Original Article

Morphological Specifications of the Bird Schistosome Cercariae and Surface Carbohydrates as Receptors for Lectins

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Abstract

Background: To determine the morphological specifications of the bird schistosomes cercaria from *Lymnaea gedrosiana* and to detect the surface carbohydrates as receptors for host lectins in the host-parasite relationship systems such as avian schistosomiasis and human cercarial dermatitis.

Methods: One hundred ninety two snails collected from Dezful areas in Khuzestan Province, in the south west of Iran, during 2005-2006 were examined for cercariae using shedding and crushing methods. In addition, surface carbohydrates on the cercariae were detected by lentil (Lens culinaris) lectins.

Results: From the total number of *Lymnaea gedrosiana*, which examined for bird schistosomes cercaria, 9(4%) snails were found to be infected with furcocercus cercaria of the bird schistosomes (probably *Gigantobilharzia* sp.). Mannose monosaccharide $CH_2OH\ (CHOH)_4CHO$ as surface carbohydrate was also detected on the cercariae.

Conclusion: Mannose carbohydrate on these cercariae may be used as receptor by lectins.

Keywords: *Bird, Mannose, Dermatitis, Iran*

Introduction

The surface carbohydrates in trematodes, cestodes and nematodes are species and stage related. In trematodes, the qualitative and the quantitative changes in the surface carbohydrate compound have been reported during the life cycle (1-3). Cercariae as larva of trematodes spend a short time in an intermediate host and then leave their host. They are covered by a thick glycocalyx coat, which serves as an osmotic protection during their free existence (4).

Khuzestan Province in the south west of Iran, has many canals and ponds which are using for bathing, drinking and washing by the people and these places are suitable for living of *Lymnaea* spp. Snails (5). Because of the presence of infected *Lymnaea* spp., water resources could be contaminated by the emerging cercariae including bird schistosomes, *Trichobilharzia* spp., and therefore consequently the bird schistosome cercariae to make an attack to the native residents directly via the skin (6).

Following our studies on cercarial dermatitis in Khuzestan Province, this research was conducted to identify the more specfications of the obtained bird schistosome cercariae as agent of cercarial dermatitis (swimmer itch) from *L. gedrosiana* and to detect the carbohydrates on their surface

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as receptors for lectins of the animal host in the cases of avian schistosomiasis and human host in cercarial dermatitis.

Materials and Methods

The Collection of the bird schistosome cercariae from examined snails

This study was performed on *L. gedrosiana* snails in Edalat and Cham golak districts in the south and east of Dezful region, Khuzestan Province, Iran. One hundred ninety two snails were collected from the drain, pond, canals and waterway and transferred to Dezfoul Health Research Center and then to Helmin-thological Unit at the School of Public Health, Tehran University of Medical Sciences as alive. The snails were keeping in the aquarium and cercariae obtained by means of the shedding and crushing methods. In the shedding method, the snails were put in the petridish containing dechlorinated tap water and placed against light for two hours or over night in the room. In the crushing method, the cercariae were collected by crushing snails in a glass plates. The collected of the bird schistosome cercariae (furcocercus cercariae) were observed as alive and then the fixed in the hot formalin (5%). The measurements and drawing pictures were prepared on the living or fixed specimens under light cover glass pressure and rather stained with neutral red or azocarmine. Cercariae were identified by systematic key references (7).

Recognition of the surface carbohydrates on the bird schistosome cercariae

To detect the surface carbohydrate, (fluorescein isothiocyanate) FITC-conjugated lectins were used. For this purpose, FITC-lectin was added to the suspension of the whole cercariae in the test tubes and added FITC-lectin to the control tubes containing 100 mM inhibitory sugar (mannose). The tubes were incubated at 4 °C for 60 min and washed three times by centrifugation (3000 rpm for two min) in PBS. Samples were mounted on slides and observed under fluorescence microscope (8).

Results

From the examined L. *gedrosiana*, 9 (4%) were infected by the bird schistosome cercariae. Good features for recognition of the furcocercus cercaria were the bifurcated tail, which was considerably longer than the body and the bifurcated parts which were less than half as long as the tail stem. The cercariae had less distinguishable eye spots and five pairs of penetration gland which were around of ventral sucker. The pharynx and oral sucker were fused together into a head organ and the intestinal system was reduced. The morphometric measurements of the bird schistosome cercariae are presented in the Table 1. The cercariae were strongly positively phototactic, i.e. they swimed towards a light source and congregated there. The cercariae were recognized as bird schistosome cercariae and probably *Gigantobilharzia* sp. (Fig. 1, 2).

Mannose monosaccharide, $CH_2OH(CHOH)_4CHO$, was detected as surface carbohydrates on the tail and body of cercariae at a 1/25 dilution. Oral sucker, ventral sucker and excretory bladder did not show localization of mannose on the body of cercariae (Fig. 3). Due to covering FITC-lectin by inhibitory sugar on cercariae in the control samples, cercariae were not observable at above dilution under fluorescence microscope.

Table 1: The morphometric measurements of the bird schistosomes cercaria

Organs of cercaria	Measurement in micrometer
Total length	380-493
Body length	210-290
Tail stem length	170-293
Bifurcate parts length	70
Body width	50
Tail stem width	30
Head organ size	35 ×10
Ventral sucker size	20 ×20

A **B**

Fig. 1: The collected cercariae of the bird schistosomes (probably *Gigantobilharzia sp.*) from *Lymnaea gedroziana* (A. Fresh sample; B. Stained sample; X 400)

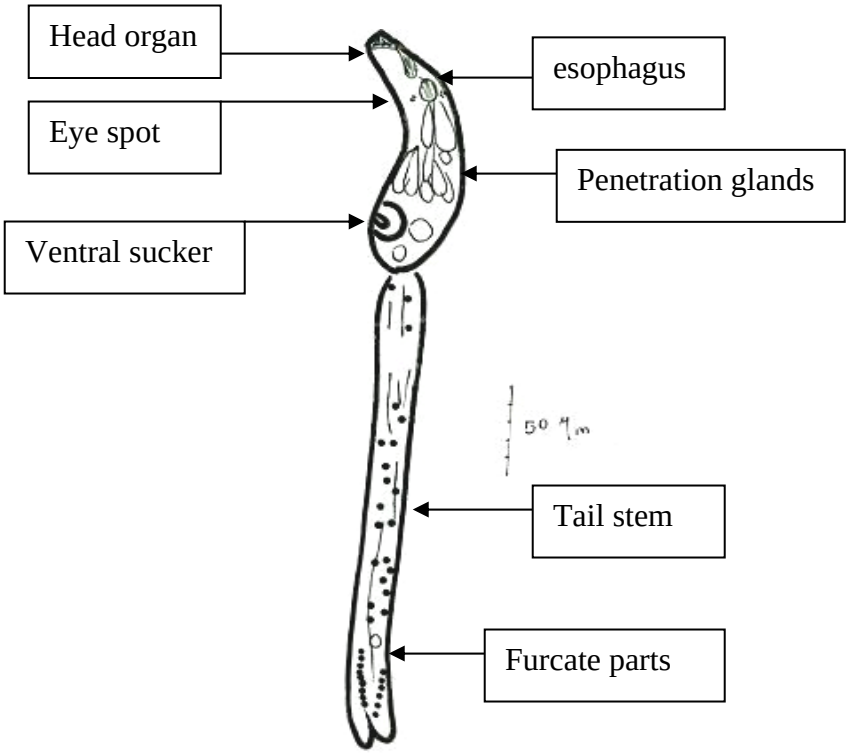

Fig. 2: The drawing picture of the bird schistosome cercaria from *Lymnaea gedrosiana* (X 400)

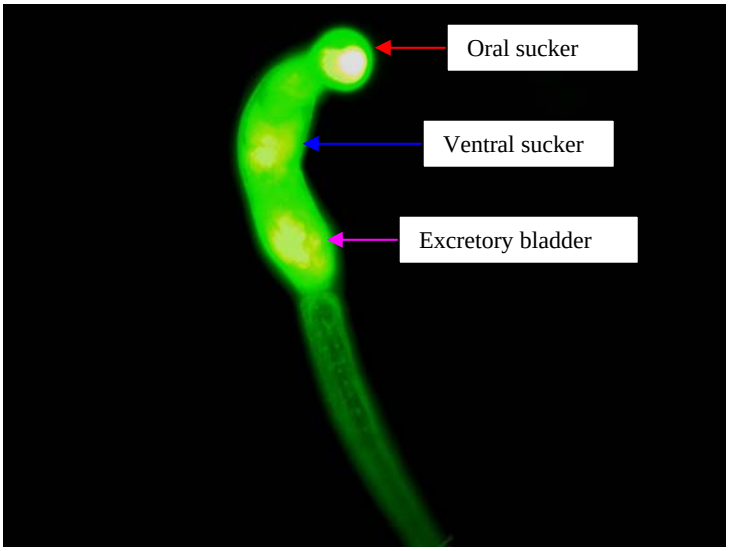

Fig. 3: The detected mannose saccharid on the surface of the bird schistosomes cercaria by the lectin (X 400)

Discussion

Many water resources around the world are contaminated with bird schistosome cercariae, which penetrate into human skin, causing an itching dermatitis called cercarial dermatitis. Gigantobilharzia cercaria reported as an agent of cercarial dermatitis in the world from more than 50 years ago and therefore, we can suppose, it may be one of the agents of cercerial dermatitis in Khuzestan Province (6, 9).

The collected bird schistosome (probably *Gigantobilharzia* sp.), could be confused with strigeid cercariae, however the former without pharynx. The obtained cercariae are belonging to none-human schistosome and PCR-RFLP analysis of the ITS2 region has been used to identify of them from human schistosomes cercariae (10).

Characterization of the carbohydrates of *Schistosoma japonicum* cercariae by analysis of lectin binding and antibody reaction have been demonstrated, lectin binding receptors are located at the same sites, where also labeled antibodies reacted (11). In addition, surface carbohydrate residues on larval stages of the avian schistosome, *Trichobilharzia* spp., have been revealed, surface saccharide residues on trematode larvae are supposed either to be the targets of the intermediate (molluscan) and final host immune systems (12).

Recently, we have detected mannose carbohydrate on xiphidiocercaria from *Lymenea gedrosiana* and mentioned their importance to entrance mechanisms of cercariae into culex larva as a model for cercarial dermatitis (13). It seems that the detected mannose saccharides on cercariae may be as receptor for human lectins in adhesion process of cercariae to animal or human host. Probably cercaria surface glycosylation patterns reflect to the interaction with their next host including human.

In Conclusion, the results of this research partially support of this theory which, mannose carbohydrate on bird schistosome cercariae could be used as receptor by lectines of animal host in the cases of animal schistosomiasis and human hosts in cercarial dermatitis.

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References

1. Horak P. Lectins of parasitic helminths: a review. Helminthologia. 1996; 33: 209-12.
2. Georgieva K, Mizinska-Boevska YA. Surface carbohydrates in helminthes (cytochemical review). Experimental Pathology and Parasitology. 1999; 3: 32-7.
3. Horak P. Developmentally regulated expression of surface carbohydrate residues on larval stages of the avian schistosome Trichobilharzia szidati. Folia Parasitologica. 1995; 42(4): 255-65.
4. Sanmuelson J, Caulfield J. The cercarial glycocalyx of Schistosoma mansoni. J Cell Biol. 1985; 100(5): 1423-34.
5. Mansoorian A. Fresh water Gastropod of Khuzestan Province, South-West of Iran. Iranian International J Science. 2001; 2(2): 96-103.

6. Farahnak A, Essalat M. A study on cercarial dermatitis in Khuzestan Province, southwestern Iran. BMC Public Health. 2003; 3: 35.
7. Morishita K, Komiya Y, Matsubayashi H. Progress of medical parasitology in Japan. Volume 1. Meguro Parasitological Museum. Tokyo; 1964. p. 429-539.
8. Maizels RM, Blaxter ML, Robertson BD, Selkirk ME. Parasite antigen and parasite genes: A laboratory manual for molecular parasitology.1st ed. Cambridge University Press UK; 1991. p. 93-5.
9. Komiya Y, Ito J. The morphology of Cercaria sturniae Tanabe, 1948 (cercaria of Gigantobilharzia sturniae Tanabe, 1951), a cause of cercaria dermatitis in Japan. Jpn J Med Sci Biol. 1952; 5(4): 215-20.
10. Barber KE, Mkoji GM, Loker ES. PCR-RFLP analysis of the ITS2 region to identify Schistosoma haematobium and S. bovis from Kenya. Am J Trop Med Hyg. 2000; 62(4): 434-40.
11. Beisler GK, Nakao M, Matsuda H, Tanaka H. Characterization of the carbohydrates of Schistosoma japonicum adult worm, egg and cercaria by analysis of lectin binding and antibody reaction. Jpn J Exp Med. 1984; 54(6): 263-73.
12. Horak P. Developmentally regulated expression of surface carbohydrate residues on larval stages of the avian schistosome Trichobilharzia szidati. Folia Parasitol. 1995; 42(4): 255-65.
13. Farahnak A, Dabagh N. Adhesion of Larva (Helminthes Parasites) to Host by Lectins-carbohydrates bonds as a Model for Evaluation of Schistosoma Entrance Mechanisms. Iranian J Publ Health (in press).