EVIDENCE FOR A LONG-TERM EFFECT OF A SINGLE DOSE OF PRAZIQUANTEL ON SCHISTOSOMA MANSONI–INDUCED HEPATOSPLENIC LESIONS IN NORTHERN UGANDA

KATHRIN FRENZEL, LORENZ GRIGULL, EMMANUEL ODONGO-AGINYA, CHRISTOPHER M. NDUGWA, TOM LORONI-LAKWO, ULRICH SCHWEIGMANN, UDO VESTER, NESTOR SPANNBRUCKER, AND EKKEHARD DOEHRING

Kinderklinik Marienhospital, Osnabrueck, Germany; Kinderklinik Medizinische Hochschule Hannover, Hannover, Germany; Uganda Virus Research Institute, Entebbe, Uganda; Department of Pediatrics and Child Health, Makerere University, Kampala, Uganda; Deutsches Kinderkardiologisches Zentrum, Berlin, Germany; Universitaetskinderklinik, Essen, Germany; Gesundheitsamt Neuruppin, Neuruppin, Germany

Abstract. Treatment with praziquantel reduces the prevalence and intensity of Schistosoma mansoni infection. However, reversibility of periporal fibrosis of the liver, which potentially leads to fatal complications, is not unequivocally substantiated. In the Nile District of Uganda, 460 patients were parasitologically (Kato–Katz method) and ultrasonographically examined during October 1991, October 1992, and May 1994. Treatment with praziquantel at a dosage of 40 mg per kilogram of body weight was given in October 1991 and October 1992 to 460 individuals (group A). Another 192 patients were seen during the baseline study in October 1991 and missed the follow-up in October 1992 but took part in the second follow-up in May 1994. Thus, they received praziquantel only once in October 1991 (group B) and had an interval of 2.7 years until the next investigation in May 1994. Periportal thickening (PT) of the liver was assessed by ultrasound at each time point. Praziquantel therapy reduced the prevalence of S. mansoni in group A from 84% in 1991 to 31% in 1992 and 30% in 1994. The respective intensities of infection (geometric means of egg output) were 81 eggs per gram (epg) of stool in 1991, 31 epg in 1992, and 30 epg in 1994. Periportal thickening was found in 46% of patients in 1991, 32% of patients in 1992, and 35% of patients in 1994. Reversibility of PT was influenced by age (markedly lower reversibility in individuals older than 30 years) and sex (women and girls responded less favorably than did men and boys). Surprisingly, no significant difference was detected between group A and group B with respect to reversibility of PT. The outcome between the 2 groups did not differ significantly. This may indicate that a single dose of praziquantel (as given to group B) may have a longer lasting effect than previously thought, that is, more than 2.5 years.

Hepatic fibrosis in patients with Schistosoma mansoni infection is of primary importance among chronic liver diseases worldwide. The macromorphology of hepatic fibrosis is highly characteristic and can be detected by ultrasound. In recent years, the official policy to combat schistosomiasis and thereby liver disease induced by S. mansoni infection, as proclaimed by the World Health Organization, has shifted from attempts to eradicate the disease to containment of morbidity. Treatment of schistosomiasis with praziquantel has proven efficient in reducing wormload, as assessed by egg excretion. Clinical trials show reductions of prevalence and intensity of infection. Ultrasonographic studies have documented improvements in periportal thickening (PT) of the liver after treatment. Homeida and coworkers provided ultrasonographic evidence for reversibility of PT after intensive community-based chemotherapy. However, ideal regimens for treatment and retreatment intervals remain elusive. Most reports on this subject apparently had retreatment intervals of 1 year. Considering that many underprivileged countries in which schistosomiasis is endemic must prioritize their resources for health, retreatment intervals longer than 1 year would be advantageous.

We hereby suggest that a single dose of praziquantel may be effective for up to 2.7 years. We encourage future trials in other endemic areas to further test our observation. Long retreatment intervals have potentially important implications on the control of the disease.

PATIENTS AND METHODS

Study participants and area. The study was performed in 2 remote settlements in the Nile District of Uganda, Rhino Camp and Obongi, each with approximately 10,000 inhabitants. The towns are located approximately 50 km apart on the River Nile (Albert Nile). The River Nile provides an important source of revenue, drinking water, fishing, and recreational activities for the inhabitants. Houses are scattered over a rather extensive area (approximately 150 km²). Despite freshwater wells drilled in 1988, most villagers prefer water from the Nile. Ecological and economic circumstances create ideal conditions for transmission of S. mansoni. During the study period (October 1991 to May 1994), the area was recuperating from previous civil unrest, some of the fiercest in all of Uganda. Each village had one health center.

Control activities against schistosomiasis had not been performed in the study area. More importantly, systematic or sporadic control activities were not carried out during the time period of the study. Praziquantel was not available in dispensaries until 1992 in the study villages of Rhino Camp or Obongi or in the district capital Arua. In 1994, praziquantel was commercially available only in Rhino Camp.

After repeated questioning, 8% of the individuals seen in 1991 reported being treated against schistosomiasis previously. Fewer than one half of these individuals remembered the approximate dates of treatment, which were usually before 1985. Intramuscular injection with a substance against schistosomiasis was frequently reported. Individuals with previous antischistosomal treatment were excluded from further follow-up but received praziquantel when they excreted eggs in their stool.

Previous reports from the study area revealed high prevalence of S. mansoni infection and severe morbidity induced...
by the parasite.\textsuperscript{11-14} In October 1991, 1,363 persons (exclusive of the patients who had received previous treatment) were initially admitted to the study. These included 388 pupils from primary schools, 412 fishermen, 59 members of the local administration, and 504 villagers, who spontaneously presented at their own initiative through the local medical assistant.

Each study participant provided 2 stool samples. The feces were processed according to a modified Kato–Katz method\textsuperscript{15} with Helm-test\textsuperscript{\textregistered} kits produced by AK Industria e Comercio Ltd. (Belo Horizonte, Brazil). Three Kato slides of 41.7 mg from each stool sample were examined per patient. Egg output was calculated as eggs per gram (epg) of stool.

When 6 slides were negative (without \textit{S. mansoni} eggs) in the field, approximately 3 g of stool was preserved in merthiolate formaldehyde solution for transport and taken to the Institute of Medical Parasitology in Bonn, Germany (152 samples in 1991 and 75 samples in 1994). Approximately 500 mg of each stool sample was examined there according to modifications of the method described by Blagg and others.\textsuperscript{16} Of the stool samples that had been found to be negative in the field, 14 and 19\% proved to still harbor \textit{S. mansoni} eggs when examined in the laboratory at Bonn in 1991 and 1994, respectively.

\textbf{Ethical considerations.} Informed consent to the study was given orally in the local language (Kakwa, Lugbara, or Madi) after full explanation of the study protocol by a local interpreter. All patients with \textit{S. mansoni} infection were treated with praziquantel at 40 mg per kilogram of body weight under close supervision of the authors. All patients found to be infected with intestinal parasites (i.e., hookworms) were treated with mebendazole. When pregnancy was detected clinically or by ultrasound, praziquantel was given to the pregnant women to be taken after delivery. Minor diseases, detected clinically or per ultrasound, were treated by the field team.

Patients with severe diseases were referred to the local health center or taken to the district hospital in Arua by the vehicle used during the study. The protocol of investigation was approved by the Assistant District Administrator of Rhino Camp and Obongi, the local health authorities in Arua, the Ministry of Health of Uganda in Entebbe, the Uganda National Science and Technology Committee in Kampala, and the Ethical Committees of the Universities of Bonn and Hannover, Germany.

Infected patients and schoolchildren received health education at the study center or in their schools, covering the disease, its transmission, and respective risk behavior. Patients visiting the study center received health education while waiting for examination.

Training of local collaborators was a strong component of the study. Because of the high prevalence of schistosomiasis, all pupils who were not enrolled into the study in Rhino Camp and Obongi were treated unselectively with praziquantel in 1991 by the local health assistant, the directors of the schools, and the authors.

\textbf{Ultrasoundographic examination.} Abdominal ultrasonography was performed in 1991 with a portable ultrasound machine (Sonoline 1300; Siemens, Erlangen, Germany) with a 3.5-MHz linear scanner and a 5-MHz sector scanner. In 1992 and 1994, an SSD 550 Hellige-Alloka (Freiburg, Germany) with a 3.5-MHz convex probe was used for ultrasonography. Because of technical problems, 2 ultrasound machines were used during this study. The Siemens Sonoline 1300 was used in many studies on schistosomiasis morbidity, providing ultrasonic technology at approximately 1985 standards. The Sonoline was damaged in shipping to Germany from Uganda after its initial use there. Therefore, another machine was used thereafter. Given the higher resolution of the SSD 550 machine, any potential bias introduced by its use would most likely be toward less reversibility because the higher resolution indicates more echogenicities, and therefore broader periportal sheathings, at follow-up.

We graded PT into 3 classes according to the Managil classification.\textsuperscript{17} Measurements according to the Cairo classification were performed in parallel. The Cairo classification was substantially modified at the second consensus conference on ultrasound in schistosomiasis convened under the auspices of the World Health Organization in Niamey, Niger, in October 1996 (WHO White Paper Report, unpublished). Therefore, the presented data rest on the Managil classification.

\textbf{Statistical analysis.} Statistical analysis was performed with the SAS system (SAS Inc., Cary, NC). Student’s \textit{t}-test and chi-square test were used where appropriate. Significance of difference was defined as \textit{P} < 0.05.

\textbf{RESULTS}

Of the 1,363 initial patients in October 1991, 460 were followed at 1 and 2.7 years after therapy (October 1992 and May 1994). At the initial investigation, and at every follow-up the infected individuals who attended received praziquantel (40 mg/kg), so that by May 1994, 2 doses of praziquantel had been given (group A, which consisted of 460 patients). In addition, 192 participants were seen at the initial investigation in October 1991 and in May 1994 only. Thus, these 192 patients (group B) missed the follow-up in October 1992 and consequently only received 1 dose of praziquantel in October 1991. The remaining 711 persons were lost to follow-up because of incomplete results or identification, or, more likely, because of high mobility in the aftermath of civil unrest.

The 460 patients in group A were not significantly different from the 192 patients in group B with regard to age (no significant differences in distribution of age), prevalence of infection (84 versus 81\%), intensity of infection (geometric mean 81 epg versus 88 epg), and sex (ratio of men and boys to women and girls, 53–47\% versus 51–49\%).

In both groups, egg output decreased significantly after therapy (Table 1). The maximum egg output was 3,288 epg in 1991, 856 epg in 1992, and 600 epg in 1994.

Comparing groups A and B, the overall rate of PT was reduced in both groups after the first treatment and reached comparable values by May 1994. Before treatment the rate of PT grade I was 27 and 32\%, respectively, and remained at comparable levels thereafter (Table 2). Periportal thickening grade II markedly reduced from 17 to 6\% (group A) and 17 to 4\% (group B), respectively. No decrease occurred in PT grade III.

Age provided the most important influence on the reversibility of PT (Figure 1). The respective highest rates of PT
Parasitologic results of 652 individuals in an area hyperendemic for *Schistosoma mansoni* in Nile District, Uganda. The 2 villages are situated in the immediate vicinity of the Albert Nile. The patients are divided into 2 groups. Infected persons in group A (n = 460) received 2 doses of praziquantel at 40 mg/kg body weight (in 1991 and 1992) until the last follow-up in May 1994. Infected persons in group B (n = 192) had only 1 cycle of treatment (1991) until May 1994.

<table>
<thead>
<tr>
<th>Prevalence of infection (%)</th>
<th>Geometric mean egg output (epg)</th>
<th>Prevalence of PT (%)</th>
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</thead>
<tbody>
<tr>
<td>1991 Group A</td>
<td>84</td>
<td>81</td>
</tr>
<tr>
<td>1992 Group A</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>1994 Group A</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>1991 Group B</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>1992 Group B</td>
<td>27</td>
<td>27</td>
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* epg = eggs per gram (of stool); PT = periportal thickening of the liver (as assessed by ultrasound).

We found that age, sex, and interval of treatment influenced reversibility of PT. The most important influence was age. Adolescents and young adults profited most from treatment. Reversibility of PT in individuals older than 30 years was markedly lower. Similarly, Homeida and others observed reversibility of periportal fibrosis in patients younger than 20 years after 2 cycles of annual and 3 cycles of biennial treatment. Our group has reported reversibility of PT in schoolchildren following praziquantel treatment. The few available studies on gender with regard to schistosomiasis have produced conflicting results. Differing water contact patterns of men and women in varying cultural settings have been proposed to explain the different degrees of morbidity due to sex. Because in the present study prevalence and intensity of infection were similar in women and men at all time points of investigation and water contact studies in 1991 showed no difference between men and women, this explanation seems unlikely to explain the lower morbidity but worse reversibility after treatment in women. Other mechanisms appear more likely. Feldmeier and others pointed out potential biases for gender and sex.

<table>
<thead>
<tr>
<th>PT in 1991 (%)</th>
<th>PT in 1992 (%)</th>
<th>PT in 1994 (%)</th>
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<tbody>
<tr>
<td>Group A</td>
<td>Group B</td>
<td>Group A</td>
</tr>
<tr>
<td>54</td>
<td>49</td>
<td>27</td>
</tr>
<tr>
<td>68</td>
<td>Not done</td>
<td>26</td>
</tr>
<tr>
<td>65</td>
<td>72</td>
<td>27</td>
</tr>
</tbody>
</table>
with regard to diagnostic categories suitable for schistosomiasis. Biological factors—for example, hormonal influence on immune responsiveness or different vascular anatomy of women compared to men—possibly may influence the expression of morbidity. Sociocultural factors may influence the varying compliance for participation in a study due to sex. We tried to avoid the potential influence of pregnancy by excluding pregnant women from the study.

A limited number of Kato slides, as performed in this study, is well known to underestimate the true prevalence of schistosomiasis in a community. De Vlas and collaborators published results of mathematical modeling efforts aimed at quantifying this underestimation. Their pocket charts provide an estimate of how high the true prevalence would be when a given number of Kato slides have been done. In our case, 6 Kato slides at 41.7 mg of stool each, as done in the field, roughly examined 250 mg of stool. The technique in Germany (revised Blagg technique) examined roughly 500 mg of stool and added approximately 15% of samples that were determined to be positive. Given the double amount of stool examined in Germany and the kinetic of egg variation as described by mathematical modeling, the results of 15% positives added are within an expected range (De Vlas SI, unpublished data).

After an unsuccessful effort to eradicate schistosomiasis under the auspices of the World Health Organization, a strategy against the disease was devised that concentrated on reduction of morbidity by chemotherapy and environmental control. However, time points of treatment and, more importantly, retreatment in case of reinfection remain unclear. In this context, a relevant finding centers around the observation that 192 individuals missed the interim follow-up in October 1992 and thereby missed the second treatment cycle with praziquantel. Because they took part in the final follow-up in 1994, they had an interval of 2.7 years between their only treatment and reexamination. Their outcome in 1994 was not significantly different from that of patients in group A, who received 2 treatment cycles. This fact may indicate that treatment cycles could possibly be spaced over longer intervals (i.e., possibly several years) than was previously practiced (treatment intervals of approximately one year). This is potentially of significant importance because treatment with praziquantel presently constitutes the most important modality on which disease containment rests. Future studies should aim at corroborating this finding in other endemic settings.

Acknowledgments: We wish to thank Angwar Sector Setoro, Peter Kapalanga, and their teams for excellent technical assistance. The friendly cooperation of the inhabitants and the authorities of Rhino Camp and Obongi is gratefully acknowledged. Mrs. Domke at the Institute of Medical Parasitology in Bonn assisted with laboratory examination. The praziquantel used in this study was donated by Bayer AG, Leverkusen, Germany.

Financial support: This investigation received financial support from Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) and from UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grant ID 900 286). Data analysis was partially supported by the Sciences and Technology for Developing Countries Programme (STD III) of the Commission of the European Communities (grant TS3-CT94-0330). Medical supplies for the field clinic were provided by Hermann Mai Stiftung (Tuebingen, Germany). Ekkhehr Doehring was a recipient of short-term lectureships to Makerere University supported by Deutschem Akademischem Austauschdienst (DAAD, German Academic Exchange Service).

Authors' addresses: Kathrin Frenzel, Kinderklinik Marienhospital, Johannistheid 2-4, 49 074 Osnabrueck, Germany. Lorenz Grigull, Kinderklinik Medizinische Hochschule Hannover, Carl Neuberg Strasse 1, 30 625 Hannover, Germany. Emmanuel Odongo-Aginya and Tom Loroni-Lakwo, Uganda Virus Research Institute, PO Box 49, Entebbe, Uganda. Christopher M. Ndugwa, Department of Pediatrics and Child Health, Makerere University, PO Box 7051, Kam-
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