ASSESSMENT OF THE EFFECTOR ANTIBODIES AGAINST *SCHISTOS-OMA MANSONI* AND THEIR IMPACT ON MEFLOQUINE EFFICACY

By

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Abstract

Host immune response against *Schistosoma mansoni* is a major contributor in the process of resistance or susceptibility to re-infection, as well as the therapeutic response towards different antischistosomal drugs. This study evaluated the role of IgG &IgG4 as immune-logical marker of cure and their effect on anti-schistosomal mefloquine efficacy. Sixty Swiss albino mice were infected with *Schistosoma* cercaria and divided into four groups; non-infected, infected not treated, infected treated with praziquantel and infected treated with mefloquine. The effect of drugs was assessed parasitologically using Kato-Katz technique and immunologically by measuring the serum level of anti-*Schistosoma* IgG & IgG4 subclass. The results showed 83.5% significant egg count reduction in MFQ-treated infected group. Also, serum anti-soluble egg antigen (SEA) total IgG & IgG4 subclass levels in MFQ treated group were significantly different from infected control group & PZQ-treated infected group.

Key words: *Schistosoma mansoni*, Mefloquine, Kato-Katz, antischistosomal IgG & IgG4

Introduction

Schistosomiasis is a widespread parasitic disease in tropical and sub-tropical areas. It occupies the second place in terms of socio-economic and public health burden (Cardoso *et al,* 2013). *Schistosoma mansoni* (*S. mansoni*) is the most prevalent *Schistosoma* species in Egypt (Helmy *et al,* 2009). Studying hosts' immune response towards *S. mansoni* infection fills the gaps of knowledge about humoral immune responses against parasitic antigens within inhabitants of the endemic areas. The role of the cellular and humoral immune response in schistosomiasis mansoni was well characterized (Butterworth *et al,* 1988). Hypergammaglobulinemia, with consistently elevated total serum IgG and IgE concentration, is a common feature of this response in chronically untreated infected individuals (Ramírez *et al,* 1996). IgG4 antibody reactivity against specific schistosoma antigens have been involved in resistance or susceptibility to re-infection (Corrêa-Oliveira *et al,* 2000). Moreover, IgE and IgG4 isotypes, could serve as markers for picking up high risk patients susceptible to re-infection. Parasite-specific IgE are ac-companying resistance to the infection/re-infection, however, parasite-specific IgG4 is supposed to be a modulator of IgE effector responses (Black *et al,* 2010). IgG4 compete with IgE receptors thus preventing their binding to the high affinity receptor on eosinophil and hence minimize their activation and defensive role in induction of eosinophil-mediating killing of schistosomula (Khalife *et al,* 1989). Therefore, the expression of protective immune response depends on the favorable balance towards IgE production in the chronic infections and IgG4 produced in acute infection. In addition, IgG coated mast cells, up on stimulation with specific antigens, would stimulate production of protective antibodies in the entire IgG sub-classes and the release of eosinophils chemotactic factors (Khalife *et al,* 1986). The relative balance between these antibodies is a key feature in humoral immunity against schistosomes (Jiz *et al,* 2009).

Several medications are used in the treatment of schistosomiasis including praziquantel (PZQ), oxamniquine, metrifonate, antimonials, hycanthone and niridazole.

Many evidences reported increased emergence of strains of *S. mansoni* resistant to PZQ (Zhang and Coultas, 2013). In recent years, antimalarial drug mefloquine (MFQ) exhibited potential effects against schistosomes (Xiao and Xue, 2012) with remarkable in vitro and in vivo activities against major *Schistosoma* species (El-Lakkany *et al,* 2011; Ingram *et al,* 2012; Zhang and Xiao, 2012; Abou-Shady *et al,* 2015; Abou-Shady *et al,* 2016). Numerous immunological tests using crude or purified egg and adult worm antigens have been developed in the last decades to detect anti-*Schistosoma mansoni* antibodies. Some of them have been recommended for post-treatment follow up.

The present study was designed to evaluate the role of specific anti-schistosomal IgG and IgG4 response in assessment of cure as well as for monitoring the efficacy of the antimalarial drug mefloquine® (MFQ).

Materials and methods

Significance of the study: To evaluate the role of specific anti-schistosomal IgG & IgG4 response in assessment of cure and for monitoring efficacy of the antimalarial drug mefloquine.

Experimental animals and parasites: CD1 Swiss albino mice of both sexes (*n* = 60), aged 5-6 weeks were obtained from the experimental unit of Schistosome Biological Supply Program, Theodor Bilharz Research Institute (Giza, Egypt). The animals were kept under favorable temperature and humidity and were fed on standard commercial pelleted diet. Animals were infected individually through percutaneous route with 60 ± 10 freshly shed Egyptian strain of *S. mansoni* cercariae from experimentally infected *Biomphalaria alexandrina* snails (Peters and Warren, 1969).

Tested drugs: MFQ (Mephaquin) tablets were purchased from Mepha Ltd., Aesch-Basel, Switzerland, and were given once as an oral suspension in 7% (v/v) Tween-80 and 3% (v/v) ethanol in a dosage of 400mg/Kg (Keiser *et al,* 2009).

PZQ (Distocide) tablets were purchased from EIPICO, El-Asher Men Ramadan, Egypt, and were crushed and given on two successive days as an oral suspension in 2 % cremophore-E1 (Sigma-Aldrich Chemical Co., St. Louis, MO) in a dosage of 500 mg/kg/day (Fallon and Doenhoff, 1994).
Both drugs were administrated to the infected mice 49 days post-infection (P.I.) using the esophageal tube.

Experimental design: The mice included in this study (*n* =60) were divided into four groups; GI: non-infected negative control group, GII: infected non-treated control group, GIII: infected PZQ-treated animal group and GIV: infected MFQ-treated animal group.

Stool samples from all groups were collected 2 weeks after administration of the tested drugs for determination of the egg count in treated and control mice. Following stool collection, mice were decapitated and blood samples from all mice were collected for immunological study.

Kato-Katz technique (cellophane fecal thick smear) for egg counting in treated and control mice (Katz *et al,* 1972): Fecal pellets were collected from each mouse 9 weeks P.I. and processed for the presence and counting of *S. mansoni* eggs/gram of feces using the Kato-Katz technique. Briefly, a small amount of feces was placed on a scrap paper and a piece of nylon screen was pressed on top so as to sieve some of the feces through the screen to be accumulated on the top. A flat-sided spatula was scraped over the upper surface of the screen and the sieved feces were collected. A template was placed on the slide and the sieved feces were added with the spatula so that the hole in the template was completely filled. The spatula was passed over the filled template to remove excess feces from the edge of the hole. The template was removed cautiously and a cylinder of feces was left on the slide. The fecal sample was covered with cellophane green coverslip soaked with malachite green (3% aqueous solution) to preserve and whit-

en the material. The slide was inverted and the fecal sample was pressed firmly alongside the hydrophilic cellophane strip to spread uniformly. The slide was placed for one hour at room temperature on the bench with cellophane upwards to permit the evaporation of water while glycerol clears the feces before microscopic examination. Three slides/mouse were made and exam ined under the light microscopy, the mean number of eggs within the three slides was calculated. The number of eggs/gram of feces was calculated by multiplying the mean number of eggs×24.

The percentage of egg output reduction in infected treated groups in comparison to infection control group was calculated according to the following equation:

$$\frac{\text{No of eggs in infected non treated control } - \text{ No. of eggs in treated}}{\text{Number of eggs in infected non treated control}} \times 100$$

Immunological study: After mice decapitation, blood samples from all animals were collected in centrifuge tubes and were centrifuged at 3000 rpm for 20min. Sera of animals were stored at -20°C until used for immunological assay.

Determination of anti-SEA IgG and IgG4 subclass were measured using indirect ELISA (Engvall and Perlman, 1971). Briefly, ELISA microtiter plates were coated with 100ul/well of 30ug/ml of SEA. Sera were diluted 1:20 and anti-mouse IgG and IgG4 (Binding site, Birmingham, UK) were used at a dilution of 1:500. Absorbance was measured at 492nm using ELISA reader (Bio-Rad microplate reader, Richomond, Co.).

Statistical analysis: Results were analyzed using SPSS (version 20.0) software. Results were expressed in mean ± standard error (SE). All data were analyzed by using ANOVA test followed by Student's t-test at 95% confidence level. P-values < 0.05 were considered as statistically significant.

Ethical considerations: The study was managed in accordance with the international valid guidelines for animal welfare and the animals were maintained under the appropriate conditions at Theodor Bilharz Research Institute.

Results

Parasitology: Assessment of *S. mansoni* egg count per gram of feces using Kato-Katz technique in MFQ-treated infected group was significantly different when compared to infected control group at $P < 0.05$, as the egg count reduction was 83.5%, without statistical significance difference as compared to PZQ-treated infected group (Tab. 1).

Table 1: *Schistosoma mansoni* eggs per gram feces using Kato-Katz technique in mice infected treated with MFQ compared to PZQ-treated and infected control mice

	Animal group	Number eggs/gm feces	Percentage of Reduction
G II	Infected non-treated control	307.33 ± 17.09	-
G III	Infected treated with PZQ	39.17 ± 5.67*	87%
G IV	Infected treated with MFQ	50.67 ± 7.26*	83.5%

Data as mean ± SEM (n = 15 in each group).*Significant different from infected control (P < 0.05).

Immunology: Since MFQ treated mice demonstrated a significant reduction in egg output, antischistosomal humoral immune response induced in experimental mice following treatment was determined in the form of total IgG & IgG4, in response to SEA. The results showed that serum anti-SEA total IgG level in mice infected treated with MFQ was significantly differed from infected control group at $P < 0.05$, without statistical significance difference when compared to PZQ treated infected group.

Meanwhile, assessment of serum anti-SEA IgG4 subclass level in mice infected and treated with MFQ was significantly different from infected control group and PZQ-treated infected group at $P < 0.05$ (Tab. 2).

Table 2: Serum anti-SEA Total IgG & IgG4 subclass levels in mice infected with *S. mansoni* and treated MFQ compared to PZQ

	Animal groups	Total IgG	IgG4
G I	Non- infected control	0.039 ± 0.01	0.151 ± 0.04
G II	Infected non-treated control	1.657 ± 0.252	0.982 ± 0.304
G III	Infected treated with PZQ	$2.063 \pm 0.436*$	1.382 ± 0.249
G IV	Infected treated with MFQ	$2.026 \pm 0.31*$	$0.676 \pm 0.158*\#$

* Significantl different from infected control ($P < 0.05$), #Significant different from infected treated with PZQ ($P < 0.05$)

Discussion

Schistosomiasis is the third most overwhelming tropical disease in the world after malaria and intestinal helminthiasis. It represents a major cause of morbidity and mortality in the developing countries in Africa, South America, the Middle East, and Asia (WHO, 2010a). More than 240 million people who live in Africa are infected with schistosomiasis with an estimated 700 million people are at risk of infection where the disease is considered endemic (WHO, 2010 a, b; Santos *et al,* 2014). The prevalence of schistosomiasis increased with age and peaks between 15-20 years. On the other hand, there was no significant change found in the prevalence of disease in older adults, but the parasite burden decreases (Hagan and Gryeels, 1994). PZQ-based chemotherapy has attained some success in schistosomiasis control but reliance on a single drug is not adequate to reduce the long-term transmission and carries the risk of development of drug resistance (Bergquist *et al,* 2008). MFQ was found to be effective against various stages of different species of *Schistosoma* both in vitro and in vivo (Keiser *et al,* 2010; Xiao *et al,* 2011; Ingram *et al,* 2012). The accepted diagnostic standard of schistosomiasis *mansoni* is evidence of viable eggs in feces or tissue biopsies (De Vlas *et al,* 1997). In the present study, *Schistosoma mansoni* egg count per gram of feces using Kato-Katz technique was evaluated with significant egg count reduction in both MFQ-treated and PZQ-treated mice compared to infected control mice. The reported finding resulted from the effect of drugs on worm burden especially, the female worm burden. This result agreed with a randomized, exploratory open-label trial done on *S.*

haematobium infected children and reported high cure rates of 61 % and egg-reduction rates of 95 % (Keiser *et al,* 2010a). Furthermore, administration of mefloquine in a dose of 400 mg/kg caused a statistical significant reduction in the number of mature female worms (El- Lakkany *et al,* 2011). Also, Abou-Shady *et al,* (2016) reported a significant reduction of tissue egg load with increased percentage of dead ova in the oogram pattern accompanied by significant reduction of the mature worm load. Serological assays have proven to be useful diagnostic tool by the detection of anti-schistosomal antibodies (Tsang *et al,* 1995). Some used isotypic assays can differentiate active from inactive infections (Grogan *et al,* 1996; Grogan *et al,* 1997; Webster *et al,* 1997). The relation between *Schistosoma*-specific IgE, eosinophils and resistance to reinfection has been detected in a variety of epidemiologic settings (Satti *et al,* 1996; Zhaosong *et al,* 1997; Jiz *et al,* 2009). Both high- and low-affinity IgE receptors on eosinophils and B cells (or in soluble form), respectively, are linked with prevention of reinfection (Ganley-Leal *et al,* 2006; Mwinzi *et al,* 2009). Dissimilarly, susceptibility to reinfection has been related to IgG4, which might act as a blocking antibody, hindering the action of IgE (Satti *et al,* 1996; Zhaosong *et al,* 1997; Oliveira *et al,* 2012). Interestingly, Anti-schistosome IgG4 & IgE responses matched their relative predisposition to reinfection (Grogan *et al,* 1996). To gain further insight on the effector function of anti-IgG4 on host immune responses and into the possible relationship between IgG4 subtype levels and response to MFQ therapy, the present study assessed the levels of total IgG &IgG4 in MFQ-treated mice and compared them with

that of infected non-treated and PZQ-treated animals. The present results revealed significantly higher IgG response to SEA in MFQ-treated mice, in comparison with both untreated and treated with PZQ groups. Similarly, IgG were shown to influence the efficacy of the drug in plasma cell-suppressed mice as well as they were found on the tegument of worms in immunocompetent mice post treatment by PZQ (Brindley' And Sher, 1987). Allam (2009) declared that *Curcumin* treatment of murine schistosomiasis *mansoni* enhanced the humoral immune response by increased serum IgG against both SWAP and SEA. It has been documented that IgG antibodies response plays a crucial rule in protection against experimental and human schistosomiasis (Hoffmann *et al,* 1999; Jankovic *et al*, 1999). Besides, Marques *et al* (2008) detected high levels of IgG with a protection of 57% against infection and hepatic granuloma significantly declined in number when mice vaccinated with recombinant P44 (fructose1, 6 bisphosphate aldolase antigen). Levels of IgG4 to SEA were significantly higher in sera from infected non-treated mice and relatively higher in PZQ treated group as compared to MFQ-treated mice. This observation could explain how the well-known property of mefloquine in eliminating juvenile stages of schistosoma, and consequently ability of resisting reinfection, is working. These results agreed with previously reported finding following treatment of human cases infected with *S. mansoni* (Walter *et al,* 2006; De Moira *et al,* 2010). They found decrease in the levels of *Schistosoma*-specific IgG4, but, *Schistosoma*-specific IgE was maintained at pretreatment levels or increases and stated that, certain *S. mansoni* adult worm-associated tegumental-allergen-like (TAL) proteins have been characterized as important potential targets of protective IgE and reinfection-associated IgG4. Thus, treatment with MFQ caused decrease in the levels of anti-SEA IgG4 which was correlated to decreased intensity of infection, as measured by reduced number of shed eggs. This suggested that the resistance to reinfection could be influenced by type and amount of antibodies produced by the antigens released from eggs. Long-standing effect of immunological change needs more investigation using specific stage antigens to establish the nature and development of acquired immunity.

Conclusion

The results showed that the serum level of antischistosomal antibodies class provoked against the parasite antigens influences prognosis and treated schistosomiasis outcome.

Contribution of each author: All manuscript authors contributed to every activity of it; idea of paper, study design, collection of materials, methodology, writing the paper and revising it.

Conflict of interest: The authors declare that they neither have competing interests nor received financial support.

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