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Alteration of extracellular collagen matrix in the myocardium of canines infected with *Dirofilaria immitis*

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Abstract

The heart consists of cardiocytes and the interstitial extracellular matrix (ECM), which is made up mainly of collagens. The ECM has been suggested to be important in maintaining the structure and function of the heart. This investigation attempted to elucidate the changes in the ECM collagens in the hearts of canines with dirofilariasis. The ECM collagen fibrils of the heart are grouped into endomysial struts, epimysial weaves, and perimysail coils. In the present study, we used the modified silver impregnation technique to stain paraffin-embedded sections to demonstrate three types of ECM. The results revealed that the ECM content of the heart was significantly reduced in heartworm-infected dogs, and became fragmented and dissociated. In addition, the amounts of collagen in the septum (Sep), RVs and LVs in canines with dirofilariasis (Sep = 11.55 ± 0.65, RV = 12.07 ± 0.59, LV = 11.72 ± 0.62 μg/mg, $n = 24$) were significantly lower ($p < 0.01$) than that in the normal canines (Sep = 15.09 ± 0.72, RV = 15.16 ± 0.83, LV = 14.91 ± 0.89 μg/mg, $n = 8$). These results indicated that heartworm infection induced the remodeling of the extracellular matrix, thus markedly altering the architecture and function of the heart.

Keywords: *Dirofilaria immitis*; Extracellular matrix; Collagen

1. Introduction

The extracellular space of the myocardium contains a collagen network that mainly comprise types I and III fibrillar collagens (Caulfield and Borg, 1979; Eghbali and Weber, 1990; Weber et al., 1994). The quantity and quality of the extracellular collagen is determined by the balance between synthesis and degradation (Tyagi, 1997; Rao and Spinal, 1999). Collagen synthesis is regulated transcriptionally and posttranslationally. Degradation is primarily mediated by matrix metalloproteinases (MMPs), and by endogenous tissue inhibitors (TIMPs) (Cleutjens et al., 1995; Tyagi, 2000; Siwik et al., 2001). The

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extracellular matrix (ECM), which is produced primarily by fibroblastic cells and surrounds the cardiac myocytes, preserves the architecture and chamber geometry of the heart (Borg et al., 1996). A disruption or loss of the collagen will result in a reduction in the tensile strength and subsequent muscle bundle slippage. Meanwhile, the three-dimensional network of the ECM comprises three basic levels, including endomysial struts that interconnect adjacent cardiocytes, epimysial weaves that surround cardiocytes, and perimysail coils that course as spring-like structures in the interstitium between cardiocytes (Borg and Caulfield, 1981; Mahbouben and Weber, 1990; Pelouch et al., 1994). Alteration of these structures may lead to the development of heart dysfunction (Weber, 1989; Factor et al., 1991).

Dirofilaria immitis, the canine heartworm, resides primarily in the pulmonary arteries and right ventricle. Heartworm infection may cause dilatation of the heart and hypertrophy of the endocardium. The aim of the present study is to investigate the changes of extracellular collagen matrix in the myocardium during the setting of dirofilariasis.

2. Materials and methods

2.1. Animals

The study population consisted of 32 mongrel dogs with body weights ranging from 15 to 23 kg. Among them, 24 dogs were diagnosed with moderate to severe dirofilariasis based on the modified Knott's test (Newton and Wright, 1956), the ELISA kit (Snap™ canine heart PF, IDEXX Laboratory, Westbrook, Maine, USA) and clinical diagnosis (Hoskins, 1996). The dogs were euthanasized using intravascular injection of overdose pentobarbital sodium (Nembutal®, Abbott Laboratories, USA), and their hearts were collected. Clinically normal dogs those free of infection with heartworm were used as controls.

2.2. Gross and tissue examination of the heart

The entire heart was removed from the thoracic cavity and washed free of blood. Heart weight, heart weight-to-body weight, and the thickness of left and right ventricles were determined. The specimen was then fixed in 10% phosphate buffered formalin for gross examination. Sections of the left and right ventricles were taken, embedded in paraffin, and stained with hematoxylin and eosin. The stained sections were then examined under a microscope for myocardial abnormalities.

2.3. Modified silver impregnation and histological examination

This study adopted the modified silver impregnation method described by Shyu et al. (1994) and Chiu et al. (1999) to detect myocardial matrix. Briefly, thick sections (25–50 μm) of formalin-fixed and paraffin-embedded cardiac tissue were deparaffinized in xylene and washed in distilled water. The washed sections were immersed in 50% Rio Hortega lithium–silver solution at 60–62 °C for 20 min. The sections were then incubated in an ammonia water-bath (10 drops of 28% ammonia in 50 ml DW) for 2 min, followed by washing with DW for 3 min. After ammonia treatment, the sections were immersed in 0.5% neutral formalin for 1 min and then the excess formalin was washed off with water. The sections were last stained with 0.2% gold chloride for 1 min. After washing and dehydration, the sections were mounted with gelatin. Finally, the collagen matrix of the heart was stained black under light microscopic examination.

2.4. Quantitation of collagen and total protein

A previously described method (López-De León and Rojkind, 1985) was used to quantify the collagen and total protein contents of the paraffin-embedded tissue sections. This method used the selective binding of Sirius red F3BA to collagen protein and Fast green FCF to noncollagen protein when both are dissolved in aqueous saturated picric acid. Briefly, individual slices with a section area of approximately 30–50 mm^2 were placed in small test tubes and covered with 0.2 ml of saturated picric acid solution that contained 0.1% Sirius red F3BA and 0.1% Fast green FCF. The tubes were incubated at room temperature for 30 min in a rotary shaker, the fluids carefully withdrawn and the sections washed repeatedly with DW until the fluid was colorless. When the sections were treated with sodium hydroxide–methanol, the eluted color was immediately read using a spectrophotometer at 540 and 605 nm.