Efficacy and Safety of Arachidonic Acid for Treatment of *Schistosoma mansoni*-Infected Children in Menoufiya, Egypt

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Abstract. Arachidonic acid (ARA), an omega-6 fatty acid, kills juvenile and adult schistosomes *in vitro* and displays highly significant and safe therapeutic effects in mice and hamsters infected with *Schistosoma mansoni* or *S. haematobium*. This study aims to examine the efficacy and safety of ARA in treatment of school-age children infected with *S. mansoni*. In total, 66 *S. mansoni*-infected schoolchildren (20–23 children/study arm) received a single dose of 40 mg/kg praziquantel (PZQ), ARA (10 mg/kg per day for 15 days), or PZQ combined with ARA. The children were examined before and after treatment for worm egg counts in stool and blood biochemical and immunological parameters. ARA proved to be as efficacious as PZQ in treatment of schoolchildren with low infection intensity (78% and 85% cure rates, respectively). For moderate-intensity infection, the ARA and PZQ combination led to 100% cure rate. Biochemical, hematological, and immunological parameters were either unchanged or ameliorated after ARA therapy.

INTRODUCTION

Schistosomiasis is a severe parasitic disease endemic in 78 countries that affects at least 243 million people, and 800 million people are at risk.¹ Work and recreational activities near fresh water bodies harboring infected snails allow exposure of the skin to the infective stage: the cercaria. On invasion of the epidermis, physiological and biochemical changes lead to transformation to the schistosomula stage characterized by replacement of the cercarial trilaminar membrane by a double lipid bilayer rich in cholesterol and sphingomyelin (SM). The schistosomula are then prepared to penetrate into the blood capillaries, which are their permanent residence.² Schistosomes show unsurpassed precision in laying eggs near the conduit for egg passage to the external environment to complete the life cycle.^{3,4} Accordingly, massive numbers of eggs daily exit from the blood capillaries to the lumen of the gut or urinary bladder, where they may be detected by analysis of stool or urine samples, respectively. Large numbers of eggs are retained in the tissues of the intestinal and urinary bladder walls and the liver and responsible for the disease's clinical manifestations by releasing egg antigens that provoke intense immunological reactions.^{1–4}

Praziquantel (PZQ) is currently the only drug of choice for treatment of schistosomiasis *mansoni*. A single oral dose of 40–60 mg/kg body weight consistently produces cure rates of 60–90%.^{1,3,5–8} The side effects are relatively mild, involving essentially headache, dizziness, rashes, nausea, abdominal pain, and diarrhea, symptoms that could be easily explained by the propensity of PZQ to bind to actin.^{9,10} Failure of cure after PZQ chemotherapy was consistently associated with high intensity of infection,^{5–8} which required repeated treatments and accelerated the impending threat of schistosome resistance to the drug.¹¹ Accordingly, use of other schistosomicides alone or combined with PZQ was sought with variable results.^{12–15}

Arachidonic acid (ARA), also termed all-*cis* 5,8,11,14-eicosatetraenoic acid, is an omega-6 fatty acid 20:4 (n-6) that is present in the phospholipids (especially phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) of membranes of the body's cells, and it was recently found to be a potent schistosomicide able to *in vitro* kill juvenile and adult male and female *Schistosoma mansoni* and *S. haematobium*.^{15–17} The proposed ARA killing mechanism was activation of parasite surface membrane-associated neutral sphingomyelinase with consequent apical bilayer SM hydrolysis and disruption of the SM-based hydrogen barrier shielding the worm from the hostile elements of the immune system.^{15–22} Expectedly, ARA was shown to display significant therapeutic effects in mice and hamsters infected with *S. mansoni* or *S. haematobium*, inducing 50–78% reductions in worm burdens and worm egg loads.^{16,17} ARA treatment of mice and hamsters was safe.

Other than being an essential constituent of membrane phospholipids, ARA is the base material used by the body to synthesize a variety of eicosanoids. Some are proinflammatory and vasoconstrictive, such as prostaglandin E₂, thromboxane A₂, and leukotriene B₄, and some are anti-inflammatory and antiaggregatory, such as prostacyclin and lipoxin A₄.^{23,24} Proper development of the brain, retina, and other body tissues depends on provision of ARA either directly in the diet (0.1–0.3 g daily) or through synthesis from linoleic acid (LA) 18:2 (n-6).²⁵ Aggregate data from randomized trials indicated that high intake of ARA or LA seems to be safe regarding human immune functions^{26–28} and particularly beneficial in decreasing risk for coronary heart disease.²⁹ Supplementation of 1 g ARA daily for 50 days was found to be well-tolerated in male athletes.³⁰ In this study, we present, for the first time, data on the efficacy and safety of the nutrient ARA in treatment of Egyptian children infected with *S. mansoni*.

MATERIALS AND METHODS

Human subjects. The study protocol was reviewed and approved by the Ethical Committee of the Egyptian Ministry of Health and Population, Central Directorate for Research

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and Health Development (Approval 34-2012/1). The study was registered with ClinicalTrials.gov (NCT02144389). Written informed consent was obtained from parents or legal guardians of all children enrolled in this study after explaining the objectives and methods of the study.

The study was carried out between January and July of 2013. Screening of about 2,000 schoolchildren took place in villages of three districts in Menoufiya governorate located about 100 km north of Cairo; two microscopic slides of stool samples were examined for each child on 3 consecutive days by the Kato–Katz method, and egg counts per gram (epg) stool were recorded.^{31,32} The study revealed that the highest prevalence was in Menouf district. Therefore, the study was conducted in Menouf district on 66 schoolchildren attending four schools in three villages (44 males and 22 females; 6–15 years old [mean $\pm$ SD = 11.7 $\pm$ 1.6 years old]) with a weight range of 25–52 kg (mean $\pm$ SD = 37.8 $\pm$ 8.6 kg) and epg range of 24–384. In total, 20 sex-, age-, and social conditions-matched parasite-free children were considered as controls.

Drugs. Praziquantel tablets (Distocide; Epico, El-Asher-Men-Ramadan City, Egypt) were provided by the Egyptian Ministry of Health and Population. ARASCO oral capsules (1 g VegCap and 396 mg ARA) containing 395 mg ARA/capsule were provided by DSM (DSM Nutritional Products, Columbia, MD). Capsules lacking ARA, Pbo, Corn-Soy, and 1 g VegCap (DSM) were used as placebo for children treated with PZQ only.

The selected children from each of four schools were divided randomly (i.e., irrespective of sex, age, weight, or baseline epg) into three groups. The first group was given a single oral dose of PZQ (40 mg/kg) on the first day of treatment and placebo of ARA for the next 3 weeks (5 doses/week). The second group orally received ARA (10 mg/kg per day) for 15 days over 3 weeks (5 days/week). The third group was given PZQ (40 mg/kg) on the first day of treatment and then received 15 doses of ARA (10 mg/kg per day for 5 doses/week). The total numbers of children were 20, 23, and 23 for PZQ, ARA, and combined PZQ and ARA study groups, respectively.

Study design. Approximately 10 mL venous blood was obtained from each child 2–3 days before start of treatment and 3 days after the end of ARA (or ARA + PZQ) treatment (corresponding to 24 days after PZQ). The blood was aseptically and equally distributed among four vacutainers, which were plain or contained sodium citrate, ethylene diamine tetraacetic acid disodium salt (EDTA), or sodium heparin (Becton, Dickinson and Company, Plymouth, United Kingdom), and the vacutainers were transported within 3 hours at 10°C to the laboratory. For the efficacy study, coded stool samples were obtained from each child on 3 consecutive days 1 week after the end of ARA treatment (corresponding to 4 weeks after PZQ) and 6 weeks after the end of ARA treatment (corresponding to 9 weeks after PZQ), and epg was assessed by two technicians entirely blind to the code (Figure 1).

Biochemical and hematological parameters. Blood samples for biochemical analysis were put in a plain tube, allowed to clot for at least 60 minutes, and centrifuged, and the serum was used to investigate the lipid profile and the parameters reflecting liver and kidney functions (Cobas Integra 400 plus, 500281; Roche Diagnostic Ltd, Rotkreuz,

FIGURE 1. Study flow chart.

Switzerland). Viral markers, including both hepatitis B surface antigen and hepatitis C virus antibody, were analyzed by Cobas e 411 (Hitachi High Technologies Corporation, Tokyo, Japan) and found to be uniformly negative. Blood in the tube containing sodium citrate was used for coagulation tests, including prothrombin time and activated partial thromboplastin time, which were analyzed by automated blood coagulation analyzer (Sysmex CA 1500; Sysmex Corporation, Kobe, Japan); 2 mL blood was dispensed in the EDTA tube for a complete blood picture and analyzed by an automated blood hematology analyzer (XT 1800i; Sysmex Corporation).

Aliquots of heparinized plasma collected at baseline and after treatment were stored at -76°C until analysis. Total lipids were extracted as described by Folch and others.³³ Fatty acid methyl esters (FAMES) were prepared as described.³⁴ The samples were purged with N₂ throughout the process to minimize oxidation. FAMES were analyzed by gas liquid chromatography (GLC) using a Hewlett Packard 6890 equipped with a flame ionization detector. The resulting peaks were identified by comparison of retention times with external FAME standard mixtures from NuCheck Prep (Elysian, MN).

The fatty acid profiles were expressed as weight percentage values for plasma phospholipids.

Levels of plasma interleukin-10 and interferon- γ . Plasma was retrieved from heparinized blood after centrifugation at $400 \times g$ for 20 minutes and stored at -76°C until assayed by capture enzyme-linked immunosorbent assay (ELISA) for levels of interleukin-10 (IL-10) and interferon- γ (IFN- γ ; ELISA MAX Set; BioLegend, San Diego, CA) according to the manufacturer's protocol.

Whole-blood cytokine responses to parasite antigen. Heparinized whole blood was diluted 1:4 in Roswell Park Memorial Institute (RPMI) -1640 medium supplemented with 200 units/mL penicillin, 200 $\mu\text{g/mL}$ streptomycin, 50 ng/mL amphotericin, and 20 $\mu\text{g/mL}$ polymyxin B (Sigma-Aldrich) as an inhibitor of any residual lipopolysaccharide contamination of antigen; 200 μL diluted blood was incubated in duplicate with 50 μL medium containing 0 or 40 $\mu\text{g/mL}$ recombinant *S. mansoni* glyceraldehyde 3-phosphate dehydrogenase (rSG3PDH) prepared as described previously.³⁵ Whole-blood cultures were incubated for 72 hours at 37°C and 3% CO_2 and then centrifuged at $400 \times g$ for 10 minutes. The cell-free supernatants were transferred into wells of sterile plates and stored at -76°C until assayed by capture ELISA for levels of released IL-4, IL-17, and IFN- γ (BioLegend) following the manufacturer's instructions.

Statistical analysis. All values were tested for normality. Student's unpaired two-tailed *t*, Mann-Whitney, Fisher's exact, and analysis of variance (ANOVA) tests were used to analyze the statistical significance of differences between experimental and control values and considered significant at $P < 0.05$.

RESULTS

Efficacy of ARA treatment. Selected *S. mansoni*-infected children were divided at random into three groups that were treated with PZQ, ARA, or PZQ + ARA (Table 1). Efficacy of ARA in treatment of school-age children with < 100 epg (baseline geometric mean egg count [GMEC] $\pm$ SE = 75 ± 8 epg for 44 schoolchildren) was highly comparable with that of PZQ + placebo, with percentage cures of 78% (11 of 14) and 85% (12 of 14), respectively, and similar levels of reduction in GMEC in uncured patients. However, therapeutic efficacy of PZQ + ARA assessed in 16 schoolchildren was not higher than for PZQ alone (Figure 2A and Table 1). The efficacy of ARA in treatment of schoolchildren with 100–400 epg (baseline GMEC $\pm$ SE = 270 ± 38 epg for 21 children) was significantly ($P < 0.0001$, Fisher's exact test) lower than for PZQ regarding

FIGURE 2. Efficacy of ARA in chemotherapy of *S. mansoni* infection in Egyptian school-age children in Menoufiya Governorate. (A) Low infection (< 100 epg) and (B) moderate infection (100–400 epg). Stool analyses and epg were recorded 4 weeks after the end of PZQ and 6 weeks after the end of ARA treatment. Percentage egg reduction is the percentage reduction in GMECs in uncured schoolchildren.

cure rate of six to nine children per group and reduction in GMEC in uncured children. However, ARA showed a highly significant ($P < 0.0001$, Fisher's exact test) additive effect to PZQ, resulting into 100% cure (7 of 7) (Figure 2B and Table 1).

Safety of ARA treatment. Not a single child reported any adverse reactions during or after treatment with ARA. A substantial majority of children treated with PZQ reported transient headache, dizziness, abdominal pain, nausea, and diarrhea. Symptoms disappeared the next day, and children received placebo or ARA capsule without any additional malaise.

Lipid profile. Therapy of *S. mansoni* patently infected schoolchildren with PZQ, ARA, or PZQ and ARA combined did not affect the serum levels of cholesterol and

TABLE 1
Effect of ARA treatment on schoolchildren's parasitological parameters

Infection level/treatment	Number	Mean age (years) $\pm$ SE	Baseline GMEC $\pm$ SE	Total cure
Light				
PZQ	6 female/8 male	11.4 ± 2.0	53 ± 26	12/14 (85%)
ARA	3 female/11 male	11.7 ± 1.1	66 ± 29	11/14 (78%)
PZQ + ARA	5 female/11 male	12.0 ± 1.2	73 ± 25	14/16 (87%)
Moderate				
PZQ	2 female/4 male	12.1 ± 1.3	182 ± 60	5/6 (83%)
ARA	4 female/5 male	11.6 ± 2.1	239 ± 94	4/9 (44%)*
PZQ + ARA	2 female/5 male	12.1 ± 1.6	282 ± 107	7/7 (100%)*

*Parameter values were statistically compared with those of PZQ-treated schoolchildren, and statistical differences with $P < 0.0001$ were measured by Fisher's exact test.

TABLE 2
Effects of ARA treatment on plasma lipid profile of schoolchildren infected with *S. mansoni*

	Mean $\pm$ SD before and after treatment		
	PZQ	ARA	PZQ + ARA
Children	20	23	23
Parameter (mg/dL)			
Cholesterol			
Before	143 $\pm$ 23	144 $\pm$ 23	141 $\pm$ 22
After	151 $\pm$ 24 (NS)	151 $\pm$ 27 (NS)	144 $\pm$ 20 (NS)
Triglycerides			
Before	66.7 $\pm$ 32.9	75.8 $\pm$ 33.9	75.7 $\pm$ 23.9
After	100.3 $\pm$ 68.8	113.4 $\pm$ 78.6	98.9 $\pm$ 36.2
P value, percentage change	0.015, 33	0.029, 33	0.016, 23
HDL			
Before	47.9 $\pm$ 13.1	43.5 $\pm$ 7.1	43.2 $\pm$ 8.4
After	45.9 $\pm$ 14.5 (NS)	41.6 $\pm$ 8.2 (NS)	42.1 $\pm$ 9.6 (NS)
LDL			
Before	84.1 $\pm$ 22.6	85.3 $\pm$ 20.2	83.3 $\pm$ 16.9
After	85.6 $\pm$ 23.6 (NS)	87.6 $\pm$ 28.1 (NS)	82.9 $\pm$ 19.1 (NS)

Values before and after treatment were analyzed by the two-tailed paired Student's *t* test. NS = not significant.

high- and low-density lipoproteins and led to significant ($P < 0.05$) increases in the levels of serum triglycerides (Table 2).

Similar results were recorded when using heparinized plasma, and total saturated fatty acids in plasma at baseline were similar between low and moderate infections with schistosomiasis and significantly lower than for healthy controls but significantly ($P < 0.001$) increased to control levels after therapy. No such variations were recorded for mono-unsaturated fatty acids. The ARA and PZQ + ARA treatment-mediated effects on plasma mono- and polyunsaturated fatty acids were equivalent, and therefore, they were combined for the purpose of statistical analysis. The percentage of ARA [20:4 (n-6)] was numerically but not statistically greater after treatment with ARA or PZQ + ARA compared with baseline and PZQ alone. The percentage of ARA was lowest in uninfected controls. Docosaehaenoic acid [DHA; 22:6 (n-3)] in plasma was lowest in subjects treated with ARA compared with PZQ only or at baseline. Plasma DHA in control subjects was 25–50% higher relative to pre- and post-treatment levels observed in study subjects. Likewise, total omega-3 long-chain polyunsaturated fatty acids were similar before and after treatment and less than levels observed in the plasma of uninfected controls (Supplemental Table 1).

Liver functions. Treatment of *S. mansoni* patently infected schoolchildren with PZQ, ARA, or PZQ and ARA combined did not affect the plasma levels of serum alanine amino transferase and alkaline phosphatase, albumin, or total bilirubin assessed 24 and 3 days after the end of PZQ and ARA treatment, respectively. The data indicated that treatment led to a significant increase in the levels of serum aspartate aminotransferase (from $P < 0.045$ to $P < 0.01$), γ -glutamyl transferase ($P < 0.0001$), and total proteins ($P < 0.01$) but a highly significant ($P < 0.001$) decrease in the levels of direct bilirubin (Table 3).

Kidney functions. Therapy with PZQ, ARA, or PZQ and ARA combined led to a significant (from $P < 0.02$ to $P < 0.0002$) decrease in levels of plasma creatinine (Supplemental Table 2).

Blood picture. Treatment with PZQ, ARA, or PZQ and ARA combined of *S. mansoni*-infected schoolchildren

resulted into a highly significant (from $P = 0.002$ to $P < 0.0001$) increase in the number of erythrocytes, the packed cell volume percentage, and the hemoglobin concentration (Table 4) and no significant changes in number of basophils or platelets. Treatment with PZQ alone but not ARA was associated with a significant ($P < 0.05$) increase in the levels of eosinophils and staff and segmented neutrophils (Supplemental Table 3).

Coagulation parameters. The three treatment regimens were similar regarding their effects on *S. mansoni*-infected schoolchildren's blood-clotting parameters (Table 5).

Effects of ARA treatment on immunological parameters. Approximately 22% of 42 schoolchildren patently infected with *S. mansoni* had 14.7 ± 4.9 (mean $\pm$ SE) pg/mL plasma IL-10, whereas all of 20 sex- and age-matched parasite-free children had no detectable circulating IL-10 (Figure 3A). Treatment with ARA alone led to the highest increase in the percentage of schoolchildren with undetectable IL-10 in plasma as early as 3 days after end of treatment compared with children treated with PZQ alone or combined with ARA. The schoolchildren who still had detectable plasma IL-10 after any of the three therapy regimens showed a significant ($P < 0.05$) decrease of 25–35% compared with levels before treatment (Figure 3B). Schoolchildren patently infected with *S. mansoni* had approximately 62 pg/mL plasma IFN- γ (double the level detected in sex- and age-matched parasite-free children) (Figure 3C). Treatment with ARA combined with PZQ alone led to the highest decrease in levels of circulating IFN- γ compared with children treated with PZQ or ARA alone (Figure 3D).

Whole-blood cultures of 64 *S. mansoni*-infected and 20 parasite-free schoolchildren were incubated in the presence of 0 or 8 μ g/mL rSG3PDH in duplicate wells for 72 hours, and supernatants were then assessed for levels of released IL-4, IL-17, and IFN- γ . No IL-4 was detected in unstimulated or rSG3PDH-stimulated cultures of parasite-free donors or infected schoolchildren before or 3 days after the end of ARA treatment that corresponds to 18 days after the end of PZQ intake. A small number of *S. mansoni*-infected schoolchildren produced IL-17 in response to rSG3PDH with no significant differences related to therapy,

TABLE 3
Effects of ARA treatment on liver function parameters of schoolchildren infected with *S. mansoni*

	Mean $\pm$ SD before and after treatment		
	PZQ	ARA	PZQ + ARA
Children	20	23	23
Parameter (units/L)			
AST			
Before	26.4 $\pm$ 5.9	23.8 $\pm$ 3.3	23.7 $\pm$ 5.6
After	30.8 $\pm$ 9.2	30.8 $\pm$ 10.8	32.7 $\pm$ 11.6
<i>P</i> value, percentage change	0.0162, 14.2	0.0035, 22.7	0.0012, 27.5
ALT			
Before	19.3 $\pm$ 9.1	18.2 $\pm$ 4.3	19.4 $\pm$ 8.4
After	19.1 $\pm$ 8.4 (NS)	17.5 $\pm$ 6.6 (NS)	18.8 $\pm$ 8.1 (NS)
ALP			
Before	257.1 $\pm$ 66.5	232.4 $\pm$ 70.7	236.1 $\pm$ 68.6
After	250.2 $\pm$ 73.2 (NS)	235.2 $\pm$ 73.9 (NS)	229.5 $\pm$ 65.5 (NS)
GGT			
Before	9.84 $\pm$ 1.95	11.38 $\pm$ 3.89	11.21 $\pm$ 2.81
After	15.76 $\pm$ 3.23	16.71 $\pm$ 5.48	17.25 $\pm$ 2.74
<i>P</i> value, percentage change	< 0.0001, 37	< 0.0001, 32	< 0.0001, 35
Total bilirubin (mg/dL)			
Before	0.45 $\pm$ 0.17	0.43 $\pm$ 0.22	0.41 $\pm$ 0.20
After	0.38 $\pm$ 0.10 (NS)	0.34 $\pm$ 0.20 (NS)	0.33 $\pm$ 0.16 (0.0023)
Direct bilirubin (mg/dL)			
Before	0.14 $\pm$ 0.04	0.16 $\pm$ 0.06	0.15 $\pm$ 0.07
After	0.07 $\pm$ 0.06	0.06 $\pm$ 0.06	0.06 $\pm$ 0.04
<i>P</i> value, percentage change	0.0008, 50	< 0.0001, 59	< 0.0001, 60
Total proteins (g/dL)			
Before	7.41 $\pm$ 0.33	7.59 $\pm$ 0.37	7.54 $\pm$ 0.39
After	7.68 $\pm$ 0.39	7.82 $\pm$ 0.33	7.74 $\pm$ 0.41
<i>P</i> value, percentage change	0.0055	0.0008	0.0052
Albumin (g/dL)			
Before	4.68 $\pm$ 0.22	4.72 $\pm$ 0.13	4.63 $\pm$ 0.23
After	4.42 $\pm$ 0.35 (0.022)	4.57 $\pm$ 0.32 (NS)	4.53 $\pm$ 0.35 (NS)

Values before and after treatment were analyzed by the two-tailed paired Student's *t* test. Normal values (units per liter) for aspartate aminotransferase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), and γ -glutamyl transferase (GGT) are 10–34, 10–44, 44–122, and 11–50, respectively. ALP values are higher than normal in *S. mansoni*-infected children before as well as after treatment. NS = not significant.

whereas whole-blood cultures from 62 of 64 *S. mansoni*-infected children produced 44–2,000 pg/mL IFN- γ in response to rSG3PDH. The levels of IFN- γ after therapy were lower than before PZQ treatment, but the differences were not statistically significant. Similarly, IFN- γ levels were (mean for 23 donors $\pm$ SE) 415 $\pm$ 121 and 237 $\pm$ 92 pg/mL before and after ARA + PZQ treatment, respectively, showing a 42% decrease but no significant two-tailed *P* value. Conversely, IFN- γ levels were (mean for 23 donors $\pm$ SE) 462 $\pm$ 74 and 193 $\pm$ 65 pg/mL before and

after ARA treatment, respectively, showing a significant (*P* < 0.01) decrease of 58%.

DISCUSSION

This study is the first to report that the nutrient ARA is as efficacious as PZQ in treatment of school-age children with low-intensity (< 100 epg in stool) *S. mansoni* infection, as judged by percentage cure rate and percentage GMEC reduction in children who were not entirely cured.

TABLE 4
Effects of ARA treatment on red blood cell parameters of schoolchildren infected with *S. mansoni*

	Mean $\pm$ SD before and after treatment		
	PZQ	ARA	PZQ + ARA
Children	20	23	23
Parameter			
RBCs ($10^6/\mu\text{L}$)			
Before	4.91 $\pm$ 0.37	4.82 $\pm$ 0.44	4.91 $\pm$ 0.44
After	5.40 $\pm$ 0.28	4.94 $\pm$ 0.42	5.09 $\pm$ 0.42
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001
PCV (%)			
Before	38.24 $\pm$ 1.57	37.55 $\pm$ 2.63	38.45 $\pm$ 2.00
After	40.12 $\pm$ 1.8	38.86 $\pm$ 2.22	40.07 $\pm$ 1.81
<i>P</i> value	< 0.0001	< 0.0001	< 0.0001
Hb (g/dL)			
Before	12.48 $\pm$ 0.75	12.27 $\pm$ 0.87	12.54 $\pm$ 0.77
After	12.92 $\pm$ 0.76 (0.002)	12.54 $\pm$ 0.86 (0.002)	12.84 $\pm$ 0.70 (0.002)

Erythrocytes (red blood cell [RBCs]), packed cell volume (PCV), and hemoglobin (Hb) values before and after treatment were analyzed by the two-tailed paired Student's *t* test. No significant changes were observed in mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) before and after treatment.

TABLE 5
Effects of ARA treatment on blood-clotting parameters of schoolchildren infected with *S. mansoni*

Children Parameter	Mean $\pm$ SD before and after treatment		
	PZQ	ARA	PZQ + ARA
Children	20	23	23
Parameter			
PT time (seconds)			
Before	11.37 $\pm$ 0.97	11.15 $\pm$ 0.90	11.21 $\pm$ 0.84
After	10.11 $\pm$ 0.79 (0.0002)	10.39 $\pm$ 0.89 (0.0091)	9.88 $\pm$ 0.64 (< 0.0001)
PT conc. (%)			
Before	103.82 $\pm$ 15.59	106.62 $\pm$ 14.00	106.09 $\pm$ 15.48
After	128.08 $\pm$ 17.34	122.28 $\pm$ 17.71	132.75 $\pm$ 15.45
P value	< 0.0001	0.0002	< 0.0001
PT/INR			
Before	0.95 $\pm$ 0.09	0.93 $\pm$ 0.06	0.94 $\pm$ 0.07
After	0.84 $\pm$ 0.06	0.86 $\pm$ 0.07	0.82 $\pm$ 0.05
P value	< 0.0001	< 0.0001	< 0.0001
PTT (seconds)			
Before	32.81 $\pm$ 6.51	31.54 $\pm$ 7.76	30.28 $\pm$ 10.33
After	31.45 $\pm$ 6.16 (NS)	31.19 $\pm$ 3.33 (NS)	31.46 $\pm$ 14.46 (NS)

Prothrombin time (PT) and PT concentration (conc.), international normalized ratio (INR), and partial thromboplastin time (PTT) values before and after treatment were analyzed by the two-tailed paired Student's *t* test. NS = not significant.

Treatment with PZQ required a single oral dose of 40 mg/kg, whereas 10 mg/kg per day ARA was given orally for 15 days over 3 weeks. Additional studies are needed to determine whether this lengthy treatment is actually required, because ARA is a nutrient other than being schistosomicidal and may increase fecundity in the surviving worms and the ability of worm eggs to exit the host. Nevertheless, ARA schistosomicidal activity in experimental animals was faithfully

reproduced in clinical trials at variance from mefloquine, which showed highly significant schistosomicidal action in mice and hamsters but poor efficacy in a randomized, exploratory human trial.^{36–40} ARA seemed a far more promising schistosomicidal remedy than myrrh (Mirazid), which showed a low (10–15%) cure rate and low egg reductions in uncured individuals, regardless of the patients' ages or pre-treatment intensities of infection.^{41–43}

FIGURE 3. Effect of ARA treatment on school-age children's plasma IL-10 and IFN- γ levels. Bars in **A** and **C** represent levels of (A) IL-10 and (C) IFN- γ in plasma of *S. mansoni*-free schoolchildren (controls) and children infected with *S. mansoni* and treated with PZQ, ARA, or PZQ + ARA. Percentage decreases in plasma (B) IL-10 and (D) IFN- γ are mean percentage decreases in geometric mean cytokine levels.

Despite that a 100% cure was reported after PZQ treatment of *S. mansoni* infections in eastern Sudan,⁴⁴ complete cures have seldom, if ever, been achieved in endemic areas after PZQ treatment.⁴⁵ Notably, 100% cure was also not achieved, even in children treated with a combination of PZQ and ARA, because two children still passed eggs. The likelihood of treatment failure necessitates careful monitoring of schistosome egg excretion rates after chemotherapy and retreatment, preferably with ARA, to avoid evolution of resistance to the massively used PZQ.^{11,45}

In contrast to PZQ, efficacy of ARA therapeutic action seemed to be related to pre-treatment intensity of infection, because cure rate in schoolchildren with moderate intensity infection (100–400 epg stool) and percentage reduction in egg excretion in uncured cases did not exceed 44%, significantly ($P < 0.0001$) lower by approximately 50% compared with PZQ. Of note, ARA showed a synergistic effect with PZQ, because complete cure of all children was achieved. Trials with higher numbers of school-aged children with low and moderate *S. mansoni* infection are required to document the excellent efficacy of the ARA and PZQ combination that seemed comparable with PZQ-oxamniquine¹² and substantially higher than PZQ-artesunate⁴⁶ and artesunate + sulfadoxine/pyrimethamine (4 mg/kg artesunate for 3 consecutive days + 25 mg sulfadoxine on day 0) in treatment of *S. mansoni* infections⁴⁴ and mefloquine-artesunate (100 mg artesunate plus 250 mg mefloquine daily for 3 days) in treatment of children concurrently infected with *S. haematobium* and *S. mansoni*.^{38,40} Final conclusions may, however, only be drawn after side-by-side trials, because otherwise, confounding factors have a great influence.

A substantial majority of children treated with PZQ reported transient headache, dizziness, abdominal pain, nausea, and diarrhea, all consistent with the documented ability of PZQ to bind to actin.^{6,7} Transient and self-limiting abdominal pain, nausea, and vomiting were also the most frequent symptoms observed in patients treated with mefloquine and mefloquine combined with artesunate.^{38,40} Conversely, not a single child reported the slightest adverse event during or after therapy with ARA, totally confirming our observations in experimental hosts^{15–17} and in accordance with the results of plasma polyunsaturated fatty acids analyses. ARA is a nutrient and could have been used to the advantage of children in these poor rural settings, because no increase in ARA or DHA plasma levels was observed 3 days after the end of treatment (Supplemental Table 1). The safety of ARA treatment was also reflected in analyses of plasma biochemical and hematological features 3 days after the end of ARA treatment (corresponding to 24 days after the end of PZQ treatment). Of note, 10 mg/kg ARA daily intake for 15 days over 3 weeks had no statistically significant effects on schoolchildren's serum levels of cholesterol or high- and low-density lipoproteins, supporting data recorded in adults given 1.7 g ARA daily for 50 days.³⁰ Similar to PZQ, ARA alone or combined with PZQ led to amelioration of the serum triglycerides, total proteins, direct bilirubin, and creatinine levels. The schoolchildren infected with *S. mansoni* showed evidence of anemia, confirming data obtained regarding *S. mansoni*-infected schoolchildren in eastern Sudan and Uganda.^{47,48} Therapy with PZQ, ARA, or PZQ + ARA elicited a highly significant ($P < 0.0001$ to $P < 0.002$) increase in the number of erythrocytes per micro-

liter, packed cell volume, and hemoglobin level, thus ameliorating the children's blood erythrocyte picture. Treatment with PZQ or ARA alone or combined with PZQ led to similar effects on platelets counts, prothrombin time and concentration, international normalized ratio, and partial thromboplastin time values, suggesting that therapy of schoolchildren with 300–600 mg/day ARA for 15 days induced no adverse changes in blood coagulation and thrombocytic tendencies, which is in accordance with findings obtained previously in healthy adults.³⁰

Therapy of *S. mansoni*-infected children with PZQ decreased the levels of circulating IL-10 and IFN- γ and the amounts of IFN- γ released in whole-blood cultures stimulated *in vitro* with rSG3PDH, giving support to the hypothesis that treatment of schistosomiasis mansoni with PZQ can significantly alter the immune response of patients.⁴⁹ Treatment with ARA alone or combined with PZQ was more effective than PZQ in altering the immune responses of children toward reducing production of IL-10 and IFN- γ as early as 3 days after the end of treatment, consistent with the observational studies showing that higher ARA consumption was associated with lower levels of inflammatory markers.⁵⁰ More importantly, ARA-associated significant decreases of the levels of the immunosuppressive IL-10 and the Th1 cytokine IFN- γ may enhance development and polarization of Th2-related immune responses to parasite antigens, which could lead to a prognosis of resistance to reinfection with schistosomes.^{51,52}

The results taken together urge additional validating studies, because if ARA effectiveness against light *S. mansoni* infections is confirmed, then it may be used instead of PZQ for travelers coming from endemic regions who show signs of infection and poor, malnourished children in areas with low endemicity. For moderate and heavy infections, it might be recommended to use ARA in conjunction with PZQ to decrease to a minimum the percentage of children still harboring worms and living usually unaware of the danger of persisting infection.

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