Artesunate Effect on Schistosome Thioredoxin Glutathione Reductase and Cytochrome c Peroxidase as New Molecular Targets in Schistosoma mansoni-Infected Mice

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Received: March, 2012
Accepted: June, 2012

ABSTRACT

Background: Schistosomiasis remains one of the most important parasitic diseases. Extensive use of praziquantel (PZQ) with concerns about possibility of drug resistance development, unavailability of an applicable vaccine, and absence of a reasonable alternative to PZQ, all represent a real challenge in control of schistosomiasis. Artemisinin derivatives are promising anti-schistosomal compounds, but their molecular mechanism of action on schistosomes is still not well defined.

Objective: This study investigated the possible effect of artesunate (ART) on schistosome thioredoxin glutathione reductase (TGR) and cytochrome c peroxidase (CcP) in Schistosoma mansoni-infected mice.

Methodology: The animals used were divided into four groups. Group I: infected untreated group (control group); group II: infected then treated with ART; group III: infected then treated with PZQ; and group IV: infected then treated with both ART and PZQ. ART was given orally in four doses, each of 300 mg/kg starting at 14th day post-infection (PI) and then every 2 weeks. PZQ was given orally in a single dose of 600 mg/kg at 42nd day PI. Then all mice were subjected to the following: adult S. mansoni worm count at 10 weeks PI, tissue egg count in liver and estimation of TGR and CcP mRNA expression in S. mansoni adult worms by semi-quantitative real time-PCR (rt-PCR).

Results: Semi-quantitative rt-PCR values revealed that treatment with ART caused significant decrease in expression of schistosome TGR and CcP in contrast to PZQ which did not cause significant change in their expression. In addition, there was more reduction in total and female worm counts in ART-PZQ treated group than in other treated groups. Moreover, complete disappearance (100%) of tissue eggs was recorded in ART-PZQ treated group with a respective reduction rate of 95.9% and 68.4% in ART- and PZQ-treated groups.

Conclusion: The current study elucidated for the first time that anti-schistosomal mechanism of action of ART is mediated via reduction in expression of schistosome TGR and CcP. Linking these findings, the addition of ART to PZQ could achieve complete cure outcome in treatment of schistosomiasis.

Keywords: Schistosomiasis, Artesunate, Praziquantel, Thioredoxin Glutathione Reductase, Cytochrome c Peroxidase.

INTRODUCTION

Human schistosomiasis remains one of the most important parasitic diseases in terms of large endemic areas with approximately 207 million infected people worldwide and an estimated 280,000 deaths annually¹-³, and another 779 million individuals at-risk of infection⁴. In the absence of a readily available vaccine for practical application⁵-⁷, chemotherapy is the recommended strategy⁸. Soon after the advent and use of PZQ as a new schistosomicidal compound, development of resistance to its therapeutic effect became a milestone in the chemotherapeutic control of schistosomiasis. Although the effectiveness of PZQ against schistosomes and other helminths is well documented⁹, it was observed that after 20 years of large-scale use, the spectra of drug-resistant parasites are looming and it is time for introducing a new therapeutic approach⁹. Despite that more than 100 million people are currently being treated for schistosomiasis with PZQ, they become rapidly re-infected and must be retreated on an annual or semiannual basis¹⁰. Low-cure rates have been recorded in many studies in Africa including Egypt where patients have yielded isolates that are tolerant to higher dosages...
of PZQ\textsuperscript{(15-13)}. It was noted that efforts to expand mass drug administration programs in these endemic countries may accelerate emergence of resistance\textsuperscript{(14)}. Moreover, it was believed that PZQ effectiveness could be limited due to its inability to kill schistosomes as early as 2-4 weeks post-infection\textsuperscript{(15)}.

Studies of schistosome life cycle have focused on the fact that it can survive for decades in the blood stream of the human host without being severely affected by assault caused by various reactive oxygen species (ROS), because it possesses a mechanism that degrades ROS\textsuperscript{(16)}. A distinction between host and parasite physiology with respect to detoxification of ROS led to identification of a novel line of potential schistosomicides. Mammals have two distinct detoxification enzymes; thioredoxin reductase and glutathione reductase, while in schistosomes these catalytic activities are performed only by the TGR molecule\textsuperscript{(17,18)}. Thus, TGR has become one of the most appealing drug targets against schistosomiasis\textsuperscript{(19)}.

In addition, CcP protects the worms from H$_2$O$_2$ generated in the mitochondria of \textit{S. mansoni} and also from exogenous H$_2$O$_2$ produced by activated phagocytes from the host. The absence of this enzyme in mammalian cells makes it a possible interesting target for therapeutic control of schistosomiasis\textsuperscript{(20)}.

Anti-schistosomal activity of artemisinin was first reported in 1980\textsuperscript{(21)}. Based on previous studies either in vitro\textsuperscript{(22)} or in vivo\textsuperscript{(23-29)}, ART was introduced as a new anti-schistosomal drug. Among several artemisinin derivatives, ART is documented to be less toxic especially when given orally owing to its pharmacokinetic properties\textsuperscript{(30-32)}. The water-soluble compound ART is safer because it is absorbed and eliminated rapidly, whereas in oil-based derivatives such as artemether or arteether, blood concentrations are sustained throughout the dosing interval\textsuperscript{(33,34)}. Until recently, it was believed that although their anti-malarial effect is basically mediated by heme-dependent cleavage of endoperoxide with subsequent liberation of cytotoxic intermediates and free radicals, yet their molecular mechanism of action on schistosomes is not yet fully defined\textsuperscript{(13,35)}. The proposal that anti-schistosomal effect of ART could be mediated via other mechanisms is supported by the observation that artemisinin analogs act against \textit{Plasmodium} and other parasites by a clear difference in concentration ranges and dosage regimen\textsuperscript{(35)}. In addition, the conclusion that severe iron-deficiency would not influence the efficacy of ART, suggests the presence of a non heme-mediated pathway as a possible mechanism\textsuperscript{(37)}.

Thus, the aim of the present work was to determine the possible effect of ART on schistosome TGR and CcP as possible targets for therapeutic control of schistosomiasis.

### MATERIAL AND METHODS

**Type of the study:** Experimental case control study.

This study was conducted from April 2011 to April 2012 in the Medical Parasitology Department, Faculty of Medicine, Tanta University.

**Parasite:** Laboratory bred 	extit{Biomphalaria alexandrina} snails were purchased from the Schistosome Biological Supply Program, Theodore Bilharz Research Institute (TBRI), Giza, Egypt. According to Lewis et al.\textsuperscript{(36)}, the snails were placed in beakers containing dechlorinated water (1 ml/snail) and exposed to direct light at 28°C for at least 4 h. \textit{Schistosoma mansoni} cercariae shed from the snails were used to infect the experimental animals of the study. The cercarial suspension was adjusted to contain 80-100 cercariae/0.1 ml dechlorinated water.

**Animals and experimental design:** A total of 200 laboratory bred male Swiss albino mice, 6-8 weeks old, weighing 20-25 g were purchased from TBRI. Mice were housed in appropriate cages and allowed commercial rodent chow and tap water \textit{ad libitum}. Each mouse was infected by subcutaneous injection of 0.1 ml cercarial suspension\textsuperscript{(37)}. They were then divided into four groups (50 mice each). Group I: infected untreated group (control group) received a vehicle of 1% sodium carbonyl methylcellulose (CMC-Na); group II: infected then treated with ART; group III: infected then treated with PZQ; and group IV: infected then treated with ART then PZQ.

**Treatment protocol:** ART (Sigma-Aldrich) was suspended in 1% CMC-Na. It was given by oral gavage in a dose of 300 mg/kg at a time schedule started 14th day PI and repeated as one dose every 2 weeks for 4 consecutive doses\textsuperscript{(37)}. PZQ (Distocide; 600 mg tablet, EIPI Co. Pharmaceuticals, Egypt) was dissolved in distilled water and given orally to mice in a single dose of 600 mg/kg at 42nd day PI\textsuperscript{(38)} (Figure 1). Drugs were administrated orally using one ml syringe equipped with a blunt, 18-gauge needle.

**Collection of adult \textit{S. mansoni} worms by animal perfusion method:** All mice were sacrificed 10 weeks PI. Hepatic and portomesenteric vessels were perfused\textsuperscript{(39)} to recover worms. All \textit{S. mansoni} adult worms in the liver and mesenteric veins of the small and large intestines were removed from the perfusion fluid, collected in Petri dish, washed, sexed and counted. Worms were then stored at -80°C for further estimation of TGR and CcP mRNA expression. The reduction rates of total and female worms were calculated by comparing the mean worm numbers in the treated groups with that of control group.

**Tissue egg count:** One gram from liver of each mouse was placed in a test tube containing 2 ml of 5% KOH and left overnight at room temperature. The second day, all test tubes were incubated at 37°C for 6 h. Each test
tube was shaken and 0.1 ml of the digest was examined microscopically for counting *S. mansoni* eggs. The average number of ova in 0.1 ml was determined and the number of ova/gram tissue was calculated. The reduction rates of tissue egg count were calculated by comparing the mean tissue egg count in the treated groups with that of control group.

**Estimation of TGR and CcP mRNA expression in *S. mansoni* adult worms by semi-quantitative rt-PCR:** *S. mansoni* adult worms were homogenized in 1 ml Trizol (Invitrogen, USA) and processed for RNA extraction using MagNa pure compact nucleic acid isolation kit I (Roche Diagnostics, GmbH, Mannheim, Germany) following manufacturer’s instructions. The yield of total RNA obtained was quantified spectrophotometrically. A total of 1 µg parasite RNA was used to prepare cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) according to the manufacturer’s instructions.

A final reaction volume of 20 µl was prepared using Light Cycler-DNA Master SYBR Green I kit (Roche diagnostics, Germany). Each mix is formed of 1 µl primer (0.5 µM), 2 µl Light Cycler-DNA Master SYBR Green I (1x), 2.4 µl MgCl₂, stock solution (4 mM), 11.6 µl H₂O sterile PCR grade and 2 µl of cDNA template (30 ng/µl). PCR reactions were done in Light Cycler (Roche, Germany). The program included an initial melting phase at 50°C for 2 min, denaturation at 95°C for 10 min followed by 45 cycles of amplification (15 seconds at 95°C, 1 min at 60°C). Using the standard curve, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control gene, semi-quantitation of genes expression was calculated as a ratio between target gene and internal control (Figure 2). The sequences of the primers are supplied in table (1).

**Statistical analysis:** Quantitative values of the measured parameters were expressed as mean± standard deviation (SD). The data were analyzed by one way-ANOVA (*F* value) to detect significance in between groups followed by post-hoc analysis to detect difference between each two means, using Statistical Package for Social Sciences (SPSS), version 14.0 for windows. The difference was considered statistically significant when *P*<0.05.

**Table (1):** Primer sets for amplification of TGR, CcP, GAPDH genes of *S. mansoni* adult worms.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thioredoxin glutathione reductase (TGR)</td>
<td>Forward: 5’-CTATTTCCGTAGACGTCTGT-3’&lt;br&gt;Reverse: 5’-AATACAGTTTCCTTCCCGTT-3’</td>
</tr>
<tr>
<td>Cytochrome c peroxidase (CcP)</td>
<td>Forward: 5’-TCCCTTTATCAAATTGAGAGG-3’&lt;br&gt;Reverse: 5’-CCAACCATAAAAACTATGATG-3’</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)</td>
<td>Forward: 5’-GTGTTGCTCATCGGGAGA-3’&lt;br&gt;Reverse: 5’-ATGCGTTAGAAACCACGGAC-3’</td>
</tr>
</tbody>
</table>
**Ethical considerations:** The experiments were conducted in accordance to the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). This research was approved by the Research Ethics committee, Quality Assurance Unit, Faculty of Medicine, Tanta University, Tanta, Egypt.

**RESULTS**

The number of total worms recovered from *S. mansoni*-infected mice 10 weeks PI was significantly reduced in ART-treated group (73.4%). The respective total worm burdens reduction in PZQ-treated group was 86.7% and 95.4% in combined ART-PZQ treated group. The reduction rate in female worms in ART-treated group and PZQ-treated group was 74.8% and 88.3%; respectively, whereas 100% reduction rate in female worms was achieved in ART-PZQ treated group (Table 2). Regarding tissue egg count, complete disappearance of tissue eggs was recorded in ART-PZQ treated group (100% reduction rate) with a respective reduction rate of 95.9 % in ART-treated group. Tissue egg count showed only 68.4% reduction in PZQ-treated group (Table 3).

Semi-quantitative rt-PCR values revealed that treatment with ART caused significant decrease in expression of schistosome TGR and CcP in comparison to the untreated group. In contrast, treatment with PZQ did not cause significant change in expression of these genes (Table 4).

**Table (2):** Effect of different treatment protocols on total and female worm burdens in mice experimentally infected with *S. mansoni* versus infected untreated mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Untreated group</th>
<th>Treated groups</th>
<th>F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td><strong>Total worm count</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>24.10±4.01</td>
<td>6.40±1.90</td>
<td>3.20±1.32</td>
</tr>
<tr>
<td>%Reduction</td>
<td>73.4</td>
<td>86.7</td>
<td>95.4</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>11.10±1.85</td>
<td>2.80±1.48</td>
<td>1.30±0.82</td>
</tr>
<tr>
<td>%Reduction</td>
<td>74.8</td>
<td>88.3</td>
<td>100</td>
</tr>
</tbody>
</table>

*P*: Significance between groups. *P*1: group II (treated with ART), group III (treated with PZQ) and group IV (treated ART and PZQ) vs group I (untreated group). *P*2: group III (treated with PZQ) vs group II (treated with ART).

**Table (3):** Effect of different treatment protocols on tissue egg count (eggs/g liver) in mice experimentally infected with *S. mansoni* versus infected untreated mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Untreated group</th>
<th>Treated groups</th>
<th>F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>2511.50±162.44</td>
<td>104.20±23.41</td>
<td>793.30±100.53</td>
</tr>
<tr>
<td>% Reduction</td>
<td>95.9</td>
<td>68.4</td>
<td>100</td>
</tr>
</tbody>
</table>

*P*: Significance in between groups. *P*1: group II (treated with ART), group III (treated with PZQ) and group IV (treated with ART and PZQ) vs group I (untreated group). *P*2: group III (treated with PZQ) vs group II (treated with ART).

**Table (4):** Semi-quantitative rt-PCR values of m-RNA expression of TGR and CcP of *S. mansoni* adult worms (10 weeks PI).

<table>
<thead>
<tr>
<th>Schistosome gene</th>
<th>Groups:</th>
<th>Untreated group</th>
<th>Treated groups</th>
<th>F value (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Group I</td>
<td>Group II</td>
<td>Group III</td>
</tr>
<tr>
<td>Thioredoxin glutathione reductase (TGR)</td>
<td>14.68±0.35</td>
<td>1.04±0.17</td>
<td>14.56±0.32</td>
<td>3644.806 (P&lt;0.001)</td>
</tr>
<tr>
<td>Cytochrome c peroxidase (CcP)</td>
<td>12.72±0.29</td>
<td>2.36±0.34</td>
<td>12.34±0.30</td>
<td>1829.279 (P&lt;0.001)</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD

*P*: Significance in between groups. *P*1: group II (treated with ART) and group III (treated with PZQ) vs group I (untreated group).

NS: Non significant.
DISCUSSION

Extensive use of PZQ with concerns about possibility of drug resistance development, unavailability of an applicable vaccine, and absence of a reasonable alternative to PZQ, all represent a real challenge in control of schistosomiasis\textsuperscript{(42)}. Artemisinins are new promising anti-schistosomal compounds with well tolerated therapeutic dosage range, but their molecular mechanism of action on schistosomes still needs to be investigated\textsuperscript{(13,35)}. Living in an aerobic environment, worms must have effective mechanisms to maintain cellular redox balance. In this respect schistosome TGR\textsuperscript{(19)} and CcP\textsuperscript{(20)} have been reviewed as possible interesting macromolecular targets for therapeutic control of schistosomiasis.

The results of the current study elucidated for the first time that anti-schistosomal mechanism of action of ART is mediated via reduction in expression of schistosome TGR and CcP. The loss of these two defensive enzymes renders the different stages of the parasite more vulnerable to assault by the host generated ROS. Regarding the functional and biochemical differences between the redox metabolism of \textit{S. mansoni} and its human host, TGR was hypothesized to be an essential parasite protein and a potentially important drug target. Characterization of \textit{S. mansoni} TGR revealed its multifunctional oxidoreductase activities with remarkably wide substrate specificity, capable of directly reducing peroxides, selenium-containing compounds, as well as thioredoxin (Trx), oxidized glutathione (GSSG) and reduced glutathione (GSH)\textsuperscript{(43,44)}. It was postulated that these substrate preferences might possibly be exploited for future TGR-directed anti-schistosome drug design\textsuperscript{(18)}.

Since schistosomes lack catalase\textsuperscript{(45)}, previous reports suggesting that glutathione peroxidase plays a pivotal role in their protection is supported by the fact that it shows the highest affinity for phospholipid hydroperoxides, a major product of lipid peroxidation\textsuperscript{(16,46)}. It is evidenced that transcription and translation of various schistosome antioxidant enzyme genes is dependent on developmental regulation, where the early skin and lung stages (3 hours and 7 days PI, respectively) exhibit the lowest level of specific mRNA. On the other hand, adult mature egg-producing worms (42 days PI) exhibit the highest transcript levels protecting them against oxidant killing\textsuperscript{(47)}. It is of interest to refer to previous studies confirming that ART caused morphological changes in \textit{S. mansoni} tegument\textsuperscript{(48)}. The fact that most of antioxidant enzymes are localized in tegument of adult schistosomes, provides an adaptive response against the host cellular response. Their localization in gut epithelium of adult schistosomes protects against ROS released from host blood cells\textsuperscript{(49)}. Although CcP is present in mitochondria in the adult worm tegment and can neutralize hydrogen peroxidase, it is unlikely released from the mitochondria to have a general effect against peroxidation\textsuperscript{(20)}. This could be debated by the fact that energy metabolism of \textit{S. mansoni} is shifted through its life cycle from being aerobic in early stages to being anaerobic in adult worms\textsuperscript{(40)}. Thus, the mitochondrial antioxidants could basically have a defensive role since they are not directed to energy production.

Although the host immune system could have more than one mechanism to eliminate parasites, argument may be raised against role of oxidative stress in parasite killing. Previously, Scott et al.\textsuperscript{(51)} demonstrated that macrophage cell lines that do not produce a respiratory burst are still able to kill schistosomula when activated with cytokines. This is most likely as a result of formation of nitric oxide through immunoregulatory mechanisms\textsuperscript{(52,53)}. However, later studies attributed the potential direct cytotoxicity of nitric oxide to production of reactive nitrogen intermediates and peroxynitrite, a toxic oxidant generated when it couples with the superoxide radical\textsuperscript{(54,55)}.

Interestingly, the current results showed that ART caused significant reduction in tissue egg count by 95.9\% versus 68.4 \% in PZQ- treated group although it caused less reduction in female worm count by 74.8\% in comparison to 88.3\% in PZQ- treated group. This observation indicates that ART impaired the fecundity of adult female worms rendering them sterile rather than affecting their count. The same result was observed by Botros et al.\textsuperscript{(28)} who found that residual worms recovered after ART treatment became sterile and incapable of laying eggs. Abdul-Ghani et al.\textsuperscript{(56)} reported that artemether, another arte mesinin derivative, induced significant reductions in the liver tissue egg load, ranging from 75.2\% to 82.6\% as well as significant alterations in oogram pattern with cessation of oviposition and increased rates of dead eggs. Araújo et al.\textsuperscript{(23)} explained the reduction in egg load by the fact that ART modified the reproductive organs of \textit{S. mansoni} female worms in the form of reduction of ovarian volume and rarefaction of the vitelline follicles. This explanation is supported by findings of Bartley et al.\textsuperscript{(57)} who described that worms became smaller (30-50\% reduction in size) with atrophic testes and ovaries. Most, if not all, remaining worms are males being not responsible for any morbidity and even their persistence in blood might be useful in eliciting concomitant immunity against schistosomiasis\textsuperscript{(58)}. 

Ashour et al.,
El-Lakkany et al.\(^5\) showed that early treatment of S. mansoni-infected mice (28 days PI) could protect the host from later damage caused by schistosome eggs. When Shaohong et al.\(^27\) used different treatment protocols of ART against experimental S. mansoni infection in mice, they found that ART not only diminished the number of loaded tissue eggs, but also affected immature worms and seemed to kill mature adults. The reduction rate of immature adult S. mansoni worms reached a maximum when ART was given at the post-lung stage that coincided with the worms peak growth profiles. In addition the researchers found that the worm reduction rate was further enhanced when additional doses were administered at 8 or 9 weeks PI. For S. mansoni, drug administration starting 14 or 21 days after infection followed by 3 repeated doses at 2-week intervals provided optimal protection.

It was reported that the effects of ART treatment on the worms appear to be partly reversible by day 56 PI and the surviving worms recovered and restarted ovi-position\(^23,57\). Considering the lengthy exposure period in heavy endemic areas, a treatment regimen with long intervals might be practical for field application\(^27\). Therefore, it was suggested that repeated doses are mandatory for effective prevention of patent schistosomal infections, and that the use of integrated treatment strategies has considerable potential for schistosomiasis control\(^25\).

Moreover, it has been established that the schistosomes become most sensitive to PZQ at 6 weeks PI timing that corresponded to the period of ovi-position and plateau phase of parasite susceptibility\(^13,60,61\). The relative lack of efficacy of PZQ against juvenile schistosomes is a potential factor in poor cure rates and treatment failures observed in some patient groups, particularly those living in areas suffering very high rates of transmission\(^62\).

Regarding this aspect, it is preferred to drugs affecting different stages of the schistosome parasites life cycle to achieve radical cure\(^63\). Artemisinin derivatives are of particular concern because they are more active against early developmental stages of schistosome\(^13\) This observation is quite interesting per se, because this is exactly the time when PZQ is ineffective\(^64,65\). Consequently, a combined treatment with PZQ together with an artemisinin derivative has been suggested as a strategy for transmission control in endemic areas in order to avoid or delay induction of parasite drug resistance, and to prevent recrudescence\(^25,26,66\).

In the present study, the treatment protocol with both PZQ and ART provided maximum reduction in total worm count (95.4%) with complete eradication of female worms and tissue egg count (100%) in comparison to the lower reduction rate achieved by monotherapy with either one of them. Previous studies reported that treatment by PZQ with any of artemesinin derivatives affects the parasites at a different stage, enables the killing of most of the schistosomules and adult worms harbored in the hosts\(^66\). Doenhoff et al.\(^13\) reviewed that PZQ monotherapy achieved cure rates that ranged from 60% up to 85-90%, but 100% cures were seldom, if ever, recorded in an endemic area. Moreover, adult S. mansoni parasites that escaped treatment could reside in the mesenteric veins of the human host, where they can survive for up to 30 years\(^67\).

In regard to its anti-schistosomal effect, experimental studies indicated that PZQ treatment comprises two aspects, first, a direct effect on schistosomes tegument, and second induction of host immune reaction in response to antigens exposed on the disrupted parasite surface\(^68-70\). Regarding the mechanism of these effects, it has been established that PZQ causes intense muscular paralysis due to a rapid influx of calcium ions\(^71\). Schistosome calcium ion (Ca\(^{2+}\)) channels are the only moiety so far identified as the molecular target of PZQ\(^13,72\).

In conclusion, the present results revealed that PZQ had no effect on expression of schistosome TGR and CeP. Linking this with the finding that ART proved to inhibit expression of these targeting enzymes as a new valuable mechanism, confirms that its addition to PZQ could achieve complete cure outcome in treatment of schistosomiasis.

**Author contribution:** DS Ashour and ZS Shoheib performed the experiment and shared writing and revision of the manuscript. AA Abdeen provided artesunate drug and the treatment protocol used and revised the manuscript.

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تأثير عقار أرتيميزينات على أنزيمى ثيوريدوكسين جلوتاثيون ريدكتيز وسيتوكروم بيرأوكسيداز كأهداف جزيئية جديدة في حيوانات التجارب المعدية بطفيل البلهارسيا المعوية

أملاء عابدين، زينب صلاح شهيب، داليا صلاح عاشور
كلية الطب، جامعة طنطا، قسمي الطفيليات الطبية والعقاقير الطبية

المقدمة: يعد مرض البلهارسيا من أهم الأمراض الطفيلية، وهذا قد أدى استخدام المفتاح لعقار البرازيكوانتل إلى احتمال حدوث مقاومة من جانب الطفيلة لهذا العقار. ويجدر بالذكر أنه لا يوجد حتى الآن علاج موثق من هذا المرض، كما أنه لا يوجد أيضاً بدائل مقبولة لعقار البرازيكوانتل. وقد أدت كل العوامل السابقة ذكرها إلى اعتبار مكافحة هذا المرض أحد التحديات التي تواجه المجتمع. إن مشتقات الأرتيميزينات تعتبر من المركبات ذات التأثير الواضح ضد طفيلة البلهارسيا، غير أنه لم يتضح حتى الآن طريقة عمل هذا العقار على الطور البالغ للطفل.

الهدف من البحث: قامت تلك الدراسة ببحث التأثير المتوقع لعقار الأرتيميزينات على أنزيمى ثيوريدوكسين جلوتاثيون ريدكتيز وسيتوكروم بيرأوكسيداز في حيوانات التجارب المعدية بطفيل البلهارسيا المعوية.

طرق البحث: تم تقسيم الفئران إلى أربعة مجموعات: (1) حيوانات معدية بالطفل ولم تتلقى أى علاج (مجموعة ضابطة)، (2) حيوانات معدية بالطفل وتمت علاجها بعقار الأرتيميزينات، (3) حيوانات معدية بالطفل وتمت علاجها بعقار البرازيكوانتل، و (4) حيوانات معدية بالطفل وتمت علاجها على طريقة الفحص السابق ذكره. تم إعطاء العلاج الأول على طريقة الفحص السابق ذكره. 300 مجم/كجم بداية من اليوم الرابع عشر للعدوى وفوق جرعة كل أسبوعين. أما عقار البرازيكوانتل، فقد تم اعطاؤه بالفم كجرعة واحدة 600 مجم/كجم في اليوم الثاني والأربعين للعدوى. وقد خضعت كل الفئران محل الدراسة لثلاث: (1) عد ديدان البلهارسيا البالغة، (2) دراسة مستوى الحمض النووي (dna) الخاص بالانزيمات محل الدراسة في ديدان البلهارسيا البالغة، وذلك باستخدام rt PCR (mRNA).

نتائج البحث: أظهرت الدراسة أن علاج عقار الأرتيميزينات قد تسبب في نقص ملحوظ في مستويات الحمض النووي (mRNA) الخاص بالانزيمات محل الدراسة في ديدان البلهارسيا البالغة مقارنة بعقار البرازيكوانتل الذي لم يسبب تغيير ملحوظ في مستويات تلك الأنزيمات. وتبين الأشارة أيضاً إلى أنه رصدت الدراسة انخفاض ملحوظ في العدد الكلى لديدان البلهارسيا البالغة، وكذلك عدد الأنثى في مجموعة (4) أكثر من باقى المجموعات. وصاحب هذا الانخفاض عدم ظهور أي بويضات في الديدان في نفس المجموعة المذكورة.

الخلاصة: أوضحت تلك الدراسة للمرة الأولى طريقة عمل عقار الأرتيميزينات على أنزيمى ثيوريدوكسين جلوتاثيون ريدكتيز وسيتوكروم بيرأوكسيداز، وذلك يمكن أن تؤدي إضافة عقار الأرتيميزينات إلى عقار البرازيكوانتل أثناء علاج الاصابة بالبلهارسيا المعوية إلى تام الشفاء.