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Specificity of Snail Derived Antigens in Diagnosis of their Trematode Parasites

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Abstract: In this study, specificity of snail feet and hepatopancreases antigens in antibody detection of their trematode parasite was evaluated via Western blot technique. Snails, of two different families, antigens; *Lymnaea cailliaudi* as intermediate host of *Fasciola gigantica* and *Biomphalaria alexandrina* as intermediate host of *Paramphistomum microbothrium*, were evaluated in detection of IgG antibodies against their trematode parasites after preparation of the required Hyper-Immune Sera (HIS) in rabbits. The results revealed higher specificity of snail feet in antibody detection than hepatopancreases antigens. Where, three of sex polypeptides of *L. cailliaudi* feet antigen; identified by *F. gigantica* HIS, showed specific positive reactivity. These polypeptides were at molecular weights of 59, 57 and 52 kDa. While, one of sex polypeptides of *L. cailliaudi* hepatopancreases antigen; identified by *F. gigantica* HIS, at molecular weight of 57 kDa was specific. Similarly, two polypeptides of *B. alexandrina* feet antigen; at molecular weights of 54 and 45 kDa, showed specific reactivity toward anti-*paramphistomum* antibodies. At a time, no specific reactivity had been shown by the antigenically active polypeptides of *B. alexandrina* hepatopancreases antigen. Owing to its cross-reactivity with incompatible trematode parasite, snail antigen can be used as a general starting antigen for immunodiagnosis of trematode infections.

Key words: *Lymnaea*, *Biomphalaria*, *Fasciola*, *Paramphistomum*, Western blot

INTRODUCTION

The concept of antigen sharing between trematodes and their intermediate hosts and the possibility of using common antigens for serodiagnosis of their target parasites are currently receiving considerable attention. This is particularly evident in the area of schistosomiasis, where common antigens between schistosomes and their intermediate hosts were described (Wide *et al*., 2006). A shared antigen between *Schistosoma mansoni*, *Fasciola hepatica* and *B. glabrata* was isolated, purified and characterized by Rasmussen *et al.* (1985). This antigen showed a limited value for the specific immunodiagnosis of schistosomiasis. Alarcon de Noya *et al.* (1989) cleared that serum from *Schistosoma* infected persons reacted versus crude *B. glabrata* antigen by ELISA; with sensitivity rate reached up to 100%. Chacon *et al.* (2002) confirmed the presence of common antigens between *S. mansoni* and its vector, *B. glabrata* and suggested that soluble

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B. glabrata antigen constituted a rich source of candidate antigens for diagnosis and prophylactic studies. Three *Bithynia* species, the snail hosts of *Opisthorchis viverrini*, provided antigenic materials for the antibody detection of human opisthorchiasis (Chanawong and Waikagul, 1991). It was found that each of whole body, head-foot and hepatopancreatic tissue antigens of *B. funiculata* was as effective as crude *O. viverrini* adult worm antigen for the ELISA detection of *O. viverrini* antibodies in infected patients.

In previous study, Shalaby (2004) demonstrated successful ELISA detection of *Fasciola gigantica* IgG antibodies in naturally infected animals using *Lymnaea cailliaudi* feet and hepatopancreases antigens and higher sensitivity of the latter antigen in antibody detection than the former. At a time, he found by SDS-PAGE that there were shared antigens between *F. gigantica* excretory-secretory, *L. cailliaudi* feet and *L. cailliaudi* hepatopancreases antigens. But, specificity of those antigens in detection of trematode infection still remains as a question. Consequently, in this study, specificity of snails, of two different families, antigens; *L. cailliaudi* as intermediate host of *Fasciola gigantica* and *B. alexandrina* as intermediate host of *Paramphistomum microbothrium*, in detection of IgG antibodies against their trematode parasites was evaluated using Western blot technique after preparation of the required hyper-immune sera in rabbits.

MATERIALS AND METHODS

The study was undertaken during the period from Oct. 2009 to Feb. 2010, at National Research Center, Egypt.

Preparation of Antigens

Snail Antigens

Snails of two different families were selected; *L. cailliaudi* as intermediate host of *F. gigantica* and *B. alexandrina* as intermediate host of *Paramphistomum microbotyrium*. Field collected snails were identified according to Brown (1994) and reared in the laboratory for production of laboratory-bred snails according to El-Bahy (1984). They were used for antigen preparation according to Khalil *et al.* (1985) and El-Bahy and Shalaby (2004). Where, feet and hepatopancreases of laboratory bred mature non-infected snails were dissected. The collected tissues were homogenized in an equal volume of 0.01 M phosphate buffered saline, pH 7.4 (PBS) and sonicated for 5 min under 150 watt interrupted pulse cycle using a sonifier cell disrupter. Then, they were centrifuged at 5000 rpm for 1 h at 4°C. The supernatant was dialyzed in 6-8 kDa dialysis tubes overnight at 4°C against 4 M urea buffer. Thereafter, they were concentrated by absorption against polyvinyl pylorridone. The protein content was measured by the method of Lowry *et al.* (1951) and stored at -70°C until used.

Parasite Antigens

F. gigantica and *P. microbothrium* crude worm antigens (CWAs) were prepared from anterior parts of fresh extracted adult worms collected from fresh condemned buffaloes' liver and rumen, respectively, at a local abattoir. The specimens were washed repeatedly in 0.01 M PBS, pH 7.4. and homogenized at 6000 rpm for 20 min and then subjected to high-speed centrifugation (10000 rpm) for 1 h at 4°C. The supernatant was separated as crude antigen after the protein content had been measured as above and stored at -70°C until used (Shalaby, 1998).

Preparation of Rabbit Hyper-Immune Sera (HIS)

Rabbit hyper-immune sera were raised against all previous snail and parasite antigens as described by Langley and Hillyer (1989) via initial subcutaneous injection in an equal volume of Freund´s complete adjuvant and three consecutive intramuscularly injections in an equal volumes of Freund´s incomplete adjuvant during 60 days. The level of specific antibodies in sera of immunized rabbits was evaluated before slaughter. Rabbits were bled before immunization as negative control sera.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS- PAGE) and Western Blot Techniques

Protein fractions of tested snail antigens, *L. cailliaudi* feet, *L. cailliaudi* hepatopancreases, *B. alexandrina* feet and *B. alexandrina* hepatopancreases, were demonstrated using 12% SDS-PAGE (100 μg/lane) according to Laemmli (1970) with the aid of high and low molecular weight standards (Pharmacia Biotech). The fractionated snail antigens were transferred onto nitrocellulose sheet for Western blot technique according to Towbin *et al.* (1979). The nitrocellulose strips blotted with snail antigens were tested in group of four as in Fig. 2:

- The first strip was used to test the recognition of the original molecules of snail antigen by homologous immune sera
- The second one was allowed to react with rabbit HIS raised against the compatible trematode parasite
- The third one was allowed to react with rabbit HIS raised against the incompatible trematode parasite
- The fourth one was allowed to react with rabbit pre-immune control sera

The molecular weight of specific and non-specific polypeptides was determined using molecular weight standard curve as described by the producer (Pharmacia).

RESULTS

Electrophoretic profile of snail antigens was resolved by SDS-PAGE into multiple components at both high and low molecular weight ranges (Fig. 1). *L. cailliaudi* feet and hepatopancreases antigens revealed at least 10 and 13 polypeptides, respectively. Those polypeptides molecular weights ranged from 16-160 kDa. Of them, the protein bands at molecular weight of about 66, 45, 34, 28 and 20 kDa in *L. cailliaudi* feet and 97, 54, 45 and 28 kDa in *L. cailliaudi* hepatopancreases were major bands (Fig. 1).

Concerning *B. alexandrina* feet and hepatopancreases antigens, there were 8 and 7 polypeptides in each antigen, respectively. Their molecular weights ranged from 21-97 kDa. The components of 97 and 30 kDa were major bands in *B. alexandrina* feet while, that of 66, 30 and 20 kDa were major bands in *B. alexandrina* hepatopancreases (Fig. 1).

To clarify specificity of the recorded snail polypeptides, the adopted Western blot technique (Fig. 2) revealed that antigenically active components in *L. cailliaudi* feet, on reaction with its homologous HIS, were eight polypeptides at molecular weights of 94, 85, 75, 70, 66, 59, 57 and 52 kDa (Fig. 2, Lane A). All those polypeptides except that of 85 and 75 kDa were recognized by *F. gigantica* HIS (Fig. 2 Lane B). While, *P. microbothrium* HIS reacted crossly with three polypeptides at molecular weights of 94, 70 and 66 kDa (Fig. 2, Lane C). At a time, no polypeptides were identified by pre-immune rabbit sera (Fig. 2 Lane D). Therefore, the three polypeptides of 59, 57 and 52 kDa were considered to be specific in

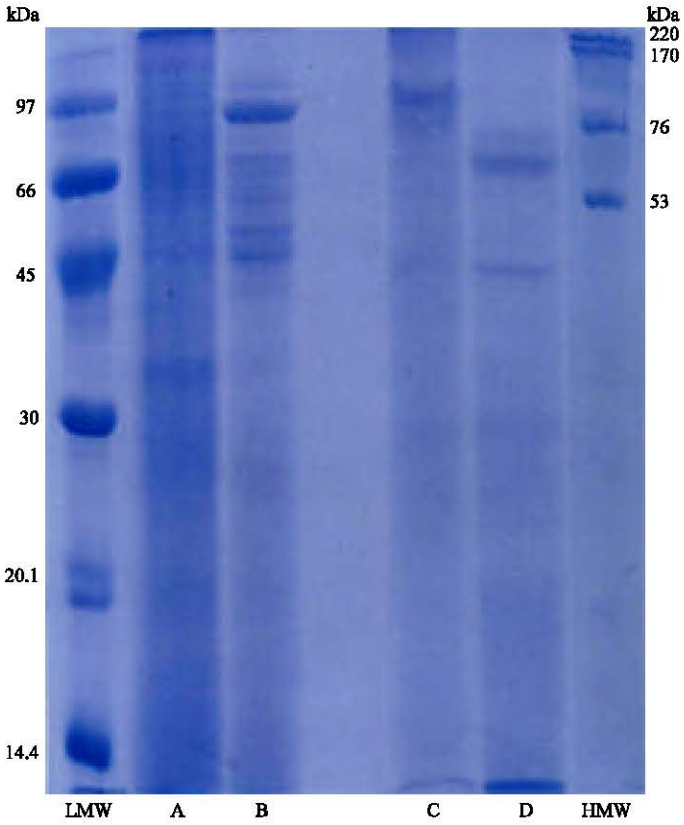

Fig. 1: SDS-PAGE of *L. cailliaudi* feet (Lane A), *L. cailliaudi* hepatopancreases (Lane B), *B. alexandrina* feet (Lane C) and *B. alexandrina* hepatopancreases (Lane D) antigens. LMW. Low Molecular Weight marker; HMW. High Molecular Weight marker

detection of anti-*Fasciola* antibodies. On the other hand, *L. cailliaudi* hepatopancreases antigen revealed seven antigenically active polypeptides at molecular weights of 97-85, 70, 57, 54, 45, 42 and 38 kDa (Fig. 2, Lane E). All those polypeptides were recognized by *F. gigantica* HIS except that of 38 kDa (Fig. 2 Lane F). From those polypeptides, five at molecular weights of 97-85, 70, 54, 45 and 42 kDa reacted crossly with *P. microbothrium* HIS (Fig. 2, Lane G). The polypeptide of 97-85 kDa was recognized by pre-immune rabbit sera (Fig. 2, Lane H). Thus, only the polypeptide at molecular weight of 57 kDa was specific in detection of anti-*Fasciola* antibodies.

Concerning *B. alexandrina* feet antigen, two polypeptides at molecular weights of 54 and 45 kDa of six antigenically active polypeptides recognized by its homologous HIS (Fig. 2, Lane I) showed specific reactivity toward anti-*paramphistomum* antibodies. Where, both polypeptides were identified by *P. microbothrium* HIS (Fig. 2, Lane J) and did not react crossly with neither *F. gigantica* HIS (Fig. 2, Lane K) nor pre-immune rabbit sera (Fig. 2, Lane L). On the other hand, the antigenically active polypeptides of *B. alexandrina* hepatopancreases antigen (Fig. 2, Lane M) had no specific reactivity toward anti-*Paramphistomum* antibodies. Where, similar antigenic profiles were observed with both *P. microbothrium* HIS (Fig. 2, Lane N) and *F. gigantica* HIS (Fig. 2, Lane O).

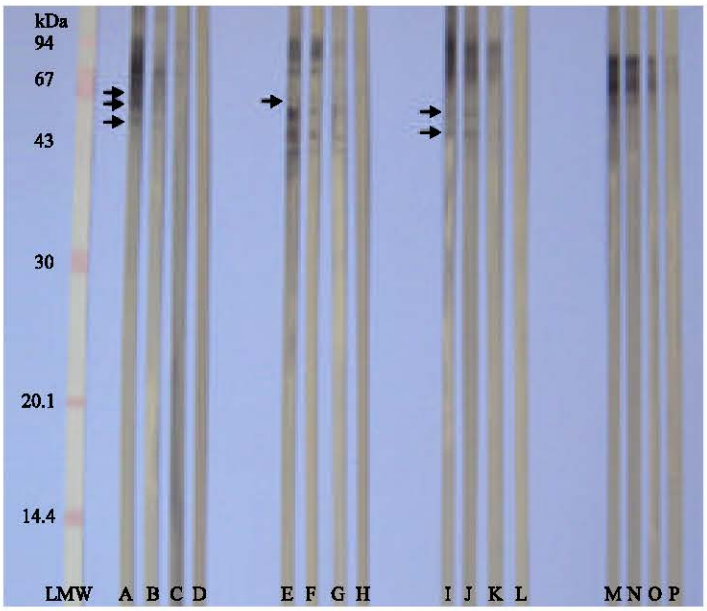

Fig. 2: Recognition of different snail antigens by homologus and trematode parasites HIS using Western blot. Lanes A-D. *L. cailliaudi* feet antigen; Lanes E-H. *L. cailliaudi* hepatopancreases antigen; Lanes I-L. *B. alexandrina* feet antigen; Lanes M-P. *B. alexandrina* hepatopancreases antigen; Lanes A, E, I and M. With homologus HIS; Lanes B, F, J and N. With compatible trematode parasite HIS; Lanes C, G, K and O. With incompatible trematode parasite HIS; Lanes D, H, L and P. With pre-immune control sera; LMW. Low Molecular Weight marker stained with Ponceau S solution; Arrows point to the specific bands

DISCUSSION

Detection of circulating anti-snail antibodies in trematode infected definitive hosts might prove of value in diagnosing trematode infection (Rasmussen *et al*., 1985). It had been proposed that the presence of common antigens between some parasites and their hosts would facilitate the search for diagnostic targets, when observing the abundance of antigenic material that could be obtained from those hosts, as demonstrated in fasciolosis. The existence of shared antigens between *F. gigantica* and *L. cailliaudi* had been shown by the presence of anti-snail antibodies in animals naturally infected with the parasite. The opposite had also been demonstrated in animals immunized with uninfected snails, which had shown anti-*Fasciola* antibodies (Shalaby, 2004). The previous study by Shalaby (2004), demonstrated higher sensitivity of *L. cailliaudi* hepatopancreases than feet antigens in detection of anti-*Fasciola* antibodies but, their specificity was not shown. That concept was extended in the present study to evaluate specificity of snail feet and hepatopancreases antigens in antibody detection of their trematode parasite via Western blot technique. The results revealed higher specificity of the former antigen in antibody detection than the latter. Where, three of the sex polypeptides of *L. cailliaudi* feet antigen; identified by *F. gigantica* HIS, showed specific positive reactivity. These polypeptides were at molecular weights of 59, 57 and 52 kDa. While, one of the sex polypeptides of *L. cailliaudi* hepatopancreases

antigen; identified by *F. gigantica* HIS, at molecular weight of 57 kDa was specific. Similarly, two polypeptides of *B. alexandrina* feet antigen; at molecular weights of 54 and 45 kDa, showed specific reactivity toward anti-*paramphistomum* antibodies. At a time, no specific reactivity had been shown by the antigenically active polypeptides of *B. alexandrina* hepatopancreases antigen. In that sense, Watthanakulpanich *et al.* (1997) evaluated crude antigens, prepared from the whole body, head-foot and hepatopancreatic tissue of *B. funiculata*, for the ELISA detection of *O. viverrini* antibodies in infected patients. Although, it was promising to find that the snail antigens could be used to detect *O. viverrini*, cross-reactions with *Paragonimus* and *Strongyloides* occurred. In a chromatographic study (Waikagul *et al*., 2002), four peaks of *B. funiculata* extract were obtained and analyzed by indirect ELISA. Each of four peaks had a sensitivity of 100, 27.5, 28.8 and 37.5%, respectively and a specificity of 36, 96, 52 and 82%, respectively. They suggested that electroeluted antigen at molecular weight of 53 kDa, notwithstanding its lower antigenicity, might have an important role in diagnosis of opisthorchiasis (Waikagul *et al*., 2002).

The extensive cross-reactivity of snail antigens with incompatible trematode confirmed the findings reported by Kemp *et al.* (1982) and Rasmussen *et al.* (1984) in which antigens obtained from *S. mansoni* adult worms by affinity chromatography through an anti-*B. glabrata* column reacted strongly with serum of humans and mice infected with *F. hepatica*. Moreover, Rasmussen *et al.* (1985) isolated, purified and characterized shared antigens between *S. mansoni* and *B. glabrata* from *S. mansoni* adult worms through a CNBr-activated Sepharose 4B column coupled with rabbit IgG prepared against a homogenate of *B. glabrata* hepatopancreases which would be of use as a specific immunodiagnostic test for schistosomiasis. The eluted antigen (designated as SMw-53P) failed to qualify as a candidate for such a reagent due to its cross-reactivity with *F. hepatica*.

The present study provided further evidence of shared antigens between trematode parasite and its intermediate snail host and extended these observations by demonstrating the specificity of these antigenic epitopes. These epitopes showed limited degree of immunologic specificity due to its cross-reactivity with incompatible trematode parasite. Therefore, snail antigen can be used as a general starting antigen for immunodiagnosis of trematode infections. Its advantage is that large amounts of material can be obtained rapidly and at comparatively lower cost than trematode antigens which require maintenance of a complex life cycle.

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