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Effect of Long Term Schistosomes Infection on Liver of the Host

N.M. SOOMRO, A.G. ARIJO, T.A. QURESHI, N.W. RUNHAM† AND M.J. DOENHOFF
Sindh Agriculture University, Tandojam-Pakistan
†*University of Wales, Bangor- UK*

ABSTRACT

Effect of infection with *Schistosoma margrebowiei* and *S. mansoni* on liver was investigated in mice. Long-term infections with low cercarial numbers showed different results compared to the acute infections. In terms of survival time, animals with chronic infections survived longer. The pathological lesions were fewer and did not differ within the two parasite infections. The pathological manifestation of *S. margrebowiei* and *S. mansoni* infections in mice are essentially similar to those reported by many workers of other species of schistosomes. Obviously, in each parasite, the severity of lesions depends on the virulence of the particular species, and/or its varity, and the number of eggs it produces. It has been suggested *S. margrebowiei* is probably more pathogenic than *S. mansoni* due to its high egg production.

Key Words: *Schistosoma mansoni*; *Schistosoma margrebowiei*; Mice; Pathology

INTRODUCTION

Schistosomiasis in animals cause significant losses, not only due to high mortality and morbidity from severe infections, but also presumably mainly due to the less easily recognizable long-term effects of moderate and long-standing chronic infection (Lawrence, 1980; Saad *et al*., 1980). The incidence of severe pathological lesions in schistosomiasis is related to intensity of infection (Von Lichtenberg *et al*., 1971; Cheever *et al*., 1974). The main pathological feature in schistosomiasis is schistosomal egg granuloma and emboli formation producing pathological changes like, phlebitis, with intimal proliferation and occasionally venous thrombosis in the host, thus interfering with circulation through the liver (Bloch *et al*., 1972). As the disease progresses, fibrosis around the granulomas and an increase in collagen deposition leads to irreversible changes in liver (Smith & Jones, 1961; Richard *et al*., 1992). The size and cellular composition of granulomas change with time and vary greatly from one host species to the other (Meleney *et al*., 1953; Hsu *et al*., 1972).

This paper describes the effect of long-term schistosomal infection on the mice.

MATERIALS AND METHODS

Experimental animals. Inbred BKTO strain mice were used throughout for the studies on schistosome host-parasite relationships.

Parasites. A Puerto Rican strain of *S. mansoni* was maintained in albino *Biomphalaria glabrata* snails and random-bred TO strain mice (Taylor *et al.*, 1969). *S. margrebowiei* (originally obtained from Lochinvar National Park, Zambia) was maintained in the laboratory using *Bulinus natalensis* as an intermediate host.

Method of infecting mice. Thirty four, age-matched 6-8 week old male mice for each group were anaesthetized and infected by 25 *S. margrebowiei* or *S. mansoni* cercariae percutaneously. Two animals from each group were killed on the week 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17 week after exposure. The liver, were examined for pathological changes. The mice were killed by cervical dislocation and their organs fixed in Susa. Three historesin sections of 4 μm thickness at 50 section intervals (200 μm) from the liver were stained by Haematoxylin and Eosin and Polychrome stains. The interpretation of the liver was made in two ways.

1. Histological examination of slides at each time interval
2. Quantitative measurements of the pathological features (percentage area of liver occupied by granulomas on the basis of 10 random fields from each slide).

RESULTS

In the infected liver at the 6th week in *S. margrebowiei* infections, there was a small amount of infiltration around blood vessels. Only one egg was observed. In *S. mansoni* infections eggs, granulomas and some infiltration was present, but most of the liver was normal. Some contracted hepatocytes, large blood spaces and enlarged bile ducts were noticed. At the 7th week in *S. margrebowiei* infections, small numbers of eggs were present otherwise it did not differ from week 6. In *S. mansoni*, many granulomas, enlarged sinusoids, some in small areas (=swollen endothelial cells) were observed. The main bile duct and smaller connections revealed little evidence of activity but there was some secretion plus

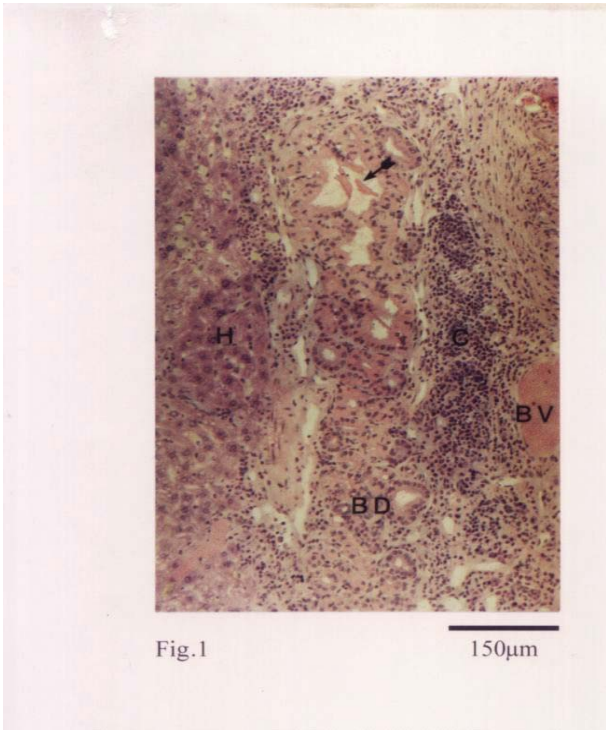
possible increases in cell size. A small amount of fatty degeneration was present in the liver. At the 8th week after infection with *S. margrebowiei* very many eggs were present with granulomas. There was shrinkage and some necrosis of hepatocytes around granulomas, massive infiltration with leucocytes and many clumps of eggs. In *S. mansoni* infections, there was enlarged bile duct with alcian blue positive staining in epithelial cells, dense masses in the cytoplasm, very strongly stained secretion. Many eggs were present in the blood vessels. Patches of infiltration and very dark slightly shrunken hepatocytes surrounded some blood vessels. From 9-11th weeks, the infection levels in both species increased, changes including shrinkage of hepatocytes, focal necrosis and enlargement of bile ducts, some of which had accumulated secretion in the epithelial cells. Liver sinusoidal spaces were found to have increased. Excessive accumulation of lipofuscin was present within granulomas and in surrounding liver tissue. In healthy areas of liver, lipofuscin was present in endothelial cells.

More extensive liver damage was observed in both groups of infected animals after 12 weeks of infection associated with more proliferation of bile ducts lined with secretory cells (Fig. 1). Fatty or mucoid degeneration had increased around granulomas and the amounts of lipofuscin had also increased. From 13 weeks, the amount of damage seems to reduce gradually up to the 17th week of infection. The bile ducts looked less swollen although secretory bile ducts were still present. Healthier hepatocytes were noticeable and sinusoids looked more normal, very patchy distribution of hepatocytes, some fairly normal, others with advanced fatty degeneration. Lipofuscin was still present in endothelial cells, but in reduced quantities. The quantitative results for this chronic infection (Tables I & II) show that at six weeks neither eggs nor granuloma were observed in *S. margrebowiei* infected animals but in *S. mansoni* infected livers eggs, granulomas and shrunken hepatocytes were present, the granulomas consisted of leucocytes without fibrosis. The number of eggs was found to be higher in *S. margrebowiei* infections than with *S. mansoni* throughout the whole infection period. The number of granulomas was higher in *S. mansoni* because characteristically *S. margrebowiei* has very few single egg granulomas, the majority being multiple egg granulomas. The relative area occupied by granulomas did not differ significantly between the species in chronic infection. In *S. margrebowiei*, the granuloma covered area was maximal at 10 weeks then it reduced gradually in succeeding weeks, indicating regeneration or recovery of the liver (Table I). In *S. mansoni*, the maximum granuloma area was present at 7 weeks but a gradual reduction was noticed from 11 weeks onward (Table II). The proportion of regeneration noticed was higher in *S. margrebowiei* than *S. mansoni*. The amount of fibrous tissue and the relative area of shrunken hepatocytes were both reduced with the duration of infection in both species of schistosome but this reduction rate was higher in *S. margrebowiei* which shows more regeneration (Tables I & II).

DISCUSSION

The observations made during this long term light infection experiment are different from those made during the acute infection. In acute infection, the intensity of infection was very high and there was a significant difference in the total area covered by granulomas. This was higher in *S. margrebowiei* infections than in *S. mansoni*. But in light infections with 25 cercariae, the intensity of the infection increased soon after the prepatent period of each species. *S. mansoni* eggs were seen one week before *S. margrebowiei*. The maximum percentage area of granuloma in *S. mansoni* infections was 35.5% at the 7th week; whereas, in *S. margrebowiei* it was 34% at the 10th week. In both cases, after 10 weeks a gradual decrease in the percentage area of granuloma was noticed which indicated regeneration or recovery of the hepatic parenchyma. In both cases, the percentage area of shrunken hepatocytes was also reduced as infection times increased after the peak period.

Fig. 1. Mouse liver 12 weeks post-infection with *Schistosoma margrebowiei*. Note the proliferated bile ducts (BD) lined with secretory cells (C=cellular reaction, H= hepatocyte, BV= blood vessel, Arrow=crystals in the bile duct); Stain: Haematoxyin and eosin



Meanwhile the egg numbers in the liver appeared to decrease as time increased. These finding are in agreement with other workers. Warren (1963) and Andrade and Warren (1964) noticed that as light infections in mice became increasingly chronic, there was a decrease in the size of the inflammatory lesion formed around mature eggs. Cheever (1965a) reported similar histological findings. Dettman and Higgins-Optiz (1994) reported a substantial drop-off in worm recovery of *S. leiperi* between 12-22 weeks after infection. In a subsequent study with *S. margrebowiei*, there was also evidence of a drop-off in worm numbers between 12-20 weeks after infection (Dettman & Higgins-Optiz, 1994). Ogbe (1985) found that worm recoveries from mice and hamsters changed dramatically with time, increasing from around 20% at three weeks (20 days) of infection, to almost 50 and 60%, respectively at 7 weeks (50 days), and thereafter dropping to less than 20% by 10 weeks (70 days). The resolution of hepatic lesions after chemotherapeutic cure of infection in rabbit was found to be impressive (Yang *et al*., 1982). The partial resolution of lesions in chronically infected but untreated rabbits was also noteworthy (Cheever *et al*., 1980). In contrast, in chimpanzees, Symmer's fibrosis did not appear to be reversible in the first few weeks after treatment (Sadun *et al*., 1974). Newly generated granulomas in 20 week infected mice achieve a maximal size which is appreciably smaller than that in 8 week infected animals. Measurement of granuloma diameters around single viable eggs in livers showed a decline of granuloma size by eight weeks with a marked decrease by 16 weeks (Warren *et al*., 1978). Studies of the mechanism of this modulation of granulomatous hypersensitivity have shown a significant diminution in cell mediated responses to soluble egg antigens coincident with a decrease in granuloma size (Colly, 1975). Evidence suggests that modulation also occurs in human infection (Doughty *et al*., 1984). In the present study, bile duct proliferation and secretory hyperplasia of biliary epithelium were frequently observed (Fig. 1). These findings are in agreement with Andrade and Cheever (1993). Bedi and Isseroff (1979) have shown hyperplasia of bile ducts in *S. mansoni* infections. They found that large amounts of proline were released into the hepatoenteric circulation. Because proline release has been linked to bile duct hyperplasia in fascioliasis. They postulated the possibility that such hyperplasia might occur in schistosomiasis. The luminal perimeter and wall thickness in bile ducts was also compared between infected and uninfected mice. In those harbouring five week old *S. mansoni* infections, there was a 180% increase in the luminal perimeter of the duct ($p<0.001$) and a 580% increase in the thickness of the duct wall ($p<0.001$). Proline production and release was apparently nearly as high in *Schistosoma mansoni* as in *Fasciola hepatica* infections (Roth & Hare, 1966; Ertel & Tsssroff, 1974). These results tend to support data linking proline to bile duct and liver fibroblast proliferation. Although blood flukes do not parasitize the bile duct nor come in direct contact with it, the results of the proline infusion studies cited above suggest that proline released by schistosomes into the portal circulation might induce bile duct enlargement.

Table I. Effect of chronic infection with *Schistosoma margrebowiei* on mouse liver

No. week	NEF	NGF	AG (%)	AWBC (%)	AFT (%)	ASH (%)
6	-	-	-	-	-	7.4
7	6.7	3.6	17.3	15.5	0.7	24.4
8	15.9	4.5	24.0	15.0	7.8	18.5
9	5.8	5.2	31.4	12.7	17.6	21.0
10	8.1	4.0	34.0	5.5	28.0	16.5
11	4.9	5.2	21.3	4.1	17.5	35.9
12	4.5	5.0	27.5	6.8	21.0	23.5
13	6.8	5.8	22.3	3.9	18.4	15.4
14	9.9	5.5	25.3	7.7	17.6	15.0
15	3.7	4.1	25.1	11.7	14.9	9.4
16	4.3	3.6	12.8	2.1	10.7	1.0
17	1.2	3.6	12.1	3.8	8.3	3.1

Table II. Effect of chronic infection with *Schistosoma mansoni* on mouse liver

No. Week	NEF	NGF	AG (%)	AWBC (%)	AFT (%)	ASH (%)
6	1.3	2.4	12.5	13.5	-	20.9
7	2.4	6.6	35.6	8.5	25.8	16.0
8	3.6	7.7	27.6	6.8	20.2	10.6
9	2.6	8.6	28.1	10.0	20.1	45.5
10	3.4	8.1	33.8	7.3	26.0	38.9
11	3.5	9.9	33.9	7.8	24.3	44.4
12	1.4	7.8	30.0	4.9	25.1	22.5
13	3.2	10.9	26.9	4.4	23.1	27.7
14	2.2	9.0	25.2	8.9	16.2	9.8
15	2.0	4.8	22.3	6.0	10.8	9.1
16	1.8	7.3	18.9	6.6	12.6	12.7
17	1.7	10.0	22.0	11.0	11.4	11.8

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