ABSTRACT. We studied the relationship between the degree of mitral protrusion and degree of mitral regurgitation in an experimental model in which the degree of mitral protrusion could be adjusted. The model was developed by dissecting the dorsal papillary muscle through a left atriotomy in 5 dogs and re-attaching the papillary muscle to the original site using a single mattress suture threading through the epicardium under cardiopulmonary bypass. By manipulating the suture from a position outside the epicardium, the degree of mitral protrusion could be adjusted. The long-axis view of the mitral valve was imaged by B-mode echocardiography with the transducer placed directly over the surface of the right ventricular outflow tract. The height (H) from the coaptation point or tip of the protruded cusp in relation to the mitral annular plane was measured as an index of mitral protrusion. Mitral regurgitation as a result of the mitral protrusion decreased the left ventricular systolic pressure, and increased the heart rate, mean left atrial pressure (LAPm), and ratio of left ventricular end-diastolic dimension to body weight (LVEDD/BW). H was negatively correlated to LAPm and LVEDD/BW ($r = -0.723$ and $-0.697$, respectively). Our results indicated that H expresses not only the degree of mitral protrusion but also the degree of mitral regurgitation, and were in agreement with the previous findings obtained on dogs with spontaneous mitral regurgitation.

KEYWORDS: B-mode echocardiography, canine, mitral regurgitation, mitral valve prolapse.

We found that the mitral valve in individual dogs with various stages of spontaneous mitral regurgitation protrudes to different degrees toward the left atrium in systole as observed by B-mode echocardiogram [6]. Autopsy studies [8, 12] have revealed, that dogs with endocardiosis have thickened chordae tendineae and valvular cusp. In the most progressive lesions there is gross distortion of the cusp, and the free edge of the valve may protrude from the ventricular side into the left atrium. This abnormality of the mitral apparatus is described as “ballooning” or “billowing” and often accompanies rupture of chordae tendineae [1, 3, 10]. Clinically, in such terminal stage of valvular disease, the mitral valve may show protrusion in systole on B-mode echocardiographic images. In spite of this clinical observation, there had been no reports regarding mitral valve protrusion in individual dogs in different disease states or with varying degrees of protrusion.

We also determined that with the progression of the degree of mitral protrusion, clinical and radiographic findings of the diseased state are prominent and the left atrial and ventricular dimensions increase. In the dogs with spontaneous mitral regurgitation, we estimated the degree of mitral regurgitation by measuring the dimensions of the left heart chambers using echocardiography. The changes of the cavities are regarded as consequences from compensatory function for the chronic volume load and the dimensions should secondarily express the degree of mitral regurgitation [2]. From our results, we theorized that the degree of mitral valve protrusion might relate to the hemodynamic condition in mitral regurgitation. In the natural disease state, mitral regurgitation in the dog is principally caused by morphologic changes of the mitral apparatus resulting from endocardiosis [1, 3, 5, 10, 12]. Perhaps the index of mitral protrusion obtained from mitral echocardiography may reflect the degree of mitral regurgitation in such cases.

In this study, we measured the acute changes of left atrial and ventricular pressure, after creating mitral regurgitation caused by mitral protrusion to confirm the relationship between the degree of mitral protrusion and degree of mitral regurgitation. In order to evaluate the relationship, we utilized a surgically prepared experimental model of mitral valvular disease in dogs. The model was specifically designed to reproduce changes of the mitral apparatus which are similar to those seen in dogs with the natural disease. In this model the degree of protrusion of the septal cusp of the mitral valve was designed to be adjustable, since most dogs with mitral regurgitation have more severe mitral valve protrusion of the septal cusp than of the parietal cusp based on observations by B-mode echocardiography [6].

MATERIALS AND METHODS

Animal preparation: Mongrel dogs were donated by a local humane society administered by a local government.
hemodilution with lactated Ringer’s solution and dextran 40 and recipient hematocrit was decreased to 25% by To optimally maintain microcirculation, the combined donor each dog, catheters were inserted via the femoral artery and with venous infusion of ketamine hydrochloride (0.4%). In premedication, and was maintained by administration of sodium thiopental (10 mg/kg, i.v.) 15 min following last thereof. Anesthesia was induced by intravenous injection of chlorpromazine hydrochloride (1.0 mg/kg, i.m.) and promethazine hydrochloride (0.3 mg/kg, i.m.) 15 min thereafter. Anesthesia was induced by intravenous injection of sodium thiopental (10 mg/kg, i.v.) 15 min following last premedication, and was maintained by administration of halothane in the recipient. Donor anesthesia was maintained with venous infusion of ketamine hydrochloride (0.4%). In each dog, catheters were inserted via the femoral artery and vein for monitoring of arterial and central venous pressure. To optimally maintain microcirculation, the combined donor and recipient hematocrit was decreased to 25% by hemodilution with lactated Ringer’s solution and dextran 40 (10 m/l/kg). In the recipient, a median sternotomy incision was made and heparin (180 IU/kg, i.v.) was administered before cannulation. The heart was supported in a pericardial cradle. Two venous cannulae were inserted via the right atrial auricle into the cranial and caudal vena cavae, and an aortic cannula was inserted into the ascending aorta. A venous cannula was inserted into the left jugular vein of the donor and subsequently connected to the recipient venous cannulae with a clamped tube. An arterial cannula was inserted into the left carotid artery of the donor and similarly connected to the recipient aortic cannula.

The venous volume in the recipient was monitored by measuring the central venous pressure during cross circulation, and venous flow was drained by gravity to the donor. The ascending aorta was cross clamped, and hypothermic cardioplegic arrest initiated. In this bypass, the recipient circulation was provided by the donor circulation. The mitral valve was exposed via a left atriotomy, and the dorsal papillary muscle was partially transected at the insertion site of the chordae tendineae from the septal cusp. The transected papillary muscle was then re-attached at the original site by a single mattress suture with No.1 nylon suture using straight needles, as illustrated in Fig. 1. To prevent tearing of the resected muscle due to force at the suture site during systole, the suture was entwined around the base of the chordae tendineae. On the pericardial side, the sutures were threaded through a plastic button, with the suture ends left loose for manipulation. Prior to closure of the atriotomy, the suture was pulled toward the epicardial side tightly enough to appose the papillary muscle and prevent any mitral regurgitation while weaning the animal from the bypass system. After adjustment of the dorsal papillary muscle position, the suture was fixed in place by clamping a mosquito forceps on the button, taking care not to injure the myocardial wall with its tip. After weaning from cross circulation, all cannulae were removed and the recipient received protamine sulfate (2.5 mg/kg, i.v.) to reverse the effect of heparin.

Measurements: Stable circulatory conditions were observed for 30 min after the preparation was complete. High-fidelity microtip catheters (Millar Instruments) were introduced into the left atrial and the left ventricular cavity through a small stab incision at the left atrial auricle and the apex for recording of mean left atrial pressure (LAPm) and left ventricular pressure. From the traces of left ventricular pressure, systolic pressure (LVPS), and end-diastolic pressure (LVEDP) were measured. Heart rate was analyzed by electrocardiography from lead II. All echocardiographic studies were performed using a commercially available system (Hitachi, EUB-165) with a 7.5 MHz transducer. B-mode echocardiographic images were obtained with the transducer placed directly over the epicardial surface with care taken not to induce arrhythmia. The mitral valve was imaged in the left ventricular long-axis view with the transducer adjacent to the surface of the right ventricular outflow tract. The image was compatible with the right parasternal long axis view used in our previous study [6]. We obtained the echocardiogram of the mitral valve which showed maximal protrusion of the mitral tip. The images of the mitral valve were recorded at 116 frames/sec by reducing the sector angle, temporarily stored in the computer.
cine-memory pre-installed in the system, and recorded with an S-VHS video tape recorder for off-line analysis. The analysis of the mitral echo was performed on play-back view using the same system. The method for measuring the mitral echo was similar to that in our previous study [6]. The dimensions of the mitral apparatus were measured at the time of the S wave on the echocardiogram. The mitral annulus was identified as the hinge point of the mitral cusp using the cine-memory, and the mitral annular diameter (MAD) was measured (Fig. 2-A). A straight line was then drawn between the mitral annulus and was identified as the mitral plane on display. We measured the dimension from the coaptation point of the cusps to the mitral plane (H). When each cusp did not coapt due to protrusion, H was measured from the tip of the protruded cusp (the septal cusp) to the mitral plane, even if the body of mitral valve protruded more than its tip (Fig. 2-B). When the tip of the leaflet was located beyond the mitral plane and extended into the left atrium, H was expressed as a negative value (Fig. 2-C). The value of H was used as an index of the degree of mitral protrusion.

The left ventricular end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were measured on the long-axis view of B-mode echocardiographic images, and LVEDD/BW and LVESD/BW were derived by dividing by the body weight (BW).

Protocol: For all measurements, control values were first obtained under the control condition of absence of mitral regurgitation by securing the suture firmly against the epicardium. Absence of mitral regurgitation was confirmed by color Doppler echocardiography, and by phonocardiography with a microphone placed on the left atrial surface. After measurement of control values, mitral protrusion and regurgitation was produced by loosening the suture. Simultaneously, observations were made of the mitral valve and regurgitant jet in the left atrium by color Doppler echocardiography. After the degree of mitral protrusion was adjusted, the suture was secured with forceps. All measurements were repeated in the presence of mitral regurgitation, then the mitral valve was returned to its normal position. A recovery period of 10 min was then provided. Serial data were collected by obtaining repeated recordings (from 5 to 8 times) in each dog under various degrees of mitral protrusion. Both the donor and recipient were euthanized immediately after completion of all measurements.

Statistical analysis: Paired values obtained under control and mitral regurgitation conditions were statistically analyzed by Student’s t-test. Linear regression analysis was determined using the least squares method. A p value < 0.05 was regarded as indicative of statistical significance. Data are presented as the means ± standard deviation (SD).

RESULTS

Manipulation of the suture was confirmed to result in adjustment of the degree of mitral protrusion (Fig. 3) in all dogs. Moreover, upon withdrawal of the suture, the mitral cusp were normally coapted, and consequently mitral regurgitation completely disappeared. The regurgitant jet,
which was observed by color Doppler echocardiography during loosening of the suture, was directed from the protruded cusp toward the lateral wall of the left atrium.

Height (H) and mitral annular diameter (MAD) when measured in the control state were $3.4 \pm 0.7 \text{ mm}$ and $14.7 \pm 1.3 \text{ mm}$, respectively. By loosening the suture, and thus allowing mitral valve protrusion, H decreased to $0.2 \pm 1.3 \text{ mm}$, and MAD increased to $16.5 \pm 1.8 \text{ mm}$ as a result of the regurgitant volume. The percent ratio of H to MAD decreased from $23.5 \pm 4.1\%$ to $1.7 \pm 7.8\%$.

Under the condition of mitral valve protrusion, in the presence of mitral regurgitation, heart rate, LAPm, LVEDP and LVEDD/BW were significantly increased (p<0.01) and LVPS significantly decreased (p<0.01, Table 1). LAPm and LVEDD/BW were negatively correlated to H ($r=-0.723$ and -0.697, respectively; Figs. 4 and 5) and LVPS positively correlated to H ($r=0.508$). LVESD/BW did not show a significant difference from the control values.

**DISCUSSION**

Previously, we found that left atrial and ventricular dilatation progresses with the degree of mitral valve protrusion (H) in dogs with mitral regurgitation [6]. Chamber dilatation of the left heart was revealed by an increase in the proportion of end-diastolic left atrial dimension to aortic dimension (LAD/AoD) and LVEDD/BW. The left heart dilatation may be caused by the volume overload of mitral regurgitation. These findings suggest that the value of H correlates with the degree of mitral regurgitation. To confirm the relationship between H and the degree of mitral regurgitation, further investigation under acute phase of mitral regurgitation is required using more direct hemodynamic indices. In this study, left atrial and ventricular pressure were measured as more definitive indices of hemodynamic consequences than left ventricular echocardiographic dimensions.

As H decreased LAPm and LVEDD/BW increased due to the left-sided volume overload of mitral regurgitation. H was negatively correlated to LAPm and LVEDD/BW. The results of this study correspond to the outcome of our previous study which suggested that the measurement of H values may be useful in the assessment of the degree of mitral regurgitation in dogs with spontaneous disease. Of the measurements, left atrial pressure in particular should indicate the relative change of mitral regurgitant volume in this acute model, because the left atrium of the normally loaded heart is less compliant than that in chronic mitral regurgitation [4]. The increase in left atrial pressure therefore depends on the volume load of the left atrium in the acute phase of mitral regurgitation [9].

This model differs from dogs with spontaneous mitral regurgitation by the absence of thickening along the mitral leaflets. Although mitral valve thickening, which exists with spontaneous mitral protrusion may yield a complex effect in the etiology of mitral regurgitation, the relationship between the degree of thickening and mitral regurgitation is currently unknown. Moreover, generally, it is difficult to clinically evaluate degree of mitral thickening even using echocardiography. Some of the reasons for the difficulty are that the mitral thickening shows a complex shape on the echocardiogram and as a result of the insertion of the chordae tendineae on the edge and ventricular surface, it may often be hard to distinguish the lesions from such attachments of the chordae tendineae, especially in mild mitral lesions [6]. For these reasons, in our previous study, H was measured arbitrarily from the midpoint of the thickened valve leaflets. However, in spite of the lack of the mitral valve thickening, the results in this study were similar to those in dogs with spontaneous mitral regurgitation. Thus, we conjectured that mitral protrusion, as one of the pathologic changes, is the major factor influencing the extent of mitral regurgitation.

This model also differs from the spontaneous disorder in

| Table 1. Hemodynamic values and left ventricular dimensions |
|----------------|--------------|
| Control        | MR           |
| HR             | 126.2 ± 12.9 | 133.1 ± 17.9** |
| LAPm           | 7.7 ± 1.6    | 13.5 ± 4.0***  |
| LVPS           | 99.1 ± 7.2   | 89.4 ± 9.0**   |
| LVEDP          | 3.7 ± 2.0    | 8.3 ± 4.2**    |
| LVEDD/BW       | 2.33 ± 0.21  | 2.79 ± 0.32**  |
| LVESD/BW       | 1.37 ± 0.18  | 1.39 ± 0.19    |

MR: Mitral regurgitation; HR: Heart rate (beats/min); LAPm: Mean left atrial pressure (mmHg); LVPS: Left ventricular systolic pressure (mmHg); LVEDP: Left ventricular end-diastolic pressure (mmHg); LVEDD: Left ventricular end-diastolic diameter (mm); LVESD: Left ventricular end-systolic diameter (mm); BW: Body weight (kg); LVEDD/BW: The LVEDD-to-BW ratio (mm/kg); LVESD/BW: The LVESD-to-BW ratio (mm/kg). ** p<0.01 vs. control.

Fig. 4. Relationship between H and mean left atrial pressure (LAPm).
that the protruding leaflet was limited to the dorsal half of the septal cusp due to manipulation of only the dorsal papillary muscle. This portion of the mitral valve is frequently subject to the degenerative changes observed in dogs with spontaneous disease, but as a matter of course, the changes may also be simultaneously observed in the parietal cusp [5]. In our previous study, the septal cusp in most dogs had more severe mitral protrusion than the parietal cusp as observed by B-mode echocardiography. Using color Doppler echocardiography, the mitral regurgitant jet was eccentric and was directed from the protruding septal cusp toward the lateral wall of the left atrium in dogs with spontaneous valvular disease [6]. The distinct direction of the jet results from the creation of a regurgitant orifice by an asymmetric gap between the edges of the valve cusps. In this model, the direction of the regurgitant jet was similar to that in spontaneous cases, and the relationship between H and degree of mitral regurgitation was also the same as that in the dogs with spontaneous mitral regurgitation. Thus, we considered that measuring the degree of mitral valve protrusion at the point of maximal protrusion may be meaningful for evaluating the degree of mitral regurgitation.

We concluded that the H values, which show the degree of mitral protrusion, reflect the degree of mitral regurgitation in this experimental model. These findings are in agreement with the outcome of our previous study in dogs with spontaneous mitral regurgitation. Therefore, measurement of H values may provide useful information about the progression of both the morphological changes of mitral valve and degree of mitral regurgitation.

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