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Galectin-3 in cardiac muscle and circulation of dogs with degenerative mitral valve disease

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Abstract *Objectives:* This study aimed to determine the association of cardiac fibrosis with the galectin-3 (Gal-3) expression, a fibrosis marker in the myocardium and to compare plasma Gal-3 levels in normal and degenerative mitral valve disease (DMVD) dogs.
Animals: Studies of muscle expression and plasma levels of Gal-3 were performed in separate groups of dogs. The tissue study was performed on cardiac tissues collected from 22 dogs. The plasma study was performed on 46 client-owned dogs.
Methods: Papillary muscle and left ventricular (LV) wall obtained from 10 normal and 12 DMVD dogs were stained with Masson trichrome and Gal-3 immunohistochemistry to determine fibrosis areas and Gal-3 expression. Plasma samples were collected from 19 normal and 27 DMVD dogs for Gal-3 measurement by ELISA.
Results: Percentage of fibrosis was higher in papillary muscle and LV wall of DMVD dogs (66.13 $\pm$ 5.58%; 52.98 $\pm$ 8.45%) than in normal dogs (35.40 $\pm$ 8.46%; 27.41 $\pm$ 7.91%; $p < 0.0001$). Gal-3 was higher in papillary muscle and LV wall of DMVD dogs (27.95 $\pm$ 6.94%; 17.25 $\pm$ 8.76%) than in normal dogs (1.08 $\pm$ 0.67%; 0.52 $\pm$ 0.42%; $p < 0.0001$). Fibrosis areas correlated strongly with the Gal-3 expression ($r = 0.821$, $p < 0.0001$). Plasma Gal-3 levels were increased in DMVD dogs (1.50; 0.87—2.36 ng/mL) compared to normal dogs (0.42; 0.27—0.63 ng/mL; $p < 0.0001$).

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Conclusions: Gal-3 expression in cardiac muscle was associated with cardiac fibrosis and was higher in DMVD dogs than in normal dogs. DMVD dogs had higher plasma Gal-3 concentrations than normal dogs. Tissue Gal-3 is a candidate of fibrosis biomarker in DMVD; however, further investigation of associations between plasma Gal-3 and myocardial fibrosis is necessary.

Abbreviations

CHF	congestive heart failure
CV	coefficient of variation
DMVD	degenerative mitral valve disease
ELISA	enzyme-linked immunosorbant assay
Gal-3	galectin-3
HRP	horse radish peroxidase
LA/Ao	the ratio of left atrium to aorta dimension
LV	left ventricular
LVEDd	left ventricular end diastolic diameter
MT	Masson trichrome
PIIINP	pro collagen type III amino-terminal propeptide
PBS	phosphate buffer saline
RPM	revolutions per minutes
SD	standard deviation

Introduction

Degenerative mitral valve disease (DMVD) is the most prevalent acquired cardiac disease and a common cause of left sided congestive heart failure (CHF) in adult small- to medium-sized dog breeds including Cavalier King Charles Spaniel, Miniature Poodle, Pomeranian, Chihuahua and Pekingese.[1] Degenerative mitral valve disease is a progressive degeneration of the mitral valve resulting in mitral regurgitation, cardiac volume overload, and cardiac structural remodeling. Echocardiography is a noninvasive technique used to assess the valve morphology, cardiac structural remodeling and function. However, echocardiography cannot directly evaluate the degree of myocardial fibrosis. Previous histopathological studies demonstrated collagen deposition in remodeled cardiac tissues of dogs affected with DMVD, similar to humans with mitral valve prolapse and other cardiovascular diseases.[2–6]

Histopathology is considered the gold standard for detecting cardiac fibrosis. However, this technique is not clinically practical. Measurement of a circulating biomarker of cardiac fibrosis might be a more suitable diagnostic tool. Currently, a few markers of cardiac fibrosis have been evaluated in veterinary medicine. These include matrix metalloproteinases, tissue inhibitors of metalloproteinases and procollagen type III amino-terminal propeptide (PIIINP). Matrix metalloproteinases and tissue inhibitors of metalloproteinases are nonspecific markers produced from several organs within the body.[7] PIIINP is widely used to detect cardiac fibrosis in humans. Several studies indicate that PIIINP may not be a good marker to determine the fibrosis in dogs with DMVD.[8,9]

Galectin-3 (Gal-3), a soluble β-galactoside-binding lectin, has been used as a biomarker of cardiac fibrosis in human patients. Gal-3 plays an important role in cardiac fibrosis by stimulating fibroblasts to change their phenotype to myofibroblasts and increase collagen synthesis.[10–13] Several studies demonstrated an up-regulation of Gal-3 in murine and human hearts with CHF.[10,13] Increased circulating Gal-3 concentration in humans directly correlated with the amount of collagen deposition in human hearts.[11] Circulating Gal-3 correlates with the incidence of CHF, disease progression and risk of mortality; and thus is an overall prognostic indicator for human patients with cardiovascular diseases.[11,14,15]

Gal-3 expression in the canine heart has not been studied and the potential of circulating Gal-3 as a biomarker for cardiac fibrosis in dogs with DMVD is not known. We hypothesized that Gal-3 would be up-regulated in cardiac muscle of dogs with DMVD and that its expression would correlate with the extent of cardiac fibrosis, similar to human patients affected with mitral valve prolapse or other cardiovascular diseases. We further hypothesized that Gal-3 would be increased in the plasma of dogs with DMVD compared to normal dogs. This study aimed to correlate the abundance of Gal-3 with the degree of cardiac fibrosis in cardiac muscle and to measure plasma Gal-3 in dogs with DMVD compared to healthy normal dogs.