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Comparative analysis of carbohydrate residues in the midgut of phlebotomines (Diptera: Psychodidae) from colony and field populations from Amazon, Brazil

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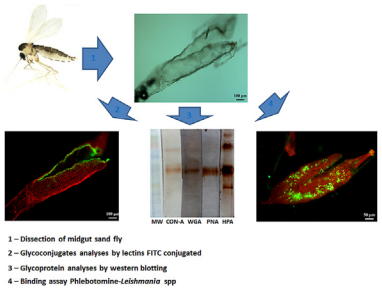
HIGHLIGHTS

- *Lutzomyia antunesi* and *Lutzomyia flaviscutellata*, vectors of cutaneous leishmaniasis, present GalNAc on midgut epithelial.
- *Lutzomyia longipalpis s.l* midgut presents residues of GalNAc, mannose, galactose and GlcNAc.
- Carbohydrate characterization improve the knowledge of Phlebotomine-*Leishmania* interaction.

GRAPHICAL ABSTRACT



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ABSTRACT

Leishmaniasis are worldwide diseases that occur in 98 countries including Brazil, transmitted by the bite of female phlebotomines during blood feeding. In Brazil it is known that some species of sand flies as *Lutzomyia longipalpis sensun latum* (vector of *Leishmania infantum chagasi*), *Lutzomyia flaviscutellata* (vector of *Leishmania (Leishmania) amazonensis*) and *Lutzomyia antunesi* [suspected vector of *Leishmania (Viannia) lindenbergi*] are incriminated of transmitting the parasite *Leishmania* for the vertebrate host. The phlebotomine-parasite is mediated by the attachment of the promastigote lipophosphoglycan (LPG) to the midgut epithelium. However, another mechanism that is LPG-independent and mediated by N-acetyl-galactosamine (GalNAc) seems to occur in some species of phlebotomines that are classified as permissive. The aim of this study was to characterize the carbohydrate residues that, probably, play a role in parasite attachment to the midgut of phlebotomine from colony and field populations from the Brazilian Amazonian region. We observed the presence of GalNAc, mannose, galactose and GlcNAc in all phlebotomine species. A binding assay between *L. (L.) amazonensis* and *L. i.chagasi* to the midguts of different species of phlebotomines was performed. The attachment of both *Leishmania* and vector species

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suggests the presence of GalNAc on the midgut surfaces. Thus, these results suggested that GalNAc is a possible binding sites of *Leishmania* in sand flies from the Brazilian Amazonian region.

1. Introduction

Leishmaniasis are infectious diseases caused by protozoan parasites of the genus *Leishmania* (Trypanosomatidae: Kinetoplastidae) that are transmitted by the bite of female phlebotomines (Diptera: Psychodidae) during blood feeding (WHO, 2015). Phlebotomines become infected when they ingest mammalian blood that contains the amastigote forms inside of macrophages that release the parasites into the midgut. Inside the vector, amastigotes differentiate to an infective metacyclic stage promastigotes (Ramalho-Ortigao et al., 2010). However, some species of phlebotomines, classified as specific or restrictive, allow the development of only one *Leishmania* species, while others, which are classified as permissive, allow the development of more than one species of *Leishmania* (Myskova et al., 2007).

The establishment of infection depends on a parasite's capacity to overcome natural barriers such as digestive enzymes produced by epithelial cells (Pimenta et al., 1992). In the early stage of development, leishmanias are protected against hydrolytic activities by peritrophic matrix (Pimenta et al., 1997). However, parasites need to escape from the endoperitrophic space and attach to the midgut epithelium to avoid being excreted with the blood remnants (Pimenta et al., 1992). This adhesion depends on the interaction between molecules present on the surface of the parasite and the midgut epithelium.

In a species-specific interaction, such as *Phlebotomus papatasi* and *Leishmania (Leishmania) major*, the adhesion is mediated by LPG, a linear chain of phosphorylated disaccharide repeating units that are bound to cell membranes by a lipid anchor. This molecule is abundantly present on flagellar forms only and presents structure variations during the life cycle of the parasite (Assis et al., 2012; Pimenta et al., 1994). During parasite-sand fly interactions, carbohydrate residues that are present on LPG are recognized by lectins as binding sites on midgut epithelial cells (Pimenta et al., 1994). In the midgut of *Phlebotomus duboscqi* and *Ph. papatasi* there is a lectin (PpGalec) that is specific for *L. (L.)major* LPG. PpGalec seems to be specific for *L. (L.) major* LPG since it was detected at low concentration in *Lu. longipalpis s.l.* and was absent in *Phlebotomus sergenti* and *Phlebotomus argentipes* (Kamhawi et al., 2004).

Some studies showed the role of LPG in the *Leishmania*-parasite interaction. Butcher et al. (1996) showed that a strain of *L. (L.)major* LPG mutant was not able to adhere on epithelial surface of its natural vector *Ph. papatasi*. In addition, parasite-phlebotomine interactions seem to occur through a LPG-independent mechanism. Experimental infections showed that *Phlebotomus perniciosus* and *Lu. longipalpis s.l.* permitted the development of *L. (L.)major* wild types and LPG mutants in the same proportion, suggesting that the interaction between the parasite and these sand fly species was not mediated by LPG (Myskova et al., 2007; Svárovská et al., 2010).

One of possible mechanism involved in the interaction of permissive phlebotomines and parasites probably depends on the carbohydrates that are present on the midgut epithelium which are recognized by lectins that are present on the surface of *Leishmania* (Azevedo-Pereira et al., 2007; Côrtes et al., 2012). Midgut biochemical analyses of the permissive phlebotomines *Lu. longipalpis s.l.*, *Phlebotomus halepensis*, *Ph. perniciosus*, *Ph.argentipes* and *Phlebotomusarabicus* showed that all of these species present N-acetyl-galactosamine (GalNAc) residues on the microvillar border whereas the midguts of specific vectors lack GalNAc (Myskova et al., 2007).

Although several studies have established the structure and the role of LPG in *Leishmania*-sand fly interactions, there are few studies about the characterization of epithelial carbohydrate residues in the midgut of sand flies. Therefore, this is the first study of carbohydrate characterization of epidemiological important species from the Amazonian region using lectins to identify GalNAc, mannose, galactose and GlcNAc residues of the midgut epithelium of sand flies.

2. Materials and methods

2.1. Sand flies

2.1.1. Sand flies from colony

Lutzomyia longipalpis s.l. specimens were obtained from a long-term closed colony (F38) of Instituto Evandro Chagas (IEC) primarily captured in the municipality of Cametá. The insects were reared and maintained in a closed colony at 25 °C, 80% relative humidity on a 50% sucrose diet.

2.1.2. Sand flies from field

Phlebotomines were captured in Barcarena (01°30′24″ S and 48°37′12″ W) and Cametá (02°14′40″ S and 49°29′45″ W) municipalities of Pará State. The capture of phlebotomines was carried out from 2013 to 2015 for five consecutive days per year using CDC light traps (Center for Disease Control and Prevention) that were installed at 5:00 p.m., and the sand flies were collected at 6:00 a.m. of the following day. The traps were installed at 1.5 m above ground level in the peridomiciliary and forested environments. Captured sand flies were transported to the laboratory and identified according to Young and Duncan (Young and Duncan, 1994).

2.2. Detection of glycoconjugates on the sand fly midgut by FITC labeled lectins

After four days of sugar feeding, female sand flies from both the colony and field populations were dissected in drops of 1% paraformaldehyde. Three midguts from different species were used for each lectin. The midguts were fixed in 4% paraformaldehyde at 4 °C for 20 min and opened longitudinally by a 1 mL needle syringe under a Zeiss stereoscopic microscope. Non-specific sites were blocked by 1% Bovine Serum Albumin (BSA) for 30 min. After that, the midguts were incubated for 1 h in a dark, humid chamber, separately, with FITC- conjugated lectins *Helix pomatia* agglutinin (HPA), concanavalin-A (CON-A), peanut agglutinin (PNA) and wheat germ agglutinin (WGA) (1:100), that bind specifically to GalNAc, mannose, galactose and GlcNac, respectively (Sharon and Lis, 2004). Subsequently, the midguts were washed three times in PBS pH 8.0 for 10 min and incubated with 4′,6-diamidino-2-phenylindole (DAPI) DNA marker and actin/myosin marker (phalloidin) for 40 min. In the negative control, HPA lectin was incubated first with 250 mM of GalNAc for 1 h. To perform the negative control of mannose, galactose and GlcNAc, the midguts were first incubated with non-fluorescent lectins. After this time, samples