Comparative study of the effects of artemether and artesunate on juvenile and adult Schistosoma mansoni in experimentally infected mice

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Abstract
Artemether and artesunate, derivatives of the antimalarial artemisinin, also exhibit antischistosomal properties. There is a need to assess comparatively the activity of both compounds against different developmental stages of schistosome parasites. Since artemisinin derivatives will be increasingly used to treat malaria, it is important to study the effects of 7-day monotherapy regimens on schistosome infections. We carried out experiments with mice, infected with juvenile or adult Schistosoma mansoni, and treated with artemether or artesunate at various doses and regimens including those currently used for monotherapy of malaria. Three doses of artemether, at concentrations of 150 or 300 mg/kg, administered to mice with juvenile S. mansoni resulted in worm reductions of 88–97%, which were significantly higher than the 67–77% obtained with artesunate (P < 0.05). Total concentrations of 600 or 800 mg/kg artesunate, administered over 2 or 4 consecutive days to mice with adult S. mansoni, reduced the worm burden significantly by 46–51% (P < 0.05). The reduction of the worm burden observed with artesunate was considerably lower, 24–33%, and not significant when compared with untreated control mice. Seven-day monotherapy regimens of artemether or artesunate given at different concentrations to mice with adult S. mansoni showed total worm reductions of 53–61% or 34–49%, respectively. We conclude that artemether and artesunate are efficacious antischistosomal agents, with artemether displaying consistently higher activities. Our findings may contribute to the current strategic discussions on the effect and use of artemisinin derivatives against schistosomes when they are used in malaria chemotherapy in areas of co-endemicity of both parasites.

Keywords: schistosomiasis, Schistosoma mansoni, experimental infections, animal models, mice, chemotherapy, artemether, artesunate, malaria monotherapy treatment regimen

Introduction
Schistosomiasis remains an important parasitic disease in the tropics and the mainstay of control is chemotherapy, with praziquantel being used as the drug of choice (WHO, 1999). Praziquantel is safe and active against all schistosome species that can infect humans, and generally results in high cure and egg reduction rates (WHO, 1993). National control programmes in Brazil, China and Egypt have achieved impressive morbidity reductions, mainly due to large-scale chemotherapy campaigns (CHITSULO et al., 2000). In recent years, the price of praziquantel has dropped substantially. By the end of 1999, the average cost of a single treatment was US$ 0.40 (CIOLI, 2000), and the costs have decreased further over the past 2 years (APPLETON & MBAYE, 2001; WHO, 2001). This low cost will further encourage widespread use of praziquantel and treatment is being proposed by new initiatives targeting praziquantel to school-aged children.

Evidence from laboratory studies and field trials has been accumulated with schistosomes exhibiting a somewhat decreased susceptibility to praziquantel (FALLON & DOENHOFF, 1994; ISMAIL et al., 1996, 1999; GEERTS & GRYSEELLS, 2000; WILLIAM et al., 2001; DANSO-APPIAH & DE VLAS, 2002). Although these observations are not of major significance so far, they indicate the importance of closely monitoring the efficacy of praziquantel in different epidemiological settings, and stress the need for research and development of novel antischistosomal drugs (CIOLI, 1998, 2000; GEERTS & GRYSEELLS, 2000; GRYSEELLS et al., 2001).

Chinese scientists have discovered that derivatives of artemisinin, which are already widely and effectively used in the treatment of malaria (MCINTOSH & OLLILARO, 2001), also display antischistosomal properties. Detailed in-vivo studies with Schistosoma japonicum revealed that the juvenile stages of the parasite were highly susceptible to artemether (XIAO et al., 1995, 1998) and artesunate (LT et al., 1996). Both drugs also showed activity against adult S. japonicum, but worm burden reductions were significantly lower. These findings were confirmed in subsequent randomized controlled trials, as artemether and artesunate reduced the incidence of patent S. japonicum infections by 60–100% (LT et al., 1996; XIAO et al., 2000a). In recent years, experimental work and clinical trials have been extended from S. japonicum to S. mansoni and S. haematobium (for review see UTZINGER et al., 2001a). Artemether proved highly active against juvenile S. mansoni (XIAO et al., 2000b) and S. haematobium (XIAO et al., 2000c). A randomized, controlled trial showed a prophylactic efficacy of artemether of 50% against reinfection with S. mansoni (UTZINGER et al., 2000). Although the susceptibility of different developmental stages of S. mansoni to artesunate has yet to be investigated, recent studies confirm that artesunate is active against S. mansoni. Administration to infected patients on 5 consecutive days resulted in parasitological cure rates of 40–54%, when evaluated 10 or 24 weeks post-treatment (DE CLERCQ et al., 2000a, 2000b). The first results from 2 field studies indicate that artesunate also shows an effect against S. haematobium, but the therapeutic efficacy is somewhat lower than against S. mansoni (BIRRITANN et al., 2001; DE CLERCQ et al., 2000b).

In the present study, we assessed comparatively the effects of different doses and treatment regimens of artemether and artesunate on juvenile and adult S. mansoni in experimentally infected mice. We also administered the 2 drugs to mice harbouring adult worms, following the recommended malaria monotherapy regimens. These data will be of importance for subsequent clinical trials in humans and will contribute to more rational discussions about the potential of artemisinins in the control of schistosomiasis.

Materials and Methods
Ethical clearance, parasites, drugs and mice
Ethical clearance for the animal studies presented here was obtained from the local government based on
Swiss national regulations. All experiments were carried out in the laboratories of the Swiss Tropical Institute.

Cercariae of *S. mansoni* (Liberian strain) were obtained from infected * Biomphalaria glabrata* snails, following routine procedures at our laboratories.

Artesunate was obtained from Mepha AG (Aesch, Switzerland) and artemether was provided by the Kunming Pharmaceutical Corporation (Kunming, China; lot No. 97080). Both pharmaceutical companies documented that their products showed very high purity: 99.4% in the case of artesunate and 99.6% for artemether. For intragastric administration, drugs were suspended in 7% Tween-80 and 3% ethanol.

A total of 165 female MORO strain mice, weighing 18–22 g, were purchased from Biotechnology and Animal Breeding Division (Füllinsdorf, Switzerland). The animals were maintained on Rodent Blox, obtained from Eberle NAFAG (Gossau, Switzerland), and water ad libitum.

**Infection and treatment**

Mice were each infected subcutaneously with 80 *S. mansoni* cercariae. Two groups of 10 mice served as control, therefore they remained untreated.

In the first series of experiments, 4 groups of 10 mice were treated intragastrically with 3 doses of artesunate or artemether at a lower concentration of 150 mg/kg or a higher one at 300 mg/kg. The first 2 doses were administered 7 and 21 days after infection, when the mice harboured juvenile *S. mansoni*. These treatments were followed by a final dose at the same concentration, 35 days post-infection.

The second set of experiments was done with mice that harboured adult *S. mansoni* worms. Ten groups of 5 mice were treated intragastrically with artesunate or artemether at different doses and treatment regimens. The first 2 groups received 100 mg/kg artesunate or artemether for 6 consecutive days, with the first dose administered 46 days after infection. The next 4 groups were treated on 4 consecutive days with 150 or 200 mg/kg artesunate or artemether, starting also at day 46 post-infection. The last 4 groups were treated twice, on day 46 and 47 after infection, with daily doses of artesunate or artemether at 300 or 400 mg/kg.

The third series of experiments also focused on adult *S. mansoni*. In this case, the treatment regimen followed that recommended by the World Health Organization for malaria monotherapy with artemisinins (WHO, 1998; McINTOSH & OLLIARO, 2001). Forty-six days after infection, 6 groups of 5 mice were treated intragastrically with artesunate or artemether at an initial dose of 200, 300 or 400 mg/kg. Mice were re-treated on 6 consecutive days by the same route of administration, but using half of the initial dose. Therefore, the different treatment groups received total doses of 800, 1200 or 1600 mg/kg. Finally, 5 groups of 5 mice were treated with a high single dose of artesunate (800 and 1200 mg/kg) or artemether (800, 1200 and 1600 mg/kg).

**Dissection of mice and assessment of the effect of artesunate or artemether**

In the first 2 series of experiments, one group of 10 infected but untreated mice served as control, and was sacrificed by blood-letting 60 days after infection. All mice in the different treatment groups were also sacrificed by blood-letting, 27 or 28 days after the final drug administration. A second group of 10 infected but untreated mice served as control in the third series of experiments. They were sacrificed 75 days post-infection. Mice from the different treatment groups were sacrificed between 24 and 31 days after the final medication.

All mice were dissected and the liver and small and large intestines were removed. The liver was placed into a 20 × 20-cm transparent plastic folder and gently compressed between 2 glass plates, so that the parenchyma became a flat and evenly dispersed layer. The layer was placed under a stereoscopic microscope and examined at ×10 magnification by one experienced microscopist. This procedure allowed the sexing and counting of all *S. mansoni* worms. The small and large intestines were placed in a Petri dish and all the mesenteric veins were examined under a stereoscopic microscope, also at ×10 magnification, by the same microscopist. All *S. mansoni* worms in the mesenteric veins were removed, sexed and counted.

In a first step, we assessed the effect of a treatment with artesunate or artemether, following the various doses and regimens, by comparing the mean number of total (and female) worms in any treatment group with that of the corresponding control group. In a second step, we compared the mean number of total (and female) worms in the different artesunate treatment groups with that of the corresponding artemether groups. For statistical analysis, we used Student’s t-test, allowing for unequal variance.

**Results**

**Effect of artesunate or artemether on juvenile *S. mansoni***

In the first series of experiments, mice previously infected with 80 *S. mansoni* cercariae received the first dose of 150 or 300 mg/kg artesunate or artemether 7 days after infection, when schistosomula have normally reached the lung. Two further doses at the same concentrations were administered 14 and 28 days later, when parasites usually have developed into organogeny and oviposition stages, respectively. Administration of artesunate or artemether at these doses and treatment regimen resulted in total and female worm reductions between 67% and 99% (Table 1, groups 2–5). The reductions in the total and female worm burden were highly significant, as compared to the untreated control group ($P < 0.001$). Total and female worm reductions were significantly greater following the administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Administration:</th>
<th>Mice (n)</th>
<th>Mean total worm burden (SD)</th>
<th>Total worm burden reduction (%)</th>
<th>Mean female worm burden (SD)</th>
<th>Female worm burden reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td></td>
<td>10</td>
<td>19.7 (7.4)</td>
<td>-</td>
<td>8.7 (3.2)</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Artesunate</td>
<td>150</td>
<td>7, 21, 35</td>
<td>10</td>
<td>6.5 (3.5)</td>
<td>67</td>
<td>2.2 (2.6)</td>
<td>70</td>
</tr>
<tr>
<td>3</td>
<td>Artesunate</td>
<td>300</td>
<td>7, 21, 35</td>
<td>10</td>
<td>2.4 (1.6)**</td>
<td>88</td>
<td>0.8 (0.6)</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Artemether</td>
<td>150</td>
<td>7, 21, 35</td>
<td>10</td>
<td>2.4 (1.6)**</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Artemether</td>
<td>300</td>
<td>7, 21, 35</td>
<td>10</td>
<td>0.6 (1.0)**</td>
<td>97</td>
<td>0.1 (0.3)</td>
<td>** 99</td>
</tr>
</tbody>
</table>

*Group 4 tested versus group 2 ($^* P < 0.05$).  
**Group 5 tested versus group 3 ($^{**} P < 0.001$).
Effect of artesunate or artemether on adult *S. mansoni*

In the second set of experiments, mice infected with adult *S. mansoni* were treated with artesunate or artemether at various doses and regimens. Administration of artesunate or artemether at 100 mg/kg on 6 consecutive days, or 150 mg/kg on 4 consecutive days resulted in total worm reductions of 15–29%, which were not significant when compared to the group of untreated control mice (Table 2, groups 6, 7, 11, 12). Higher artemether concentrations of 200, 300 or 400 mg/kg, administered on 2 or 4 consecutive days (groups 13–15), revealed total worm reductions of 46–53%, which were significant when compared to the untreated control group (P < 0.05). However, administration of artesunate at the same concentrations and regimens had no significant impact on the total worm burden (groups 8–10). In all different treatment groups, female worm reductions were consistently higher than the total worm reductions, resulting always in significantly lower female worm burdens when compared to artesunate (P = 0.045), while the difference in the mean female worm burden showed borderline significance (P = 0.059).

Comparison of the total and female worm reductions obtained by a 7-day monotherapy using artesunate or artemether revealed artesunate being consistently more effective; however, the differences were statistically not significant. It should be noted that 2 mice died in 2 of the 6 treatment groups: (1) artemether at 1 ≤ 300 mg/kg plus 6 × 150 mg/kg (group 23); and (2) artesunate: 1 × 400 mg/kg plus 6 × 200 mg/kg (group 19).

In the third series of experiments, a first initial dose of 1200 mg/kg was administered to adult *S. mansoni* at single high doses. Four out of 5 mice died when artesunate was administered at 800 mg/kg (group 20) and all 5 mice were killed by a single dose of 1200 mg/kg (group 21). Mice tolerated higher doses of artemether; however, 2 out of 5 mice died when a single dose of 1200 mg/kg was administered (group 26).

**Discussion**

The results of our in-vivo studies clearly showed that artemether and artesunate are highly active against the juvenile stages of *S. mansoni*, whereas adult worms are considerably less susceptible. The similarities in susceptibility of different developmental stages of *S. mansoni* to artemether and artesunate might be explained by the fact that the 2 compounds are remarkably related in terms of chemical structures and also pharmacodynamic properties (ZIFFER et al., 1997; LI et al., 1998; VROMAN et al., 1999). In this connection, it could be speculated that other artemisinin derivatives should exhibit similar antischistosomal properties, which has indeed been confirmed using artemether (GROUPS 22–24) and also dihydroartemisinin (ABDEL AZIZ & EL-BADAWY, 2000).

Artemether showed consistently higher antischistosomal activities than artesunate. Differences in the metabolic pathways of the 2 compounds might account for

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**Table 2. Mice infected with adult *Schistosoma mansoni* and treated with artesunate or artemether at different doses and treatment regimens (n = number of mice)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Administration: day after infection</th>
<th>Mice (n)</th>
<th>Mean total worm burden (SD)</th>
<th>Mean female worm burden reduction (%)</th>
<th>Mean female worm burden (SD)</th>
<th>Female worm burden reduction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>19.7 (7.4) *</td>
<td>8.7 (3.2)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Artesunate</td>
<td>100</td>
<td>46, 47, 48, 49, 50, 51</td>
<td>5</td>
<td>14.8 (3.9)*</td>
<td>5.2 (1.6)*</td>
<td>25</td>
<td>5.2 (1.6)*</td>
</tr>
<tr>
<td>7</td>
<td>Artesunate</td>
<td>150</td>
<td>46, 47, 48, 49</td>
<td>5</td>
<td>15.6 (5.1)*</td>
<td>5.2 (1.6)*</td>
<td>21</td>
<td>5.2 (1.6)*</td>
</tr>
<tr>
<td>8</td>
<td>Artesunate</td>
<td>200</td>
<td>46, 47, 48, 49</td>
<td>5</td>
<td>14.2 (6.3)*</td>
<td>3.8 (1.9)*</td>
<td>28</td>
<td>3.8 (1.9)*</td>
</tr>
<tr>
<td>9</td>
<td>Artesunate</td>
<td>300</td>
<td>46, 47, 48</td>
<td>5</td>
<td>15.0 (8.3)*</td>
<td>4.2 (3.6)*</td>
<td>24</td>
<td>4.2 (3.6)*</td>
</tr>
<tr>
<td>10</td>
<td>Artemether</td>
<td>400</td>
<td>46, 47</td>
<td>33</td>
<td>13.2 (5.2)*</td>
<td>4.0 (2.8)*</td>
<td>33</td>
<td>4.0 (2.8)*</td>
</tr>
<tr>
<td>11</td>
<td>Artemether</td>
<td>100</td>
<td>46, 47, 48, 49, 50, 51</td>
<td>15</td>
<td>16.8 (6.6)*</td>
<td>5.2 (0.8)*</td>
<td>15</td>
<td>5.2 (0.8)*</td>
</tr>
<tr>
<td>12</td>
<td>Artemether</td>
<td>150</td>
<td>46, 47, 48, 49</td>
<td>5</td>
<td>14.0 (4.7)*</td>
<td>4.4 (1.5)*</td>
<td>29</td>
<td>4.4 (1.5)*</td>
</tr>
<tr>
<td>13</td>
<td>Artemether</td>
<td>200</td>
<td>46, 47, 48, 49</td>
<td>53</td>
<td>9.2 (1.1)*</td>
<td>1.6 (0.9)**</td>
<td>53</td>
<td>1.6 (0.9)**</td>
</tr>
<tr>
<td>14</td>
<td>Artemether</td>
<td>300</td>
<td>46, 47</td>
<td>46</td>
<td>10.6 (4.9)*</td>
<td>1.8 (2.5)**</td>
<td>46</td>
<td>1.8 (2.5)**</td>
</tr>
<tr>
<td>15</td>
<td>Artemether</td>
<td>400</td>
<td>46, 47</td>
<td>51</td>
<td>9.6 (2.3)*</td>
<td>1.2 (1.1)**</td>
<td>51</td>
<td>1.2 (1.1)**</td>
</tr>
</tbody>
</table>

*Groups 6–15 tested versus group 1 (*P < 0.05; **P < 0.001).*
this finding. Previous studies assessing the biotransformation in rats revealed that artesunate had an extremely short terminal half-life of only 2–4 min and was converted rapidly into its major active metabolite dihydroartemisinin (Maggs et al., 2000; Davis et al., 2001). In contrast, pharmacokinetic studies showed that artemether has a considerably longer terminal half-life of 1–2 h and is converted more slowly into dihydroartemisinin and to a smaller extent also into several hydroxylated metabolites (Vangtamel et al., 1999; Maggs et al., 2000). Therefore, it is possible that not only the mother compounds artemether and artesunate, but also metabolites could play an important role in explaining the differences in antischistosomal properties.

Our findings are in agreement with previous studies assessing the susceptibility of different developmental stages of S. mansoni to artemether. Large worm reductions of 56–90% were observed when a single dose of 300 or 400 mg/kg artemether was administered to mice infected with 7–28-day-old schistosomula, whereas drug administration to adult S. mansoni resulted in considerably lower worm reductions of 30–51% (Xiao & Catto, 1989; Xiao et al., 2000b). Artemether, given at a single dose of 15 mg/kg to rabbits infected with 5–21-day-old S. japonicum, also proved highly active, with worm reductions of 69–93%. The effect of a single dose administered to adult S. japonicum was still marked, but worm reductions were significantly lower (Xiao et al., 1995). In the first series of in-vivo studies with juvenile S. haematobium harboured in hampsters, the schistosomicidal activity of artemether was also confirmed (Xiao et al., 2000c). These experimental findings have been confirmed in a series of randomized controlled trials against S. japonicum (Xiao et al., 2000a), which is considered as one of the most important contributions to schistosomiasis control in China in recent years (Yuan et al., 2000). Following a successful field trial against S. mansoni (Utzinger et al., 2000), the relevance for areas endemic for S. mansoni and S. haematobium is currently being discussed (Utzinger et al., 2001a).

Although detailed studies on the susceptibility of S. mansoni parasites of different ages to artesunate have yet to be conducted, it is likely that schistosomula are more susceptible than adult worms. In fact, first in-vivo studies with adult S. mansoni harboured in mice have shown that treated once or repeatedly with artesunate at 300 or 500 mg/kg only resulted in small numbers of dead worms (Araujo et al., 1999). These experiments should be repeated with juvenile S. mansoni and are expected to result in large worm reductions, as has already been observed after administration of artesunate to juvenile S. japonicum in different host animals (Li et al., 1996). First studies in human populations have confirmed that artesunate is safe and effective in the prevention of S. japonicum infections (Li et al., 1996), as well as in the cure of S. mansoni infections (De Clercq et al., 2000a, 2000b). Although therapeutic efficacy of artesunate against S. haematobium was considerably lower than against S. mansoni, the infection intensities and the proportion of heavy infections decreased significantly (Borkmann et al., 2001; De Clercq et al., 2002).

The observation that artemisinin derivatives show highest activities on early developmental stages of schistosome parasites is quite interesting per se, because this is exactly the time when praziquantel is ineffective (Andrews, 1981; Sabah et al., 1986). Consequently, a combined treatment with praziquantel together with an artemisinin derivative has been proposed, as this covers the whole period of the parasitic in its vertebrate host (Utzinger et al., 2001a). Recent laboratory studies with S. japonicum and S. mansoni parasites of different ages confirmed that a combination therapy with praziquantel and artemether showed significantly larger
worm reductions than each drug administered singly (Xiao et al., 2000a; Utzinger et al., 2001b). In addition, first clinical observations among S. mansoni-infected patients in Senegal also report an additive effect by combining praziquantel with artesunate, when assessing parasitological cure rates 5 weeks post-treatment (De Clercq et al., 2000b). In a recent study done in Gabon, combination therapy with praziquantel and artesunate resulted in significantly higher egg reduction rates than praziquantel administration alone (Borrmann et al., 2001).

More important is the finding that 7-day artemisinin monotherapy regimens, recommended for patients with intolerable antimalarial drugs (WHO, 1998; McIntosh & Olliaro, 2001), have a significant effect on S. mansoni. This result is of great significance, since artemisinins are increasingly used in the treatment of severe and uncomplicated malaria, especially in areas where parasites have developed resistance to nearly all currently available antimalarials (Price et al., 1998, 1999). Therefore, we suggest that in areas where malaria and schistosomiasis are co-endemic and artemisinins will be used in the treatment of malaria—in monotherapy or in combination with other antimalarial drugs (WHO, 1999)—the impact on the prevalence and intensity of schistosome infections should be monitored and evaluated, as recently stressed at a WHO-TDR (2001) meeting about the potential of artemisinins in the prevention and control of schistosomiasis.

Acknowledgements

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Swiss Tropical Institute. We are grateful to Mejha AG and the Kumming Cooperative for providing artesunate and artemether. We thank Ms Yvette Endris for assistance in the laboratory and Dr Jennifer Keiser for useful comments on the manuscript. Jürg Utzinger acknowledges financial support from the Swiss National Science Foundation and the Centre for Health and Wellbeing at Princeton University.

References


Field test of the ‘dose pole’ for praziquantel in Zanzibar

A. Montresor, D. Engels, M. Ramsan, A. Foum, and L. Savolli

Abstract

A graduated pole for height measurement, estimating the number of praziquantel tablets needed for treatment, was field-tested on 1289 children in Zanzibar. A bathroom-type scale performed better than the dose pole in delivering the optimal dose (40–60 mg/kg) and the 2 methods performed similarly in delivering a dose considered appropriate (30–60 mg/kg).

Keywords: schistosomiasis, Schistosoma mansoni, praziquantel, dosage determination, ‘dose pole’, Zanzibar


Introduction

The World Health Organization (WHO) advocates the control of schistosomiasis by regular treatment of vulnerable groups with praziquantel, delivered through schools and health services. One of the major disadvantages of wide delivery of praziquantel is the fact that its dosage has to be calculated according to weight. HALL et al. (1999), recognizing that the provision of sufficient weighing scales to maintain long-term mass dosing programmes in the rural areas of developing countries was a major problem, prepared the use of a graduated pole to assess height and so determine the number of tablets of praziquantel to be used for each individual. A ‘dose pole’ was tested on height and weight data of more than 25000 children from 10 countries with positive results (MONTRESOR et al., 2001). The aim of our study was to field-test the dose pole in Zanzibar and compare its performance with that of a bathroom-type scale. A digital scale was used as a reference standard.

Material and Methods

A wooden pole was locally produced, with thresholds and corresponding dosages as described by MONTRESOR et al. (2001): 110–125 cm = 1.5 tablets, 125–138 cm = 2 tablets, 138–150 cm = 2.5 tablets, 150–160 cm = 3 tablets and 160–178 cm = 4 tablets. The cost of producing the wooden dose pole and that of purchasing a new scale was similar, approximately US$6. The field test was conducted in April 2001 on 1289 children in Kinyasini School, Zanzibar, United Republic of Tanzania. Each child received 3 independent evaluations of the number of tablets of praziquantel needed for treatment: the first (the reference standard) using a digital scale (Seca Inc., Columbia, Maryland, USA), the second using a new bathroom-type scale (Camry Inc., China), and the third using the dose pole.

The results were recorded separately so that the