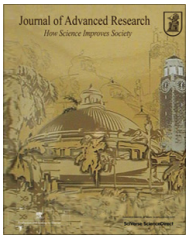
Journal of Advanced Research (2015) 6, 851–857

Cairo University

Journal of Advanced Research



ORIGINAL ARTICLE

Effect of artemether on cytokine profile and egg induced pathology in murine *schistosomiasis mansoni*

Neveen A. Madbouly [a], Ibraheem R. Shalash [b], Somaya O. El Deeb [a], Azza M. El Amir [a,*]

[a] *Zoology Department, Faculty of Science, Cairo University, Egypt*
[b] *Theodore Bilharz Research Institute, Giza, Egypt*

ARTICLE INFO

Article history:
Received 17 April 2014
Received in revised form 19 July 2014
Accepted 19 July 2014
Available online 29 July 2014

Keywords:
Schistosomiasis
Artemether
IFN-γ
IL-4
IL-10

ABSTRACT

Artemether (ART), the methylated derivative of artemisinin, is an efficacious antimalarial drug that also displays antischistosomal properties. This study was designed to evaluate the immunomodulatory action of a single intramuscular dose (50 mg/kg body weight) of ART in comparison with PZQ treatment (42 days PI). ART administration was 7, 14, 21 and 45 days PI. ART effect was studied parasitologically, histopathologically and immunologically. It was found that maximum effect was reached when ART treatment interfered with 14 or 21 days old schistosomula. ART treatment 14 or 21 days PI was associated with shift from Th2 to Th1 predominancy (decrease in IL-4 and upgrading of serum IFN-γ levels). In conclusion, ART is a promising drug in control of *schistosomiasis mansoni* due to its reductive effect on worm burden and its role in improvement of hepatic granulomatous lesions.

Introduction

Schistosomiasis is a common intravascular trematode infection. It is one of the most prevalent parasitic diseases in the world, after Malaria [1]. Currently; praziquantel (PZQ) is the drug of choice for mass treatment of schistosomiasis. PZQ is active against all five human *Schistosoma* species [2]. To be left with only one drug for schistosomiasis treatment is a very dangerous situation; especially that PZQ does not prevent reinfection, mainly in high transmission areas. Furthermore, there is increasing concern about the possible development of parasite resistance and tolerance against PZQ. Recent attempts are directed toward natural products to design novel drugs that avoid the side effects of the synthetic medications. These may be from plant extracts and even camel milk [3]. One of these plant extracts is artemisinin, derived from the herb; *Artemisia annua*. Artemether (ART) is the methylated

* Corresponding author. Tel.: +20 1001649556 (mobile), +20 38556875 (Home).
E-mail address: azzaelamir@yahoo.com (A.M. El Amir).
Peer review under responsibility of Cairo University.

Production and hosting by Elsevier

2090-1232 © 2014 Production and hosting by Elsevier B.V. on behalf of Cairo University.
http://dx.doi.org/10.1016/j.jare.2014.07.003

derivative of artemisinin. In addition to the amazing antimalarial effect of ART, it showed anti-parasitic properties toward many protozoan parasites such as *Leishmania*, *Toxoplasma gondii* and *Trypansoma* spp. [4].

Metazoan parasites as, *Schistosoma* spp., *Echinostoma caproni*, the liver fluke *Opisthorchi sviverrini*, *Clonorchis sinensis* [5] and *Fasciola hepatica* [6] are also greatly susceptible to ART.

One of the great advantages of ART therapy is its prophylactic action. The prophylactic effect of ART is defined by its ability to eradicate the developing stages of schistosomula, so that the egg-laying mature female worms are not allowed to develop in the vasculature [7].

The aim of the present study was to evaluate the immunomodulatory effect of a single intramuscular ART dose (50 mg/kg) on the cytokine profile in experimental *Schistosoma mansoni* infection.

Material and methods

Animals

Laboratory bred female, Swiss albino mice *Mus musculus* (CD-1 strain), each weighing 18–20 g, were used in this study. Experimental animals were obtained from Schistosome Biological Supply Center (SBSP) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Mice were kept for 8 weeks (experiment duration) in air-conditioned animal house at 20–22 °C and maintained on food containing 24% protein. Mice were also given carrot, lettuce and milk as source of vitamins. *Animal experiments were carried out according to the internationally valid guidelines and in an institution responsible for animal ethics.*

Parasites and infection

The Egyptian strain of *S. mansoni* cercariae was obtained from SBSP at TBRI. Infection was performed by subcutaneous injection (s.c.) of *S. mansoni* cercariae (80 ± 10/mouse) [8].

Drugs

PZQ, was obtained in the form of Tablets (600 mg/Tablet) (Distocide, Epico, Corporation, Cairo). The drug was freshly prepared and administered orally as a suspension in 2% Cremophor (Sigma) in a dose of 500 mg/kg/b.wt. for two consecutive days, 42 days postinfection (PI). **ART**, suspended in ground-nut oil, in the form of intramuscular (i.m.) ampoules (80 mg/ampoule) with documented purity of 99.6% was purchased from Kunming Pharmaceutical Corporation (Kunming, China). This preparation is stable at room temperature for 4 years [18]. The drug was administrated i.m. as a single dose of 50 mg/kg/b.wt. according to the experimental design [9].

Experimental design

The experimental groups are illustrated in Table 1. Mice were euthanized 8 weeks postinfection (PI) by decapitation. Then, the blood was collected individually in plastic tubes without anticoagulant. Blood was allowed to stand for 1 h at 37 °C, then overnight at 4 °C and centrifuged at 2500 rpm for 15 min. The serum was obtained and kept in aliquots at −20 °C for cytokine assessment.

Worm burden

Individual worm burdens were examined after perfusing the hepatic and portomesenteric vessels of each animal. Infected mice were perfused.

Histopathology and granuloma measurement

Liver sections were microscopically studied to evaluate the pathological changes including portal tracts and schistosomal granulomatous reactions. Pieces of mice livers were fixed in 10% phosphate-buffered formalin, pH 7.2, processed into Paraffin blocks. Transverse sections (5 μm in thickness) were taken, 5 sections from each liver, using a microtome (Bright 5030, UK). Each section was at a distance of at least 300 μm from the proceeding one. Sections were mounted on glass slides. Deparaffinization was performed by dipping slides in 100% xylene and descending ethanol series (100%, 95%, 80% and 70%) for rehydration. Sections were stained with Hematoxylin and Eosin (H&E) and Masson Trichrome.

Mean hepatic granuloma number (MGN) and diameter (MGD)

Measurements were taken only for granuloma containing single egg in the center using an ocular micrometer. The number of granuloma in 5 successive low power fields (10 × 10) was counted and recorded for MGN [10]. The MGD of each granuloma was calculated by measuring two diameters of the lesion at right angles to each other [11].

Cytokine assay

Levels of the cytokines IL-4, IL-10 and IFN-γ were measured in serum using sandwich ELISA. Briefly, plates (Nunc, Roskilde, Denmark) were coated with capture antibodies with 100 μl of serum sample or recombinant cytokine. Following addition of the biotinylated detection antibody and streptavidin–alkaline phosphatase conjugate, the reaction was developed with p-nitrophenyl phosphate (PNPP) (Sigma). Absorbance at 405 nm was measured with a Benchmark reader (Bio-Rad Laboratories Inc., Hercules, Calif.). Assays were performed in duplicates. The cytokine concentration was obtained from a regression curve prepared with the help of Microplate Manager Software (Bio-Rad). The results were expressed as pg/ml.

Statistical analysis

The data were presented as mean ± standard error of mean (mean ± S.E). Statistical analysis of results was carried out using one-way analysis of variance (ANOVA). Comparison between two groups was done by the Student's *t*-test. All statistical analysis was performed with the aid of the SPSS computer program (version 13.0 Windows). The data were considered significant if ($P < 0.05$), highly significant if ($P < 0.01$) and very highly significant if ($P < 0.001$). Percent

Table 1 Experimental layout, indicating times of infection, PZQ administration, ART injections and perfusion of mice.

Animal group	*n*	Time (days)							
		−2[a]	0[b]	7	14	21	42	45	56
a. Uninfected-untreated	10	–	–	–	–	–	–	–	Perfuse
b. Infected-untreated	15	–	(80 ± 10) cercariae	–	–	–	–	–	Perfuse
c. Infected, PZQ	15	–	(80 ± 10) cercariae	–	–	–	PZQ	–	Perfuse
d. Infected, ART 7 day PI	15	–	(80 ± 10) cercariae	ART	–	–	–	–	Perfuse
e. Infected, ART 14 day PI	15	–	(80 ± 10) cercariae	–	ART	–	–	–	Perfuse
f. Infected, ART 21 day PI	15	–	(80 ± 10) cercariae	–	–	ART	–	–	Perfuse
g. Infected, ART 45 day PI	15	–	(80 ± 10) cercariae	–	–	–	–	ART	Perfuse
h. ART pretreated-infected	15	ART	(80 ± 10) cercariae	–	–	–	–	–	Perfuse

[a] ART injection 2 days preinfection.
[b] Time of infection.

reduction (PR) in all parameters was calculated according to Fonseca et al. [12] using the following equation:

$$PR = \frac{1 - \text{Mean value of IT group}}{\text{Mean value of IU control group}} \times 100$$

where IT is the infected treated group and IU is the infected untreated group.

Results

The effect of the treatment with PZQ or ART on worm burden and distribution

Table 2 shows the worm burden and distribution for all infected groups. PZQ-treated mice showed a very highly significant reduction ($P < 0.001$) in the mean worm burden for males, females and couples with PR: 72.15%, 75.26% and 60%, respectively. The total worm burden was reduced by 92.67% in comparison with infected-untreated mice. Moreover, ART injection 7, 14 and 21 days PI induced a very highly significant decrease ($P < 0.001$) of both total and mean worm burden in comparison with infected-treated group, and the percentage of total worm reduction was 73.10%, 77.43% and 87.15%, respectively. On the other hand, infected mice treated with ART 45 day PI showed only 25.35% of total worm reduction. It was clear from Table 2 that pre-treatment has a very highly significant reductive ($P < 0.001$) effect on total worm burden of 68.75% compared with infected-untreated group.

Histopathological features

Liver sections of uninfected-untreated, infected-untreated and different treated groups were studied for MGN, MGD and cellular profiles (Fig. 1)

The effect of the treatment with PZQ or ART on mean granuloma number (MGN)

The MGN in 5 successive low power fields (10 × 10) was determined in the different infected groups and tabulated in Table 3. Hepatic tissues of infected-untreated mice showed the maximum MGN, while mice treated with PZQ showed a very highly significant decrease ($P < 0.001$) in MGN with PR of 78.33%. The schistosomicidal effect of ART injection on the developing schistosomules was reflected as a decrease in MGN with time from the 7 days old schistosomula to 21 days old *S. mansoni* larvae. This decrease in MGN was even lower than that recorded after the PZQ treatment. When infected mice were injected with ART 45 days PI MGN showed highly significant reduction ($P < 0.01$) with PR: 44.64%. In the ART pretreated-infected mice, there was a very highly significant decrease ($P < 0.001$) in the MGN with PR: 69.44%, compared with the infected-untreated mice.

Table 2 The effect of single intramuscular dose (50 mg/kg) of artemether (ART) in comparison to praziquantel (PZQ) treatment on worm distribution and worm burden in *Schistosoma mansoni*-infected mice.

Animal groups	Mean worm burden ± S.E. (PR)			Total worm burden ± S.E. (PR)
	Males	Females	Couples	
Infected-untreated	11.28 ± 1.20	7.52 ± 0.4	5.0 ± 0.7	28.8 ± 1.1
Infected, PZQ	1.4 ± 0.53*** (87.58)	0.67 ± 0.29*** (91.09)	0 ± 0*** (100)	2.11 ± 0.79*** (92.67)
Infected, ART 7 day PI	3.88 ± 0.67*** (65.59)	1.88 ± 0.40*** (75)	1 ± 0.38*** (80)	7.75 ± 1.46*** (73.10)
Infected, ART 14 day PI	4.3 ± 0.83*** (61.86)	1.2 ± 0.33*** (84.04)	0.5 ± 0.22*** (90)	6.5 ± 0.93*** (77.43)
Infected, ART 21 day PI	1.8 ± 0.51*** (84)	1.1 ± 0.23*** (85.37)	0.4 ± 0.22*** (92)	3.7 ± 0.95*** (87.15)
Infected, ART 45 day PI	9.25 ± 1.44 (17.96)	3.25 ± 0.84*** (56.78)	4.5 ± 0.50 (10)	21.5 ± 1.4*** (25.35)
ART pretreated-infected	3.14 ± 0.59*** (72.15)	1.86 ± 0.34*** (75.26)	2 ± 0.22*** (60)	9 ± 1.25*** (68.75)

Significance from infected-untreated group: *Significant ($P < 0.05$); **Highly significant ($P < 0.01$).
*** Significance from infected-untreated group: Very highly significant ($P < 0.001$).

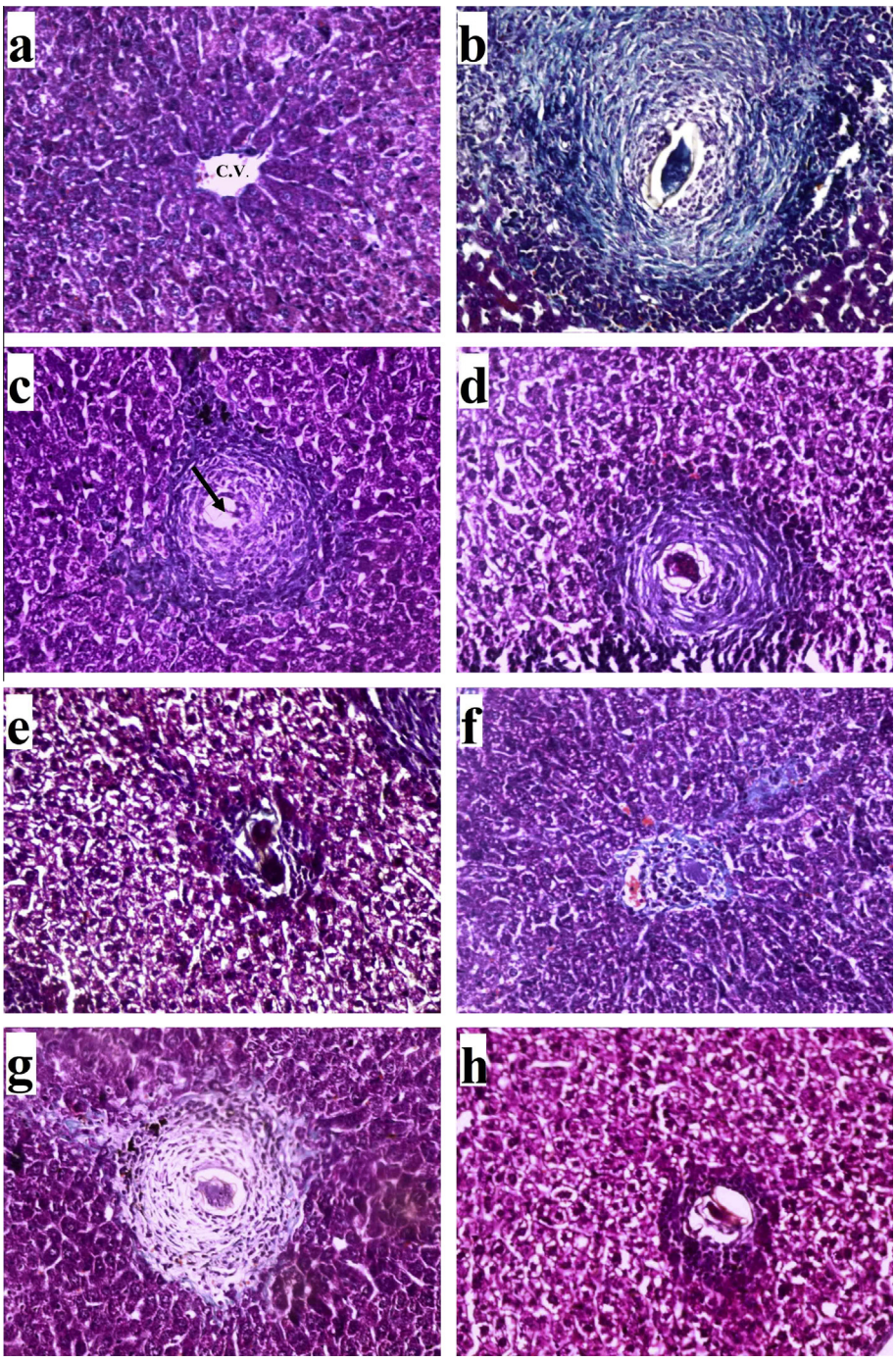


Fig. 1 Masson trichrome stained liver sections (400×) of (a) uninfected-untreated mouse showing normal hepatic architecture surrounding central vein (C.V.). (b) Infected-untreated mouse with massive fibrocellular granuloma consisting of collagen fibers and inflammatory cells surrounding living-intact egg. (c) Infected, PZQ-treated mice 42 days post infection (PI) showing medium sized fibrocellular granuloma with degenerated egg (arrow). (d). Infected, ART-treated 7 days PI showing medium sized granuloma with starting ovum degeneration. (e) Infected, ART-treated 14 days PI showing small sized granuloma with less collagenous fibers and inflammatory cells surrounding degenerated ova. (f) Infected, ART-treated 21 days PI showing reduced granuloma with little collagen fibers and inflammatory cells surrounding degenerated ovum. (g) Infected, ART-treated 45 days PI showing large fibrocellular granuloma with living ovum. (h) ART-treated 2 days preinfection showing small sized granuloma with low collagenous fibers and low inflammatory cell infiltration.

Table 3 The effect of single intramuscular dose (50 mg/kg) of artemether (ART) in comparison to praziquantel (PZQ) treatment on mean granuloma number (MGN) and mean granuloma diameter (MGD) in *Schistosoma mansoni*-infected mice.

Animal groups	MGN (mean ± S.E.)	PR (%)	MGD (μm) (mean ± S.E.)	PR (%)
Infected-untreated	9.23 ± 1.92	–	179.98 ± 28.99	–
Infected, PZQ	2.00 ± 0.86***	78.33	144.14 ± 00.87*	19.90
Infected, ART 7 day PI	2.50 ± 0.81***	72.91	168.87 ± 30.02	6.61
Infected, ART 14 day PI	1.30 ± 0.65***	85.92	146.33 ± 42.51*	18.69
Infected, ART 21 day PI	1.64 ± 0.80***	82.23	125.05 ± 33.88**	30.51
Infected, ART 45 day PI	5.11 ± 1.48**	44.64	179.66 ± 24.58	00.02
ART pretreated-infected	2.82 ± 1.44***	69.44	132.81 ± 30.35*	26.20

* Significance from infected-untreated group: Significant ($P < 0.05$).
** Significance from infected-untreated group: Highly significant.
*** Significance from infected-untreated group: Very highly significant.

The effect of the treatment with PZQ or ART on mean granuloma diameter (MGD)

In comparison with infected-untreated group, almost the same MGD reduction was obtained when infected mice received either PZQ treatment 42 days PI or ART treatment 14 days PI. The maximal reduction of MGD was found in mice treated with ART 21 days PI or 2 days pre-infection. These results go beyond that revealed from that in the case of PZQ treatment on MGD (Table 3).

Effect of PZQ or ART on cytokine profile

IFN-γ: In comparison with the infected-untreated mice the infected, PZQ-treated group showed a very highly significant increase ($P < 0.001$) in serum level of IFN-γ. ART treatment affected the serum level of IFN-γ in a deferential manner depending on the time of injection. This increase was very highly significant ($P < 0.001$) 14 or 21 days PI. The early (7 days PI) or late ART treatment (45 days PI) induced non-significant effect on the IFN-γ level ($P > 0.05$). ART injection 2 days pre-infection showed a very highly significant increase ($P < 0.001$) in IFN-γ level (Table 4).

IL-4: In comparison with the infected-untreated control the infected, PZQ-treated mice showed a very high significant decrease ($P < 0.001$) in serum IL-4. ART treatment showed a gradual decrease in IL-4 level from day 7 to day 14 and then maximally by day 21 PI. On the other hand, treatment with ART at late time (45 days PI) showed no significant ($P > 0.05$) decrease in serum IL-4 level. ART treatment 2 days pre-infection as a prophylactic agent recorded a very high significance decrease ($P < 0.001$) in IL-4 level as shown in (Table 4).

IL-10: In comparison with the infected-untreated control the infected, PZQ-treated mice showed a very highly significant decrease ($P < 0.001$) in serum IL-10. The level of IL-10 was directly proportional with that of IL-4 and inversely proportional with that of IFN-γ in the groups which received a single i.m. The treatment with ART at late time (45 days PI) showed highly significant decrease ($P < 0.01$) in serum IL-10 level. Finally, ART pre-treatment showed a very highly significant decrease ($P < 0.001$) in IL-10 level (Table 4).

Discussion

Schistosomiasis mansoni occupies the first place in the list of endemic diseases in Egypt [13]. Although PZQ is the only drug available for the treatment of human schistosomiasis, many scientific views worry about the complete dependence on it as a sole antischistosomal drug. This is because of evidences of decreased susceptibility. ART, the methylated derivative of the naturally occurring artemisinin, is efficient toward 3–21 days old schistosomulae [14]. In the present study, mice treated with a low single i.m. ART dose (50 mg/kg) at different timings pre- and post-*S. mansoni* infection found to modulate the course of schistosomiasis infection.

The present study confirmed earlier observations which concluded that ART prominently affect the early stages of *Schistosoma*. Reduction in worm burden was improved by the treatment at 7 days PI and reached 73.1%. On the other

Table 4 The effect of single intramuscular dose (50 mg/kg) of artemether (ART) in comparison to praziquantel (PZQ) treatment on the serum levels of Interferon gama (IFN-γ), Interleukin-4 (IL-4) and Interleukin-10 (IL-10).

Animal group	IFN-γ (pg/ml)	IL-4 (pg/ml)	IL-10 (pg/ml)
Uninfected-untreated	238.60 ± 6.96	227.00 ± 5.13	322.17 ± 4.54
Infected-untreated	273.33 ± 13.99	587.20 ± 22.92***	888.00 ± 6.93***
Infected, PZQ	638.10 ± 6.99***,ΦΦΦ	307.80 ± 15.30**ΦΦ,Φ	617.90 ± 14.38***,ΦΦΦ
Infected, ART 7 day PI	283.29 ± 12.50*	688.40 ± 35.84***,Φ	719.56 ± 24.47***,ΦΦΦ
Infected, ART 14 day PI	531 ± 24.56***,ΦΦΦ	464.20 ± 29.55***,ΦΦ	637.88 ± 45.65***,ΦΦΦ
Infected, ART 21 day PI	664.63 ± 19.03***,ΦΦΦ	274.00 ± 10.55*,ΦΦΦ	621.30 ± 19.23***,ΦΦΦ
Infected, ART 45 day PI	351.75 ± 27.34**,ΦΦ	623.75 ± 24.04***	844.57 ± 9.85***,ΦΦ
ART pretreated-infected	477 ± 7.73***,ΦΦΦ	425.43 ± 23.28***,ΦΦΦ	767.6 ± 26.17***,ΦΦΦ

* Significance from normal group, Φ Significance from infected-untreated group: *Φ Significant ($P < 0.05$), **ΦΦ Highly significant, ***ΦΦΦ Very highly significant.

hand, maximal PR was recorded when the treatment was interfering with the 14–21 day old schistosomula stage. This was in consistence with the data recorded by El-Beshbishi et al. [15]. The susceptibility of juvenile worms to ART may be due to the fact that they have a lower capacity of detoxification than the adult worms. ART was found to cause inhibition of glutathion-s-transferase and to some extent supereoxide dismutase enzymes. These enzymes are involved in passive detoxification of antischistosomal drugs and elimination of superoxide radicals produced during metabolism. Such inhibition aims finally to the protection of the free radicals that formed by ART from interfering with the action of these enzymes [16].

By comparing the PR in total worm burden after PZQ treatment 42 days PI to that obtained after ART treatment 45 days PI, data showed that the PR was 92.67% and 25.35%, respectively. These results confirm suggestions of many earlier studies e.g. Araújo et al. [17] that ART and PZQ act against different developmental stages of the parasite and can act well together. As the anti-oxidant system in adult worms is stronger than that in immature worms [18], less susceptibility of adults compared to juveniles, under the same dose level of ART, is clarified.

Xioa et al. [19] suggested that adult female worms are more susceptible to ART treatment rather than adult male worms. The present study confirmed this suggestion as ART treatment caused dramatic decrease in female worm burden even with such low dose.

In addition, results recorded a striking reduction in worm pairs in all ART treatment regimens before sexual maturity. The reduction rate reached its top value for 14–21 days old worms with 90–92% PR. This may be attributed to the ultrastructural toxic effect of ART on schistosme worms as ART was proved to induce tegumental damage, muscular paralysis and even sustained shrinkage, atrophy and degeneration of the worm's reproductive glands (the testis in males and ovary as well as vitelline gland in females) [20].

Infection with *S. mansoni* induces chronic disease as a result of the ongoing host immune response toward the tissue trapped eggs. Granuloma is the main pathology of such chronic disease which is induced by $CD4^+$ T-cell programmed inflammations under stimulation of soluble egg antigens (SEA). Granulomas contain eosinophils, macrophages, lymphocytes and are also characterized by collagen deposition. MGN and MGD were reduced by 78.33% and 19.9%, respectively with PZQ treatment. Similar observations were recorded by Andrade and Grimaud [21] who found that granulomas around eggs decreased 2–3 folds in volume 3 weeks after PZQ treatment and in the subsequent weeks.

Records in the present work revealed dramatic reduction in MGN especially in groups which received ART treatment at 14 or 21 days PI (PR: 82.23% and 85.92%, respectively). An interesting observation was that the PR in MGN for these two groups exceeded that recorded in PZQ treated group which was 78.33%. This reduction in tissue granuloma may be attributed to the recorded reduction in total worm burden and female worm burden. The correlation between worm burden, egg load and granuloma formation was studied and was proved by Botros et al. [22].

The present results demonstrated that MGD diminished significantly when ART was administrated 14–21 days PI this was parallel to the histopathological reduction in collagen deposition surrounding eggs. The correlation between the reduction in MGD and the reduction of type III procollagen was suggested by Badawy et al. [23].

In murine schistosomiasis, Th1 reaction (with a predominant secretion of IFN-γ, minimal level of IL-4 and IL-5) occurs during prepatency and then shifts to a Th2-based profile which develops after the onset of oviposition and persists throughout the acute phase of infection (with high IL-4 and IL-5, but low IFN-γ) [24].

The shift from Th2- to Th1-like immune response is essential for the down modulation of granuloma reaction and disease control. The relation between Th1 cytokine profile and the development of smaller granulomas was also reviewed by Brunet and his colleagues [25].

The antischistosomal action of PZQ was proved to be dependent on T cell mediated immunity [26]. In contrast, the role of T-cell immunity in ART action is not clearly understood. While some studies suggesting immunosuppressive action of ART by inhibition of T cell progression [27], other studies recorded T-cell independent action of ART [28].

The current study revealed that ART treatment may cause a switching over effect from Th2 to Th1 predominancy (decrease in IL-10, IL-4 and upgrading of serum IFN-γ levels). We suggest two probable hypothesizes explaining Th1 predominancy. The first is in light of Wang et al. [27] study which proved the immunosuppressive properties of ART. So it prevents the ongoing of TH2 response which is responsible for granuloma progression.

The second hypothesis is that ART helminthotoxic to schistosomulae itself, regardless to immune response of the host, preventing their development to egg-laying adult worm pairs. The reduction in worm burden and fecundity in turn down regulates the egg-induced Th2 response and maintains Th1 predominant cytokine profile characterized by high levels of IFN-γ and low IL-4 and reduction in liver pathology.

The involvement of IFN-γ in protective immunity to schistosomiasis is well documented in the murine model [29]

In conclusion, after i.m. administration of ART to *S. mansoni* infected mice, it was shown that i.m. ART has promising prophylactic properties on acute schistosomiasis. ART administration is associated with shift to Th1 response and reduction in liver pathology. But ART use in schistosomiasis control still facing many challenges in both basic and applied research. Further research as well as clinical studies will determine the successes of ART as antischistosomal drug.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgments

This study was funded by Theodor Bilharze Institute and Faculty of Science, Cairo University.

References

[1] WHO. Report of the third global meeting of the partners for parasite control Deworming for Health and Development. Geneva, 29–30 November 2004, WHO/CDS/CPE/PVC/2005.14; 2005.

[2] Xiao SH, You JQ, Mei JY, Jiao PY. Early treatment of schistosomal infection with praziquantel in mice. Acta Pharmacol Sin 1993;14:533–8.
[3] Maghraby AS, Mohamed MA, Abdel-Salam AM. Anti-schistosomal activity of colostral and mature camel milk on *Schistosoma mansoni* infected mice. Asia Pacific J Clin Nutr 2005;14:432–8.
[4] Mishina YV, Krishna S, Hynes RK, Meade JC. Artemisinins inhibit *Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense* in vitro growth. Antimicrob Agents Chemother 2007;51:1852–4.
[5] Keiser J, Xiao SH, Xue J, Chan ZS, Odermatt P, Tesana S, et al. Effect of artesunate and artemether against *Clonorchis sinensis* and *Opisthorchis viverrini* in rodent models. Int J Antimicrob Agents 2006;28:370–3.
[6] Keiser J, Rinaldi L, Veneziano V, Mezzino L, Tanner M, Utzinger J. Efficacy and safety of artemether against a natural Fasciola hepatica infection in sheep. Parasitol Res 2008;103: 517–22.
[7] Xiao SH, Booth M, Tanner M. The prophylactic effects of artemether against *Schistosoma japonicum* infections. Parasitol Today 2000;16:122–6.
[8] Liang YS, John BI, Boyed DA. Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. Proc First Sino-Am Symp 1987;1:34–48.
[9] Lescano SZ, Chieff PP, Canhassi RR, Boulos M, Neto VA. Antischistosomal activity of artemether in experimental schistosomiasis mansoni. Rev Saúde Pública 2004;38:71–5.
[10] Lichtenberg EV. Host response to eggs of *Schistosoma mansoni*. Granuloma formation in the unsensitized laboratory mouse. Am J Pathol 1962;41:711–5.
[11] Mahmoud AA, Warren KS. Anti-inflammatory effects of tartaremetic and niridazole suppression of schistosome egg granuloma. J Immunol 1974;112:222–8.
[12] Fonseca CT, Brito CFA, Alves JB, Oliveira SC. IL-12 enhances protective immunity in mice engendered by immunization with recombinant 14 kDa *Schistosoma mansoni* fatty acid-binding protein through an IFN-γ and TNF-α dependent pathway. Vaccine 2004;22:503–10.
[13] El-Khoby T, Galal N, Fenwick A, Barakat R, El-Hawey A, Nooman Z, et al. The epidemiology of schistosomiasis in Egypt: summary finding in nine governorates. Am J Trop Med Hyg 2000;62:88–99.
[14] Xiao SH, Chollet J, Weiss NA, Bergquist RN, Tanner M. Preventive effect of artemether in experimental animals infected with *S. mansoni*. Parasitol Int 2000;49:19–24.
[15] El-Beshbishi SN, Taman A, El-Malky M, Azab MS, El-Hawary AK, El-Tantawy DA. *In vivo* effect of single oral dose of artemether against early juvenile stages of *Schistosoma mansoni* Egyptian strain. Exp Parasitol 2013;135:240–5.
[16] Hamza RS, Metwaly AS, Abo EL-Maaty DA. Effects of artemether treatment on prepatant and patent *Schistosoma mansoni* infection in experimentally infected mice. PUJ 2012;5: 147–54.
[17] Araújo N, Kohny A, Katz N. Activity of the artemether in experimental schistosomiasis mansoni. Mem Inst Oswaldo Cruz, Rio de Janeiro 1991;86:185–8.
[18] Nare B, Smith JM, Prichard RK. *Schistosoma mansoni*: levels of antioxidants and resistance to oxidants increase during development. Exp Parasitol 1990;70:389–97.
[19] Xiao SH, Binggui S, Utzinger J, Chollet J, Tanner M. Transmission electron microscopic observations on ultra structural damage in juvenile *Schistosoma mansoni* caused by artemether. Acta Trop 2002;81:53–61.
[20] Pearce EJ. Priming of the immune response by schistosome eggs. Parasite Immunol 2005;27:265–70.
[21] Andrade ZA, Grimaud JA. Morphology of chronic collagen resorption. A study on the late stages of schistosomal granuloma involution. Am J Pathol 1988;132:389–99.
[22] Botros SS, Hammam M, Bergquist R. Praziquantel efficacy in mice infected with PZQ non-susceptible S. *mansoni* isolate treated with artemether: parasitological, biochemical and immunohistochemical assessment. APMIS 2010;118:692–702.
[23] Badawy AA, EL-Badrawy M, Nada JM, EL-Garem AA, Ebeid F, Abdel-Hady AM, et al. Effect of PZQ on hepatic murine schistosomiasis: histological study, immunolocalization of type III procollagen and serological analysis. Egypt J Bilh 1991;13: 117–29.
[24] Davies SJ, Lim KC, Blank RB, Kim JH, Lucas KD, Hernandez DC, et al. Involvement of tumor necrosis factor in limiting liver pathology and promoting parasite survival during schistosome infection. Int J Parasitol 2004;34:27–36.
[25] Brunet LR, Dunne DW, Pearce EJ. Cytokine interaction and immune responses during *Schistosoma mansoni* infection. Parasitol Today 1998;14:422–7.
[26] Ammann P, Waldvogel A, Breyer I, Esposito M, Muller N, Gottstein B. The role of B- and T-cell immunity in toltrazuril-treated C57BL/6 WT, microMT and nude mice experimentally infected with *Neospora caninum*. Parasitol Res 2004;93:178–87.
[27] Wang JX, Tang W, Sh LP, Wan J, Zhou R, Ni J, et al. Investigation of the immunosuppressive activity of artemether on T-cell activation and proliferation. Br J Pharmacol 2007;150: 652–61.
[28] Keiser J, Vargas M, Doenhoff MJ. Short report: activity of artemether and mefloquine against juvenile and adult *Schistosoma mansoni* in athymic and immunocompetent NMRI mice. Am J Trop Med Hyg 2010;82:112–4.
[29] Hewitson JP, Hamblin PA, Mountford AP. Immunity induced by the radiation-attenuated schistosome vaccine. Parasite Immunol 2005;27:271–80.