EFFICACY OF PRAZIQUANTEL AGAINST SCHISTOSOMA HAEMATOBIUM INFECTION IN CHILDREN

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Abstract. A study was performed to determine the efficacy of praziquantel (PZQ) against Schistosoma haematobium. Children (n = 592) infected with S. haematobium received either a single treatment with PZQ (40 mg/kg) or two or three treatments with PZQ at three-week intervals after the initial treatment and efficacy was monitored for nine weeks. Cure rates at three-weeks post-treatment were low (< 50%), suggesting either that worms are killed very slowly or, more likely, that eggs continue to be released from tissues after worm death. Interestingly, a single dose of PZQ showed high efficacy (cure rate > 83% and egg reduction rate > 98%) when assessed from six weeks post-treatment onward. There were no significant differences in cure rates or intensity of infection between the three cohorts at any point in the study, despite the different treatment regimens. Since children were in contact with transmission sites during the study period, the results suggest good efficacy of PZQ against all stages of S. haematobium, including the immature worms.

INTRODUCTION

Praziquantel (PZQ) is currently the drug of choice for the treatment of schistosomiasis and is rapidly becoming the only commercially available antischistosomal drug. It is highly effective against all five schistosome species that infect humans. With the advent of this safe drug, morbidity control has become the mainstay of schistosomiasis control.1 Praziquantel has been used extensively and successfully in national control programs in Brazil, China, Egypt, and the Philippines, and there is little evidence of the development of clinically relevant resistance. The renewed impetus for extending schistosomiasis control throughout sub-Saharan Africa will probably result in greater use of PZQ than ever before. This welcome development is set against the background of limited knowledge on many aspects of this drug.2

A critical aspect in the assessment of PZQ efficacy is its activity against the different parasite developmental stages. Indeed, experimental laboratory studies have shown that the activity of PZQ is stage dependent. This drug is active primarily against the adult worm stages, whereas immature schistosomes (2–4 weeks old) are less susceptible.3,4 Thus, doses of drug that are curative against mature adult infections are sub-curative against developing worms. In high transmission areas, the removal of adult worms by treatment will not result in normal levels of cure rates due to the development of immature worms into egg-producing adults by the time of the follow-up assessment of cure, as observed in Senegal for S. mansoni.5-9

Despite the extensive use of PZQ against S. haematobium, which accounts for 67% of the schistosome infections in sub-Saharan Africa,10 we know very little on its efficacy against the immature stages of this species. A recent study of efficacy of PZQ against S. haematobium showed a low worm reduction at 4-weeks post-infection, i.e., 56%, whereas high reductions were obtained at 8- and 12-weeks post-infection.11 A key point yet to be clarified concerns the activity of PZQ during the very long prepatent period of this species, i.e., approximately 10–12 weeks compared with 5–7 weeks for S. mansoni.12 Therefore, a study was conducted to compare a single treatment with PZQ with two or three treatment regimens at three-week intervals. The objective of the repeated treatments was to determine the efficacy of PZQ against all development stages of S. haematobium. The children were expected to be infected with all stages because they were in contact with active transmission sites before and during the study period. The cure rate and reduction in egg counts were monitored every three weeks over a nine-week period, i.e., within the pre-patent period of S. haematobium.

MATERIALS AND METHODS

Study site and children. The town of Loum, known from previous studies as an active transmission focus of S. haematobium,13 was selected for the study. The parasitologic surveys of school children were conducted between April and June 2002 in quarters adjacent to the Mbette River and its tributaries where the main transmission sites of schistosomiasis were identified.13 These transmission sites are permanent streams, and schistosomiasis transmission in the focus of Loum is known to occur all year, with the study being conducted during one of the high transmission periods.

With the approval of the school inspector, directors, and teachers, four schools were sampled (Table 1). The school children were invited to participate in the study, and were registered only after explanation of the objectives of the study to them and to their parents or guardian, and after full informed consent had been obtained. The study protocol was reviewed and approved by the National Ethics Committee of Cameroon. Initially, 1,613 pupils from these four schools were registered and provided urine samples. Of these, 674 were positive for S. haematobium, giving an overall infection prevalence of 41.8%. All school children present at schools were treated with 40 mg/kg of PZQ (Shin Poong, Seoul, South Korea).

A total of 592 infected pupils were treated with PZQ, and only this subset of children was selected for the follow-up studies. Initially, these 592 children were divided into four cohorts with care being taken that all cohorts had a similar ratio of light and heavy infections (low infections: < 50 eggs/10 mL of urine, heavy infections: ≥ 50 eggs/10 mL of urine), a
similar age, a similar sex ratio, and a similar distribution between schools. The original aim of the study had been to follow the children for 12 weeks (with one cohort, no. 4, receiving four treatments). However, the study was ended nine weeks after initial treatment due to changes in school schedules. As a consequence, the treatment regimen for the original cohorts 1 and 4 was the same for these nine weeks; these results were therefore combined. Throughout the rest of this report, cohort 1 refers to the combined original cohorts 1 and 4. The three cohorts were followed-up for nine weeks (i.e., within the pre-patent period of *S. haematobium*) at three-week intervals. Children in cohort 1 were treated again at three and six weeks after the initial dose; children in cohort 2 were re-treated only once, at three weeks after initial treatment, whereas children in cohort 3 were not re-treated. At the end of the study, all infected children were treated with PZQ.

**Parasitologic techniques.** For each survey, urine samples were collected from each participant on two consecutive days and transported to a local field laboratory where they were all examined on the same day of collection. No preservative was used. These urine samples were collected in 50-mL plastic screw-cap vials between 11:00 AM and 1:00 PM. Each urine sample was agitated to ensure adequate dispersal of eggs, 10 mL of urine was filtered through a filter (Nucleopore, Pleasanton, CA), and the filters were examined by microscopy for the presence of eggs.

**Statistical analysis.** Egg counts of the two consecutive days were averaged (arithmetic mean) at the individual child level. The parasitologic cure rates were calculated as the proportion of children excreting eggs at the first survey before treatment and who were not excreting eggs in their urine after treatment. Ninety-five percent confidence intervals (CIs) were calculated for proportions. Geometric mean (GM) values of all individuals were used to assess average egg counts of each group. The GM was calculated as the antilogarithm of the mean of all log transformed egg counts + 1. The intensity reduction rate was calculated as \[1 - \frac{\text{GM egg counts per 10 mL of urine after treatment}}{\text{GM egg counts per 10 mL before treatment}}\] × 100. Prior to treatment, egg counts were all positive and log-normally distributed, therefore to check that individuals had been assigned to random groups, analysis of variance of the log-transformed egg counts was used. To assess whether differences in egg counts post-treatment (i.e., cured) were associated with treatment regimens, and/or other parameters, generalized linear models with binomial errors were used. To assess whether differences in egg counts between schools at week 9 were due to the subset of individuals present at week 9, chi-square tests on proportions were carried out. In all cases, a *P* value < 0.05 was taken to indicate significance.

**RESULTS**

Of the 592 children selected for the study, 515 (230 males and 285 females) participated in the first three surveys (week 0, week 3, and week 6), giving a participation rate of 87%. However, only 245 of these 515 children participated in the fourth survey (week 9), due to a change in the school schedules, leading to an early holiday for some classes and to the absence of children at week 9. Therefore, the analysis for weeks 0, 3, and 6 was also repeated throughout with only these 245 individuals to ensure that the results for week 9 were not due to the subset of individuals present at week 9. The overall male:female ratio was 0.81:1.

Before the treatment, approximately 25% of the children were heavily infected (> 50 eggs/10 mL) in each of the three cohorts (range = 21.5–25.6%). The overall GM of egg counts was 219.6 eggs/10 mL of urine.

<table>
<thead>
<tr>
<th>Intensity class</th>
<th>Before treatment</th>
<th>Three weeks after the first treatment</th>
<th>Three weeks after the second treatment</th>
<th>Three weeks after the third treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>GM*</td>
<td>Cure rate (%)</td>
<td>GM eggs/10 mL</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>183</td>
<td>9.50</td>
<td>87</td>
<td>47.5</td>
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<tr>
<td>≥ 50</td>
<td>63</td>
<td>129.05</td>
<td>11</td>
<td>17.5</td>
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<tr>
<td>All classes</td>
<td>246</td>
<td>19.00</td>
<td>98</td>
<td>39.8</td>
</tr>
</tbody>
</table>

* GM = geometric mean egg count per 10 mL of urine.
† See Materials and Methods for definition.
‡ Number of children who participated in all four surveys.
§ Comparing egg counts of those individuals that provided four samples.
Cure rates, geometric mean egg counts, and intensity reduction rates in school children infected with *Schistosoma haematobium* after one and two treatments with praziquantel at three-week intervals in Loum, Cameroon (Cohort 2)

Table 3

<table>
<thead>
<tr>
<th>Intensity class before treatment</th>
<th>Before treatment</th>
<th>Three weeks after the first treatment</th>
<th>Three weeks after the second treatment</th>
<th>Six weeks after the second treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>GM* eggs/10 mL</td>
<td>No. of subjects cured</td>
<td>Cure rate (%)</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>100</td>
<td>7.30</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>≥ 50</td>
<td>34</td>
<td>173.02</td>
<td>4</td>
<td>11.8</td>
</tr>
<tr>
<td>All classes</td>
<td>134</td>
<td>16.96</td>
<td>55</td>
<td>41</td>
</tr>
</tbody>
</table>

* GM = geometric mean egg count per 10 mL of urine.
† See Materials and Methods for definition.
‡ Number of subjects who participated in four surveys.
§ Number of individuals who provided four samples.

before treatment was 18 eggs/10 mL. There was no significant change in log-transformed egg counts with age, sex, or school class (F > 0.727, P < 0.603). While there were significant differences in egg counts between the schools (F3,511 = 2.824, P = 0.038; GM ranging from 13.3 for School St. Jean Marie Vianney 2 to 21.7 for Public School Bonkeng), there was no significant difference in egg counts within schools between the three cohorts (F6,500 = 0.424, P = 0.822), indicating that the randomization of individuals into the three cohorts had not resulted in a bias in terms of school individuals.

**Cure rates.** Three weeks after treatment, the cure rates in the three cohorts varied from 39.8% (95% CI = 33.7–46.3%) to 50.4% (95% CI = 42.0–58.7%) (Tables 2–4). However, there was no significant difference in cure and cohort (χ² = 4.180, degrees of freedom [df] = 2, P = 0.124, Figure 1a). Furthermore, there was no significant difference in cure and age or sex (males = 46.9%, females = 39.6%) either overall (χ² < 2.773, df = 1, P > 0.096) or within the cohorts (χ² < 1.042, df = 2, P > 0.594). There were however, highly significant differences in cure between heavily and lightly infected individuals (χ² = 48.407, df = 1, P < 0.001; Tables 2–4), and schools (χ² = 15.217, df = 3, P < 0.002), but there was no difference in cure in these groups between the three cohorts (P > 0.627). Excluding those individuals who were not present at the final sampling made no difference in terms of significance for cohort, age, sex, or heavy/light initial infection.

Three weeks after the second treatment of cohorts 1 and 2, cure rates in cohort 1 and 2 had significantly increased to 82.1% (95% CI = 76.6–86.6%) and 79.1% (95% CI = 71.0–85.5%), respectively (χ² > 38.892, P < 0.001). However, the cure rate for the third cohort (which had not received a second treatment) had also significantly increased to 83.0% (95% CI = 75.3–88.7%, χ² = 30.817, P < 0.001). As a result, there was no significant difference in individuals cured between all three cohorts (χ² = 0.751, df = 2, P < 0.688, Figure 1a), or if cohort 3 was compared with cohorts 1 and 2 combined (χ² = 0.245, df = 1, P < 0.621). There was still a significant difference in cure between initially heavily (69.8%, 95% CI = 60.9–77.4%) and lightly (85.5%, 95% CI = 81.4–88.8%) infected individuals (χ² = 14.636, df = 1, P < 0.001), but there was no difference in these groups between cohorts (χ² = 0.510, df = 2, P < 0.775; Tables 2–4). In addition, there was still no significant difference in cure in age with age, sex, or school (χ² < 2.512, P > 0.473). Excluding those individuals who were not present at the final sampling made no difference in terms of significance for cohort, age, sex, or heavy/light initial infection.

Three weeks after the third treatment of cohort 1, the cure rate had increased to 87.8%; however, this increase was not significant (χ² = 1.496, P < 0.222). In addition, the cure rate in cohort 2 (which had not received the third treatment) also increased marginally to 80%, and the cure rate for cohort 3 (which had not received the second or the third treatments) increased to 88.6% (Tables 2–4), neither of which was significant (χ² < 0.735, P > 0.391). Despite the different treatment regimens, there was no significant difference in cure between the three cohorts nine weeks after the first treatment (χ² = 2.352, P < 0.309, Figure 1a). There was still a significant difference in cure between initially heavily (76.7%, 95% CI = 65.0–85.5%) and lightly (90.1%, 95% CI = 84.4–94.0%) infected individuals (χ² = 7.255, df = 1, P < 0.008), but there was no difference in these groups between cohorts (χ² = 4.219, df = 2, P < 0.122; Tables 2–4). In addition, there was still no significant difference in cure in age with age, sex, or school (χ² < 2.831, P > 0.092).

**Intensity reduction rates.** Three weeks after the first treat-

Table 4

<table>
<thead>
<tr>
<th>Intensity class before treatment (eggs/10 mL)</th>
<th>Before treatment</th>
<th>Three weeks after treatment</th>
<th>Six weeks after treatment</th>
<th>Nine weeks after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>GM* eggs/10 mL</td>
<td>No. of subjects cured</td>
<td>Cure rate (%)</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>103</td>
<td>8.22</td>
<td>60</td>
<td>58.3</td>
</tr>
<tr>
<td>≥ 50</td>
<td>32</td>
<td>115.92</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>All classes</td>
<td>135</td>
<td>15.83</td>
<td>68</td>
<td>50.4</td>
</tr>
</tbody>
</table>

* GM = geometric mean egg count per 10 mL of urine.
† See Materials and Methods for definition.
‡ Number of children who participated in four surveys.
§ Number of children who participated in all four surveys.

Comparing egg counts of those individuals that provided four samples.
ment, the GM intensity of infection had significantly decreased \( (\chi^2 = 526.2, df = 1, P < 0.001) \) by more than 91% to 1.51, ranging from 1.36 to 1.63 in the three cohorts (Tables 2–4), with no significant difference between the three cohorts \( (\chi^2 = 2.106, df = 2, P < 0.349, \text{Figure 1b}) \). There was also no significant difference in intensity with age or sex \( (\chi^2 < 0.954, P > 0.329) \). In contrast there was a significant relationship between intensity and school \( (\chi^2 = 26.691, df = 3, P < 0.001) \). In addition, there was still a significant difference in intensity 3 weeks after treatment between initially heavily and lightly infected individuals \( (\chi^2 = 58.14, df = 1, P < 0.0001) \), but these differences were not cohort specific \( (\chi^2 = 1.033, df = 2, P < 0.597) \).

Six weeks after the first treatment (3 weeks after cohorts 1 and 2 had had their second treatments), intensities had decreased significantly further \( (\chi^2 = 54.58, df = 1, P < 0.001, \text{Figure 1b}) \). However, there were no significant differences in intensities between the three cohorts (Figure 1c) or initially heavily and lightly infected individuals, sex, or school \( (\chi^2 < 3.160, P > 0.075; \text{Tables 2–4}) \). In contrast, there was a significant effect of age \( (\chi^2 = 5.250, df = 1, P < 0.022) \), with burdens peaking at age 10.

Nine weeks after the first treatment (3 weeks after cohort 1 had received its third treatment and six weeks after cohort 2 had received its second treatment), intensities in the 245 individuals who were sampled the fourth time had not significantly changed \( (\chi^2 = 0.624, df = 1, P < 0.430) \). In addition, there were no significant differences in intensity between cohorts (Figure 1c), age, sex, or school \( (\chi^2 < 4.373, P > 0.066) \). However, initially heavily infected individuals had significantly greater intensities than initially lightly infected individuals \( (\chi^2 = 14.207, df = 1, P < 0.001) \), and this difference was not treatment regimen specific \( (\chi^2 = 1.678, df = 2, P < 0.432) \).

DISCUSSION

The results of the present study indicate that a single treatment (cohort 3) with PZQ possesses a high efficacy since at six and nine weeks post-treatment the cure rate was 83–88.6% and the egg reduction rate was > 98%. Furthermore, a second and third treatment at three-week interval resulted in no difference in the patterns observed. The low cure rates at three-weeks post-treatment, compared with the significantly higher cure rates at six- and nine-weeks post-treatment, suggest that either worms are killed very slowly or, more likely, that eggs continue to be released from tissues after worm death. In mice infected with *S. mansoni*, it has been shown that PZQ completes its antischistosomal action in one week, and a similar rate of action may well apply to *S. haematobium*. The idea of a slowly tapering egg excretion is indirectly reinforced by the much lower cures rates (at three weeks) in heavily infected children (< 25%) compared with children with low infection levels (58%). The viability of excreted eggs was not determined in the present study, but it has been reported that dead eggs may be present for months in the urine of treated patients infected with *S. haematobium*. Continued excretion of viable *S. haematobium* eggs has been previously reported in sporadic cases, while it appears to be a recurring phenomenon in this study. In any event, the persistent egg excretion at three and six weeks, especially in the high-intensity group of children that showed cure at nine weeks, raises two important questions, at least for *S. haematobium* areas. 1) What is the optimal time after treatment at which PZQ efficacy should be assessed? 2) Could previously reported low cure rates at 4–6 weeks be linked to a passive egg excretion phenomenon?

The persistent high cure rate during the nine-week follow-up in children receiving a single treatment and the lack of differences in cure rates in children receiving repeated treatments suggests good efficacy for PZQ against the immature stages of the parasite. Schistosomiasis transmission in the focus of Loum is known to occur all year and the study started during one of the high-transmission periods. Under these conditions, one could have expected that recent infections before treatment would become patent during the nine-week follow-up of the study, resulting in a decreased cure rate pattern in the case that PZQ lacked efficacy against immature stages.

In conclusion, this study showed that in Cameroon PZQ has a high cure rate against *S. haematobium* and suggests an efficacy of PZQ against the immature stages.

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