NOTE
Pathology

Histopathological Comparison of Pulmonary Artery Lesions Between Raccoon Dogs (Nyctereutes procyonoides) and Domestic Dogs Experimentally Infected with Dirofilaria immitis

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(Received 24 May 2007/Accepted 21 November 2007)

ABSTRACT. Five raccoon dogs (Nyctereutes procyonoides) and two domestic dogs (Canis familiaris) were subcutaneously infected with 100 infective larvae (L3) of Dirofilaria immitis. Two and five worms, respectively, were collected from two of three raccoon dogs. Villous endarteritis was found in the raccoon dog with five worms and two dogs at 116 days after infection. The number of recovered worms in the raccoon dog was significantly smaller than that of the domestic dogs having 22 and 29 worms, while histopathological features and the severity of the lesions in the raccoon dogs were similar to those in the domestic dogs. The vascular lesions in two chronically-infected raccoon dogs turned into much severe at 565 and 590 days after inoculation.

KEY WORDS: Dirofilaria immitis, raccoon dog, villous endarteritis.

The prevalence of canine heartworm in free-ranging raccoon dogs (Nyctereutes procyonoides) is lower than that in domestic dogs kept in same areas [13]. This situation of raccoon dog dirofilariasis is similar to that of domestic cats. Holmes et al. [8] mentioned that the cat might be a definitive host for the canine heartworm but not perfect because of its susceptibility and microfilaria. Histopathological changes of the lung in dirofilariasis have been reported in many species of animals. Villous endarteritis with luminal occlusion caused by the villous intimal proliferation and medial hypertrophy has been characteristically observed in dogs, cats and red fox [7, 9, 11, 14]. However, there are few reports on pulmonary lesions of the raccoon dogs infected with D. immitis.

In this study, we compared the pulmonary artery lesions between in the domestic dogs and the raccoon dogs experimentally infected with D. immitis.

First, infective larvae (L3) were produced to infect D. immitis successfully in the experiments. L3 were separated from Aedes togoi that fed the blood from a highly microfilaremic dog and washed several times in Hank’s balanced salt solution containing penicillin and streptomycin. In the experiment I, three raccoon dogs, aged 6 to 12 month, provided by Mr. Eiji Kanda (Wildlife Research Center Co., Ltd., Itsukaichi, Tokyo, Japan) and 2 female domestic dogs (beagle, 12 month-old) (Nihon Nosan Kougyo, Co., Ltd.) were subcutaneously infected four times each with twenty-five L3 during a period of three weeks. These animals were sacrificed by overdose of sodium pentobarbital (100 mg/kg) at 116 days after the last inoculation. The experiment II was designed to examine the microfilaria production in the long-term infection, and two raccoon dogs were infected once with 100 of L3 larvae. After 565 and 590 days, the animals were sacrificed by overdose of sodium pentobarbital. The experiments were performed during the wet season (May to October in the Honshu island of Japan) and the animals were fed dog chows twice a day and given water in a mosquito-proof room. The animals were necropsied and the caudal lobes of the right lung were dissected, fixed in 10% buffered formalin and embedded in paraffin. The paraffin sections were cut and stained with hematoxylin-eosin (H-E) and elastica van Gieson. The present studies were performed following the instructions of the Experimental Animal Care Committee of Nippon Juichikusan University.

In the experiment I, although 22 and 29 worms, respectively, were recovered from the right ventricle and pulmonary arteries in two dogs, only 2 and 5 worms, respectively, were recovered from the right ventricle in two of three raccoon dogs. The mean size of the male and female worms was 13.0 ± 3.35 cm and 17.4 ± 6.38 cm, respectively. There were no differences in the worm length between that recovered from the raccoon dogs and the domestic dogs.

Histopathologic examination revealed villous endarteritis with intimal infiltration of mononuclear cells and eosinophils, and medial hypertrophy in the large pulmonary arteries (Fig. 1) in one raccoon dog with 5 worms and two dogs. The internal elastic laminae and the elastic fibers in the media of the affected vessels were partially disrupted (Fig. 2). These were no differences in the character and intensity of these vascular lesions between the raccoon dogs and domestic dogs. No thrombi were observed in the arteries in.
Fig. 1. Villous endarteritis in the large pulmonary artery of the raccoon dog at 116 days after the last inoculation of infective larvae (L3) of *D. immitis* in the experiment I. The villi were edematous and composed of mononuclear cells and eosinophils. Hematoxylin and eosin (H-E) stain. PA: pulmonary artery. Bar=150 µm.

Fig. 2. Disruption of the internal elastic lamina and elastic fibers (arrow) of the pulmonary artery in the raccoon dog at 116 days after the last inoculation of L3 of *D. immitis* in the experiment I. Elastica van Gieson stain. PA: pulmonary artery. Bar=200 µm.

Fig. 3. Severe villous endarteritis in the large pulmonary artery in the raccoon dog at 590 days after the inoculation of L3 of *D. immitis* in the experiment II. Note that the elastic fibers were completely disappeared and that the medial smooth muscle layer was replaced by the collagen fibers (arrow). Elastica van Gieson stain. PA: pulmonary artery. Bar=200 µm.

Fig. 4. Partial occlusion of the lumen of the pulmonary artery due to sever proliferative lesion seen in the raccoon dog at 590 days after the inoculation of L3 of *D. immitis* in the experiment II. Elastica van Gieson stain. Bar=200 µm.

Fig. 5. Interstitial fibrosis associated with infiltration of mononuclear cells and eosinophils (arrow) in the raccoon dog at 590 days after the inoculation of L3 of *D. immitis* in the experiment II. H-E stain. Bar=100 µm. Inset indicates marked hypertrophy of smooth muscle layers of the terminal bronchioles (arrow). H-E stain. Bar=200 µm.
the lung of all the animals examined. In addition to the lesions observed in the lung, there was a mild infiltrate of mononuclear cells and eosinophils in the pulmonary trunk valve in the heart, which was observed in the raccoon dog and the domestic dogs.

In the experiment II, 3 (2 males and a female) and 6 (three males and three females) worms, respectively, were recovered from the right ventricle of each raccoon dog.

Histopathologically, some pulmonary arteries were severely dilated, and the medial smooth muscle layer became thinner and was accompanied with proliferation of collagen fibers (Fig. 3). These proliferative lesions resulted in the partial occlusion of the lumen of some pulmonary arteries (Fig. 4). Intimal proliferation was also detected in smaller branches of the pulmonary arteries. These histopathological findings in the pulmonary arteries were more severe than those seen in the experiment I. No thrombi were observed in the arteries of the lung in 2 raccoon dogs. In addition to the vascular lesions, multifocal interstitial fibrosis associated with infiltration of mononuclear cells and eosinophils, and marked hypertrophy of smooth muscle layers of the terminal bronchioles and alveolar ducts was seen in the lung of both raccoon dogs (Fig. 5). In association with the findings observed in the lung, the wall of right ventricle of the heart was thickened with the vascular degeneration of the myocardium. The valves of pulmonary trunk were edematous and irregularly thickened with the infiltrate of mononuclear cells and eosinophils, which resulted inervilleous appearance.

In the present study, worm number in the raccoon dogs was significantly lower than that in the domestic dogs. Similarity, it has been observed that the prevalence of infection in cats was very low and the worm number is lower in comparison with that of domestic dogs [12]. These observations suggest low susceptibility to D. immitis in raccoon dogs similar to cats.

Histopathological lesions were seen in one of three raccoon dogs in the experiment I, and were similar to those described in the red fox, the cat and the dogs [7, 9]. It has been suggested that the severity of the lesions are related to the localization of the worms rather than the worm number in dogs. No correlation has been noted between worm number and severity of pulmonary vascular resistance [6]. The results in the present study might indicate that the vascular tissues of raccoon dogs are more sensitive and reactive against D. immitis than those of domestic dogs. Villous endarteritis that extended to the small branches of the pulmonary arteries and medial hyperplasia of the raccoon dogs seen in the experiment II were more severe than those seen in the experiment I. In addition, severe infiltration of the inflammatory cells in the pulmonary trunk valve and endarteritis of the pulmonary trunk were observed only in the raccoon dogs examined in the experiment II. The difference might be due to that of the term of parasitism of D. immitis in the ventricle or pulmonary arteries as seen in domestic dogs.

Vascular diseases caused by the D. immitis infection lead to the periarteritis, which is accompanied by the intense inflammation into the adjacent alveoli [6]. It is shown that the inflammatory progression is associated with a secretary compound from D. immitis that triggers the production of nitric oxide, which is said to be an important factor for the inflammatory reaction [2, 3]. In the present study, the multifocal fibrosis was observed only in the raccoon dogs in the experiment II, suggesting that the reaction of the pulmonary tissue might be associated with the term of parasitism of D. immitis.

In humans, the smooth muscle proliferation of the lung could be seen in the patients with the pulmonary hypertension [10]. Hypoxia is an important factor for airway smooth muscle proliferation in the pulmonary diseases [4, 5]. In the present study, the raccoon dogs in the experiment II showed the severe smooth muscle hypertrophy in the alveolar septa. In the lung, the vascular disease, extending into the adjacent alveoli and the interstitial tissue, lead to the difficulty of breathing and hypoxia. Therefore, the hypertrophy of the smooth muscle of the alveolar septa may be due to the long-term infection of D. immitis that made the pulmonary lesions severer in the raccoon dogs.

Typical histopathological lesion seen in the lung of cats infected with D. immitis is severe medial hyperplasia of the small arteries [6]. Also a few D. immitis causes severe lesions and sudden death in the cats [1, 11]. The lesions of the raccoon dogs seen in the experiment I are similar to those found in cats. These might indicate that the raccoon dogs fall into the pathophysiologic condition similar to the cats in dirofilariasis.

REFERENCES