Basic study on serum fatty acid compositions in dogs with mitral insufficiency

Summary of Doctor Thesis

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Mitral insufficiency (MI) is caused by myxomatous transformation degeneration, and is the most common chronic heart disease in dogs. A variety of supplements for the MI, have become popular, in addition to the general heart disease therapeutic agents. Products mainly composed of fatty acids are also used for MI patients, but studies on the pathogenesis of MI with blood fatty acid composition are small. Fatty acids are a major energy source for normal myocardium, which occupies 60-90% of the ATP production in the myocardium. However, if the heart muscle is subjected to a load, the energy is known to change to utilization of glucose from fatty acids. Changes in the fatty acid metabolism in heart failure caused by the aberrance of the regulation of calcium ion in myocardial cell construction and of the cardiac muscle cell membrane, by the accumulation of fat in the myocardium are considered as further worsen the heart failure.

The gas chromatograph (GC) and high-performance liquid chromatography are commonly used for the measurement of canine serum fatty acid. The GC method is suited with can be measured by small sample and the capacity of separation is high. However become this method requires the processes of extraction and methylation of the fatty acids from serum. These processes require specialized equipment and high-temperature heating. Because of compensation, serum fatty acid measurement has not become common in the veterinary field. This is one of the reasons, the relationship between serum fatty acid composition and

pathophysiology in dogs have not been investigated.

In this study we 1) examined the method with the fatty acid methylation kit and the methylated fatty acid purification kit, to measure serum fatty acids in dogs, 2) examined the circadian variation in serum fatty acids to determine the optimal blood sampling time point for measuring in healthy dogs, 3) set a criterion range for serum fatty acid compositions in healthy dogs and 4) compared serum fatty acid compositions in dogs with MI by the stage of the classification and then correlations between fatty acids and echocardiographic parameters were confirmed.

1. Methods for measuring canine serum fatty acids (Chapter 2)

We examined the practicality of fatty acid measurements by gas chromatography (GC) method using the kits for the methylated fatty acid and purification of methylated fatty acid. First, the results of this method were compared with the conventional method. Secondly, reproducibility of the within - run and between-run, and the inter class reliability was confirmed. As a result, a total of 13 kinds of fatty acids were quantifiable; the saturated fatty acids (SFA) 2 types, monounsaturated fatty acids (MUFA) 2 kinds and polyunsaturated fatty acids (PUFA) 9 types.

This method demonstrated a high correlation with all kinds of fatty acids between the conventional method (correlation coefficient: 0.875 to 1.000). Range of coefficient of variance (CV) in within-run reproducibility was 2.0% to 7.4%. In addition, CV in the

between-run reproducibility was 0.4% to 2.8%. Further, no significant differences were observed between examiners. The current method can be used safely and conveniently with high accuracy as compared with the conventional method.

2. Blood sampling point for measuring the serum fatty acid level of dogs. (Chapter 3)

We examined the circadian variation in serum fatty acids to determine the optimal blood sampling time point for measuring in healthy dogs. Six healthy male beagles were fed the same food that meets the criteria of the Association of American Feed Control Officials for more than 2 months. They were fed twice daily at 7:00 and 19:00. The blood samples collected immediately before food provision at 7:00 am (Pre) and every 3rd hour for 24 hours. The results indicate that the total MUFA of 3 hours after the Pre, the level of total n-9 fatty acid and oleic acid increased significantly ($P < 0.05$) than the Pre. There were no significant differences from Pre, however. In the n-3 fatty acid, the levels of α-linoleic acid (ALA) at 3 hours after the meal in the morning were significantly higher than the corresponding Pre levels ($P < 0.05$), and there were no significant difference of 6 hours or more after. In the serum fatty acid weight ratios, 3h and 6h eicosapentaenoic acid (EPA) and 3h docosapentaenoic acid (DPA) decreased significantly ($P<0.05$) than the Pre. There were no significant changes at 9 hours or more. These results indicate that the optimal timing of blood sampling is

when the animals are hungriest, i.e., before breakfast, and that it is desirable to interpose a 9-hour or more interval when sampling is performed after morning meal.

3. Setting a criterion range for serum fatty acid compositions in healthy dogs (Chapter 4)

The level, weight ratio and proportion of each serum fatty acids of 105 clinically healthy dogs were examined for setting criterion range. The dogs were divided into groups of puppy, young adults and mature adults. Further, these groups were subdivided into an uncastrated male group, a castrated male group, an unsterilized female group, and a sterilized female group. The variable factors of fatty acids were the technical, the intraindivisual and interindivisual. By excluding factor of the blood sampling point, sexual cycle and dietary habits no significant differences by age or sex were observed in the level, weight ratio and the ratio of serum fatty acids. Therefore, the criterion range of the fatty acid level was considered to be fixed regardless of age or sex. However, in order to compare the serum fatty acids of individuals, it is necessary to confirm the effects of various fluctuation factors. Because of the small number of dogs used in this study, further data needs to be accumulated to determine the effect of food and breed. Therefore, these 95% intervals were to be used as a reference range in the present study.

4. Serum fatty acid compositions in dogs with MI (Chapter 5)

We divided 30 dogs with MI into groups I, II, and III, based on MI severity, based guideline with the International Small Animal Cardiac Heart Council and compared levels, weight ratios and the ratios of serum fatty acid among these groups and a healthy control group. The changes in serum fatty acid composition of MI dogs were examined and compared. In addition, correlations of serum fatty acid compositions with echocardiographic parameters in dogs with MI were analyzed. In order to differentiate healthy dogs and dogs with MI, we analyzed and determined the cutoff values, EPA and the ratio of EPA and arachidonic acid (EPA/AA) by the ROC. As a result, arachidonic acid (AA) level in groups I and II were significantly lower than that in the healthy group ($P < 0.01$, $P < 0.05$, respectively). Serum level and weight ratio of EPA in groups II and III were significantly lower than that in the healthy group ($P < 0.05$, $P < 0.01$, respectively). In addition, the EPA / AA ratio in groups II and III were significantly lower than that in healthy group ($P < 0.05$, $P < 0.01$, respectively). In group I, with low AA, the structural change of myocardium activated AA metabolism and in group II, the decrescence of serum EPA and AA levels is caused by the metabolic activation of EPA following AA. These changes might be concerned in the cytokines as tumor necrosis factor-alpha and interleukin 1 that increased in chronic heart failure. Furthermore, the decrease of AA in group III is thought to be caused by the activation of EPA metabolism by these cytokines.

Significant positive correlations of serum AA and docosatetraenoic acid levels by the left ventricular end-diastolic diameter index were noted in the MI group. A significant negative correlation was noted between the DPA level and fractional shortening (FS). The DPA weight ratio had a significant negative correlation with both left atrial to aortic root ratio (LA/Ao) and FS. Furthermore, a significant negative correlation was noted between EPA/AA and LA/Ao. From these results, the expansion of the left ventricle increases the n-6 fatty acids in the serum fatty acid that are substrates of pro-inflammatory eicosanoids. Additionally, increase of FS and LA/Ao that suggested to develop of mitral regurgitation, decrease n-3 fatty acids and EPA/AA which is a substrate of anti-inflammatory eicosanoids. Therefore, serum fatty acids suggested that reflect changes in myocardial energy metabolism associated with the progress of the heart failure.

Then, the cutoff value of EPA level, determined to timing of administration of the fatty acid supplements used as a therapeutic adjunct to MI, was 47.5 μg/mL, that sensitivity and specificity were both 83.33%. On the other hand, the cutoff value of EPA/AA was 0.029, with sensitivity and specificity of 83.33% and 66.67%, respectively. Therefore, these values were likely to be the indicative of the timing of treatment of EPA.

The GC method with kits for the purification of methylated fatty acid and methylated fatty acids proved to be a stable and

convenient method, with comparable accuracy of the conventional method, for measuring the fatty acid composition in the blood that is to be associated with the pathology of MI. The recommended timing of blood sampling for measuring the serum fatty acids is in the morning feeding before or at least 9 hours thereafter. The reference value of serum fatty acids of healthy dogs, age or sex, showed no significant difference. Further studies with more subjects and different breeds are recommended. The serum fatty acid composition in dogs with MI was different from the healthy dogs, and it the reflected the grade of cardiac function. The correlation between fatty acid ratio and the echocardiographic parameters were observed. It was suggested that changes in serum fatty acid value reflect the abnormal form of the myocardium. The cutoff values of EPA level and EPA/AA were likely to be the indicative of the timing of treatment of EPA. We need to find out the association of clinical effect and serum fatty acid composition by the administration of EPA based on these indicative.