PULMONARY PATHOLOGY IN RABBITS INFECTED WITH THE BALING AND KOYAN STRAINS OF *SCHISTOSOMA MALAYENSIS*

K Chandra Shekhar[1] and R Pathmanathan[2]

Departments of [1]Parasitology and [2]Pathology Faculty of Medicine, University of Malaya, Kuala Lumpur 59100, Malaysia.

Abstract. Two distinct strains of *Schistosoma malayensis* exist in Malaysia (designated the Baling and Koyan strains). Both these strains show intraspecific variations in pathology (Greer *et al,* 1988). To evaluate the differences in the pulmonary pathology resulting from infections of the two different strains of Malaysian schistosome, a total of 20 experimental rabbits were infected, 10 each with cercariae of the Koyan strains. Pathological changes were studied over a period of 28 weeks. Granulomas in the lung occurring as a result of infection with the Baling strain were compared with those caused by infection with the Koyan strain. Although both strains produced parenchymatous and alveolar lesions, granulomas caused by the Baling strain of Malaysian schistosome were more numerous and larger (when comparing mean diameter as well as area of granuloma, $p < 0.05$). In addition, pulmonary vascular hypertensive changes were present in Baling strain infected rabbits. These comprised of pulmonary arteriolar endothelial swelling and damage, intimal elastosis and medial hypertrophy. Angiitis and pulmonary periphlebitis were also noted occasionally. In contrast, Koyan strain infection resulted in fewer and smaller granulomas. Pulmonary vascular changes were minimal.

INTRODUCTION

The pulmonary lesions in schistosomiasis occur in both the early stages of schistosomal infection (during the passage of larvae through the lungs) as well as in the late chronic state of infection, following the deposition of ova and migration of worms into the lungs. Pulmonary involvement is one of the most frequent extra-hepatic-intestinal complications of infection with *Schistosoma mansoni* and *S. haematobium* in man, with severe forms leading to cor pulmonale (Kucsko, 1953; Kuida *et al,* 1957; Chaves, 1966). This involvement of the lungs is almost invariably preceded by severe hepatosplenic disease with the establishment of portal systemic collateral circulation (Shaw and Ghareeb, 1938; Faria, 1954). Although Inokuchi *et al* (1979) reported on the occurrence of schistosomiasis japonica outside the hepato-intestinal tract in man, the development of cor pulmonale is rare (Ninomiya, 1970).

Experimental animals such as mice, rabbits and monkeys have been used to investigate the detailed pulmonary pathophysiology of schistosomiasis. Literature on pulmonary schistosomiasis in experimental animals infected with *S. japonicum* is rare. The lesions in rabbits have been reported by Miyasato *et al* (1984), Ninomiya (1970), Cheever *et al* (1980) and Inokuchi *et al* (1979). Miyasato *et al* (1984) carried out a detailed pathological analysis of pulmonary schistosomiasis japonica in rabbits and found that these animals served as excellent experimental models. There workers classified lung diseases into four categories according to the severity and extent of the pulmonary lesions. The existence of two strains of *S. malayensis,* a new species, has recently been published (Greer *et al, 1980; Yong et al,* 1985; Shekahar and Pathmanathan, 1987; Greer *et al,* 1988; Shekhar, 1987). Our work has shown that significant pathological differences exists between the two strains particularly with reference to pathological changes in the liver, spleen, intestines and kidneys (Shekhar, 1990). The purpose of this investigation is to document the pulmonary lesions occurring in rabbits resulting from infections from both strains of *S. malayensis* in rabbit models and to determine how these compare with the lesions caused by other schistosome species.

MATERIALS AND METHODS

The Koyan strain of *S. malayensis* was obtained from *Robertsiella kaporensis* snails and the Baling

strain from *Robertsiella* species maintained at the Division of Parasitology, Institute for Medical Research, Kuala Lumpur. All snails were field collected and the cercariae used represented pools from many maintained in several cultures. They had been maintained under 12 hours of darkness and 12 hours of daylight. Ten rabbits were infected with the Baling strain and another ten with the Koyan strain. 3 rabbits were used as controls. All rabbits were six-month old female inbred local strains, 2 - 2.5 kg in weight and were maintained on pellet diets and water. The animals were anesthetized with Ketalar (Ketamine hydrochloride-Parke Davis) at an anesthetic dose of 25 mg/kg body weight. Using a stereoscopic microscope (Nikon), 250 cercariae from the same pool were counted, picked up using a wire loop, and placed on the shaved abdomen of these rabbits. In order to ascertain whether or not an equal distribution of the sexes of the parasite has been achieved, 20 mice for each rabbit were infected with 25 cercariae each from the same pool of petri dishes. The mice were killed 42 days after exposure. The worms recovered from the mice were sexed and totalled by group. The feces were collected daily 30 days after infection to determine patency. Animals were killed at the end of 28 weeks with an overdose of sodium pentothal. The lungs were dissected, observed grossly, fixed in buffered 10% formalin, and subsequently processed and embedded in paraffin (Paraplast + +). Sections of lungs were cut at 4-5 microns and stained in Haematoxylin and Eosin. In addition, multiple sections of the same organ were stained with Masson's trichrome, Martius scarlet blue, Van Giesen and Periodic Acid Schiff.

RESULTS

Pathological observations

Gross pathology: The lungs of all infected rabbits appeared edematous, congested and hemorrhagic with focal atelectasis. The lungs were pinkish white and firm in consistency. Numerous miliary granulomas ranging in size from 0.1 - 2 mm were visible sub-pleurally, especially in the mid and lower zone of both lungs. No adult worms were detected when lung tissue was sliced, crushed and examined under the stereoscopic microscope.

Microscopic pathology: Lesions were present in both groups of infected rabbits but large florid epithelioid granulomas were prominent in animals infected with the Baling strain. Maximum and minimum granuloma areas and diameters for both the strains are shown in Table 1. Mean granuloma sizes induced by the Baling strain was significantly larger ($p < 0.05$). Other pulmonary lesions consisted of alveolar septal thickening, with diffuse interstitial inflammation and a polymorphous infiltrate of neutrophils, epithelioid macrophages and eosinophils. Many foreign-body type giant cells with phagocytosed ova were present in the epithelioid granulomas, surrounded by eosinophils, lymphocytes and plasma cells (Fig 1). Heavy pigment deposition was seen extracellularly in the alveolar septae as well as within macrophages. Fibrosis of the periphery of granulomas was already established at 28 weeks.

A significant pathological change in Baling strain infected rabbits was noted in the pulmonary arterioles, with the majority of blood vessels showing medial hypertrophy. Many granulomas were located adjacent to blood vessels, particularly around small pulmonary arteries and arterioles. Vaxculitis and periarteritis and occasional phlebitis were present. Rarely, epithelioid granulomata were noted encroach upon the wall of a pulmonary artery (Fig 2). The blood vessel walls showed muscular thickening and the adventitia was infiltrated by eosinophils and mononuclear cells. Mild medial hypertrophy of the small caliber vessels, particularly arterioles was evident. Angiitis and endothelial hyperplasia were observed in many vessels. In advanced lesions, the intima was thickened with fibroblastic proliferation, and disruption and of the elastic lamina often best demonstrated with

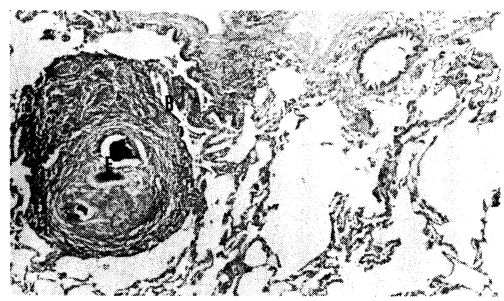

Fig 1—Rabbit lung, hematoxylin and eosin, × 400. Early granuloma formation with multi-nucleate foreign body type-giant cells phagocyto sing ova (E). A peripheral collar of eopsinophils, plasma cells and lymphocytes is present.

Table 1

Measurements of pulmonary granulomas in rabbits infected with the Baling and Koyan strains of Malaysian schistosomes.

Strain	Minimum area (μ^2)	Maximum area (μ^2)	Mean area (μ^2)	Minimum diameter (μ^2)	Maximum diameter (μ^2)	Mean diameter (μ^2)
Baling	69.14	1225.5	432 ± 253.8	10.17	54.16	26.9 ± 9.98
Koyan	440.28	8217.5	2140 ± 1940.9	25.81	118.94	59.0 ± 24.88

Number of granulomas measured for Baling strain = 115
Number of granulomas measured for Koyan strain = 68
*$p < 0.05$

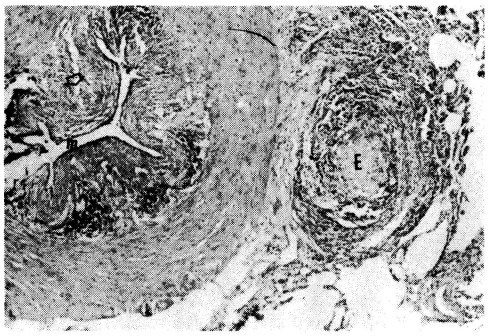

Fig 2—Lung, hematoxylin and eosin, × 200. Epitheloid granulomata (E) are seen encroaching onto the adventitia of a pulmonary artery. Intimal hyperplasia with medial hypertrophy (m) with chronic lymphocytic infiltrate at the media and intima interphase is seen (⇩)

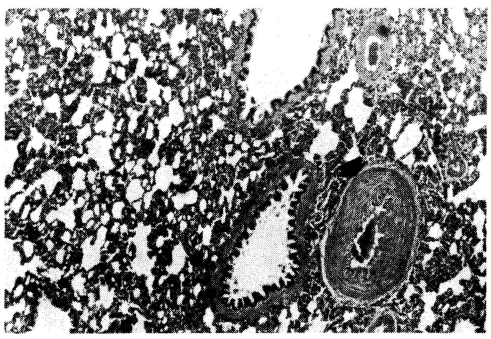

Fig 3—Small pulmonary artery, hematoxylin and eosin. × 400. Medial hypertrophy with intimal proliferation of fibrous tissue ⬇ The lumen is narrowed and slit-like (E).

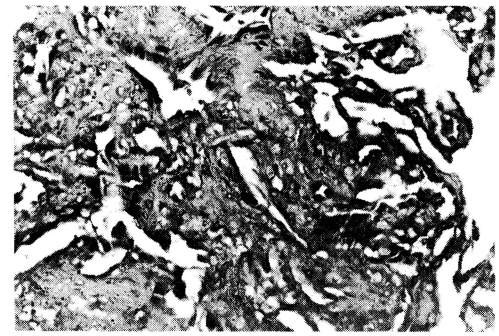

Fig 4—Hematoxylin and eosin, × 200. Pulmonary arteriole with plexiform lesion in Baling strain infected rabbit.

Masson's trichrome (Fig 3). Often there was associated medial muscular loss, atrophy and replacement fibrosis. Angimatoid and plexiform lesions were present (Fig 4) and intraluminal extension of mural granulomata occasionally resulted in thrombosis. Granulomatous pulmonary arteritis other than that involving terminal pulmonary arterioles were rarely present. Clinically, no animal appeared to have cor pulmonale.

In the Koyan strain infected animals, granulomas were fewer and septal inflammation mild. Most epithelioid granulomata were smaller, were composed primarily of epithelioid cells, and did not have eggs within them.

DISCUSSION

Pulmonary schistosomiasis is usually associated with severe liver lesions and an established

porto-systemic collateral circulation (Shaw and Ghareeb, 1938; Faria, 1954). The occurrence of hepatosplenic schistosomiasis including porto-systemic collateral circulation in mice infected with *S. mansoni* has been reported (Warren and De Witt, 1958; De Witt and Warren, 1959). Partial portal vein ligation four weeks after exposure to cercariae of *S. mansoni* caused the formation of many granulomata and severe pulmonary arteritis in mice (Warren, 1964). In the lungs, it is assumed that the pathological lesions result as a consequence of widespread egg embolization. Pulmonary lesions are predominantly vascular (Shaw and Ghareeb, 1938). The obliterative endarteritis and arteriolitis are considered the dominant cause of the pulmonary hypertension and pathological lesions associated with it (Faria, 1954). Medial hypertrophy of the small and medium - sized muscular arteries and muscularization of the arterioles were common findings in human cases infected with *S. mansoni* (Spencer, 1950). The striking lesion of severe hypertension was a plexiform and angiomatoid lesion. In the present finding, the macroscopic features of the lungs infected each with both strains of *S. malayensis* were similar to the findings of Miyasato *et al* (1984) of pulmonary lesions induced by *S. japonicum.* Microscopically, rabbits infected with the Baling and Koyan strains of *S. malayensis* developed parenchymatous granulomatous pulmonary lesions and the granulomas produced by the Baling strain were larger and more florid. However, vascular hypertensive features were noted with regularity in only Baling strain infected rabbits. Similar findings were noted in rabbits infected with *S. japonicum* (Japanese strain) using a cercariae dose level of 300-2000 and sacrificed 48 to 400 days after infection (Miyasato *et al,* 1984). Cheever *et al* (1980) reported that arterial changes suggesting pulmonary hypertension were rarely present in schistosomiasis and there was no evidence of diffuse arterial lesions were reported in rabbits infected with *S. japonicum* (Japanese strain). In our study, however, as noted earlier, the Baling strain induced vascular changes in infected rabbits. Increase of pulmonary pressure resulting from diffuse granulomatous inflammation, obliteration of the vascular bed and fibrotic occlusion of artieruioles appear to be the principal pathogenetic pathways leading to the vasculopathy observed in Baling strain infected animals.

ACKNOWLEDGEMENTS

Our sincere thanks to Dr Ow Yong and Dr George Greer of the Division of Parasitology, Institute for Medical Research, Kuala Lumpur for their generous supply of cercariae for this study. Thanks are also due to the staff of the Department of Animal Resources, Faculty of Medicine, University of Malaya for the maintenance of the infected animals. Finally, the University of Malaya is thanked for granting us the research vote to carry out this study.

REFERENCES

Chaves E. Necrotizing and healing pulmonary arteritis in schistosomal cor pulmonale. A retrospective study of ten cases. *Am J Trop Med Hyg* 1966; 15 : 162-7.

Cheever AW, Duval RH, Minker RG. Extra-hepatic pathology in rabbits infected with Japanese and Philippine strains of *Schistosoma japonicum* and the relation of intestinal lesion to passage of eggs in the feces. *Am J Trop Med Hyg* 1980; 29 : 1316-26.

DeWitt WB, Warren KS. Hepato-splenic schistosomiasis in mice. *Am J Trop Med Hyg* 1959; 8 : 440-6.

Faria JL. Pulmonary vascular changes in schistosomal cor pulmonale. *Am J Pathol Bact* 1954; 58 : 589-602.

Greer GJ, Ow-Yong CK, Singh KI, Lim HK. Discovery of a site of transmission and hosts of a Schistosoma japonicum-like schistosome in Peninsular Malaysia. *Trans R Soc Trop Med Hyg* 1980; 74 : 425.

Greer GJ, Ow-Yang CK, Yong HS. *Schistosoma malayensis* N SP: A *Schistosoma japonicum* - complex schistosome from Peninsular Malaysia. *J Parasitol* 1988; 74 : 471-80.

Inokuchi T, Lo CT, Ito Y. Pulmonary schistosomiasis in Japan. *Keio Igaku* 1979; 34 : 345-52.

Kucsko L. Uber arerіovenose verbindungen und der menschlichen lunge und ihre funktionelle Bedeutung. *Frankfurt Ztschr Path* 1953; 64 : 54-9.

Kuida H, Dammin GJ, Haynes FW, Rapaport E, Dexter L. Primary pulmonary hypertension. *Am J Med* 1957; 23 : 166-9.

Miyasato M, Kakizoe Y, Lida H, Hirano Y, Tsutsumi. Experimental pulmonary embolism with *Schistosoma japonicum* eggs. II. Pulmonary lesions in rabbits infected with *S. japonicum. Kurumme Med J* 1984; 31 : 47-53.

Ninomiya T. A comparative histopathology of *Schistosoma japonicum. Igakkai Zasshi* 1970; 40 : 460-503.

Shaw AFB, Ghareeb AA. The pathogenesis of pulmonary schistosomiasis in Egypt with special reference to Ayerza's disease. *J Pathol Bact* 1938; 46 : 401-24.

Shekhar KC, Pathmanathan R. Schistosomiasis in Malaysia - A Review. *Rev Infect Dis* 1987; 9 : 1026-37.

Shekhar KC. A comparative parasitological study of Malaysian schistosomes (Koyan and Baling strain) with *Schistosoma japonicum* (Philippine strain) and *S. mekongi* (Thai strain) in various laboratory animals. *Trop Biomed* 1987; 4 : 132-44.

Shekhar KC. *Schistosoma malayensis:* The pathology of experimental infection. *Trop Biomed* 1990; 7 : 107-11.

Spencer H. Primary pulmonary hypertension and related vascular changes in the lungs. *J Pathol Bact* 1950; 62 : 75-8.

Warren KS, DeWitt WB. Production of portal hypertension and oesophageal varices in the mouse. *Proc Soc Exp Biol Med* 1958; 98 : 99-101.

Warren KS. Correlation between experimental and human infection with *Schistosoma mansoni. Nature* 1964; 201 : 899-901.

Yong HS, Ooi CS, Greer GJ, Lai KPF, Ow-Yang CK. Biochemical, genetic differentiation of three species of *Robertsiella* snails (Gastropa: Prosobranchia: Pomatiopsidae), the intermediate host of a Malaysian schistosome. *Trop Biomed* 1985; 2 : 113-20.