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Effect of praziquantel administration on hepatic stereology of mice infected with *Schistosoma mansoni* and fed a low-protein diet

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Abstract

A study was undertaken to investigate the effect of administering praziquantel (PZQ), focusing on the liver stereological findings of malnourished mice infected with *Schistosoma mansoni*. Thirty female Swiss Webster mice (age: 21 days; weight: 8-14 g) were fed either a low-protein diet (8%) or standard chow (22% protein) for 15 days. Five mice in each group were infected with 50 cercariae each of the BH strain (Brazil). PZQ therapy (80 mg/kg body weight, per day) was started on the 50th day of infection and consisted of daily administration for 5 days. Volume density (hepatocytes, sinusoids and hepatic fibrosis) was determined by stereology using a light microscope. Body weight gain and total serum albumin levels were always lower in undernourished mice. Our stereological study demonstrated that treatment increased both volume density of hepatocytes in mice fed standard chow (47.56%, treated group and 12.06%, control) and low-protein chow (30.98%, treated group and 21.44%, control), and hepatic sinusoids [standard chow (12.52%, treated group and 9.06%, control), low-protein chow (14.42%, treated group and 8.46%, control)], while hepatic fibrosis was reduced [standard chow (39.92%, treated group and 78.88%, control) and low-protein chow (54.60%, treated group and 70.10%, control)]. On the other hand, mice fed low-protein chow decreased density volume of hepatocytes and hepatic fibrosis. In conclusion, our findings indicate that treatment with PZQ ameliorates hepatic schistosomiasis pathology even in mice fed a low-protein diet.

Key words: *Schistosoma mansoni*; Low-protein diet; Stereology; Praziquantel

Introduction

Over the last several years, experimental studies in mice have demonstrated that host nutritional status may influence the outcome and progression of schistosomiasis infection, since it interferes with the dynamics of connective tissue changes occurring in hepatic lesions (1). Undernourished mice exhibit diminished periovular granuloma and minimal liver fibrosis compared to control animals (2).

Chemotherapy with praziquantel (PZQ) plays a very important role in reducing the morbidity of *Schistosoma mansoni*-infected mice, effectively killing adult worms and mature eggs trapped in the host tissues (3). Additionally, it decreases inflammation and progressive matrix degradation (4). In contrast, protein-deficient mice cannot effectively clear adult worms after PZQ compared to nourished mice (5).

Although proved relevant in clinical studies (6), stereological data on parasitic infection are rather scarce (7). In a previous investigation, stereology also proved to be useful for experimental evaluation of the nutritional effects of a high-fat diet in murine schistosomiasis (8). On the basis of the considerations, the objective of the present study was to test liver status following PZQ administration to *S. mansoni*-infected mice fed a low-protein diet, using stereology parameters.

Material and Methods

Animals

Thirty female Swiss Webster mice (age: 21 days; weight:

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8-14 g) were obtained from The FIOCRUZ Central Animal House breeding stock (Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil). The animals were housed under standard caging conditions, i.e., temperature of 21 ± 1°C, 60 ± 10% humidity, a 12-h light and dark cycle, and permitted *ad libitum* consumption of water and pellet chow. All animal experiments were conducted in accordance with valid international guidelines for animal experimentation.

Diets. After weaning, experimental mice were fed a low-protein diet with an 8% protein content and standard chow mice were fed a normal chow (Nuvilab®, Brazil) with a 22% protein content throughout the investigation.

Nutritional status evaluation. Body weight was measured twice a week throughout the experiment and serum albumin concentration was measured after 15 days of the low-protein diet.

Infection. Mice were exposed subcutaneously to ~50 *S. mansoni* cercariae (BH strain, Brazil) 15 days after ingesting their respective diets. The cercariae were obtained from laboratory-raised and -infected *Biomphalaria glabrata*, as described (8).

Treatment. PZQ (80 mg/kg; Merck, Germany) was orally administered to mice by stomach gavage in 2% Cremophor EL (Sigma, USA) for 5 consecutive days, beginning on day 50 after infection.

Experimental groups. *S. mansoni*-infected mice were allocated to six groups of 5 mice each, as follows: group I (IUSC): infected untreated mice fed a standard chow. Group II (IULC): infected untreated mice fed a low-protein chow. Group III (ITSC): infected treated mice fed a standard chow. Group IV (ITLC): infected treated mice fed a low-protein chow. Group V (USC): uninfected mice fed a standard chow. Group VI (ULC): uninfected mice fed a low-protein chow.

Autopsy. Seventeen days after the last PZQ administration, all mice were euthanized by cervical dislocation. A midline incision was made in the thorax and abdomen of the mice to remove the liver.

Tissue processing. Liver volume was measured by the immersion method (9). Livers were fixed in freshly prepared 4% formaldehyde, w/v, in 0.1 M phosphate buffer, pH 7.2, and embedded in paraffin. Histologic sections (5 µm) were obtained and stained with Masson's trichrome.

Stereological study

The liver was analyzed considering hepatocytes, sinusoids, and hepatic fibrosis. Details of the procedures are given elsewhere (8). Briefly, hepatic fragments (minimum of three fragments per animal) were embedded together and oriented to provide a random cut when sectioned. Several sections were cut and five microscopic fields were randomly analyzed using a video-microscopic system (Olympus BX 50, Japan) and a test system consisting of 42 points (P_T), i.e., volume density of hepatocytes (V_v [h]), sinusoids (V_v [s]) and hepatic fibrosis (V_v [hf]), was determined as follows: $V_v = P_P / P_T$ (%), where P_P represents the points hitting the structures (10).

Statistical analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 9.0. Data are reported as means ± SD and were analyzed by the Mann-Whitney test and/or the Kruskal-Wallis test followed by post-test to determine which groups were divergent. The

Table 1. Biochemical and biometrical characteristics of schistosomiasis-infected Swiss Webster mice fed low-protein or standard chow and treated or not with praziquantel.

Parameters	Groups						P
	USC	ULC	IUSC	IULC	ITSC	ITLC	
AL (g/dL)	4.4 ± 0.3	3.7 ± 0.5	-	-	-	-	0.02 [a]
BM^1 (g/cm)	13.5 ± 0.9	11.0 ± 2.9	13.2 ± 1.2	12.0 ± 1.7	12.5 ± 2.5	11.0 ± 1.5	-
BM^{15} (g/cm)	35.6 ± 5.0	20.3 ± 6.0	32.8 ± 4.5	25.3 ± 3.0	33.7 ± 4.4	22.0 ± 3.6	0.0001 [acefhmp]
BM^{50} (g/cm)	45.2 ± 5.5	33.7 ± 5.3	42.9 ± 4.5	37.4 ± 2.8	44.4 ± 3.7	34.0 ± 2.2	0.0002 [aefhmp]
BM^{72} (g/cm)	53.5 ± 5.0	39.2 ± 4.5	50.5 ± 4.7	39.0 ± 2.6	52.8 ± 4.6	37.3 ± 1.8	0.0001 [acefhjmnp]
LV (10^3 mm^3)	2.2 ± 0.1	1.7 ± 0.1	2.8 ± 0.4	1.9 ± 0.1	2.5 ± 0.2	1.8 ± 0.1	0.0001 [abefhjmnp]

Data are reported as means ± SD for 5 animals in each group. USC = uninfected mice fed standard chow; ULC = uninfected mice fed low-protein chow; IUSC = infected untreated mice fed standard chow; IULC = infected untreated mice fed low-protein chow; ITSC = infected treated mice fed standard chow; ITLC = infected treated mice fed low-protein chow; AL = total serum albumin level 15 days after ingestion of the respective diet. BM^1, BM^{15}, BM^{50}, BM^{72} = body mass index after 1, 15, 50, and 72 days of the experiment, respectively; LV = liver volume. Statistical comparisons (P ≤ 0.05, Mann-Whitney test or Kruskal-Wallis test): [a] USC *vs* ULC; [b] USC *vs* IUSC; [c] USC *vs* IULC; [d] USC *vs* ITSC; [e] USC *vs* ITLC; [f] ULC *vs* IUSC; [g] ULC *vs* IULC; [h] ULC *vs* ITSC; [i] ULC *vs* ITLC; [j] IUSC *vs* IULC; [l] IUSC *vs* ITSC; [m] IUSC *vs* ITLC; [n] IULC *vs* ITSC; [o] IULC *vs* ITLC; [p] ITSC *vs* ITLC.

level of significance was set at P < 0.05.

Results

Animal biometry and nutritional data

Body weight gain and total serum albumin levels were lower (P < 0.05) in the mice fed the low-protein diet compared to well-nourished mice (Table 1).

The liver weight ratios of malnourished mice (IULC and ITLC) were always lower than those of their counterparts (well-fed mice - IUSC and ITSC). Liver weight after PZQ therapy was significantly (P < 0.05) lower in the ITLC group than in well-fed mice (Table 1).

Table 2. Stereological densities of hepatocytes, sinusoids and hepatic fibrosis in schistosomiasis-infected Swiss Webster mice fed low-protein or standard chow and treated or not with praziquantel.

Groups	Hepatocytes (%)	Sinusoids (%)	Hepatic fibrosis (%)
USC	81.18 ± 1.11	18.82 ± 1.12	-
ULC	71.7 ± 1.05 [a]	28.36 ± 1.17 [a]	-
IUSC	12.06 ± 1.06 [a,b]	9.06 ± 1.20 [a,b]	78.88 ± 1.51
IULC	21.44 ± 0.86 [a,b,c]	8.46 ± 0.36 [a,b]	70.10 ± 1.09 [c]
ITSC	47.56 ± 1.12 [a,b,c,d]	12.52 ± 1.55 [a,b,c,d]	39.92 ± 1.42 [c,d]
ITLC	30.98 ± 0.44 [a,b,c,d,e]	14.42 ± 1.68 [a,b,c,d]	54.60 ± 1.34 [c,d,e]

Data are reported as means ± SD in percent. For abbreviations, see legend to Table 1. Statistical comparisons (P < 0.05, Kruskal-Wallis test): [a] *vs* USC group; [b] *vs* ULC group; [c] *vs* IUSC group; [d] *vs* IULC group; [e] *vs* ITSC group.

Stereological study

Table 2 presents the stereological findings.

Hepatocytes. The volume density of hepatocytes was larger in animals fed the standard diet compared to mice fed the low-protein diet: 81.18% (USC) and 71.70% (ULC). Our stereological study demonstrated that treatment increased the percentage of hepatocytes in mice fed the standard chow (47.56%) compared to mice fed the low-protein chow (30.98%). IUSC, IULC, ITSC, and ITLC values differed significantly (P < 0.05) from control (USC and ULC).

Sinusoids. The volume density of sinusoids differed (P = 0.001) between uninfected mice [18.82% (USC), 28.36% (ULC)], infected mice [9.06% (IUSC), 8.46% (IULC)], and treated mice [12.52% (ITSC), 14.42% (ITLC)]. However, no significant difference (P > 0.05) was observed between IULC *vs* IUSC and ITSC *vs* ITLC groups.

Hepatic fibrosis. Mice fed the low-protein chow showed a decreasing percentage of hepatic fibrosis: 78.88% (IUSC), 70.10% (IULC). The volume density of hepatic fibrosis was significantly different (P = 0.001) between IUSC *vs* IULC and ITSC *vs* ITLC groups.

Discussion

Experimental models have been extensively used to assess the effects of nutritional status on schistosomiasis mansoni infection (11,12). Previous research has shown that feeding a low-protein diet leads to a reduction in total body weight gain and serum albumin levels in mice infected with *S. mansoni* (13). Indeed, we have found that feeding mice a low-protein diet induces malnutrition. One explanation for this effect may lie in some liver-specific proteins like albumin that are known to respond to dietary management, such as protein malnutrition, by reducing hepatic protein synthesis (14).

Although proved relevant in clinical studies (6), very little is known about stereological data in hepatic schistosomiasis (7,8). The diet of the host has been suggested to be a stimulation factor to hepatic regeneration (15). Schistosomiasis also represents a stimulus during the acute phase of the infection (8,15). The present findings showed that both malnutrition and schistosomiasis led to adverse effects on liver morphology. The volume density of hepatocytes was smaller in uninfected mice fed low-protein chow than in those fed a 22% protein diet.

Granuloma formation in schistosomiasis is a manifestation of delayed-type hypersensitivity to antigenic material released by *S. mansoni* eggs that peaks at 8 weeks post-infection (16). Liver fibrosis is a complex process resulting from increased synthesis and deposition of extracellular matrix components (17). Histopathological and morphometric data indicate that undernourished mice develop smaller circumoval granulomas, less intense portal inflammation, and minimal liver fibrosis when compared with control animals. Additionally, decreased total liver fibrosis may have resulted from an impaired mechanism of repair, a lower antigen load or a low antibody production due to a low protein content (2). In the present study, V_v fibrosis was not statistically different between the groups of infected mice. However, in contrast to the present study, undernourished mice failed to develop liver fibrosis as extensively as well-nourished mice (1,11).

PZQ chemotherapy eliminates parasites and kills mature eggs trapped in the host tissues (3). However, there are no reports so far of its efficacy in malnourished hosts. Results from the present study provide additional strong evidence for the beneficial effects of praziquantel. The liver responds to injury with regulated tissue regeneration every time there is a major loss of hepatic tissue (18). Treatment reduces the morbidity of *S. mansoni*-infected mice because periovular granulomas very rapidly regress in size after reduction of the amount of collagen (4,19).

Our stereological study demonstrated that V_v hepatocytes and V_v sinusoids were significantly increased, whereas V_v fibrosis was reduced after chemotherapy. It is clear that V_v fibrosis reduction was larger in the well-fed than in the

low-protein-fed infected mice. This result seems to contradict other studies demonstrating that malnourished infected mice present a lower inflammatory response than well-fed mice (2). Our findings confirm studies demonstrating that protein deficiency impairs the schistosomicidal action of PZQ (5). Based on stereology, our findings indicate that treatment with PZQ ameliorates the hepatic disease caused by schistosomiasis in mice fed a low-protein diet.

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