimentally infected rats (*Rattus norvegicus*) (approved by the Ethics Committee on Use of Animals; Protocol 075/2008). Feces were mixed with equal part of charcoal, and incubated at 28 °C for 3 days. L3 were collected and concentrated using Rugai apparatus (Rugai et al., 1954) and then washed 5 times in phosphate-buffered saline (PBS, 0.01 M, pH 7.2), and stored at −20 °C in PBS.

Saline extract (SE) was obtained according to Gonzaga et al. (2013). Briefly, S. venezuelensis filariform larvae were suspended in PBS containing protease inhibitors cocktail and disrupted by cycles of freezing, maceration, and thawing/sonication until no integral larvae were found after microscope examination. After a 2 h incubation period at 4 °C under constant shaking, the suspension was centrifuged (12400*g*, 30 min, 4 °C) and the supernatant – SE – was analyzed for protein content according to Lowry et al. (1951), subdivided into aliquots and stored at −20 °C until use.

2.3. Metaperiodate treatment and eletrophoretic analyses

2.3.1. Larvae

Intact filarioid larvae were incubated with sodium metaperiodate ($NaIO_4$; 200 mM; Sigma, Germany) diluted in acetate buffer (200 mM; pH 4.5; Synth, Brazil) for 30 min, 1 h or 2 h. Control sample consisted of larvae incubated with PBS only. All steps, as described for SE, were performed in microtubes, with centrifugation (2000*g*, 1 min) cycles for solution exchange. Larvae were washed with PBS, to remove salt excess, and stored (−20 °C).

2.3.2. Saline extract

SE was mixed with sodium metaperiodate ($NaIO_4$; 200 mM; Sigma, Germany) diluted in acetate buffer (200 mM; pH 4.5; Synth, Brazil). Solution was kept in the dark for 1 h at room temperature (RT). To stop reaction and to reduce the oxidized aldehydes sodium borohydride ($NaBH_4$; final concentration 200 mM in PBS; 30 min; RT; Isofar, Brasil) was added. Both periodate and borohydride solutions were prepared immediately before use. SE treated with sodium metaperiodate was dialyzed (PBS, 4–8 °C) and concentrated using ultra centrifugal filter units (Amicon Ultracel – 3 K; Millipore, Ireland), according to the manufacturer recommendations. After three centrifugation cycles, the content was recovered and stored (−20 °C) named as MP. Other two aliquots were incubated (without MP-treatment), and then tested as controls of: treatment time (SE; 1 h30 min, RT) and acid buffer effect (SE'; incubation with acetate buffer; 1 h30 min, RT). Protein (Lowry et al., 1951) and carbohydrate' (Masuko et al., 2005) contents were analyzed.

2.3.3. Gel electrophoresis

Electrophoretic profiles were analyzed for SE, SE' and MP – prepared after sodium metaperiodate oxidation (MP 1) and sodium borohydride reduction (MP 2). Protein profile was carried out under discontinuous buffer, denaturing and non-reducing conditions in 17% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli, 1970). Polypeptides were visualized after Friedman (1982) silver staining protocol, modified by heating solutions in microwave oven. Gel analysis was performed by using Image J version 1.44 software (National Institutes of Health, Bethesda, USA) to generate protein lane graphical plot, peaks were compared with a protein standard marker (RECOM Blue Wide Range Prestain Marker, Real Biotech, Taiwan) to estimate relative molecular weight in kiloDalton (kDa).

2.4. Larvae histochemical characterization

Fehling test, that detects monosaccharides and reducing sugars, oxidizing aldehydes groups, was performed to detect differences between untreated and MP-treated larvae. Larvae were placed on slides and covered with a freshly prepared mixture (A- copper sulfate pentahydrate 6.8% in distilled water; B- sodium hydroxide 10% + potassium sodium tartrate tetrahydrate 35% in distilled water; 1:1, v/v) and then boiled on hot plate.

2.5. Enzyme-linked immunosorbent assay (ELISA) to detect IgG and subclasses anti-Strongyloides

ELISA for serum IgG detection was performed according to Gonzaga et al. (2011), with modifications. Briefly, polystyrene microplates (Greiner Bio-one, Germany) were used as support for adsorption of SE, SE' or MP (5 μg/mL, to a final volume 50 μL/well in carbonate-bicarbonate buffer (0.06 M, pH 9.6), plates were kept at 4 °C for 18 h in a humid chamber. After washing (5 min; PBS + 0.05% Tween 20 - PBS-T), serum samples were added (50 μL/well), diluted 1:160 in PBS-TM 3% (PBS-T + 3% skimmed milk), and incubated (1 h at 37 °C). After a wash cycle (PBS-T, 3x, 5 min), anti-human IgG Fc specific peroxidase conjugate (1:2000 in PBS-T; Sigma, Germany) was added for IgG detection. Plates were kept for 1 h at 37 °C and after another washing cycle (PBS-T, 3x, 5 min), the reaction was revealed with chromogen solution of o-phenylenediamine and hydrogen peroxide (10 mg o-phenylenediamine (Sigma, USA) + 10 μL hydrogen peroxide 30% (Merck, Germany) + 25 mL citrate-phosphate buffer, 0.1 M, pH 5.5). Plates were kept in the dark (15 min, RT) and the reaction was stopped by adding 2 N sulfuric acid (25 μL/well). Optical density (OD) was obtained in microplate reader (TP Reader, Thermo Plate, Brazil) with 492 nm filter.

Detection of anti-*Strongyloides* seric IgG1, IgG2, IgG3 and IgG4 by ELISA was performed as described for IgG, with modifications. High affinity microtiter plates (Costar 3590, Corning, USA) were coated with SE, SE' or MP (10 μg/mL, 50 μL/well). Serum samples were diluted 1:40 for IgG1 and IgG3 detection and 1:10 in IgG2 and IgG4 assays (1 h 30 min, 37 °C). Then, biotinylated secondary anti- human IgG subclasses (Fc-specific) antibodies (IgG1 and IgG4 - Calbiochem, USA; IgG2 and IgG3 - Sigma, USA) were added at a dilution of 1:1000 in PBS-TM