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Histopathological changes in the pancreas due to decreased pancreatic blood flow in a canine tachycardia-induced cardiomyopathy model

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ABSTRACT. In dogs, pancreatic acinar cell injury is thought to be caused by decreased pancreatic blood flow due to heart failure. In previous our report, it demonstrated that decreased heart function causes a significant decrease in pancreatic blood flow in heart failure dog model caused by rapid ventricular pacing (RVP). However, the types of histopathological changes remain unclear. We aimed to verify the types of histopathological changes occurring in the pancreatic tissue due to decreased heart function. After RVP for 4 weeks, atrophy of pancreatic acinar cells, characterized by a decrease in zymogen granules, was observed in all areas of the pancreas. In conclusion, the result of this study suggests that attention should be paid to ischemia/hypoperfusion injury in the pancreas.

KEY WORDS: atrophy, dog, heart failure, ischemia, pancreatic acinar cell

Heart failure is a clinical syndrome characterized by edema, dyspnea, and fatigue, caused by the breakdown of the compensation mechanism due to reduced function of the cardiac pump and structural and/or functional cardiac abnormalities [8]. In particular, when cardiac output is decreased due to contractility dysfunction, the heart is unable to supply enough blood to peripheral tissues and various organs, thereby causing various disorders due to hypoperfusion and ischemia [1, 9, 10].

Previously, we have evaluated and reported changes in cardiac function and pancreatic blood flow using heart failure model dogs by performing rapid ventricular pacing (RVP). As a result, 4 weeks after the start of RVP (4W), the left ventricular fraction shortening decreased by approximately 70% (mean ± standard deviation. baseline vs. 4W; 38.5 ± 5.2% vs. 10.7 ± 2.3%), cardiac index decreased by approximately 25% (4.4 ± 0.5 l/min/m^2 vs. 3.2 ± 0.8 l/min/m^2), and mean blood pressure decreased by approximately 30% (102.7 ± 8.8 mmHg vs. 74.0 ± 10.2 mmHg), compared to that before the start of RVP (baseline). Furthermore, when pancreatic blood flow was measured using contrast-enhanced ultrasonography, a decrease of about 50% in pancreatic blood flow was observed using the area under the curve, compared to that at baseline (369.7 ± 129.0 vs. 188.7 ± 99.7) [14]. However, the pathological changes caused in the pancreatic tissue by reduced blood flow remain unclear.

In this study, we aimed to verify by optical microscopy the types of histopathological changes occurring in the pancreatic tissue due to decreased pancreatic blood flow. For this purpose, we used dogs with heart failure induced by RVP for 4 weeks. This study is a further study of our preceding article, which demonstrated that pancreatic blood flow decreased due to decreased cardiac function, and some of the data related to this study have already been published [14].

The method of creating heart failure model, the method of evaluating cardiac function and pancreatic blood flow, and those results will be omitted since this study was performed using the same animals as in the preceding article [14]. RVP was initiated with 4 volts of stimulation, at a frequency of 260 beats per min, for 4W. This prospective study was conducted according to the regulations of the Animal Experimentation Committee of the Tokyo University of Agriculture and Technology, Tokyo, Japan

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At baseline, a portion of the left or right lobe of the pancreas was randomly sampled 1 cm from its margin under laparotomy during pacemaker implantation (right lobe: four dogs; left lobe: three dogs). At 4W, general anesthesia was performed; the animals were then euthanized with pentobarbital sodium by intravenous injection (Somnopentyl®, Kyoritsu Seiyaku Corp., Tokyo, Japan), and the whole pancreas was removed. These tissues were fixed with 10% formalin neutral-buffer solution.

These tissues were embedded in paraffin wax by routine histological methods and sectioned at a thickness of 2 µm. The prepared paraffin sections were stained with hematoxylin and eosin, and histopathological examination of the pancreatic tissue was performed using an optical microscope. At each site, the degree of zymogen granules in pancreatic acinar cells was scored. Scoring was performed by examining 20 consecutive adjacent areas using a 40 × objective lens: "0" for less than 5% pancreatic acinar cells containing zymogen granules, "1" for 5–25%, "2" for 26–50%, "3" for 51% or more. At 4W, each site in the right lobe, left lobe and pancreatic body was scored, and the average value was measured.

For immunohistochemistry, paraffin sections were deparaffinized and then immersed in a freshly made solution of 0.3% hydrogen peroxide in methanol for 30 min to block endogenous activity. Sections were then treated with 10% normal goat serum for 30 min to block non-specific reaction. Anti-trypsin antibodies (×50, rabbit monoclonal, Abcam Plc, Cambridge, UK), which also reacts with trypsinogen in pancreatic acinar cell, were applied as the primary antibodies and incubated overnight at 4°C. Each section was then incubated in Envision solution (Dako, Glostrup, Denmark) against rabbit immunoglobulin G for 30 min at room temperature. Tris-buffered saline (0.15 M NaCl, 0.05 M Tris-HCl, pH 7.6) was used for rinsing. Antibody binding was visualized using 3.3-diaminobenzidine chromogen and counterstained with Mayer's hematoxylin.

As for the level of zymogen granules, histological scoring data at baseline and after 4W were expressed as mean ± standard error. The normality of data distribution was analyzed using the Shapiro–Wilk test. The statistical analysis was performed using Wilcoxon signed-rank test. All statistical analyses were performed using statistical software (BellCurve for Excel, Social Survey Research Information Co., Ltd., Tokyo, Japan). A $P<0.05$ was considered statistically significant.

In the baseline preparations of the pancreas, there were no abnormal histopathological findings. In the 4W preparations, although the degree was not uniform among individual dogs, atrophy of pancreatic acinar cells, with a decrease in zymogen granules, was observed in all areas of the pancreas in all 7 dogs (baseline vs. 4W; 2.7 ± 0.0 vs. 1.3 ± 0.0, $P<0.01$) (Fig. 1). In addition, there was no difference in each site regarding the extent of the lesion. There were no congestive findings in the pancreas, such as dilation of capillaries or leak of blood components.

Immunohistochemically, at baseline, a trypsinogen-positive reaction was observed in the zymogen granule region of the pancreatic acinar cells, which was confirmed by hematoxylin-eosin staining. However, at 4W, a tendency to attenuate the trypsinogen-positive reaction was observed in all 7 dogs (Fig. 2).

In this study, atrophy of pancreatic acinar cells with a decrease in zymogen granules was observed in the entire pancreas in all dogs, which were experimentally made to have low-output heart failure. Diseases in which atrophy of pancreatic acinar cells are observed include chronic pancreatitis and exocrine pancreatic insufficiency [3, 4]. In particular, in addition to atrophy of pancreatic acinar cells, chronic pancreatitis is characterized by infiltration of inflammatory cells and fibrosis [3]. Previously, it has been reported that histopathological findings closely resembling chronic pancreatitis, such as infiltration of inflammatory cells and fibrosis following atrophy/shedding of pancreatic acinar cells, were observed in a pancreatic chronic ischemic dog model created by ligation/cutting of the pancreatic branch that flows into the left lobe of the pancreas [13]. Furthermore, these pathological changes such as atrophy/shedding of pancreatic acinar cells are enhanced over time [13]. Therefore, it is considered that the atrophy of pancreatic acinar cells observed in this study is in the initial stage of infiltration of inflammatory cells and fibrosis. Furthermore, if the duration and/or degree of further ischemia is severe, the pathological changes are considered to be more severe and clinical symptoms also manifest.

Various digestive enzymes are abundantly stored in the inactivated form in the zymogen granules in pancreatic acinar cells [5]. Among them, trypsinogen, which is an enzyme precursor of trypsin, which has a proteolytic action, is one of the important enzyme precursors responsible for the exocrine function of the pancreas [5]. In this study, as an immunohistochemical result, a tendency to attenuate the trypsinogen-positive reaction was observed. Therefore, this reaction observed by immunohistochemistry supported the decrease of zymogen granules in pancreatic acinar cells observed by hematoxylin-eosin staining.

Similar pathological findings were clearly observed in all 7 dogs this time, but with a degree of difference. This could be due to the individual differences in the resistance of pancreatic acinar cells to decreased blood flow. The possibility of a postprandial physiological decrease in zymogen granules is negative as sampling of the pancreatic tissue was performed under fasting conditions.

The present study had several limitations. First, in this study, the decrease in cardiac output reduced blood flow to the pancreas and pathological changes in pancreatic acinar cells were observed; however, the hypoxic state of the tissue could not be verified. Therefore, the relationship between decreased blood flow and pancreatic acinar cell atrophy can be evaluated by measuring the partial pressure of oxygen in the pancreatic blood vessels and pancreatic tissue by blood gas analysis. Second, in this study, partial resection of the pancreas for sampling at baseline was performed under general anesthesia. Appropriate biopsy of the pancreas has not been shown to influence histopathological effect in the pancreas of healthy dogs, except for transient mild inflammation during the healing process at the biopsy site [6]. However, since the occurrence of surgery-related pancreatic acinar cell injury has been reported in dogs, this effect cannot be completely ruled out [7, 11]. Third, the type of heart failure adopted in this study was low-output heart failure, and the hemodynamics of the test animals were clinically very similar to that seen in dilated cardiomyopathy [12]. The most common type of heart disease in dogs is mitral regurgitation due to myxomatous mitral valve disease (MMVD).

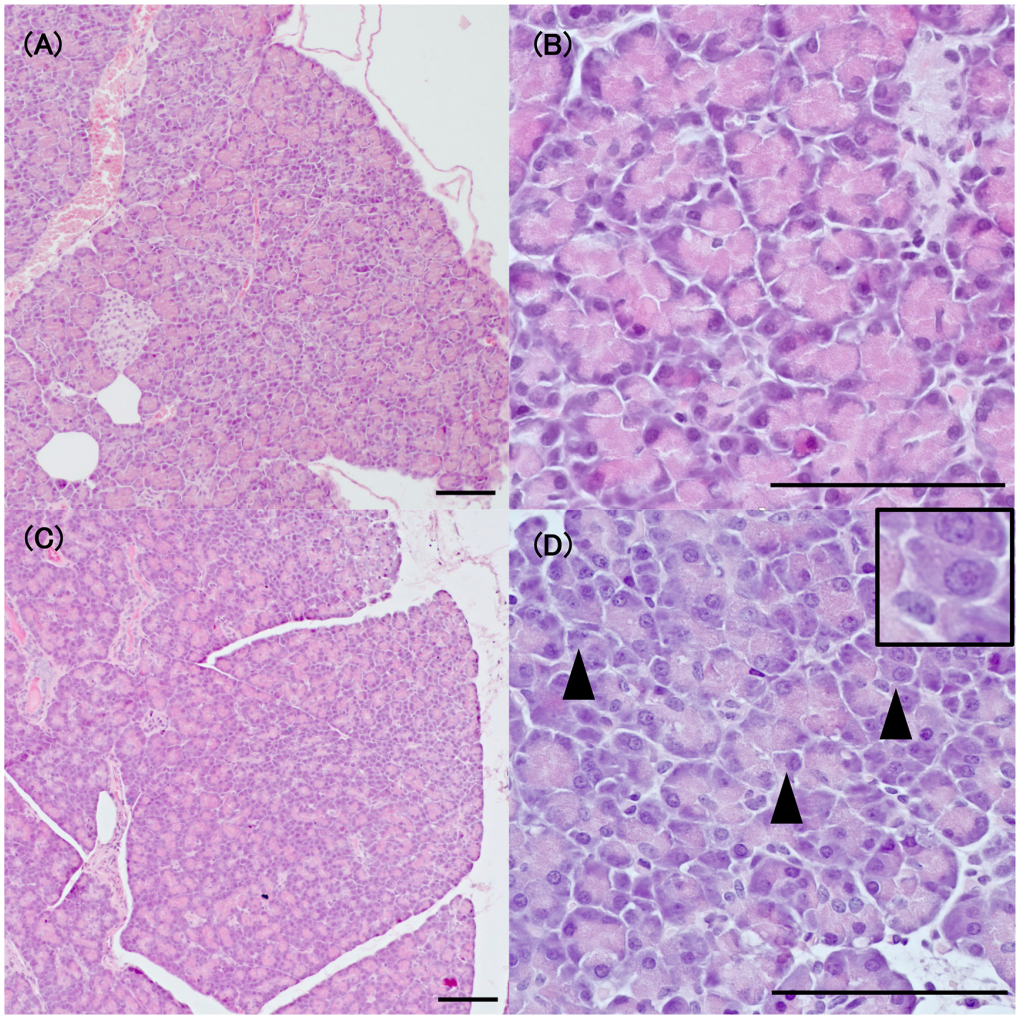

Fig. 1. Representative histopathological image in pancreas. (A), (B): Before the initiation of rapid ventricular pacing. Eosinophilic zymogen granules are found in the cytoplasm in the pancreatic acinar cell. (C), (D): 4 weeks after the initiation of rapid ventricular pacing. Atrophy of pancreatic acinar cells, characterized by a decrease in zymogen granules, is observed in all areas of the pancreas when compared with baseline (arrowheads). Inset showed atrophic acinar cells at high magnification. Bars=100 µm.

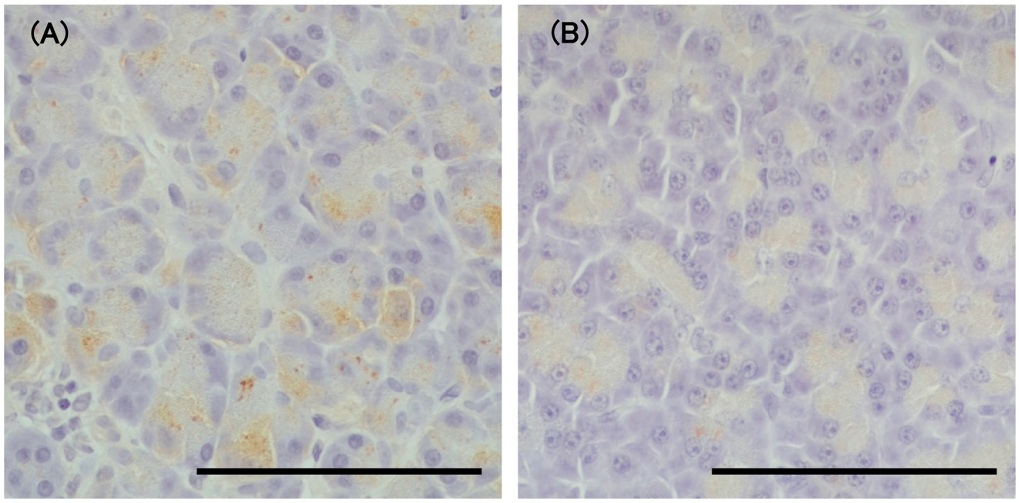

Fig. 2. Representative immunohistochemical image in pancreas. (A): Before the initiation of rapid ventricular pacing. A trypsinogen-positive reaction was observed in the zymogen granule region in the pancreatic acinar cells. (B): 4 weeks after the initiation of rapid ventricular pacing. A tendency to attenuate the trypsinogen-positive reaction was observed. Bars=100 µm.

MMVD has a chronic course, and cardiac output is said to decrease after a much later stage [2]. Therefore, it is necessary to carefully extrapolate the results of canine MMVD patients. Finally, our study included only a small number of animals. Therefore, it is necessary to conduct further studies in the future.

In conclusion, it was demonstrated that decreased pancreatic blood flow due to cardiac dysfunction caused histopathological changes to pancreatic acinar cells in the form of atrophy, characterized by decreased zymogen granules. The result of this study supports the hypothesis that heart failure has a direct effect on pancreatic acinar cells. This study provides a useful basis for designing further studies in this field.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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