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Comparison of cellular changes in Cavalier King Charles spaniel and mixed breed dogs with myxomatous mitral valve disease

C.-C. Lu, DVM [a], M.-M. Liu, PhD [a], G. Culshaw, BVM&S [a], A. French, PhD [b], B. Corcoran, PhD [a,*]

[a] *Royal (Dick) School of Veterinary Studies and The Roslin Institute, The University of Edinburgh, Easterbush, Roslin, Mid-Lothian, Scotland, EH25 9RG, UK*
[b] *School of Veterinary Medicine, The University of Glasgow, Garscube Campus, Bearsden Road, Glasgow, Scotland, G61 1QH, UK*

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Abstract *Introduction:* The aim of this study was to determine if there are differences in cellular changes in Cavalier King Charles spaniel (CKCS) myxomatous mitral valves compared to non-CKCS dogs.
Animals: Cavalier King Charles spaniels (n = 6) and age-matched mixed breed (n = 6) with severe myxomatous mitral valve disease (MMVD), and normal mixed breed (n = 4) dogs.
Materials and Methods: Immunohistochemistry staining and qualitative and quantitative analysis of mitral valves sections, examining for the presence of CD11c and CD45, vimentin, alpha smooth muscle actin (α-SMA) and embryonic smooth muscle myosin heavy chain (Smemb), von Willebrand factor and CD31 and Ki-67.
Results: Vimentin positive cell numbers were increased in the MMVD dogs and distributed throughout the valve with greatest density close to the endothelium. There were no significant differences in cell marker expression for the two diseased groups, but cell numbers were significantly increased compared to controls for α-SMA (CKCS only) and Smemb (CKCS and mixed breed: $p < 0.05$). Alpha smooth muscle actin+ cells were primarily located at the valve edge, with Smemb+ cells similarly located, but also present throughout the valve stroma. A small number of cells

* Corresponding author.
E-mail address: Brendan.Corcoran@ed.ac.uk (B. Corcoran).

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close to the valve edge co-expressed α-SMA and Smemb. Endothelial von Willebrand factor expression was identified in all valves, with evidence of disrupted endothelium in the diseased, but was also found in diseased valve stroma. There was no staining for CD11c, CD45 or CD31 in any valve. Ki-67+ cells formed linear clusters at the leaflet tip and were sparsely distributed throughout both myxomatous valve groups.
Conclusions: The cellular changes notes with advanced stage MMVD appear similar for CKCS when compared to mixed breed dogs.

Abbreviations

α-SMA	alpha smooth muscle actin
CKCS	Cavalier King Charles spaniel
MMVD	myxomatous mitral valve disease
PBS	phosphate-buffered saline
Smemb	embryonic smooth muscle myosin heavy chain
VEC	valve endothelial cell
VIC	valve interstitial cell
vWF	von Willebrand factor

Introduction

The Cavalier King Charles spaniel (CKCS) breed is recognised to be particularly predisposed to the development of myxomatous mitral valve disease (MMVD) in terms of age of onset, progression and severity [1—3]. Myxomatous mitral valve disease is highly heritable in the CKCS breed and it is reasonable to suppose heritability also has a role in disease appearance and progression [4,5]. There are CKCS-specific traits such as differences in platelet numbers and platelet aggregation tendency and higher serum magnesium and 5-hydroxytryptamine(serotonin) levels compared to other dogs that have been examined for correlation with development of MMVD, with varying results [6—12]. Furthermore, the CKCS is more predisposed to chronic fibrosing pancreatitis and syringomyelia than other breeds and with MMVD this might reflect a breed-specific global connective tissue abnormality [13—15]. Although there is no clear causal link established between these findings in the CKCS and MMVD it still points toward breed-specific phenotypic characteristics that might have bearing on disease appearance.

Cellular changes that occur with MMVD are well described. There is an increase in α-smooth muscle actin (α-SMA) positive valve interstitial cells (activated myofibroblasts, activated valve interstitial cells [aVICs]) which locate mainly toward the valve distal free-edge (valve tip) and appears to be related to disease severity [16—19]. It is activation of the VICs that is believed to drive the extra-cellular matrix damage seen in MMVD through a combination of increased catabolic activity and aberrant repair and replacement. In normal valve stroma most cells are quiescent vimentin positive VICs (qVIC), with a few desmin-positive cells [16,17]. In overtly myxomatous areas of affected valves some cells also appear to be activated myofibroblasts or with increased differentiation capacity based on their expression of embryonic smooth muscle myosin heavy chain (Smemb), with appearance of cells in clusters close to the endothelium and deeper in the valve stroma [20,21]. There is no evidence that there is active cell proliferation and although some cells exhibit a pro-apoptotic status they do not undergo programmed cell death [16,22]. However, there are regional changes in cell numbers; reduced in overtly myxoid stroma and increased in sub-endothelial zones [21]. A small increase in mast cell numbers is also noted, but there is no increase in macrophages numbers and no inflammatory cells are reported [16,17]. Changes in endothelial cell (EC) morphology, coupled with EC (valve EC [VEC]) loss are also reported [16,23,24]. Changes include increased expression of endothelin receptors, evidence of VEC activation, apoptosis, basement membrane loss and damage with cell detachment [25,26]. The interaction between the VECs and VICs in the context of the pathogenesis of MMVD is not understood, but the characteristic accumulation of activated myofibroblasts close to the endothelium and in particular areas of damaged endothelium suggest some sort of interaction does occur [26].

Of the studies to date, all have presented data from a mix of pedigree and cross-bred dogs. Considering the susceptibility of the CKCS to MMVD, the aim of this study was to determine if there are