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ORIGINAL PAPER

Structural changes of *Schistosoma mansoni* adult worms recovered from C57BL/6 mice treated with radiation-attenuated vaccine and/or praziquantel against infection

Enayat S. Reda · Allal Ouhtit · Sherif H. Abdeen · Eman A. El-Shabasy

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Abstract Although the current treatment of schistosomiasis relies largely on praziquantel (PZQ), it has not significantly reduced the overall number of disease cases, perhaps due to inevitable resistance to PZQ. Previous studies showed that radiation-attenuated vaccine gives protection levels for *Schistosoma mansoni* in host various species. In the present study, we evaluated the effect of various vaccination strategies in C57BL/6 mice, including single or multiple vaccination strategy, subcurative dose (20 mg/kg) of PZQ, and a combination of single vaccination with subcurative dose of PZQ. Groups of five mice were sacrificed postinfection in 42 days and schistosomes were collected by perfusion and examined by scanning electron microscopy. Treatment either with subcurative dose of PZQ or with a single vaccination of attenuated cercariae (500 per mouse), caused significant reduction in total worm burden, hepatic and intestinal ova counts 43.03%, 73.2%, 59.5% and 37.97%, 52.02%, 26.3%, respectively. Furthermore, tegumental changes were observed, including severe swelling, fusion of tegumental folds, vesicle formation, and loss or shortening of the spines on the tubercles. However, multiple vaccination strategy resulted in much higher reduction in total worm burden, hepatic and intestinal ova count. However, multiple vaccination strategy resulted in high reduction of worm burden, hepatic and intestinal ova counts 72.5%, 90.7%, 65.79%, respectively, and further causing swollen, disruption of tubercles teguments and erosion, extensive peeling, fusion of tegumental folds. Our findings suggest that multiple vaccination strategy is the most effective strategy to clear schistosomal infection, indicating its potential in guiding the design of appropriate therapeutic strategy against schistosomes.

E. S. Reda (✉) · S. H. Abdeen · E. A. El-Shabasy
Department of Zoology, Faculty of Science, Mansoura University, Mansoura, Egypt
e-mail: enayatsalem40@yahoo.com

A. Ouhtit (✉)
Department of Genetics, College of Medicine and Health Sciences, Sultan Qaboos University,
Muscat, Oman
e-mail: aouhtit@squ.edu.om

Introduction

Schistosomiasis is a disease caused by the blood fluke *Schistosoma* spp. (class Trematoda, family Schistosomatidae). *Schistosoma haematobium, Schistosoma japonicum*, and *Schistosoma mansoni* are the three main species parasitizing humans (Utzinger and Keiser 2004; Gryseels et al. 2006). It has been estimated that more than 207 million people are infected worldwide, with 780 million at risk of infection (Steinmann et al. 2006; Caffrey 2007). *S. mansoni* is the most prevalent, being endemic in 54 countries, mostly in Africa and parts of South America (Chitsulo et al. 2000). The annual mortality rate in Africa is estimated to be as high as 280,000 (van der Werf et al. 2003).

Due to unavailability of schistosomiasis vaccine (Bergquist and Colley 1998; Katz 1999; Bergquist et al. 2002; Hagan and Sharaf 2003), the current strategy for the control of schistosomiasis depends only on a drug namely praziquantel (PZQ), which is used in the treatment of three major species of schistosomes infecting humans (Fenwick et al. 2003; Doenhoff et al. 2009). However, drug resistance has been a challenging issue (Bergquist 2002; Doenhoff et al. 2002; Sangster 2001). Therefore, the development of a vaccine against *S. mansoni* would provide a powerful tool to prevent and cure the disease. Radiation-attenuated (RA) schistosome

vaccine is highly effective under laboratory conditions, but for ethical and practical reasons, it cannot be used in humans. However, it serves as a compelling model for the development of a recombinant vaccine (Coulson 1997; Wilson and Coulson 1999). The protective levels of RA vaccine were originally established in mice (Dean 1983) and subsequently extended to primates (Soisson et al. 1993; Yole et al. 1996a, b; Eberl et al. 2001).

The tegument of adult *Schistosoma* is a protective sheath that provides defense as well as a role in the uptake of nutrients, osomoregulation, and excretion. Hence, the importance of ultrastructural studies which can potentially clarify aspects of drug or vaccine induced damage. Furthermore, protective immunity could not be assessed by reduction of recovered worm burdens only, but also via indirect estimates of reduction in hepatic and intestinal ova count as well as the level of schistosome antigens in the bloodstream.

The data generated from our recent study (Abdeen et al. 2011) suggest that high levels of antibodies were developed in the vaccinated rabbit sera after multiple exposures to UV-attenuated cercariae of *S. mansoni;* this might explain a passive transfer of protection from the infection and elimination of the worms. Therefore, as a continuation of our recent study, the present study aims to compare vaccine efficacy of single or multiple vaccination strategy, subcurative dose (20 mg/kg) of PZQ, and a combination of single vaccination with subcurative dose of PZQ on the surface topography of adult *S. mansoni*.

Material and methods

Parasites and mice

S. mansoni cercariae (Egyptian strain) were collected from infected *Biomphalaria alexandrina* snails. Female C57BL/6 mice weighing 35–40 g were obtained from the Biological Production Unit of Theodore Bilharz Research Institute (Giza, Egypt). All the experiments described in this study were approved by the local ethical committee.

Drug

PZQ in the form of tablets was ground to white powder and suspended in 2% Cremophore-EL, as it is insoluble in water. The drug was freshly prepared before oral administration using oral canola. The dose given was 20 mg/kg body weight at the fifth week postinfection.

Experimental design

Mice (n=50) were immunized with 500 attenuated cercariae per mouse or infected percutaneously for 1 h by tail immersion to 200 normal *S. mansoni* cercariae. Prior to mice infection or immunization, *S. mansoni* cercariae were shed from experimentally infected *B. alexandrina* after exposure to artificial light for 4 h, by looping method as previously reported by Sornmani et al. (1973). Fifty mice were used in the experiment, divided into four main groups as follow:

1. The naive-infected group of ten mice (NC) which takes infection with normal cercariae (200 cercariae per mouse).
2. The UV-attenuated group which include two sub-groups (ten mice each) as follow:
   a. Vaccinated-challenged group (VC) which was immunized in the first week and challenged in the sixth week then sacrificed at 6 weeks postchallenge.
   b. The multiply vaccinated-challenged group (V4C) which was immunized in the 1st, 5th, 11th, and 17th and challenged in 23 weeks then sacrificed at the sixth week postchallenge.
3. Chemotherapy group which include two sub-groups (ten mice each):
   a. Naive-infected chemotherapy group (NCC) which takes infection (200 cercariae per mouse) in the first week and chemotherapy at the fifth week after infection and sacrificed at week 6 postinfection.
   b. The vaccinated-challenged chemotherapy group (VCC) which takes UV-attenuated 500 cercariae per animal in the 1st week, challenged (200 normal cercariae per mouse) in the 6th week then chemotherapy at 11th week and sacrificed at week 6 postchallenge (infection).

All animals were sacrificed 6 weeks (42 days) postinfection (Table 1). Adult worms were collected from mice by perfusion method (Sornmani et al. 1973) using 0.1 M citrate in 0.15 M NaCl solution. The worms were washed several times with normal saline solution and fixed in glutaraldehyde–phosphate buffer for scanning electron microscopic examination.

Scanning electron microscopic examination

Eight worms (seven to eight) were fixed in 2.5% glutaraldehyde–phosphate buffer (0.1 mol/L, pH 7.4) at 4°C for 24 h and postfixed in 1% osmium tetroxide buffer for 1 h. They were dehydrated through a graded series of ethanol and dried using liquid carbon dioxide as a transitional medium. After drying, they were mounted on stubs and coated with gold in an ion-sputtering apparatus. They were examined and photographed using the Joel Electron Microscope (Japan) operating at 25 kV. All measurements were taken in micrometers (μm).

Table 1 Experimental layout, indicating times of infection, single or multiple vaccinations with attenuated cercariae, PZQ administration, and with combination with single vaccination and perfusion

Groups	Subgroup	*n*	Time (weeks)										
			0	5	6	11	12	14	17	18	20	23	29
Control (NC)	–	10	Infection		Perfusion								
Chemotherapy	NCC	10	Infection	PZQ	Perfusion								
(PZQ)	VCC	10	1st vacc.		Infection	PZQ	Perfusion						
Attenuated cercariae	VC	10	1st vacc.		Infection		Perfusion					Infection	Perfusion
	V4C	10	1st vacc.	2nd vacc.		3rd vacc.			4th vacc				

Vacc. vaccination

Quantification of *S. mansoni* eggs in the liver and large intestine (ova count)

The method of Cheever (1970) was followed with minor modifications. Hepatic and intestinal tissues were removed from mice after perfusion, washed with normal saline, dried, weighed, and placed in 5 ml of 5% potassium hydroxide. Tissues were homogenized on ice for 20–30 s using a Polytron homogenizer (Brinkmann Instruments, Wesbury, NY, USA). The mixtures were allowed to incubate at 37°C for 24 h. At the end of the incubation period, the mixture was shaken well, 100 μl samples were removed, and the total number of eggs in the sample was counted under low power magnification. The results were reported as the mean total egg number of ten independently counted samples per gram tissue ± standard deviation.

Statistical analysis

Comparison between experimental and control groups was carried out using the Student's *t* test. The data were considered significant when $p \leq 0.05$ and highly significant when $p \leq 0.001$. The percent reduction of the number of worms was determined for each group according to the formula described below (Fonseca et al. 2004):

$$\% \text{ reduction} = 1 - \frac{\text{Mean number of IM group}}{\text{Mean number of IU group}} \times 100$$

where IU = mean number of worms in the control group and IM = mean number of worms in the immunized group.

Results

Effects of single subcurative dose of PZQ alone and in combination with single vaccination

The mean number of surviving worms was 47.43± 2.19, 27±2.77, and 82.4±1.6 in normal control (NC), single subcurative PZQ (NCC) and vaccinated chemotherapy (VCC) groups, respectively (Table 2). Moreover, the reduction of hepatic and intestinal ova counts were 73.2% and 59.5%, respectively in NCC group but 40.2% and 20.4% in VCC group were respectively (Table 3).

Effect of single and multiple vaccinations with attenuated cercariae

The mean number of surviving worms was 29±2.16 (percent reduction=37.97%) and 13±1.5 (percent reduction=72.57%) in vaccinated one time (VC), and vaccinated four times (V4C) groups, respectively (Table 2). Moreover, the reduction of hepatic and intestinal ova count were 52.02% and 26.3%, respectively in VC group

Table 2 Effect of single sub-curative dose of PZQ, single vaccination, in combination with single vaccination and multiple vaccination on the number of hepatic and intestinal ova count in *S. mansoni*-infected mice

Animal groups	Hepatic ova count (Mean±S.E.)	PR(%)	*P* value	Intestinal ova count (Mean±S.E.)	PR(%)	*P* value
NC	26,712.8±7,614.02	–	–	14,859±5,526.55	–	–
NCC	7,162.6±1,118.04	73.2	0.056	6,011±386.689	59.5	0.173
VC	12,815.6±1,698.629	52.02	0.164	10,954±1,939.637	26.3	0.485
VCC	15,976.2±5,854.62	40.2	0.141	21,254.6±5,218.19	20.4	0.285
V4C	2,486±93.62[a]	90.7	0.033[a]	5,083±548.09	65.79	0.137

[a] Significant difference to NC group

Table 3 Effect of single sub-curative dose of PZQ, single vaccination, in combination with single vaccination and multiple vaccination on the number of worm burden in *S. mansoni*-infected mice

Group of mice	Normal mice control group	NCC group	VCC group	VC group	V4C group
Mean	47.428	41.428	27.857	29.4	13
Std. deviation	6.877	6.205	3.507	4.83	3.39
±SE	3	2.77	1.6	2.16	1.5
t test	–	3.831	7.926	5.112	9.207
$P<0.05$	–	0.019[a]	>0.05	0.007[a]	0.001[a]

[a] Significant difference to normal control group

while in V4C group were 90.7% and 65.79%, respectively (Table 3).

Surface topography of *S. mansoni* from the normal mice control group (NC)

The adult male worm (M) appeared thicker and shorter (male length was 1,571.42 μm) than the female (F), and had a longitudinal cleft and the gynaecophoral canal, in which the female was hold during copulation. The surface between the oral and ventral suckers was devoided of spines but ciliated sensory papillae were detected (Fig. 1). The tegumental tubercles were arranged regularly in transverse rows (Fig. 2), with the presence of sensory papillae, and few dome-shaped papillae (DP) which were arranged irregularly between tubercles (Fig. 2). The average number of spines per tubercle was 74. Just after the ventral sucker, the genital pore (GP) was detected and its surface was porous, spineless, and surrounded with sensory dome-shaped papillae (Fig. 3). The genital pore was small and satellite in shape (Fig. 3). The left edge of the gynaecophoral canal was provided firstly with ciliated (CP) and dome-shaped (DP) papillae in addition to small spines on outer rim then spines were more crowded with ciliated and dome-shaped papillae (Fig. 4a, b). Along the canal, spines were observed not protruded in first region then protruded to extent then fully protruded as in Fig. 4b.

The length of the female worm reached 3,000 μm. The surface between the oral and ventral suckers was smooth and free of spines but had sparse sensory papillae on parallel transverse folds (Fig. 5).

Surface topography of worms collected from mice treated with subcurative dose of PZQ (NCC group)

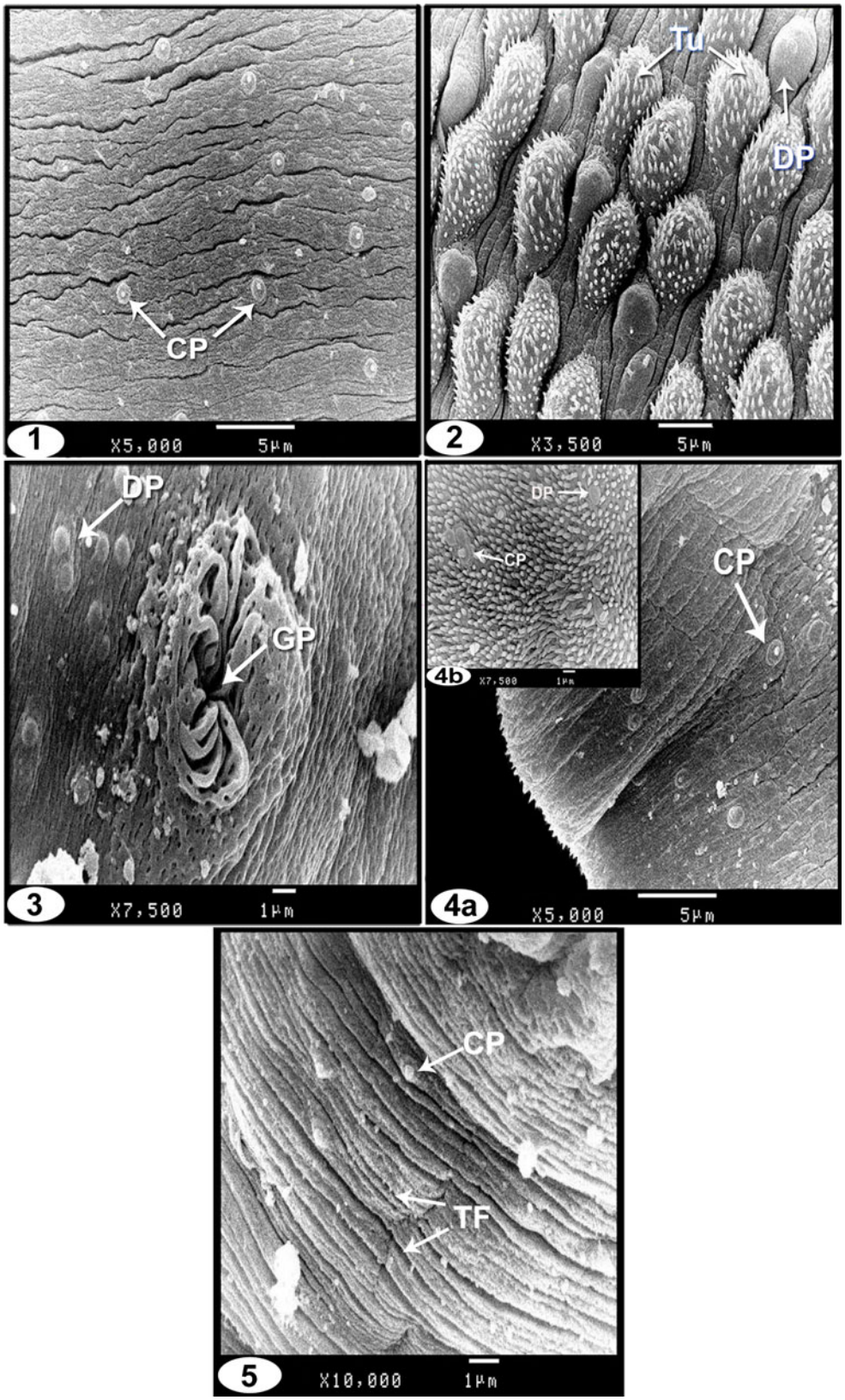
Two weeks after treatment with 20 mg/kg of PZQ to mice infected with 42-day-old *S. mansoni*, all adult males and females examined showed clear changes in shape. These were characterized by intensive contraction and bending of the worm body backwards beneath the ventral sucker (Fig. 6).

The male worms were shorter little than that of normal group, they reached 1,385.71 μm. In most male worms, the oral sucker contracted and lost many spines but no apparent alteration in the sensory papillae was seen. The ventral sucker was flattened. In the surface between the two suckers sever damage were seen; there was alteration in sensory papillae, fusion of folds, swollen of tegument, and appearance of cracks, leading to exposure of the sub-tegumental structures (Fig. 7). Severe damage to middle region of the gynaecophoral canal surface was observed, revealed by fusion of swollen folds, local collapse, and erosion of tegument as well as the emergence of many small vesicles (Ve) inside the canal surface (Fig. 8). The left edge of the gynaecophoral canal in some worms was swollen accompanied by the damage of sensory structures as well as a disordered arrangement of folds and fusion of swollen folds (Fig. 9). In higher magnification, the left edge of the gynaecophoral canal in other worms cold be divided into two regions; the first region appeared full of tegumental folds became like cracks and ciliated papillae were founded (Fig. 10a) and the second region became with very minute, non-protruded spines, and ciliated papillae (Fig. 10b). The tegumental tubercles were arranged in irregular rows and had dome-shaped papillae (Fig. 11). The changes in tubercles were namely swelling and shortening, or even loss of the spines on the surface (Fig. 11). The number of spines on tubercles was 57/one.

In female worms, there were also, intensive contraction and bending of body in many regions (Fig. 12). It shorter than normal female, its length reached to 1,928.57 μm. In certain regions between the two suckers and on lateral tegument, there was extensive damage of sensory papillae raised between normal surfaces similar to those in Fig. 9.

Surface topography of worms collected from mice vaccinated one time (VC group)

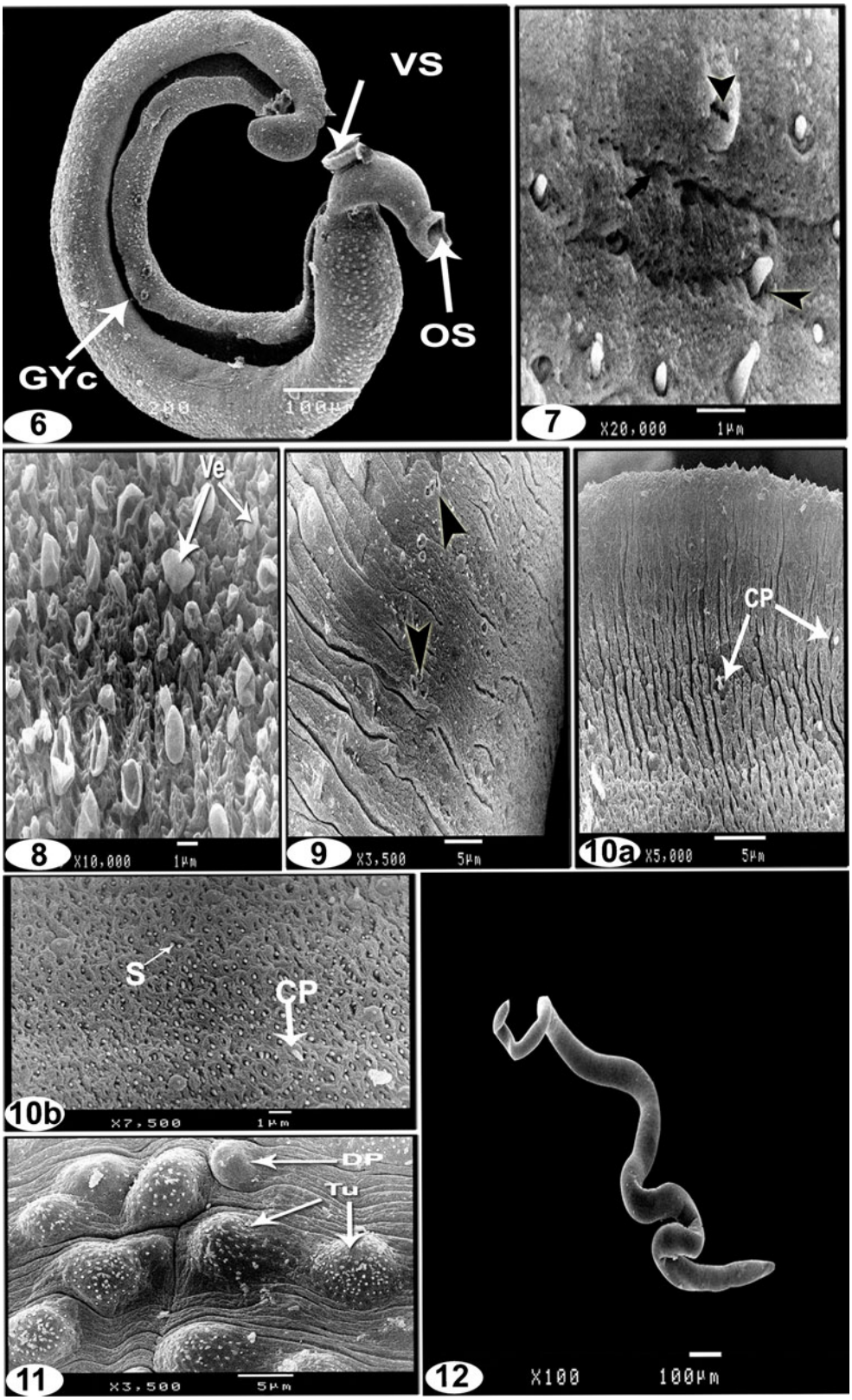
Male worms were recovered in copulation with female mostly and some of males were observed single. The length of male was shorter than male of control group and reached to 1,428.57 μm. No changes happened to the two suckers but the surface between them was observed with spongy appearance without tegumental folds and with spines (Fig. 13). Outer left edge of the gynaecophoral canal lost its normal shape and became with spongy shape in addition to presence of ciliated papillae (Fig. 14). Although no changes were detected along the inner surface of the



Figs. 1–4 Micrographs of adult male *S. mansoni* from control group. *1* Smooth surface between the two suckers with ciliated sensory papillae (*CP*). *2* Dorsal tegument with large, numerous, and spiny tubercles. Note presence of dome-shaped papillae (*DP*). *3* Magnified genital pore (*GP*) satellite in shape surround by porous surface and dome-shaped papillae (*DP*). *4a* Left edge of the gynaecophoral canal with smooth surface and ciliated papillae (*CP*). *4b* The outer surface of the gynaecophoral canal from left side showing spines, ciliated (*CP*) and dome-shaped papillae (*DP*).

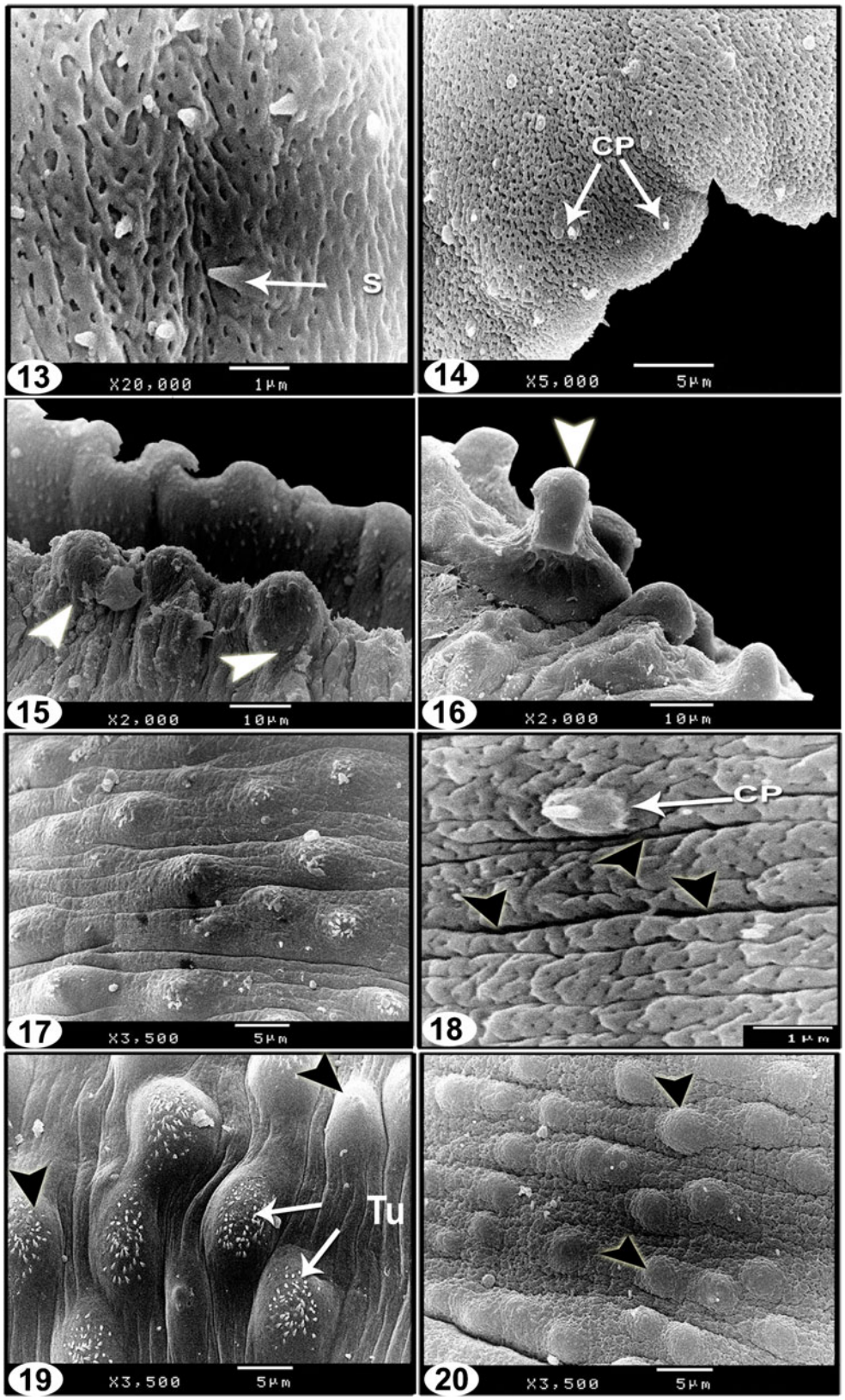
Fig. 5 Micrograph of adult female *S. mansoni* from control group showing the surface between the two suckers, note parallel transverse tegumental folds (*TF*) and ciliated papillae (*CP*)

gynaecophoral canal, there were changes on right edge of the gynaecophoral canal were observed. There was swollen of tegument and tubercles on the right edge of the canal and lost their spines (Fig. 15). In other specimens, severe



Figs. 6–12 Micrographs of adult male and female. *12 S. mansoni* from group treated with sub-curative dose of PZQ *6* Whole mount of male. Note bending of body after the ventral sucker (*VS*) and appearance of peeling on the gynaecophoral (*GYc*) canal edge. *OS* oral sucker. *7* Enlargement part of the surface between the two suckers. Note swollen and fusion of tegumental folds in addition to appearance of cracks (*thick arrow*) and damage of sensory papillae (*arrow heads*). *8* Middle inner surface of the gynaecophoral canal. Note erosion, fusion of swollen folds and emergence of small vesicles (*Ve*) from the surface. *9* Left edge of the gynaecophoral canal. Note damage of sensory papillae (*arrow heads*). *10a* The outer edge of the gynaecophoral canal from left side. Note the tegument filled of cracks and ciliated papillae (*CP*) appeared. *10b* The inner surface of left edge of the gynaecophoral canal. Note very minute, non-protruded spines (*S*), and reticular appearance of tegument with ciliated papillae (*CP*). *11* Dorsal tegument with large, numerous and spiny tubercles (*Tu*). Note irregular arrangement of them and presence of dome-shaped papillae (*DP*). *12* Whole mount of female

swelling and erosion in tegumental tubercles on the edge of the gynaecophoral canal was detected in addition to appearance of finger-like projection (Fig. 16). Dorsally, the tegument was swollen, led to changes in tegumental folds and tubercles, namely shortening and loss of spines (Fig. 17).



Figs. 13–17 Micrographs of adult male *S. mansoni* from group vaccinated one time. *13* The surface between the two suckers with spongy appearance. Note presence of small number of spines (*S*). *14* Left edge of the gynaecophoral canal with spongy surface in appearance. Note ciliated papillae (*CP*) and very minute spines on outer rim. *15* Right edge of the gynaecophoral canal with swollen tegument and tubercles (*arrow heads*). Note the tubercles without spines completely. *16* Finger-like projection of swollen tubercles (*arrow head*) on the right edge of the gynaecophoral canal. *17* Dorsal tegument with swollen tubercles. Note loss and shortening of all or most of tubercles spines

Figs. 18–20 Micrographs of adult male *S. mansoni* from group that treated with subcurative dose of PZQ post vaccination one time. *18* The surface between the two suckers with interlocking furrows and pits on tegumental folds (*arrow heads*). Note presence of ciliated papillae (*CP*) (*19*). Dorsal tegument with swollen tubercles (*Tu*). Note shortening and loss of tubercles spines (*arrow heads*). *20* Dorsal swollen surface with extensive erosion of tubercles (*arrow heads*). Note all tubercles lost all spines

Surface topography of worms collected from mice vaccinated one time and treated with subcurative dose of PZQ (VCC group)

All females were founded in copulation with male worms. Male worms were longer than males of control group. The length of the male reached to 1,417.29 μm. The surface between the two suckers appeared with interlocking furrows and pits on tegumental folds in addition to presence of ciliated papilla (Fig. 18). All worms showed moderate to severe changes of the dorsal surface of the tegument. The most prominent changes were swollen of tegument and tubercles in addition to loss and shortening of tubercular spines (Fig. 19). The number of tubercular spines was 53/one. Some tubercles lost their spines totally (Fig. 20).

Surface topography of worms collected from mice vaccinated four times (V4C group)

Males of this group had large change in length. The length of male ranged from 1,546.67 to 2,186 μm (Fig. 21). The surface surrounded the genital pore was characterized by erosion and swelling of tegument, also, the sensory papillae which surrounded it in normal male were disappeared (Fig. 22). There were severe changes to the internal surface of the gynaecophoral canal. The anterior region of the gynaecophoral canal had swelling, fusion of tegumental folds, the spines and ciliated papillae were disappeared totally (Fig. 23). Middle and posterior regions had severe damage to tegumental surface; most of spines disappeared in addition to erosion and peeling of tegument (Fig. 24). The damage also, was extended to the edge of the gynaecophoral canal. There was erosion, swelling, and damage to the ciliated papillae of the left edge of the canal (Fig. 25). There was moderate to severe damage recorded in dorsal surface of the tegument. There was damage to tegumental folds observed; it looked like crosslinks around tubercles (Fig. 26). Severe peeling, disruption of the tubercles, and tegumental folds were observed (Fig. 27). Also, tubercles became deformed, swollen with shortening of their spines and tegumental folds were deformed, become like parallel fibers, and ciliated papillae were chelated from its place (Fig. 28).

Female worms had a very large shortening in length and appeared very thinner than normal female. The length of female reached to 1,142.86 μm (Fig. 29). Extensive erosion and swelling of the outer oral sucker was recorded in female. The damage was extended to the surface between the two suckers (Fig. 30). The ventral sucker also was affected. It appeared very contracted and pulled to inside in all worms (Fig. 31). Fusion of tegumental folds, erosion of tegumental surface, and appearance of pores were recorded dorsally. Extensive peeling and swelling in the middle dorsolateral tegument (Fig. 32).

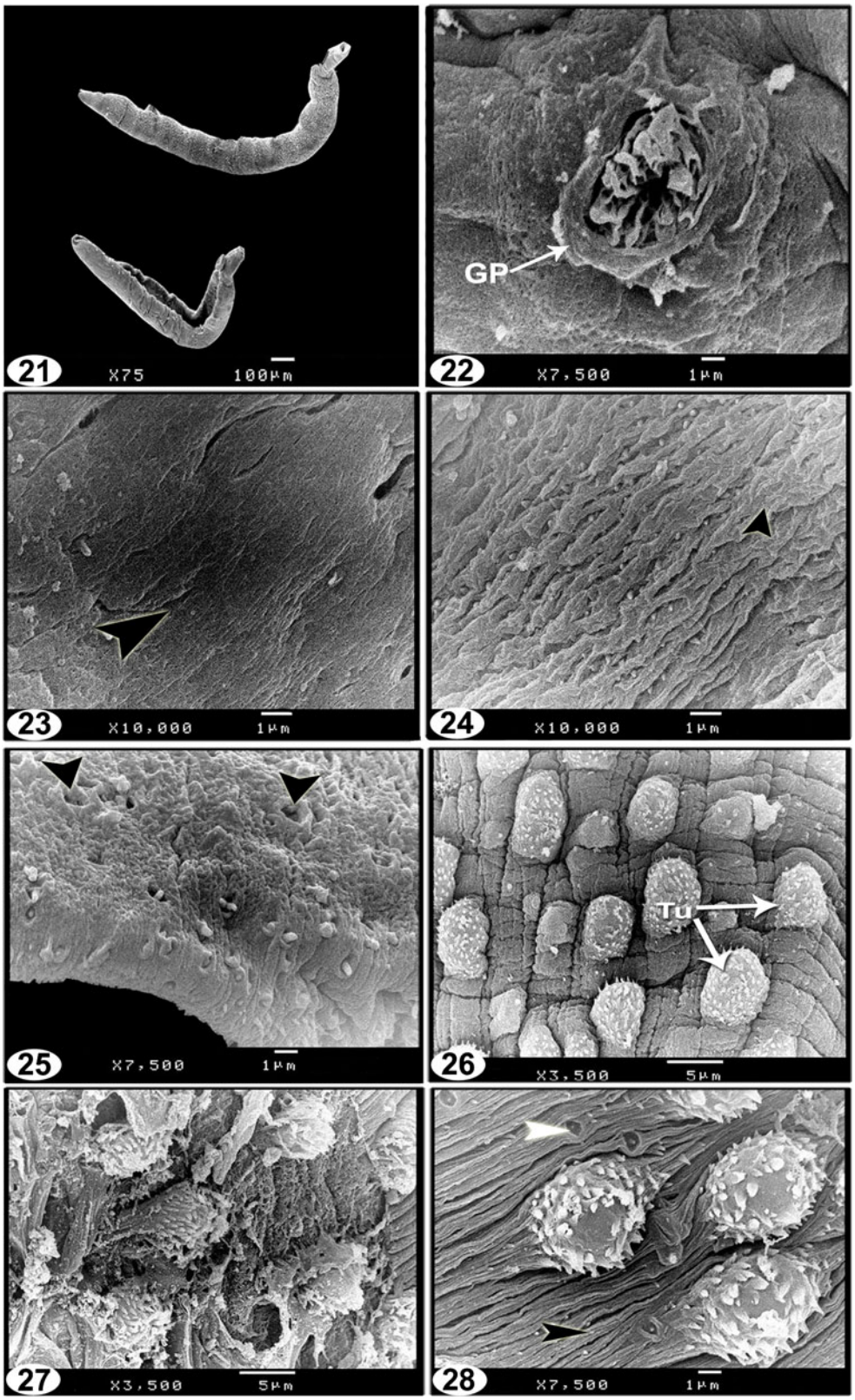
Discussion

The present study is the first to report the effects of vaccination with RA vaccine one or multiple times and subcurative dose of PZQ or/and in combination with one time vaccination against adult *S. mansoni* infection, by revealing unique ultrastructural alterations supporting the hypothesis that the RA vaccine is the most effective experimental vaccine available so far, might transfer protection against *S. mansoni* infection.

Using C57BL/6 strain of mice is in accordance with previous studies which showed that this strain was more susceptible to the infection with Egyptian strain of *S. mansoni* than BALB/c mice strain (Bin Dajem et al. 2008), and is known to respond better to UV-irradiated cercariae (Murrell et al. 1979; Richter et al. 1993; Mountford et al. 2001). In addition, Incani et al. (2001) reported that the strain of the host appeared to influence the susceptibility to infection, the fecundity, and the percentage of eggs distributed in liver and in intestine during chronic stage of infection.

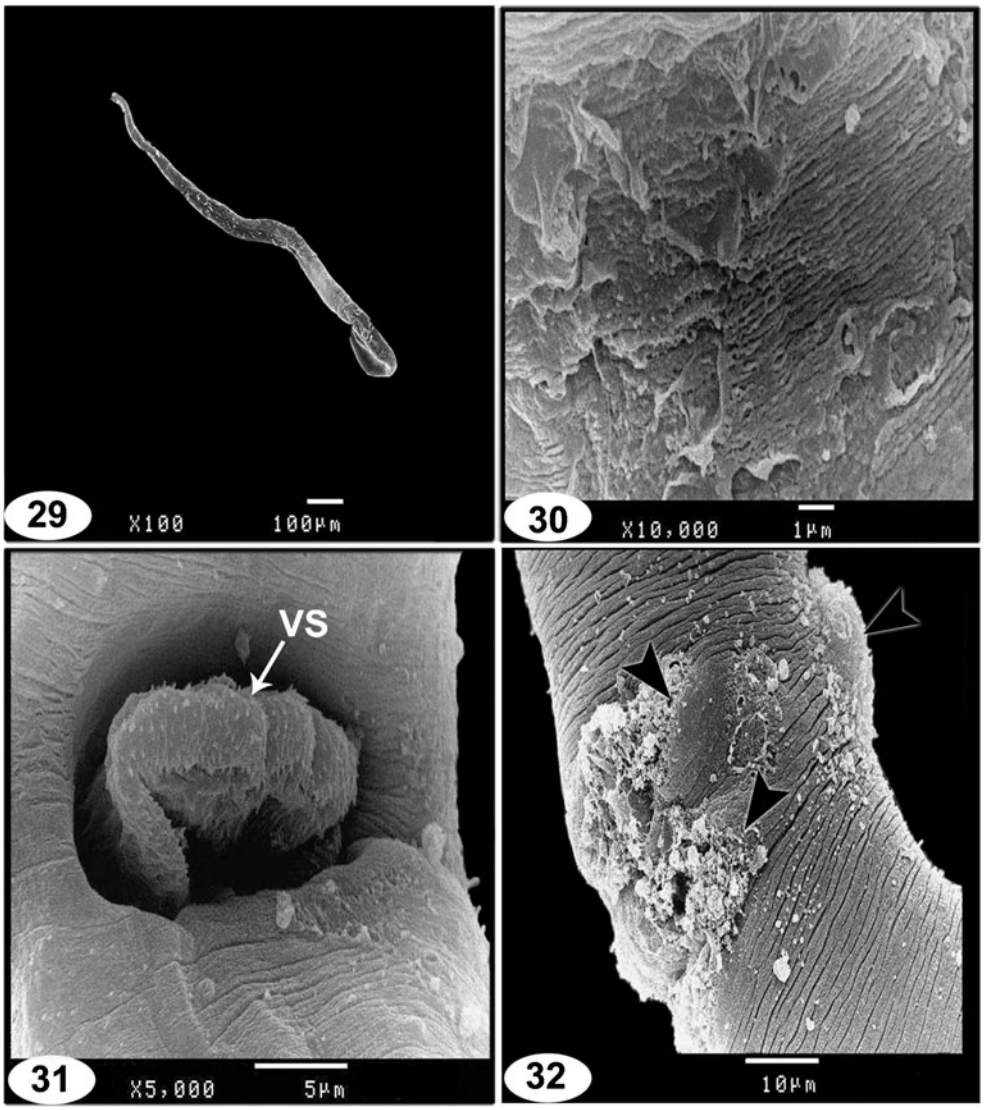
For the assessment of anti-schistosomal agent or drug efficacy in *S. mansoni*-infected mice, it is important to study several criteria related to the parasitic intensity, stages and distribution through the host tissues. Among these criteria, the worm burden, the percentage of ova pattern in parts of the intestine and ova count in the liver and intestine were considered (Abdel-Ghaffar 2004; Abdel-Ghaffar et al. 2005). The reduction of these parameters was considered as a strong evidence of efficiency of the anti-schistosomal drug (El-Mahdy 2006; Mostafa et al. 2011). The death of the worms due to treatment with anti-schistosomal drugs was attributed to metabolic disorders, mechanical destruction, and muscular contraction of the treated worms (Harder et al. 1987).

Treatment with low single (subcurative) dose of PZQ (20 mg/kg) postinfection or post-vaccination with *S. mansoni*-attenuated cercariae, reduced schistosomiasis infection by 43.03% in NCC group. This is in agreement with Fallon and Doenhoff (1994) and Utzinger et al. (2001). Araujo et al. (2004) reported that in high prevalence of parasite infections, they adapted to the host immune response, so it could not destroy them. The present work showed a diverse effect in vaccinated chemotherapy group (VCC group) as there is suggestion that the combination between chemotherapy and one vaccination strategy resulted in immunosuppression to the immune response in mice than the normal level giving a high number of recovered worms than control group.



Figs. 21–28 Micrographs of adult male *S. mansoni* from group that vaccinated four times. *21* Whole mount of male. Note changes in length. *22* Magnified genital pore (*GP*). Note swelling and erosion of tegumental surface surround it and absence of sensory papillae. *23* Anterior inner surface of the gynaecophoral canal showing fusion of tegumental folds, no spines, and swelling of the surface (*arrow head*). *24* Middle and posterior inner surface of the gynaecophoral canal showing severe swelling and peeling of the tegumental surface (*arrow heads*). *25* Left edge of the gynaecophoral canal. Note damage of some ciliated papillae (*arrow heads*), swelling and erosion of the tegumental surface. *26* Dorsal tegument in other specimens. Note cross-linked tegument around the swollen tubercles (*Tu*). *27* Severe damage and disruption of tegument and tubercles surface in some specimens. *28* Damage of the tegumental folds (*black head arrow*) and sensory papillae (*white head arrow*) between tubercles. Note tegumental folds appeared like fibers

Moreover, a primary aim of vaccination is to prevent or reduce infection of the host. In terms of vaccine-induced protection, multiple doses of RA vaccine appear to induce high titers of antibody and high levels of immunity (Kariuki et al. 2004), and significantly, this vaccine is highly effective even when used to vaccinate animals that had



Figs. 29–32 Micrographs of adult female *S. mansoni* from group that vaccinated four times. *29* Whole mount of female. Note that it is very thin than normal. *30* Extensive peeling and erosion of the tegumental surface between the two suckers. *31* The ventral sucker (VS) was very contracted and pulled into cavity. *32* Extensive peeling and swelling in the middle dorsolateral tegument (*arrow head*)

been previously infected. This is important as any vaccine that might be deployed in endemic areas is likely to be given to individuals that have a prior history of schistosome exposure.

As explained by Bickle et al. (1985), Mangold and Dean (1986), and Delgado and McLaren (1990), the first exposure to attenuated cercariae which gave a large increment in protection may reach to 60–70% in the C57BL/6 mice strain. However, in one study, Hsu et al. (1981) reported one, three, and five vaccinations with X-ray-attenuated cercariae induced progressively high levels of immunity (maximum 91%) independent of the attenuating dose used. This may imply that the method of attenuation selected and thus the nature of damage inflicted on the parasite, has no effect on the way that immune system is primed.

In the present study, one vaccination and four times vaccination pre-infection are founded to modulate the schistosomiasis infection. This modulation is indicated by decline in parasitological parameters (worm burden, ova count, and oogram).

In the infected untreated group, the percentage of infecting cercariae that developed into mature worms ranged between 23.7% and 24.7%. The subcurative dose of PZQ reduced mean worms burden by 43.03%. Moreover, the result could be compare with the result obtained by Xiao et al. (1999) which recorded reduction of worm burdens by only 50% post lower dose of levo-PZQ (50 mg/kg). Mossallam et al. (2007) recorded 92.68% and Botros et al. (2007) recorded 95.1% post using curative dose of PZQ.

While the treatment with subcurative dose of PZQ, post one vaccination in a preliminary study did not result in reduction of worm burdens. Similar results were observed in treatment of infected mice with mefloquine in combination with artemether at smaller dose of 50 mg/kg and when infected mice were treated with mefloquine (MQ) com-

bined with PZQ at single dose of 50 mg/kg, no apparent improvement in efficacy was seen (Xiao et al. 2011).

In one time vaccinated (VC) group, the reduced mean worms' burden reached to 37.97%, while in that vaccinated four times, the percent reduction (PR) reached to 72.5%. The results are comparable to that observed by Dean et al. (1996) from four experiments post two immunizations with mean (63.5%) in C57BL/6 mice.

The current studies reported that RA vaccine gave protection levels for *S. mansoni* in host species such as rats, mice and non-human primates including baboons and chimpanzees (Yole et al. 1996a, b; Mountford et al. 2001).

The number of ova per gram tissue is also an important criterion for the evaluation of the effect of anti-schistosomal agent on the infection (Mostafa 2005). The successfully developed worms produce thousands of eggs which are mostly trapped in hepatic and intestinal tissues (Warren 1975). The high count of ova in the intestinal tissue as compared with that of liver may be due to the increased proportion of pairing among schistosomes in the portal mesenteric tract (Ghaleb et al. 1979).

There is significant reduction by comparing V4C group with control group. These data pulls us to suggest that using attenuated cercariae in vaccination-induced ovarian toxicity caused not only a reduction in egg production, but also a qualitative egg defect (Xiao et al. 1995).

Scanning electron microscope is an important tool in studying the surface of the parasite when considering the host–parasite relationship (Hockley 1986). The tegument of normal *S. mansoni* is an essential interface between the parasite and its intravascular environment in the host, and the tegumental surface of adult *S. mansoni* has already been described in a number of SEM studies (Miller et al. 1972; Hockley 1973; Hockley and McLaren 1973). Through this specialized tegument, adult worms perform three basic activities for their survival: (1) assimilate blood nutrients from the host, (2) escape from the immune response of the host against their response (Kalapothakis et al. 1988; Abath and Werkhauser 1996), and (3) regenerate from induced lesions (Popiel et al. 1985). The spines on the borders of the oral sucker are considered to function in attaching the helminthes and scarping the tissues of blood vessel walls (Kruatrachue et al. 1979).

The ventral sucker is more related to the displacement of the specimen, considering that this structure is bigger than the oral sucker and with sensorial papillae that are mechano-receptor structures (Hockley 1973). In our study, unlike the anterior region of the body, the middle region showed a wide range of spines distribution on the ventral and dorsal surface of the body of both the male and female schistosomes as previously reported (Khon et al. 1982; Machado-Silva et al. 1997). Spines on the top of the tubercles were visible arranged in parallel manner as previously described by Manneck et al. (2010).

Treatment with subcurative dose of PZQ on the adult *S. mansoni* worms showed high contract of worms as reported by Mehlhorn et al. (1981). Many distinct differences appeared in comparison with control group, such as swelling and loss or shortening of spines; it has been also documented for PZQ (Xiao et al. 1981).

This drug has two major effects: Firstly, stimulation of motor activity by the influx of calcium from external sources causes spasms and paralysis of the muscles (Pax et al. 1978). Secondly, there is damage to the tegument revealed by the formation of vacuoles and later vesicles (Irie et al. 1989). In addition, PZQ acts synergistically with host immune response as it depends on the host antibody response (Brindley and Sher 1987). The damage to tegument along the worm's body would have impaired the functioning of the tegument and also destroyed the defense system of the worm, so that it could easily be attacked by the host's immune system (Xiao et al. 2000a).

In the group treated with a single little dose of PZQ, medium and large vesicles were observed protruded from the tegument of the gynaecophoral canal, most likely due to swelling of cytoplasm (Xiao et al. 1981). The tegument of male worms showed a higher sensitivity to PZQ than female worms in vivo; male worms showed the same result to PZQ in vitro (Pica-Mattoccia and Cioli 2004) and exhibited more extensive tegumental damage than female worms following PZQ treatment (Shaw 1990). It can be suggested that either a sex-specific interference of the drug with the target might occur or there are different targets for PZQ in females compared to males. This conclusion is in agreement with Keiser et al. (2009).

In the present study, PZQ treatments resulted in morphological changes similar to that caused by artemether (Xiao et al. 2002) and artesunate (Shaohong et al. 2006).

The tegumental alterations in VC group showed a similarity to the results obtained by Mohamed (1999) and Mohamed and Kamel (2000). Some worms showed only slight damage or even normal tegument, suggesting that they could have recovered from the initial tegumental damage.

In VCC group, no reduction in worm burdens but many tegumental alterations were appeared due to chemotherapy effect. Furthermore, extensive peeling of tegument occurred rapidly, resulting in exposure of worm surface antigens and severe disruption of tegumental functions. In vivo, such damage may render worms vulnerable to attacks by the host's immune system.

These results are in agreement of that induced by MQ (Manneck et al. 2010), that induced by praziquantel (300 mg/kg and 80 μg/ml) (Jiraungkoorskul et al. 2005, 2006, respectively), praziquantel enantiomers (Xiao et al.

2000b), and that induced by atorvastatin on *S. haematobium* (Soliman and Ibrahim 2005).

In summary, it is obvious that vaccination with attenuated cercariae multiple times induced high reduction of worms' burden, ova count, and severe ultrastructural alterations to both male and female worms. This result in comparing with that results obtained by Abdeen et al. (2011). As RA vaccine generate high level of protection against subsequent challenge is not acceptable for use in human, reaching to vaccine depends on attenuated cercariae with indirect way as further target. This may represent important agent in getting a promising strategy for control of schistosomiasis mansoni.

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