A study of the cardiac effects of long-term high-dose prednisolone administration in dogs

Summary of Doctoral Thesis

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Hyperadrenocorticism (HAC) is recognized as a major endocrine disorder in dogs. Cushing syndrome (CS) caused by HAC in dogs presents with systemic changes due to excess cortisol, a glucocorticoid (GC) in the blood. It is known that in patients with HAC in human medicine, as in dogs, excessive production of cortisol causes systemic changes. The presence of left ventricular hypertrophy (LVH) and diastolic dysfunction (DD) has also been reported in human patients with HAC. In addition, histopathological changes in the heart of HAC patients have been reported to include hypertrophy of cardiomyocytes and increased collagen fibrosis in the myocardium. On the other hand, it has recently been reported in veterinary medicine that left ventricular wall thickness (ILVWT) and DD occur in HAC-affected dogs. It has also been reported that ILVWT and DD occurred in healthy beagle dogs treated with high dose (2 mg/kg, every 12 hours) of prednisolone, a synthetic GC preparation, for 28 days. Furthermore, left ventricular remodeling in human and canine HAC cases, has been reported to be affected by the duration of hypercortisolemia. However, to the best of our knowledge, the structural and functional effects of long-term prednisolone administration in dogs on the myocardium, histopathological changes in myocardial tissue, and the mitral valve tissue, the atrioventricular valve of the left heart system, have not been reported when hyperglucocorticism (HGC) is sustained for a long time. Therefore, the purpose of this study was to investigate the effects of long-term administration of high-dose prednisolone on the heart by evaluating the morphological and functional evaluation and the histopathological changes by administering high-dose prednisolone for 84 days.

Chapter 2

The effects of long-term prednisolone administration of HGC to dogs on cardiac

morphology and function had not been investigated. Therefore, we conducted a study to evaluate the morphological and functional effects of long-term prednisolone-induced HGC on the heart over time. The results showed that morphologically, ILVWT occurred, and functionally, left ventricular diastolic function was reduced. None of the common echocardiographic findings correlated with systolic blood pressure (SBP), and these results were consistent with recent reports in veterinary medicine. The new finding of a negative correlation between SBP and peak velocities of left ventricular myocardium at early diastole (Em) on the ventricular septal side, suggests that glucocorticoid-induced hyper blood pressure may have an effect on reduced left ventricular DD.

Chapter 3

Chapter 2 revealed that morphological and functional changes in the heart in dogs with sustained HGC caused by long-term prednisolone administration, but the causes of these changes were not clear. Therefore, in Chapter 3, we aimed to investigate the causes of ILVWT and DD in dogs with persistent HGC by examining the histopathological changes in the hearts of the model dogs created in Chapter 2 and correlating them with the results of Chapter 2. The results showed that the percentage of collagen fibers in the left ventricular free wall and ventricular septum. There was a positive correlation between the percentage of area occupied by collagen fibers in the ventricular septum and the end-diastolic ventricular septal wall thickness and left ventricular mass on echocardiography, and a negative correlation between the percentage of area occupied by collagen fibers in the ventricular septum and the Em on the ventricular septum side in the same dogs reported in Chapter 2. Therefore, it is speculated that left ventricular fibrosis may be associated as one of the causes of LVH and DD reported in HAC

dogs and in dogs receiving prednisolone experimentally. The percentage of glucocorticoid-receptor (GCR) in left ventricular myocardium was significantly decreased in prednisolone-treated dogs (P group) compared to healthy dogs (C group), and the mineralocorticoid receptor (MCR) was significantly increased in the P group compared to the C group. Thus, it is possible that excess GC downregulated the GCR, and more GC bound to and activated the upregulated MCR. Histological studies on mitral valve tissue, revealed possible histological changes in the P group, including decreased collagen fiber and acidic sulfated mucosubstances deposition and increased trabecular layer thickness with associated increased fat cell occupied areas. Decreased sulfated mucus material and collagen deposition may lead to mechanical abnormalities such as primarily decreased resistance to mechanical loading.

Chapter 4

Chapter 2 revealed that fibrosis of the left ventricular myocardium occurs in dogs with persistent HGC due to long-term prednisolone administration. In Chapter 4, we aimed to investigate the mechanism by which HGC causes fibrosis of the left ventricular myocardium. The results showed that the percentage of angiotensin II type 1 receptor (AT1R), 8-hydroxy-2′ -deoxyguanosine (8OHdG), and transforming growth factor β1 (TGFβ1) in myocardium and aorta in P group was significantly higher than that in group C. In the present study, the increase in AT1R in the myocardial tissues of the P group may have resulted in increased Angiotensin II stimulation, leading to an increase in 8OHdG, an oxidative stress marker. These results may support the hypothesis that in left ventricular myocardium in dogs with HGC, increased oxidative stress in the myocardium activates the GC-MCR complex, which increases the

expression of TGF-β1, a potent inducer in myocardial fibrosis, resulting in fibrosis. On the other hand, a significant increase in SBP at 84 days after the start of prednisolone medication in the P group, suggests that the increase in TGF-β1 expression in the myocardium may have been caused by increased Angiotensin II stimulation due to increased AT1R, which is associated with increased blood pressure. Although the mechanism of GC-induced hypertension has not been fully elucidated, it has been reported that increased oxidative stress in the blood of hypertensive rats plays an important role in the development of hypertension by causing vasoconstriction through inactivation of nitric oxide. Thus, it is possible that in the P group, the increase in vascular oxidative stress itself led to the increase in blood pressure, and the increase in TGF-β levels in the myocardium and aorta tissue that accompanied the increase in blood pressure.

In summary, this study was conducted to investigate the effects of HGC on the heart. The results indicate that HGC may cause ILVWT and DD associated with fibrosis of the left ventricle, and may be involved in the degeneration of mitral valve tissue. The results also revealed that the mechanism by which HGC induces myocardial fibrosis may involve increased Angiotensin II stimulation and oxidative stress. These results obtained in this study may contribute to the elucidation of the cardiovascular effects of HGC, following recent reports, and further research in this area is expected in the future.