STUDIES ON SCHISTOSOMIASIS IN WESTERN KENYA: II. EFFICACY OF PRAZIQUANTEL FOR TREATMENT OF SCHISTOSOMIASIS IN PERSONS COINFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS-1

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Abstract. Praziquantel is the drug of choice for schistosomiasis chemotherapy. Although the exact mechanism of how praziquantel kills schistosomes remains poorly understood, the immune response of the host is an important factor in drug efficacy. It is thus possible that disease states of humans that lead to immunodeficiencies, such as infection with human immunodeficiency virus-1 (HIV-1), may render praziquantel less effective in treating schistosomiasis. To test this hypothesis, persons with high levels of Schistosoma mansoni infection who were or were not also infected with HIV-1 were treated with a standard regimen of praziquantel and monitored by quantitative fecal examination and plasma circulating cathodic antigen. Both groups responded to praziquantel therapy equally and individuals with low percentages (<20%) of CD4+ T cells did not differ from individuals with higher CD4 cell percentages. These data demonstrate that persons with HIV-1 infection can be treated effectively for schistosomiasis with praziquantel.

The most widespread drug currently used for treatment of schistosomiasis is praziquantel. Although the exact mechanism(s) of action of praziquantel on schistosomes is not understood, this drug appears to function by rendering the parasite more susceptible to elimination by the immune response of the host.1,2 Experimental studies have demonstrated that schistosome infections in immunodeficient animals are impervious to treatment with praziquantel and that a functional immune response is necessary for drug efficacy.3,4 Because drug alone is not sufficient for cure of experimental infections, it is possible that praziquantel may be less effective in persons with schistosomiasis who are also immunosuppressed. To address this possibility, we examined praziquantel efficacy in a group of individuals with high levels of Schistosoma mansoni infections who were or were not also seropositive for human immunodeficiency virus-1 (HIV). As measured by decreased egg excretion and lowered levels of circulating adult worm antigens, praziquantel was useful for treating schistosomiasis infections, even in persons with HIV-1 coinfection and lowered CD4+ lymphocyte percentages.

MATERIALS AND METHODS

Patient population and parasitology. These studies were approved by the institutional review boards of the Kenya Medical Research Institute and the Centers for Disease Control and Prevention. The patients involved in this study have been described previously5 and included 15 individuals who had both schistosomiasis and HIV infection and 32 individuals who had schistosomiasis alone. Briefly, these individuals worked as car washers in Kisumu, Kenya and are exposed to infectious water in Lake Victoria for 5–7 days a week, 8–10 hr a day. Biomphalaria spp. snails are present in this water, with a high percentage of the tested snails shedding bifurcate cercariae (Karanja DMS, Secor WE, unpublished data). After giving informed consent, car wash workers provided stool and urine samples for schistosome ova examinations. A single stool sample was examined in duplicate by the Kato-Katz method to quantitate the number of S. mansoni eggs per gram (epg) of feces. Kato-Katz slides were read within 24 hr and other parasite ova in the stool were also noted. Urine was tested for presence of blood using Ames Hemasix reagent strips (Miles, Inc., Elkhart, IN) and midday 10-ml urine samples were filtered through 12-μm Nucleopore polycarbonate membranes (#110416; Costar, Cambridge MA) and examined microscopically for ova of S. haematobium. As part of the consent process, individuals were asked to donate blood for studies involving both schistosomiasis and HIV. Medical histories were obtained and physical examinations and HIV counseling were conducted in the native language (Luo) by qualified clinicians. Patients who agreed to have their blood tested for the presence of antibodies to HIV were given pretest counseling and were offered post-test counseling. Confidential counseling was provided by trained counselors from western Kenya. Each blood sample was assigned a patient identification number to maintain confidentiality. Testing for antibodies against HIV-1 was performed with a commercial ELISA kit (Genetic Systems Corp., Redmont, WA) and confirmed with a commercial Western Blot Kit (Murex Diagnostic Ltd., Kent, United Kingdom).

Patient treatment and follow-up. Persons with schistosomiasis were offered treatment with praziquantel in a single dose of 40 mg/kg. Four weeks following treatment, stool and urine samples were re-examined for the presence of schistosome eggs as described above. Individuals not cleared of schistosomiasis were offered a second dose of praziquantel. At six months after initial enrollment, a third set of urine and stool samples and a second blood sample were obtained from willing study participants. Again, persons found to be positive for schistosome ova were offered treatment with praziquantel.

Assay for circulating antigen. Patient plasma samples
were tested for the presence of adult worm circulating cathodic antigen (CCA) using the monoclonal antibody 5H11 sandwich assay as previously described.5, 6 Briefly, plates were coated with 5 μg/ml of monoclonal antibody 5H11 in 0.05M sodium carbonate buffer, pH 9.6. Following blocking, phosphate-buffered saline (PBS) containing 0.3% Tween 20, plasma samples (that had been pretreated [v/v] with 4% trichloroacetic acid for 20 min, centrifuged at 14,000 rpm for 15 min, and had supernatants removed and neutralized with an equal volume of 0.25 M sodium carbonate buffer, pH 9.6) were added and incubated for 1 hr. Following washing with PBS containing 0.05% Tween 20, monoclonal antibody 5H11 that had been derivatized with biotin hydrazide (#21339X; Pierce Chemical Company, Rockford, IL) was added to all but blank wells for 1 hr. The plates were then washed with PBS containing 0.05% Tween 20, streptavidin peroxidase (#14-30-00; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was added to all but blank wells for 1 hr. The plates were then washed with PBS containing 0.05% Tween 20 and developed with 3, 3′, 5, 5′-tetramethylbenzidine peroxidase substrate (#850-76-04; Kirkegaard & Perry Laboratories, Inc.), stopped with 2 M H₂SO₄, and read at 450 nm on a microplate reader (Dynatech, Chantilly, VA). Absorbance levels from patient plasma samples were compared with those obtained on a schistosome worm antigen preparation standard curve run in parallel to obtain a score that could be used to compare results from samples run on different days.

**Determination of percent CD4⁺ cells.** From each patient, 100 μl of heparinized peripheral blood was incubated with 5 μl each of phycocerythrin-conjugated anti-human CD4 and fluorescein isothiocyanate–conjugated anti-human CD8 (#30155X and #30324X; PharMingen, San Diego, CA) at 4°C in the dark. After 30 min, 2 ml of red blood cell lysing solution (#92-0002; Becton-Dickinson, San Jose, CA) were added to each tube; the tubes were briefly vortexed and incubated for 10 min at room temperature in the dark. The tubes were then centrifuged at 300 x g for 7 min at 10°C, supernatants were aspirated, and cells were resuspended in 1 ml of PBS containing 2% fetal bovine serum and 0.1% sodium azide. Following a second centrifugation, cells were resuspended in 0.5 ml of cold PBS containing 1% paraformaldehyde and stored at 4°C in the dark. The percentage of CD4⁺ cells was determined by flow cytometry as the percentage of cells within the lymphocyte population positive for phycoerythrin staining.

**Western blots.** Western blots were performed as previously described.7 Briefly, protein that had been expressed from the ECL-3 cDNA encoding the 5’ end of a 200-kD adult worm tubercle antigen was loaded at 40 ng/mm onto a 10% polyacrylamide gel for sodium dodecyl sulfate–polyacrylamide gel electrophoresis, followed by Western blotting onto nitrocellulose (BA85 Protran; Schleicher & Schuell, Keene, NH). The nitrocellulose was blocked with 5% nonfat milk in PBS plus 0.3% Tween 20, cut into strips, and incubated with a 1:100 dilution of individual patients’ serum in PBS plus 5% nonfat milk and 0.3% Tween 20 for 1 hr. Following four 5-min washes in PBS plus 0.3% Tween, strips were incubated with IgG or IgG isotype-specific peroxidase conjugates for 1 hr. Following additional wash steps, strips were developed with 0.06% 4-chloro-1-napthol (Sigma, St. Louis, MO) in Tris-buffered saline (0.2 M Tris, 0.5 M NaCl, pH 7.5) containing 0.01% H₂O₂ (Sigma). Peroxidase-conjugated anti-human IgG (#A90404) was obtained from Biosource International (Camarillo, CA). Peroxidase-conjugated anti-human IgG3 (#9050-05), IgG2 (#9080-05), and IgG4 (#9190-05) were obtained from Southern Biotechnology Associates, Inc. (Birmingham, AL).

**Statistical analyses.** Statistical analyses for comparisons of groups were performed by the nonparametric (Mann-Whitney) t-test. P values < 0.05 were considered significant. All statistical analyses were performed with the aid of the InStat computer program (GraphPAD Software, San Diego, CA).

**RESULTS**

Throughout this study, only one person was found to have one *S. haematobium* egg present in their urine. Therefore, the results presented will only address *S. mansoni* infections. At four weeks following initial praziquantel treatment, stools from 59% (19 of 32) of HIV-1-negative and 53% (8 of 15) of HIV-1-positive individuals were negative for *S. mansoni* eggs (Table 1). Although many persons were still excreting eggs four weeks after initial drug treatment, these individuals had an average 93% decrease in their *S. mansoni* egg regardless of HIV-1 serostatus. All but one individual (who...
was HIV-negative) had decreased egg excretion as a result of a single praziquantel treatment (Figure 1).

Within HIV serostatus groups, there were no differences in percent CD4+ cells between those individuals that did or did not cease *S. mansoni* egg excretion after treatment with praziquantel (Table 1). Similarly, there were no differences in the overall decrease in egg count when patients were grouped by % CD4 cells (Figure 2). Two persons still positive for *S. mansoni* egg excretion after initial praziquantel treatment were cleared of infection, based on stool examination, with subsequent treatments.

Because individuals positive for HIV-1 appear to excrete eggs less efficiently than HIV-1-negative individuals and egg counts derived from a single stool examination may not be a completely accurate reflection of a patient’s infection status (either the presence of infection or the level of infection if present), we used CCA levels in patients’ plasma as a secondary measure of infection status and evaluation of praziquantel efficacy. Initial and six-month plasma samples were tested for CCA using the monoclonal antibody 5H11 sandwich ELISA (Figure 3). As with egg counts, all patients demonstrated decreased CCA levels following praziquantel therapy. However, some patients’ six-month plasma CCA levels were only moderately lower than their initial CCA levels. This was not necessarily surprising since participants in this study continued to work as car washers after treatment for their schistosome infections and the six-month fecal examinations of many individuals who were not excreting eggs at four weeks after initial treatment showed renewed egg excretion (Table 1), suggesting that these individuals had become reininfected.

Praziquantel is not an effective treatment for schistosome infections in immunodeficient mice. However, passive transfer of infected mouse sera or a monoclonal antibody that recognizes a 200-kD adult worm tubercle glycoprotein restores the efficacy of praziquantel to levels seen in immunocompetent animals. These data suggest a role for antibody in praziquantel-mediated schistosome clearance. The presence of the appropriate antibodies in the sera of both HIV-positive and HIV-negative individuals may explain in part the absence of an observed difference in praziquantel efficacy in these populations. To test this, we used a recombinant antigen (ECL-3) that expresses the amino terminal half of the 200-kD tubercle protein. Using Western blots, we found that total IgG (Figure 4) or IgG isotypes (Table 2) from HIV-positive and HIV-negative schistosomiasis patient sera recognized ECL-3 with similar reactivity patterns. Furthermore, two patients who were HIV seronegative at initial enrollment but subsequently became HIV seropositive maintained their IgG reactivity with ECL-3 (Figure 4).

**DISCUSSION**

We have previously demonstrated that schistosomiasis patients with HIV-1 coinfections have reduced egg excretion to adult worm ratios compared with non-HIV-1-infected schistosomiasis patients. This result was predicted by experimental models using immunodeficient mice. Because
studies using B or T cell-depleted animals have also demonstrated that praziquantel requires a functional immune system to eliminate experimental schistosome infections,\textsuperscript{3, 4} we believed it important to determine whether HIV coinfection altered praziquantel efficacy in human schistosomiasis.

The results presented in this study demonstrate that praziquantel is an effective treatment for schistosomiasis regardless of the patient’s HIV-1 serostatus. Persons with schistosomiasis infections who were also positive for HIV-1 responded to praziquantel therapy in an almost identical manner to those who were HIV-1 seronegative. Although the percent of individuals in either group that cleared infection with a single praziquantel treatment was low (< 60%), these patients had very high levels of \textit{S. mansoni} infection at the time of enrollment. Although the differences were not significant, those individuals who did not cease to excrete eggs after a single treatment tended to have higher initial egg counts than those individuals who did (Table 1). Egg reduction following a single praziquantel treatment was no different over a range of CD4 levels (Figure 2). When circulating antigen was used as an additional measure of infection, similar results were obtained since all patients had lowered CCA levels following praziquantel therapy (Figure 3).

These results do not necessarily conflict with previous findings using experimental models that demonstrated that praziquantel was inefficient for treating \textit{S. mansoni} infections of immunodeficient mice.\textsuperscript{3, 4} Although the individuals positive for HIV-1 had decreased levels of CD4\textsuperscript{+} lymphocytes as compared with individuals negative for HIV-1, they still have some remaining T cells, unlike experimental models in which the T cell component of the immune response is virtually eliminated. A second explanation for the apparent discrepancy between experimental studies and the current study is that although functional CD4\textsuperscript{+} cells are necessary for the development of immune responses necessary for praziquantel efficacy, elimination of parasites in the presence of praziquantel is actually mediated by antibodies.\textsuperscript{2, 9, 13} Two proteins, a 200-kD glycoprotein and a 27-kD esterase, have been implicated in the immune-dependent action of praziquantel in experimental \textit{S. mansoni} infection. Antibodies specific for these target antigens can confer praziquantel efficacy in immunodeficient mice.\textsuperscript{10, 14} Thus, if individuals acquired and developed the critical antibodies to schistosomes prior to their infection with HIV-1 (which would be predicted by standard age-prevalence curves), their ability to eliminate schistosomes when given praziquantel might not be affected by subsequent CD4\textsuperscript{+} lymphocyte reductions. Here we have shown that antibodies reactive with a recombinant protein representing the amino terminus of the 200-kD tubercle-associated glycoprotein are no different between HIV-positive and HIV-negative schistosomiasis patients. Furthermore, two patients who converted from HIV negative to HIV positive during the course of the study did not show any diminution of their ECL-3 response, confirming that acquisition of HIV infection does not cause loss of ECL-3 reactivity. Interestingly, sera from some schistosomiasis patients in both HIV groups who were effectively treated with praziquantel failed to recognize ECL-3, suggesting that antibody against the 200-kD tubercle glycoprotein is not the only immunoreactivity that can confer praziquantel efficacy in humans.

The results of this study demonstrating that praziquantel is effective against schistosomiasis in HIV-1 infected individuals is encouraging. This is especially important with the increasing coincidence of these two infections in developing

\textbf{TABLE 2}

IgG subtype reactivities of patient sera with ECL-3 as determined by western blot\textsuperscript{*}

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<th>Patient no.</th>
<th>HIV Negative</th>
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\textsuperscript{*} HIV = human immunodeficiency virus. Symbols represent relative band intensities on western blots. – = negative; + = weak positive; ++ = positive; +++ = strong positive; ++++ = very strong positive.
countries and the hypothesis that HIV-1 may replicate more readily in T helper 2 cell (Th2) environments,\textsuperscript{15,16} such as those caused by schistosome infections.\textsuperscript{17,18} If the hypothesis that helminth infections provide an environment favorable for viral replication is true,\textsuperscript{19} treating patients’ schistosomiasis infections may lead to a reduction in viral burden. This possibility is the subject of ongoing investigations.

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