TRIGGERING OF HIGH-LEVEL RESISTANCE AGAINST SCHISTOSOMA MANSONI REINFECTION BY ARTEMETHER IN THE MOUSE MODEL

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Ingerod 407, Brastad, Sweden; Swiss Tropical Institute, Basel, Switzerland; National Institute of Parasitic Diseases, Chinese Centre for Disease Control and Prevention, Shanghai, People’s Republic of China

Abstract. Artemether, a methyl ether derivative of dihydroartemisinin, not only exhibits antimalarial properties, but also possesses strong activity against schistosomula, the immature stages of a parasitic worm that can cause schistosomiasis. To test if the effect would be similar to that of irradiation with respect to the induction of immunologic protective responses, groups of mice were infected with Schistosoma mansoni cercariae and treated with artemether at 1–3 weeks post-infection. Control mice were either infected with normal cercariae or with cercariae exposed to radiation that permitted early development but not maturation of the parasites. The mice were challenged six weeks after the initial infection, and the mean numbers of schistosomes recovered in the various groups were calculated upon dissection eight weeks post-challenge. The administration of artemether two weeks after the initial infection resulted in 58% protection, while giving the drug three weeks post-infection increased the level of protection to 81%. This level of protection is as high as that normally obtained by immunization with irradiated cercariae (84% in the present study) and is superior to the level of resistance obtained with any individual schistosome vaccine candidate antigen thus far reported.

INTRODUCTION

In the early 1980s, it was discovered that artemisinin, artemether (a methyl ether derivative of dihydroartemisinin), and artesunate (artemisinin hemisuccinate), in addition to their excellent antimalarial properties, also exhibit activity against Schistosoma japonicum. Laboratory studies have now firmly established that the larval migratory stages of the three major species infective for humans, i.e., S. japonicum, S. mansoni, and S. haematobium, are all highly susceptible to these drugs. Peak efficacy varies from two weeks after cercarial skin penetration for S. japonicum to three weeks for S. mansoni and up to four weeks for S. haematobium, respectively. Particular progress has been made with artemether, including its effect on biochemical metabolism, antioxidant systems, potential long-term toxicity, and possible mechanism of action against schistosomes. Importantly, randomized controlled trials in different epidemiologic settings found that artemether, orally administered at a dose of 6 mg/kg once every 2–4 weeks, significantly reduces the incidence of infection, thereby opening additional avenues for the treatment and control of schistosomiasis.

Vaccine development in schistosomiasis remains a major, yet elusive, goal. No single schistosome antigen is capable of inducing a level of protection as strong as that achieved with cercariae attenuated by electromagnetic radiation adjusted to permit early worm development, but not full maturation and oviposition. This effect is measurable approximately two weeks after exposure, reaches a plateau with respect to effect a few weeks later, and remains high for an undetermined period. The unusual situation of a narrow window of almost exclusive effect against the lung-stage parasite offers the possibility of emulating the irradiated cercariae model ensuring maximum immunologic impact. The idea of inducing resistance through drug-abbreviated infections goes back more than 40 years, but has received new contemporary interest.

Earlier investigators, however, did not have access to a drug with the characteristics of artemether. A first attempt is made here to assess the level of protective immunity induced by early administration of artemether to mice experimentally infected with S. mansoni.

MATERIALS AND METHODS

Mice, parasites, and artemether. The laboratory investigations reported here were reviewed and approved by the Institutional Review Board of the Swiss Tropical Institute (Basel, Switzerland), and were performed in compliance with Swiss national regulations. All experiments were done with MORO (SPF) mice, weighing 18–22 grams, obtained from Biologic Research Laboratories Ltd. (Füllinsdorf, Switzerland). Animals were given Rodent Blox diet obtained from Eberle NAFAG (Gossau, Switzerland) and water ad libitum throughout the study.

Schistosoma mansoni cercariae (Liberian strain) were collected after release from laboratory-bred intermediate host snails (Biomphalaria glabrata) following exposure to artificial light for at least four hours. Artemether, a gift from the Kunming Pharmaceutical Corporation (Kunming, People’s Republic of China), was suspended in 7% Tween-80 and 3% ethanol before use. The volume of drug suspension administered to the mice was 10 mL/kg and the final dosage of artemether used was 400 mg/kg of body weight.

Infection, treatment, challenge, and dissection. The experimental design is shown in Table 1. Briefly, four groups of 12 mice each were infected with 100 S. mansoni cercariae per mouse by subcutaneous injection in the back at the root of the tail. The first group was kept untreated to serve as control, while groups 2–4 were given a single dose of 400 mg/kg of artemether administered intragastrically at one, two, or three weeks post-infection. The four groups were divided into subgroups of equal sizes, one for challenge infection at week 6 followed by dissection at week 14, and one for dissection at week 10 without challenge. A fifth extra control group of 12 mice were kept naive, but challenged after six weeks and also dissected at week 14 along with the treated and challenged groups. Mice were killed by blood-letting and dissected as follows. First, the liver was removed, placed into a transparent plastic folder, gently compressed between two glass plates, and the flat layer of parenchyma was quantitatively examined for S. mansoni worms under a stereoscopic microscope at low magnification. Second, the small and the large intestines were also removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms were removed, placed in a Petri dish, and all S. mansoni worms.
Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>Initial infection</th>
<th>Arteether dosing</th>
<th>Week</th>
<th>Mean number of worms at dissection (SE)</th>
<th>Protective efficacy against challenge infection (95% CI)*</th>
<th>Significance test of protective efficacy against challenge infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>12</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control 2</td>
<td>6</td>
<td>Normal cercariae</td>
<td>–</td>
<td>1</td>
<td>20.3 (2.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>6</td>
<td>Normal cercariae</td>
<td>Arteether</td>
<td>2</td>
<td>9.2 (0.6)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>6</td>
<td>Normal cercariae</td>
<td>Arteether</td>
<td>3</td>
<td>18.2 (3.1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>6</td>
<td>Normal cercariae</td>
<td>Arteether</td>
<td>6</td>
<td>11.2 (1.6)</td>
<td>81% (42, 121)</td>
<td>F1,21 = 13.3, P = 0.0015</td>
</tr>
<tr>
<td>Irradiation</td>
<td>6</td>
<td>Irradiated cercariae</td>
<td>–</td>
<td>6</td>
<td>1.5 (0.7)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* CI = confidence interval.
† The mean number (including SE) of worms in this control group corresponds to \( \bar{x}_2 \).
‡ One mouse in this control group died before completion of the schedule.

Table 2

Worm burden in the different treatment and control groups and estimated protective efficacy to reinfection with *Schistosoma mansoni* in relation to the timing of arteether administration or induced by irradiated cercariae

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial infection</th>
<th>No. of mice</th>
<th>Arteether dosing</th>
<th>Mean number of worms at dissection (SE)</th>
<th>Protective efficacy against challenge infection (95% CI)*</th>
<th>Significance test of protective efficacy against challenge infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>–</td>
<td>12</td>
<td>–</td>
<td>21.4 (2.7)†</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Control 2</td>
<td>Normal cercariae</td>
<td>6</td>
<td>–</td>
<td>20.3 (2.5)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>Normal cercariae</td>
<td>6</td>
<td>Week 1</td>
<td>18.2 (3.1)</td>
<td>53% (16, 100)</td>
<td>F1,21 = 6.1, P = 0.02</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>Normal cercariae</td>
<td>6</td>
<td>Week 2</td>
<td>11.2 (1.6)</td>
<td>81% (42, 121)</td>
<td>F1,21 = 13.3, P = 0.0015</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>Normal cercariae</td>
<td>6</td>
<td>Week 3</td>
<td>1.5 (0.7)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Irradiation</td>
<td>Irradiated cercariae</td>
<td>6</td>
<td>–</td>
<td>4.8 (1.9)</td>
<td>84% (46, 123)</td>
<td>F1,21 = 15.0, P = 0.0009</td>
</tr>
</tbody>
</table>

† The mean number (including SE) of worms in this control group corresponds to \( \bar{x}_2 \).
‡ One mouse in this control group died before completion of the schedule.
**Effect of artemether or irradiated cercariae on resistance to reinfection.** The results of the protective efficacy against a challenge infection following differently timed artemether administration or due to an initial infection with irradiated cercariae are summarized in Table 2. Mice infected with normal cercariae, treated with a single dose of 400 mg/kg of artemether one week post-infection and challenged at week 6, produced a mean worm burden of 44.2 (SE = 9.2). Infected mice that were given artemether at the same dose and schedule, but remained unchallenged, had a mean worm burden of 20.3 (SE = 2.5), leading to the conclusion that the administration of artemether one-week post-infection did not induce any protective efficacy against a challenge infection.

Administration of artemether two weeks after the initial infection showed a protective efficacy of 58% (95% CI = 16, 100), which was significant \(P = 0.02\). Mean schistosome worm burdens recovered from both infected groups of mice that were either challenged or unchallenged were more than halved when compared with the corresponding groups receiving artemether one week post-infection.

In the third treatment experiment, artemether was administered three weeks after the initial infection, resulting in mean worm burdens in the challenged and unchallenged groups of 11.2 (SE = 1.6) and 7.2 (SE = 1.8), respectively. These numbers were considerably lower than those obtained in the groups given artemether two weeks after the initial infection, demonstrating a highly significant protective efficacy against the challenge of 81% (95% CI = 42, 121, \(P = 0.0015\)).

Mice infected with irradiated cercariae but not treated at any time showed a very similar protective efficacy to that seen with artemether given three weeks post-infection. The protective efficacy was highly significant, namely 84% (95% CI = 46, 123, \(P = 0.0009\)). Concurrently, this experiment resulted in the lowest recorded mean worm burdens both in the challenged and the unchallenged groups of mice.

**DISCUSSION**

To our knowledge, this is the first experimental study estimating the protective efficacy against a challenge infection with \(S. mansoni\) following early dosing of artemether. The 81% resistance to reinfection achieved with administration of artemether three weeks after the initial infection of mice with normal \(S. mansoni\) cercariae reaches the level obtained regularly with immunizations with irradiated cercariae (e.g., 84% in our experiments). At the present time, the basis for the strong protection associated with truncated infection remains unclear. Possible explanations include superior antigen presentation in the lungs by the attenuated parasites and/or radiation-associated release of particularly protective antigens. In our hands, artemether administration exhibited no protective effect against the challenge infection one week after primary infection, but this increased during the following two weeks indicating the role of the lung stage parasite with regard to inducing resistance to reinfection. In view of these encouraging results, further investigations are warranted with an emphasis on replicating these outcomes, and accompanying immunologic studies to investigate whether serum or T cells from mice treated in this way confer protection. It should also be noted that our findings correlate with previous results demonstrating highest \(S. mansoni\) worm burden reductions when artemether was administered three weeks after infection. Cycling A can induce the same level of protection to challenge infection, but this effect might rather be due to immune modulation than a direct schistosomulocidal effect. This mechanism is unlikely with regard to artemether since it has not been seen thus far in any patient, and we have never encountered it experimentally. For this reason, and since the half-life of artemether is less than two hours, a control for a possible alternative effect of the drug alone was omitted in our experiments. The minimum time between drug administration and challenge infection was three weeks, ensuring that the drug alone could not have affected the observed outcome.

Using mathematical models, Woolhouse and Hagan have argued that protection in non-treated humans develops slowly since it requires antigens released at the end of the life span of the parasite, which can be several years. It has been suggested that praziquantel, the current drug of choice for schistosomiasis, accelerates this effect by contributing to the premature release of such antigens. Further support for this hypothesis comes from several sources. These reports demonstrate an association between treatment with praziquantel and the development of resistance to reinfection. In this regard, it is interesting to note that \(S. mansoni\)-infected patients in Senegal who were treated with artesunate showed a parasitologic cure rate 10 weeks post-treatment that was considerably higher than that reached after only five weeks. Since there was ample opportunity for reinfection, the cure rate should have decreased over time, as was observed in the same study population with praziquantel.

The results presented in this report, stemming from a single but large experiment, show that appropriately timed artemether administration produces resistance against schistosomes by selectively destroying invading parasites at a site that is preferential for the generation of protective immunity. However, whether the combination of this stimulus with that emanating from disintegrating adult worms caused by praziquantel is beneficial or not must be further investigated. If our findings are confirmed in subsequent experiments, and are supported by respective immunologic studies, they might become of considerable relevance. Artemether is thus not only one of the most effective anti-malarial drugs, and also a compound that prevents the establishment of patent schistosome infections, but may emerge as a new tool for the elucidation of how protective anti-schistosomal immune responses are generated.

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