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# Modification of tissue egg load in *schistosomiasis mansoni* as a tool for morbidity reduction

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## Introduction

In *Schistosoma mansoni* the major pathogenic factors are the parasite egg-induced T-cell mediated granulomatous inflammations and subsequent tissue fibrosis (WARREN [14]). Most of the disease symptoms are directly attributable to egg granuloma formation. There is conclusive epidemiological evidence that the intensity of infection expressed as eggs/g tissue, determines the severity of the disease (WILKINS [15], CHEN and MOTT [4]).

Modification of schistosome-induced pathology has been observed following intervention using a variety of treatments. The use of schistosomicides and steroids on mice infected with *Schistosoma mansoni* resulted in a significant reduction in faecal egg output and eggs recovered from the intestine and liver (LAMBERTUCCI et al. [10]). Immunosuppressive drugs, thymectomy, antilymphocyte serum and antimacrophage serum reduce granuloma size (DOMINGO et al. [6], DOMINGO and WARREN [7, 8] , BOROS and WARREN [1]). However, immunological intervention can expose the host to the risk of establishment of opportunistic and other infectious agents. HILLYER and CANGIANO (9) reported defective cell responses towards schistosome eggs in an *S. mansoni* infected patient who was undergoing immunotherapy following a renal transplant operation.

We are reporting on the efficacy of different treatment regimes on the reduction of morbidity due to *S. mansoni* and *S. haematobium* in man and in a murine *S. mansoni* model. Data are presented on:

long term pattern of egg excretion and egg deposition following praziquantel (PZQ) treatment of patients with schistosomiasis;

the pattern of faecal egg production, tissue egg load and egg viability following PZQ treatment of mice infected with *S. mansoni;*

the dynamics of granuloma formation and attrition following exposure of *S. mansoni* infected mice to a collagen-controlling substance in conjunction with PZQ treatment.

## Material and methods

The study population was derived from a cohort of 18 to 23 year-old men who came for a long term stay to Czechoslovakia and proved positive in tests for schistosomiasis. PZQ was administered orally with two doses six hours apart under nurse supervision. The total dose PZQ was 40 mg/kg. Biopsies for histological examination were taken from the rectum and from urinary bladder. Samples of urine and stool as well as bioptical samples, were examined 23 to 26 months following chemotherapy.

The Puerto Rican strain of *S. mansoni,* maintained in laboratory, was used to infect mice. Outbread mice of ICR strain were infected transcutaneously with 200 cercariae.

To assess the efficacy of PZQ on egg load in faeces and tissues and on egg viability, mice received two oral doses of PZQ (800 mg/kg) 9 days apart. The first dose was administered 60 days post infection. Only mice passing more than 100 eggs per gram in the faeces were selected for the experiment. Mice determined to have the higher eggs per gram of faeces (EPG) level, were selected for the treatment group and those with lower EPG (but $>$ 100 EPG) were used as controls. Control and treatment groups contained 10 mice per group.

To assess the effect of PZQBAPN and beta-aminopropionitrile (BAPN), a lysyl oxidase inhibitor, on granuloma formation in *S. mansoni*-infected mice the following experimental design was used.

Group 1:
Untreated infected mice.

Group 2:
BAPN (5 mg/mouse/day) was administered to mice subcutaneously for 20 consecutive days, starting on day 40 post infection.

Group 3:
PZQ (800 mg/kg) was administered on day 45 and day 49 post infection.

Group 4:
Mice were treated with both BAPN and PZQ, using the combined protocols as described for groups 2 and 3.

All mice were autopsied 60 days post infection.

Tissue egg load was determined according to the method of CHEEVER (2). Maturity and viability of eggs in the tissues (oogram) was determined by the method of PELEGRINO et al. (11).

## Results and discussion

The efficacy of the standard dose of PZQ in human schistosome infections revealed that 40 mg/kg of PZQ reduced the worm burden and viable eggs could not be detected in patient excreta. Egg layed by surviving worms may continue to be deposited in the tissues. Details are shown in Table 1. Our results are in concordance with CUNHA and PEDROSA (5) who found 91.7% cure rate for PZQ assessed by stool examination and 29.2% by oogram in *S. mansoni* infection. The results from the human study, particularly those from histological assessement of PZQ efficacy focused our interest on further studies of morbidity reduction in schistosome infections.

As only mature eggs are affected by PZQ (CHEEVER and ANDERSON [3], RICHARDS et al. [12]) 2 doses of this drug, 9 days apart, were administered. The second dose was applied after the time required for egg maturation. The first dose reduced markedly

TABLE 1

**Parasitological assessment of praziquantel efficacy in human schistosomes**

| Schistosoma mansoni | Criteria | | | | | Total cure rate |
|---|---|---|---|---|---|---|
| | Ova in stool/urine | | Cure rate | Ova in biopt. excision from rectum/bladder | | |
| | <1 year | >1 year | | <1 year | >1 year | |
| S. mansoni n = 6 | 0 | 1 | 83% | 1 | 1 | 50% |
| S. haematobium n = 2 | 0 | 0 | | 0 | 1 | 50% |
| S. mansoni plus<br>S. haematobium n = 5 | 1 | | 90%<br>85% | 1 | 3 | 80%<br>20% |
| Total<br>S. mansoni n = 11<br>S. haematobium n = 7 | 0<br>1 | 1<br>0 | 90%<br>85% | 2<br>0 | 1<br>4 | 63.6%<br>28.5% |

the number of eggs in faeces (Fig. 1) but the percentage of eggs recovered from the faeces which hatched (Fig. 2) was equal to the control. It may indicate that the first dose of PZQ affected only mature eggs and the remaining immature eggs survived, reached maturity and hatched normally 9 days later. There was no reduction particularly in liver tissue egg load 9 days after the first dose of PZQ (Fig. 3), however, the proportion of dead eggs in oograms from small intestine and liver were significantly elevated (data not shown). Nine days after the second dose of PZQ, no eggs were found in faeces even when a sensitive concentration technique (merthiolate-iodine-formaldehyde concentration) was used (Fig. 1).

It indicates that the second dose of PZQ killed mature eggs which reached maturity after the first dose of PZQ. The drop in mean EPG in the control group may be as a result of examining 2 control mice with low EPG (168, 240 EPG respectively). Figure 3 demonstrates only partial decline of liver egg load after the second dose of PZQ and elevation of egg load in intestinal tissue. Only 1.3% of the eggs were alive after the second dose of PZQ as determined by the intestinal oogram, and 4.2% were observed to be alive in the liver oogram.

The results from this experiment enabled us to better understand the impact of PZQ treatment on the fate of *S. mansoni* eggs in faeces and tissues. It would seem that the therapeutic potency of PZQ correlates more closely with the duration of treatment rather than the absolute dose. Treatment with repeated standard dose, spanning the time required for egg maturation should be considered as a treatment schedule for human *S. mansoni* as proposed by RICHARDS et al. (loc. cit.).

Beta-aminopropionitrile (BAPN) is an inactivator of lysyl oxidase. Inactivation of lysyl oxidase leads to protection of cross-linking of the new formed collagen (SIEGEL [13]). Efficacy of modulation of egg granuloma formation by BAPN alone or in association with PZQ was assessed by the tissue egg load and the viability of *S. mansoni* egg in 4 groups of mice with different treatment regimes (Fig. 4). Application of both drugs (BAPN + PZQ) decreased, very significantly, the number of eggs trapped in the liver (86% reduction as compared with control) and in intestine (99.1% reduction as compared with control). Statistically significant reduction of tissue egg load was also achieved

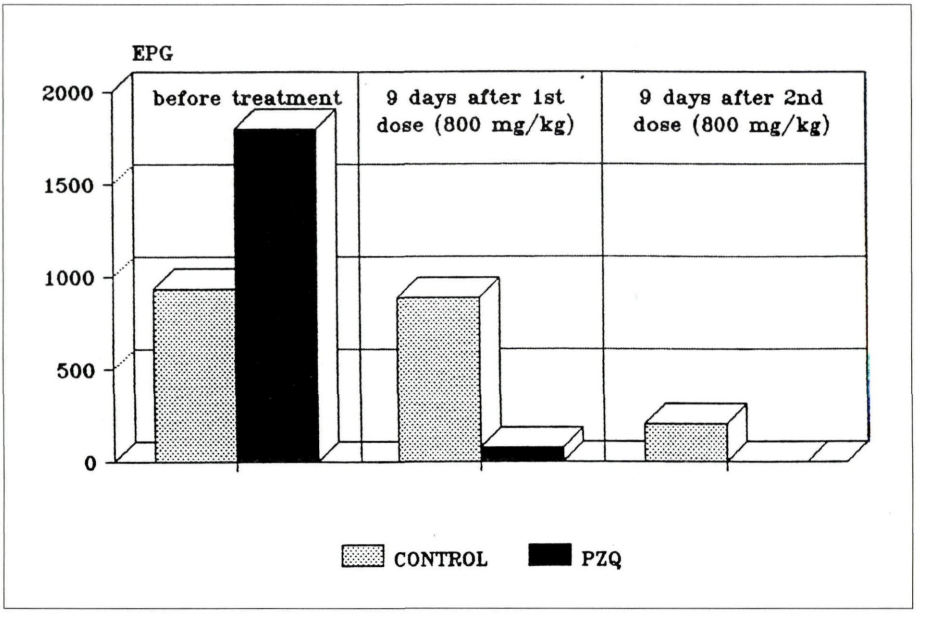


Figure 1: Eggs per gram (EPG) of faeces influenced by praziquantel (PZQ) treatment. The first dose of PZQ was administered 60 days post-infection (p. i.). EPG value before treatment was determined in control and treatment groups at the same day as treatment was started. Mice received second dose of PZQ 69 days p. i., and at the same day efficacy of first dose of PZQ on EPG was evaluated. Impact of 2nd dose of PZQ on EPG was measured 78 days p. i. (9 days after 2nd dose of PZQ). Each measurement represents 2 mice in both control and treated groups.

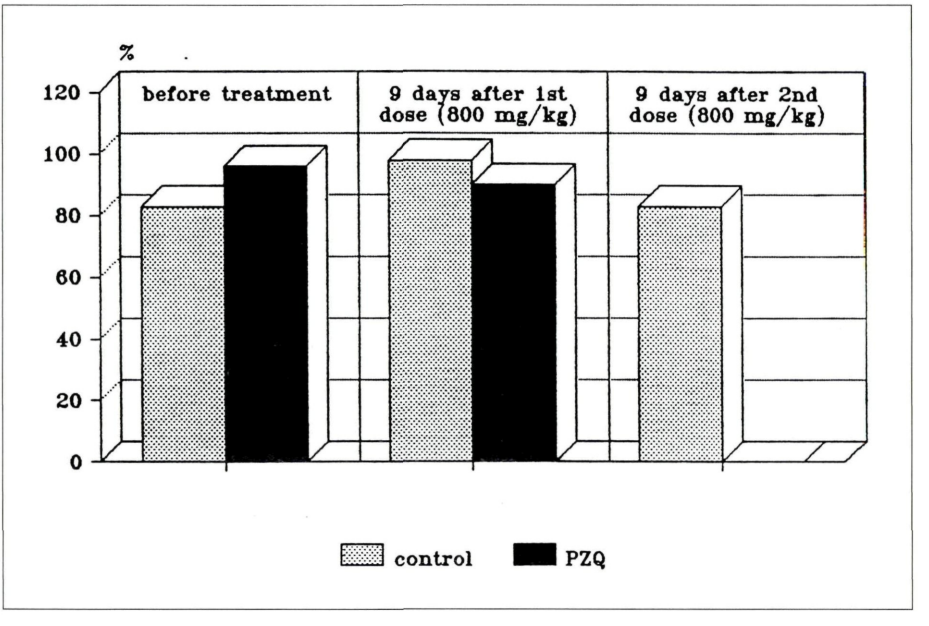


Figure 2: Egg hatching percentage from faeces after PZQ treatment. The treatment schedule was the same as described in the legend to Fig. 1. Hatchingtest was performed at the days as indicated over the seperate graphs of results.

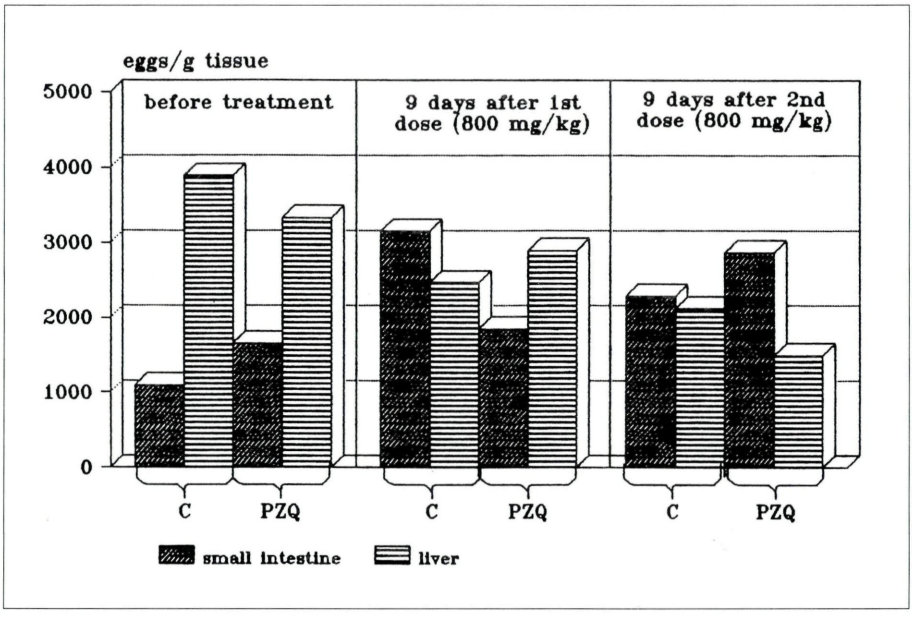


Figure 3: Tissue egg load after PZQ treatment C-control, PZQ-praziquantel. The treatment schedule was the same as described in the legend to Fig. 1. Control and treatment groups were kept separately already before the treatment. Dates of tissue egg load determination are indicated over the separate graphs of results.

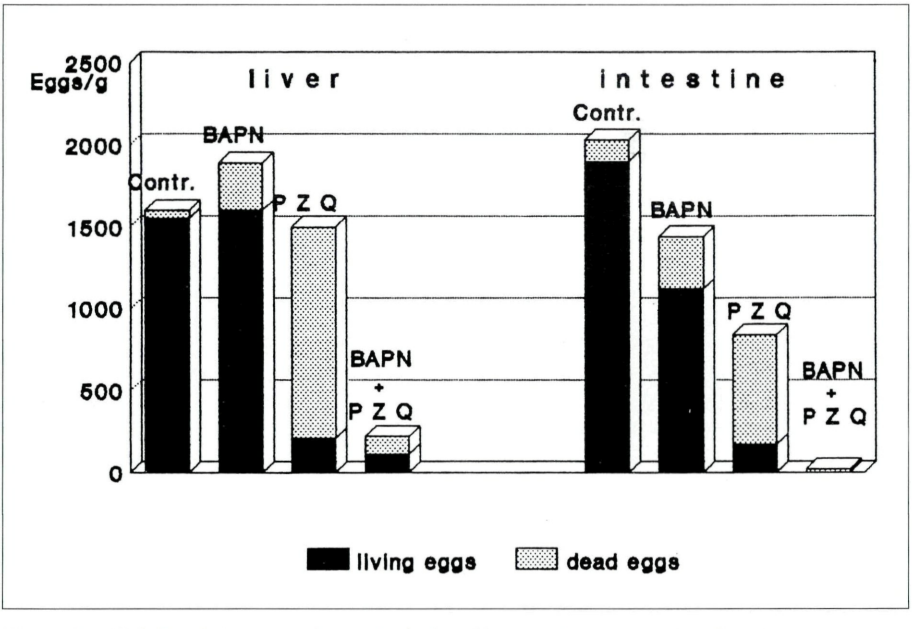


Figure 4: Viability of S. mansoni eggs (calculated from mature eggs) after β-aminopropionitrine (BAPN) treatment or praziquantel (PZQ) treatment or with their combination by the same protocol. Mice were sacrified 1 day after BAPN aplication and 11 days after 2nd dose of PZQ; total dose of BAPN = 100 mg/mouse, total dose of PZQ = 1600 mg/kg.

when compared with the group of mice treated with PZQ alone ($P < 0.01$ for liver, $P < 0.05$ for intestine). The lowest number of living eggs found in 1 g of liver or intestine was in the BAPN + PZQ treated group of mice (Fig. 4).

Those results traced different modes of action of both drugs tested. PZQ kills the mature eggs in the tissues of infected mice while BAPN appears to facilitate the release of eggs trapped in host tissues.

Intervention which prevents the development of irreversible life-threatening pathology, rather than only reducing egg output, would seem to be a desirable goal. The regulation of collagen synthesis, among others, by inhibition of cross-link formation between the fibrils may be an important adjunct to the chemotherapeutic control of schistosomiasis.

**Summary**

Efficacy of different treatment regimes on the reduction of morbidity due to *S. mansoni* and *S. haematobium* in man and in a murine *S. mansoni* were performed. The standard dose of praziquantel (PZQ) in human schistosome infections reduced the worm burden. Examination of human excreta as well as of bioptical excisions from rectum and bladder for schistosome eggs revealed a cure rate of PZQ of 63.6% for *S. mansoni* and of 28.5% for *S. haematobium*. The pattern of faecal egg excretion, tissue egg load and egg viability following PZQ treatment of mice with *S. mansoni* revealed that therapeutic potency of PZQ correlate more closely with the duration of treatment rather than the absolute dose. It should be considered as a treatment schedule for human schistosomiasis due to *S. mansoni*. The dynamics of granuloma formation and attrition following exposure of *S. mansoni* infected mice to a collagen — controlling substance in conjuction with PZQ treatment indicated that inhibition of cross-linking of collagen may effectively support the chemotherapeutic control of schistosomiasis.

**Key words**

Schistosomiasis, morbidity, tissue egg load, interventions.

**Zusammenfassung**

## Die Beeinflussung der Gewebsbelastung mit Eiern von *Schistosoma mansoni* als Maßnahme zur Verringerung der Morbidität

Die Behandlungsergebnisse von Patienten mit *Schistosoma mansoni* und *Schistosoma haematobium* durch eine Standardkur mit 40 g Praziquantel/kg für einen Tag ergab eine Heilungsrate von 63% für *S. mansoni* und 28,5% für *S. haematobium* gemessen an der Eiausscheidung und dem Gehalt an Eiern in Biopsien von Darm- und Harnblasenschleimhaut 23 bis 26 Monate nach der Behandlung. Die Chemotherapie von experimentell mit *S. mansoni* infizierten Mäusen zeigte, sowohl im Hinblick auf die Eiausscheidung als auch auf das Oogramm in der Leberhistologie, daß die Infestation wirksamer kontrolliert wurde, wenn Praziquantel in zwei Einzeldosen im Abstand von 9 Tagen verabreicht worden war als nach einer einzigen Gabe. Die zusätzliche Verabreichung des Kollageninhibitors Beta-aminopropionontril verbesserte die Praziquantelwirkung in dem Sinn, daß die Zahl von Schistosoma-Eiern in der Leber signifikant verringert wurde. Offenbar erleichtert die Kollagenhemmung das Ausschwemmen der Schistosoma-Eier aus den Geweben durch Verminderung der Granulombildung.

**Schlüsselwörter**

Bilharziose, *Schistosoma mansoni, Schistosoma haematobium,* Chemotherapie, Kollageninhibition.

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