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SPONTANEOUSLY ARISING DISEASE

Pathological Features and Pathogenesis of the Endomyocardial Form of Restrictive Cardiomyopathy in Cats

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Summary

This study reports pathological and molecular features in 41 cases of feline restrictive cardiomyopathy (RCM). Grossly, there were patchy or diffuse areas of endocardial thickening affecting the left ventricle. The more common patchy endocardial lesions occurred as large trabecular or irregular broad bands of fibrous tissue bridging the left ventricular free wall and ventricular septum. Microscopically, regardless of the gross pattern, the thickened endocardium contained various numbers of stellate, spindle-shaped or elongated mesenchymal cells surrounded by fibrous connective tissue. Immunohistochemical findings were indicative of smooth muscle differentiation in mesenchymal cells. These cells proliferated vigorously and produced alcian blue-positive ground substance and collagen fibres; it was considered that the mesenchymal cells contributed to the formation of the endocardial lesions. In addition, multiple left ventricular 'false tendons' were invariably included within the trabecular or broad fibrous bands, providing a framework for formation of those bands. Evidence of endocarditis or endomyocarditis was lacking in all 41 cases, and no viral genomes were detected in any of the DNA or RNA samples obtained from 14 of the hearts. These observations suggest that any relationship between feline RCM and a virus-induced inflammatory response seems unlikely.

Keywords: cat; endomyocardial fibrosis; pathogenesis; restrictive cardiomyopathy

Introduction

Restrictive cardiomyopathy (RCM) in man is characterized by restrictive filling and reduced diastolic volume of either or both ventricles, with normal or near-normal systolic function and wall thickness (Richardson *et al.*, 1996). A variety of specific pathological processes can cause RCM, including endomyocardial scarring, myocardial fibrosis or infiltrative disorders (Gallo and d'Amati, 2001). These conditions are divided into two distinct groups in man: those with predominant endocardial involvement (endomyocardial form) and others with predominant myocardial involvement (myocardial form) (Gallo and d'Amati, 2001). This classification of RCM also appears to be applicable to cats (Ferasin *et al.*, 2003; Fox, 2004) and the endomyocardial type, also known as endomyocardial fibrosis, is the most prevalent form of feline RCM (Bond and Fox, 1984; Kimura *et al.*, 2016).

A prominent pathological feature of the endomyocardial form of feline RCM is severe and extensive

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fibrous thickening of the endocardium and chamber deformity (Fox, 2004). This process primarily affects the left ventricle (LV) or, rarely, both ventricles. There are two basic morphological patterns of endocardial fibrosis, although a degree of overlap in features may occur (Fox, 2004). In the first pattern, the LV shows diffuse, marked fibrosis, which appears as an opaque, white–grey, firm covering involving substantial portions of the left ventricular cavity. In the second pattern, large trabecular or irregular broad bands are observed bridging the left ventricular free wall and ventricular septum (VS) and often causing fixed stenosis or a fibrotic tube in the mid to apical left ventricular chamber. This is the most common form reported in cats with endomyocardial RCM (Fox, 2004).

The aetiology of endomyocardial RCM in cats is unknown, but is thought to be multifactorial, as there is a wide spectrum of clinical manifestations and pathological phenotypes (Fox, 2004). Possible hypotheses include viral or immune-mediated endomyocarditis followed by reparative fibrosis, or a consequence of the end stage of myocardial failure and infarction from hypertrophic cardiomyopathy (Fox, 1999, 2004).

The present report provides a fuller description of the range of pathological features in the endomyocardial form of RCM and presents a hypothesis to explain the pathogenesis of this disease in the cat.

Materials and Methods

Animals

As described in our previous report (Kimura *et al.*, 2016), the materials for the present study were obtained from consecutive feline necropsy examinations conducted at the Laboratory of Veterinary Clinical Oncology, Tokyo University of Agriculture and Technology, Tokyo, Japan, during the period 2005–2014. A total of 327 necropsy examinations were performed on cats with heart disease, and 41 cats were diagnosed as having the endomyocardial form of RCM. In these cases, the diagnosis was based on characteristic gross features of marked fibrosis focally or diffusely involving the endocardium of the LV. The hallmark gross findings included an enlarged heart with marked left atrial and often right atrial dilation and hypertrophy; severe and extensive endocardial fibrosis, frequently bridging the left ventricular free wall and VS; left ventricular hypertrophy commonly associated with wall and chamber deformity; and mural thrombi in the left atrium (LA) or LV (Fox, 1999, 2004).

Pathological Studies

A complete necropsy examination was performed on all 41 cats within 12 h of death. The hearts were examined and weighed, and the heart weight to body weight (HW/BW) was calculated (i.e. HW in g/BW in kg). The hearts were then placed in 10% neutral buffered formalin for a minimum of 5 days. After fixation, the ventricles were sliced transversely into serial sections approximately 5 mm thick, from the mitral valve to the cardiac apex. Each transverse ventricular slice was embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin (HE). Selected sections were also stained with Masson's trichrome for collagen fibres, elastic van Gieson for elastic fibres and alcian blue (pH 2.5) and toluidine blue for acidic glycosaminoglycans. The microscopical examination focused on any morphological changes in the endocardium of the LV.

Immunohistochemistry (IHC) was performed for identification of the endothelial marker CD34 (mouse monoclonal anti-human CD34 class II, Clone QBEnd-10; 1 in 100 dilution; Dako, Glostrup, Denmark), vimentin (mouse monoclonal antibody against vimentin, clone Vim 3B4; 1 in 100 dilution; Dako), α-smooth muscle actin (α-SMA; mouse monoclonal antibody against human smooth muscle actin, clone 1A4; 1 in 100 dilution; Dako), caldesmon (mouse monoclonal antibody against human caldesmon, clone h-CD; 1 in 100 dilution; Dako), platelet-derived growth factor (PDGF; rabbit polyclonal antibody against PDGF BB; 1 in 50 dilution; Abcam, Cambridge, UK) and transforming growth factor-β (TGF-β; rabbit polyclonal antibody against TGF-β; 1 in 100 dilution; Abcam) in the endocardium of the LV. The avidin–biotin–peroxidase method (Vectastain, Vector Laboratories, Burlingame, California, USA) was employed in order to identify the reaction product, with haematoxylin as a counterstain.

Molecular Biological Studies

Real-time polymerase chain reaction (PCR) was used to determine whether viral nucleic acids were detectable in cardiac tissues including the endocardium, myocardium and epicardium. DNA and RNA samples were extracted from wax-embedded tissue blocks of 14 cat hearts with endomyocardial RCM collected between 2012 and 2014 using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Life Technologies, Carlsbad, California, USA). Screening for a total of six viruses, including feline herpesvirus (FHV), feline panleukopenia virus (FPLV), feline calicivirus (FCV), feline coronavirus (FCoV), feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV), was carried out according to their

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