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Prophylactic effect of the anti-inflammatory drug diclofenac in experimental schistosomiasis mansoni

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Hepatic granuloma measurement

Summary
Objectives: This study was a trial to demonstrate the prophylactic effect of diclofenac, a widely used anti-inflammatory drug (diclofenac potassium, CAS-15307-81-0, Ciba Geigy, 334.2) in experimental schistosomiasis mansoni. Two different dose regimens were used to explore the effects upon worm load, tissue egg load, and hepatic granuloma size.
Methods: In this study, a group of 50 Swiss albino mice was used. This group was divided into five subgroups: subgroup I constituted infected untreated control mice; subgroup II, infected mice given 0.5 mg diclofenac orally 24 h post infection, then sacrificed three weeks later; subgroup III, infected mice given 0.5 mg diclofenac orally six weeks post infection and sacrificed one week later; subgroup IV, infected mice administered 1 mg diclofenac orally 24 h post infection and sacrificed three weeks later; and subgroup V, infected mice given 1 mg of the drug orally six weeks post infection and sacrificed one week later.
Results: Mice given the high dose regimen (1 mg orally/mouse) 24 h post infection, then sacrificed three weeks later, demonstrated a significant reduction in the immature worms recovered, compared to the untreated controls. Animals receiving the high dose of the drug six weeks post infection, then sacrificed one week later, revealed a drop in the number of mature worms and in the tissue egg load (hepatic and intestinal), and the smallest hepatic granuloma measurement compared to the untreated controls. These findings were less conspicuous in animals given the low dose regimen.
Conclusion: Diclofenac could be used successfully as a preventive agent against schistosomiasis mansoni infection in endemic areas.

Introduction

Egypt without schistosomiasis? It sounds like a dream. The disease continues to rank second to malaria in the world's parasitic diseases in terms of the extent of endemic areas and the number of infected people. There is as yet no vaccine

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available and the current mainstay of control is chemotherapy with praziquantel.[1] In view of concern about the development of tolerance and/or resistance to praziquantel,[2] there is a need for novel drugs for the prevention and cure of schistosomiasis.[1] Diclofenac is a commonly used anti-inflammatory drug in gout, musculoskeletal and periarticular disorders.[3] Farag et al.[4] previously tried ibuprofen or diclofenac sodium in schistosomiasis mansoni. The authors found that the drug succeeded in reducing the severity of infection with attenuation of hepatic fibrosis, particularly when the treatment was initiated early.

The objectives of this study were to investigate the use of diclofenac, 0.5 and 1.0 mg, in a trial to demonstrate its prophylactic effect upon the immature and mature stages of experimental schistosomiasis mansoni, and also pathological parameters of assessment including hepatic granuloma measurement in animals sacrificed seven weeks post infection. This study was performed to include the immature worm count, mature worm load, tissue egg loads, and hepatic granuloma size.

Material and methods

A group of 50 Swiss male albino mice of C.D. strain, weighing 18—20 g each were infected with an Egyptian strain of *Schistosoma mansoni* cercariae. The strain used originated from the Nile Delta in Egypt, and has been bred through mice and *Biomphalaria alexandrina* for six years. Animals were supplied and bred through the Schistosome Biological Supply Program, Theodor Bilharz Research Institute, Imbaba, Cairo, Egypt. The experimental animals were maintained for 112—124 days in air-conditioned rooms at 21 °C and given food with a 24% protein content. The animal experiments were carried out according to the internationally valid guidelines in an institution responsible for animal ethics (Theodor Bilharz Research Institute).[5]

Diclofenac (diclofenac potassium, (0-(2,6-dichloroanilino)phenyl)acetate, Ciba Geigy, 334.2) was supplied by the Theodor Bilharz Research Institute. The usual human dose for inflammatory conditions is 100 mg/day given orally in 2—3 divided doses. The 50 mice were divided equally into five subgroups of 10: subgroup I received 80 *Schistosoma mansoni* cercariae per mouse by subcutaneous injection, and served as control infected untreated mice sacrificed three weeks later; subgroup II constituted infected mice given 0.5 mg diclofenac orally per mouse as a single dose 24 h post infection, and sacrificed three weeks later; subgroup III constituted infected mice treated with diclofenac orally, 0.5 mg per mouse as a single dose six weeks post infection, and sacrificed one week later; subgroup IV constituted infected mice given 1.0 mg of the drug orally per mouse as a single dose 24 h post infecton, and sacrificed three weeks later; and subgroup V constituted infected mice administered diclofenac 1.0 mg orally as a single dose six weeks post infection, and sacrificed one week later.

The drug was diluted in distilled water and was given through an esophageal syringe. After the animals had been sacrificed by cervical dislocation, perfusion was done taking care to separate hepatic from portomesenteric worms.[6] Livers were collected and fixed in 10% formalin solution for subsequent preparation of hematoxylin and eosin-stained sections for granuloma measurements.[7]

Bilharzial hepatic granulomas were measured in stained sections by selecting lesions containing single eggs in their centers. The mean diameter of each lesion was obtained by measuring perpendicular diameters using an ocular micrometer. The volume of each lesion was calculated from its mean diameter using the formula:

$$V = r^3 \times \pi \times \frac{4}{3}$$

The mean volume per group represents the mean of 70 lesions and is expressed in mm^3.

Statistics

Comparison was done between each treated group and the untreated control group. The percentage change between the two groups to be compared was assessed using the formula:

$$\frac{(\text{mean value of the first group} - \text{mean value of the second group})}{\text{mean value of the first group}} \times 100$$

Differences between the mean scores of any of the two groups to be compared were tested for significance using an unpaired two-tailed Student's *t*-test. The data were considered significant if p values were <0.05.

Results

Immature worm count

The mean immature worm count in the control and treatment groups, groups II and IV, as shown in Table 1 were 36.5, 21.5, and 16.5 worms, respectively. Group IV had the lowest immature worm count. The difference was statistically significant from untreated control mice at $p < 0.001$.

Table 1 Effect of diclofenac on the immature stages in *Schistosoma mansoni*-infected mice treated one day post infection and sacrificed three weeks later

Animal group	Mean no. of immature worms ± SE	Percentage immature worm reduction
Group I — infected controls	36.5 ± 0.4	
Group II — infected, given low dose regimen 0.5 mg/mouse	21.5 ± 0.8[a]	41.1
Group IV — infected, given high dose regimen 1.0 mg/mouse	16.5 ± 0.5[a]	54.8

[a] Statistically significant difference from infected untreated controls at $p < 0.001$.

Table 2 Effect of diclofenac on the mature worm and tissue egg loads in *Schistosoma mansoni*-infected mice treated six weeks post infection and sacrificed one week later

Animal group	Mean total worms ± SE	Percentage mature worm reduction ± SE	Hepatic egg load ± SE	Percentage reduction	Intestinal egg load ± SE	Percentage reduction
Group I — infected controls	30.8 ± 4.1		4664.2 ± 126.3		4535.6 ± 1212.6	
Group III — infected, given 0.5 mg/mouse	23.7 ± 4.2	23.1	4588 ± 1337	1.6	3183 ± 739	29.8
Group V — infected, given 1.0 mg/mouse	24.3 ± 2.5	21.1	4207 ± 1216	9.8	2787.5 ± 927[a]	38.5

[a] Statistically significant difference from infected untreated controls at $p < 0.001$.

Table 3 Effect of diclofenac on the hepatic granuloma measurements in *Schistosoma mansoni*-infected mice treated six weeks post infection and sacrificed one week later

Animal group	Percentage mature worm reduction	Mean hepatic granuloma diameter in μm ± SE	Percentage reduction
Group I — infected controls		140.7 ± 3.1	
Group III — infected, given 0.5 mg/mouse	23.1	137.3 ± 2.8	2.4
Group V — infected, given 1.0 mg/mouse	21.1	123.4 ± 0.7[a]	11.8

[a] Statistically significant difference from infected untreated controls at $p < 0.05$.

Mature worm load and tissue egg loads

Group V had the lowest worm and tissue (hepatic and intestinal) egg loads. The intestinal egg load was statistically significantly lower than in untreated control mice at $p < 0.001$ (Table 2).

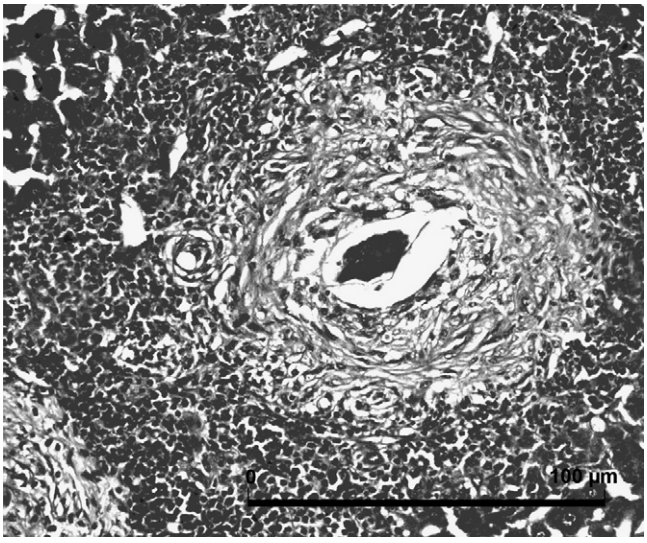

Figure 1 Liver section of a representative of the infected control group of mice (8 weeks post infection), illustrating large cellular granuloma composed of a mixture of chronic inflammatory cells rich in eosinophils. The intact cellular miracidium is seen inside the ovum. Adjacent hepatocytes show hydropic change and focal atypical hyperplasia (H&E × 200) (mean diameter = 140 μm).

Hepatic granuloma measurements

Group V had the smallest hepatic granuloma mean diameter (123.4 ± 0.7). This value, when compared with the untreated controls, was statistically significant at $p < 0.05$ (Table 3, Figures 1—3).

It was noted that animals tolerated the treatment very well. The dose given succeeded in performing the optimal antischistosomal activity without any undesirable side effects.

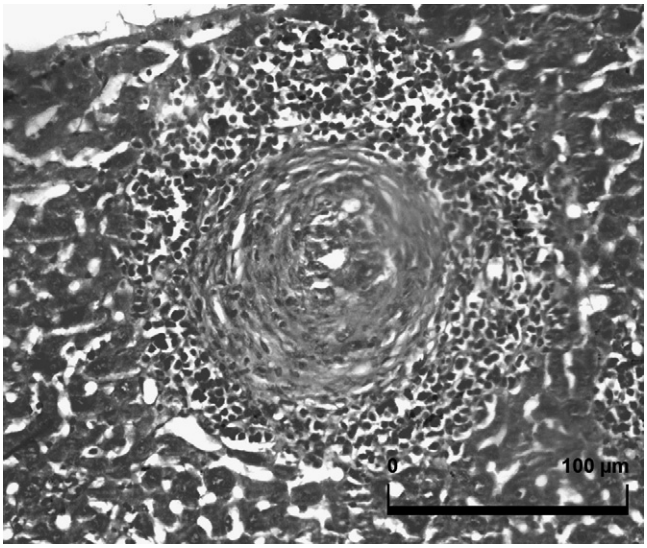

Figure 2 Liver section of a representative of the infected and orally treated low dose diclofenac mice showing a small hepatic fibrocellular granuloma (H&E × 200) (mean diameter = 137 μm).

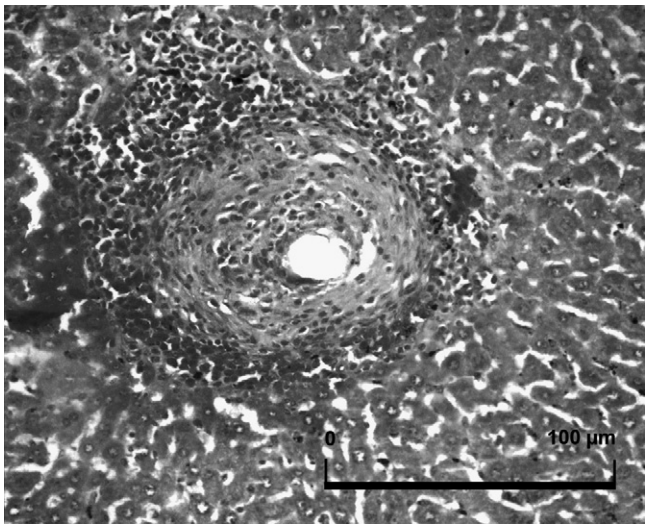

Figure 3 Liver section of a representative of the infected and orally treated high dose diclofenac mice showing a small hepatic fibrocellular granuloma (H&E × 200) (mean diameter = 123 µm).

Discussion

From this study, it could be concluded that diclofenac exerts potent prophylactic activity in experimental schistosomiasis mansoni infection, concurrent with its anti-inflammatory action. This is evident by the lower level of immature worms recovered in the group given 1.0 mg of the drug 24 h post infection and sacrificed three weeks later (group IV). The drug also produced a deleterious effect on adult schistosomes, evidenced by lowered worm recovery in the group given 1.0 mg diclofenac six weeks post infection and sacrificed one week later (group V). This group also exhibited a lowered hepatic and intestinal egg load, and the smallest hepatic granuloma mean diameter.

Diclofenac was previously tried in experimental schistosomiasis mansoni by Farag et al.[4] The authors postulated that arachidonic acid metabolites participate in skin penetration by schistosome cercariae and in the pathogenesis of schistosomiasis. They treated mice with ibuprofen or diclofenac sodium (2.5 mg/kg/day) for seven days before infection. The authors noted a significant drop in the liver weight, worm load, and hepatic hydroxyproline content compared to the untreated mice. Again, if treatment was continued for two days post infection, a significant drop in hepatic gamma-glutamyl transpeptidase activity was noted.[4] Furthermore, when diclofenac sodium was administered in a smaller dose of 1.2 mg/kg/day, all the measured parameters of infection were significantly lower when the treatment was initiated seven days post infection. Farag et al.[4] concluded that treatment with ibuprofen or diclofenac sodium was effective in reducing the severity of infection and in attenuating hepatic fibrosis particularly when the treatment was initiated early.

More recently, Ojewole and Adewunmi[3] found that the fruit of *Tetrapleura tetraptera* was used in African traditional medicine for the management and/or control of an array of human ailments such as arthritis, asthma, diabetes mellitus, hypertension, epilepsy, and schistosomiasis. The authors tried diclofenac 100 mg/kg p.o. and chlorpropamide 250 mg/kg p.o. as reference anti-inflammatory and hypoglycemic agents for comparison. They found that the *Tetrapleura tetraptera* fruit produced a dose-related, significant reduction of the fresh albumin-induced acute inflammation of the rat hind paw edema. The plant extract also produced a dose-dependant significant drop in the blood glucose level of both fasted normal and fasted diabetic rats. The results indicate that this plant extract could be used as an anti-inflammatory and hypoglycemic agent in some communities in West Nigeria.[3]

The use of anti-inflammatory or antifibrotic agents in schistosomiasis mansoni has been previously tried by Giboda et al.[8] and Hassan et al.[9] The authors suggested the use of an antifibrotic agent, beta-aminopropionitrile (BAPN) in conjunction with praziquantel to inhibit cross-linking of newly deposited collagen in murine schistosomiasis mansoni, by blocking lysyl oxidase. Mansy et al.[10] reported that early praziquantel therapy after the 8th week of infection decreased hepatic collagen content, while later treatment at the 18th week of infection increases hepatic collagen. Badawy et al.[11] stated that the antifibrotic agent colchicine postpones granulomatous reaction healing and diminishes collagen deposition rather than inhibiting collagen formation or degradation. Recent trials with interferon alpha demonstrated an antifibrotic effect that is independent from its antiviral effect.[12] The product can reduce and prevent deposition of fibrotic tissue in the liver by increasing its capacity to degrade type I and type III collagen, and by increasing the activity of plasminogen activator. This antifibrotic activity of interferon may be mediated by cytokines that bring about histologic improvement of the liver. Gross et al.,[13] postulated that among the cytokines that appear to orchestrate fibrogenic changes, transforming growth factor beta (TGF beta) appears to be predominant. When the activity of TGF beta has been altered experimentally by gene therapy, hepatic fibrosis has been prevented or even reversed.[13]

In this study, mice given the high dose diclofenac regimen (1.0 mg/mouse) 24 h post infection, revealed a recovery of the lowest number of immature worms. As regards previous trials of antischistosomal drugs in different time periods prior or post infection, Nessim et al.,[5] tried the broad spectrum anthelmintic drug flubendazole on *Schistosoma mansoni*-infected mice. The authors postulated that mice given the drug 25 days post infection revealed a significant reduction in the recovery of adult schistosomes after portal perfusion, and the smallest granuloma mean diameter. These data were less notable in mice treated 4 h after and 24 h before infection. Again, in this study, the smallest hepatic granuloma measurement was recorded in the group given diclofenac 1.0 mg/mouse six weeks post infection.

Reduction in granuloma size after treatment has previously been reported by Botros et al.,[14] concerning praziquantel. The authors noted diminution in the number of T-helper cells in the granulomata. Botros et al.,[14] and Hassan et al.[15] stated that this reduction in granuloma size after treatment could be the result of inhibition of the inflammatory mediators released locally at the site of granulomatous inflammation. Moreover, suppression of this reaction as a T-cell mediated response cannot be ruled out. In favor of their conclusion was the reduction in the number of Lyt1 T-lymphocytes in the granu-

lomata after praziquantel treatment. The reduction in the granuloma size after flubendazole treatment is consistent with the assumption of Pancera et al.[16] The authors stated that the mechanism of action of mebendazole may be due to unspecific immunomodulation.

Chatterjee and Van Marck[17] explored the possible modulatory role of the neuropeptide somatostatin in the outcome of Schistosoma morbidity in man. The authors stated that somatostatin has an inhibitory effect on hormone, immune, and physiological body functions, like growth hormone, interferon gamma production, collagen I and III formation, and hepatic stellate cell activation. Therefore, somatostatin may determine pre-disposition to "Schistosoma" morbidity in man.[17]

More recently, Doenhoff et al.,[18] stated that aqueous extracts of *Schistosoma mansoni* eggs have been shown to have fibrinolytic activity inhibitable by a serine protease inhibitor. Last but not least, Pyrrho et al.[19] investigated the possible use of immunomodulators as coadjuvants in the treatment of chronic *Schistosoma mansoni* infection. The authors evaluated the effect of dexamethasone in chronic experimental schistosomiasis. They found that the drug induced a decrease in the number of hepatic granulomata without affecting the alanine aminotransferase profile. It also reduced splenomegaly and hepatomegaly associated with the disease and improved hemoglobin concentration, hematocrit values, and reduced the percentage of reticulocytes, preventing the development of anemia that occurs in the chronic phase of the infection. These data suggest that treatment with dexamethasone results in a mild course of murine schistosomiasis and point to this drug as a promising agent to complement *Schistosoma mansoni*-specific treatment.[19]

Conflict of interest: No conflict of interest to declare.

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