FULL PAPER *Surgery*

# Long-Term Clinical Evaluation of Mitral Valve Replacement with Porcine Bioprosthetic Valves in Dogs

Kazuaki TAKASHIMA[1)]*, Aiko SODA[2)], Ryou TANAKA[2)] and Yoshihisa YAMANE[1,2)]

*[1)]Animal Clinical Research Foundation, 214–10 Yatsuya, Kurayoshi, Tottori 682–0025 and [2)]Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai, Fuchu, Tokyo 183–8509, Japan*

(Received 13 July 2007/Accepted 28 November 2007)

ABSTRACT. This study evaluated the long-term clinical performance of newly developed porcine bioprosthetic valves cross-linked with glutaraldehyde and polyepoxy compound for mitral valve replacement (MVR) in dogs. Five beagle dogs underwent MVR using the porcine bioprosthetic valves during cardiopulmonary bypass. Antithrombotic drugs were administered only for one month after MVR. Six months after MVR, transvalvular regurgitation was not observed in all dogs, paravalvular leakage was seen only in one dog. Twelve months after MVR, mild transvalvular regurgitations were observed in two dogs. Although diastolic atrioventricular pressure gradient was increased gradually, no significant differences were observed. Pressure half-time and valve area were within normal ranges as the bioprosthetic value. There was no clinical symptom of the thrombosis and the thrombogenesis was not observed in the porcine bioprosthetic valve and the annulus in all dogs for twelve months after MVR. The clinical findings suggest that antithrombogenicity of the valves were maintained, though the duability might not be enough in the long-term period.

KEY WORDS: antithrombogenicity, bioprosthetic valve, canine, clinical evaluation, mitral valve replacement.

*J. Vet. Med. Sci.* 70(3): 279–283 2008

Mitral regurgitation (MR) is the most common acquired heart disease that increases with aging in dogs. MR is caused by myxomatous degeneration with disorganized collagen fibers in the mitral valve and increased content of acid mucopolysaccharide. In myxomatous degeneration, the valves are thickened and the valve leaflets become redundant; secondary dilation of the valve annulus contributes to MR. Progressive congestive heart failure and death are the inevitable consequences of severe MR despite any optimal medical treatment [13, 19].

Surgical treatment for MR has not been established in veterinary medicine, although radical operation is generally performed in human medicine. Mitral valve replacement (MVR) is one of the surgical treatments for MR, and bioprosthetic valves are recommended for dogs, because of their antithrombogenic property [24]. In human medicine, the xenograft valve is a mainstream of the bioprosthetic valve. There are different types of xenograft bioprosthetic valves that are routinely cross-linked during manufacturing using glutaraldehyde because of the antigenicity and sterility [4, 8]. Bioprosthetic valves have a possibility of prosthetic valve failure due to calcification, pannus formation and tissue degeneration; several reagents have been extensively investigated to inhibit them [5, 6, 18]. Polyepoxy compound (Denacol EX-313) was developed in order to reduce calcification, degeneration and thrombosis [17, 22].

In a previous study, the porcine bioprosthetic valves cross-linked with glutaraldehyde and polyepoxy compounds were newly developed for MVR. MVR procedures under cardiopulmonary bypass (CPB) and the use of the newly developed porcine bioprosthetic valves were shown to have been effective in dogs during short-term evaluation [21]. The aim of this study was to evaluate the long-term clinical performance of newly developed porcine bioprosthetic valves for mitral valve replacement (MVR) in dogs.

* CORRESPONDENCE TO: TAKASHIMA, K., Animal Clinical Research Foundation, 214–10 Yatsuya, Kurayoshi, Tottori 682–0025, Japan.
e-mail: takashima@ncn-k.net

## MATERIALS AND METHODS

*Animals*: Five apparently healthy one year-old male beagle dogs, weighing 10–14 kg were used in this study. The dogs were subjected to clinical examinations, complete blood counts, heartworm antigen tests, blood chemistry, thoracic radiography, electrocardiography, phonocardiography and echocardiography. No abnormal findings were observed in the dogs.

The dogs were housed in individual runs. Standard environmental conditions of 22–23°C ambient temperature and 40–50% relative humidity were maintained. The dogs were given balanced maintenance diets and fresh water. During all phases of this study, the laboratory animals were handled and care for in accordance with the standards established by the Tokyo University of Agriculture and Technology as described in its "Guide for the care and use of laboratory animals".

*Bioprosthetic valves and MVR*: All beagle dogs underwent MVR using the porcine bioprosthetic valves cross-linked with glutaraldehyde and polyepoxy compounds during CPB. The Porcine bioprosthetic valves were made from Yorkshire breed, 70–80 kg. The valves, with similar dimensions to that of canine mitral valves, were used (22.5 ± 0.9 mm). The valves and the protocols followed for MVR were in accordance with previous study [21, 26].

*Antithrombotic therapy*: Heparin sodium, 50–100 U/kg SC (TID-QID),was administered postoperatively. Oral

administration of dipyridamole, 150 mg/dog (SID), and warfarin were initiated 2–5 days after MVR, and administering of heparin sodium was halted. Warfarin was administered at 0.1 mg/kg (BID) and its dose was adjusted according to the results of the Thrombotest values of 20% [7]. The antithrombotic drugs were administered for only about one month after MVR.

*Clinical examination*: The examinations conducted included thoracic radiography, phonocardiography, electrocardiography, echocardiography and blood tests. Cardio thoracic ratio (CTR) and vertebral heart size (VHS) were calculated by thoracic radiography [13, 19]. Diastolic atrioventricular pressure gradients (Bernoulli equation) were obtained from the continuous-wave Doppler signal of mitral flow velocity [3]. Pressure-half time (PHT), which is the time interval during which the atrioventricular pressure gradient falls to half its initial value, was calculated from these tracings. Mitral valve area (MVA) was calculated by the continuity equation (MVA=220/PHT) [3]. Left ventricular end-diastolic diameter (LVEdD), left ventricular end-systolic diameter (LVEsD), left ventricular posterior wall thickness in diastole (LVPWd), left ventricular posterior wall thickness in systole (LVPWs), interventricular septum thickness in diastole (IVSd), interventricular septum thickness in systole (IVSs) and fractional shortening (FS) were obtained by M-mode echocardiography [3, 19].

The examinations were performed before MVR and seven days, six months and twelve months after MVR. Differences in the data were evaluated using one way repeated measures ANOVA followed by the Steel-Dwass multiple comparison test. P values less than 0.05 were considered statistically significant. The values at one week after MVR of the diastolic atrioventricular pressure gradients, PHT and MVA for prosthetic valve function, were established as the control and compared with values obtained six months and twelve months after MVR.

## RESULTS

In electrocardiography, no significant difference in R wave height in lead II was observed before MVR and seven days, six months and twelve months after MVR (2.08 ± 0.55 (mean ± SE)mV, 2.18 ± 0.24 mV, 2.42 ± 0.28 mV and 2.86 ± 0.29 mV, respectively), although the height tended to increase gradually. The mean electrical axis was not significantly different before MVR and seven days, six months and twelve months after MVR (38.0 ± 5.3°, 34.0 ± 4.2°, 34.4 ± 13.3° and 29.6 ± 6.8°, respectively) A slight systolic regurgitant murmur was observed in one dog at 6 months after MVR and was also seen in 2 dogs at twelve months after MVR.

In thoracic radiography, no significant difference in CTR was observed before MVR and seven days, six months and twelve months after MVR (62.96 ± 0.68%, 63.70 ± 1.76%, 60.06 ± 2.05% and 63.14 ± 1.27%, respectively ). VHS was not also significantly different between those observed before MVR and seven days, six months and twelve months after MVR (9.98 ± 0.16v , 10.04 ± 0.17v, 10.48 ± 0.26v and 10.78 ± 0.35v, respectively). CTR and VHS values were within normal ranges. Pulmonary edema was not observed six months after MVR, although slight pulmonary edema was seen in two dogs at twelve months after MVR. Blood examination values were not significantly different between groups and were within normal ranges in all examinations conducted.

In echocardiography, thrombogenesis was not observed in the bioprosthetic valves for twelve months. Six months after MVR, transvalvular regurgitation was not observed in all dogs; paravalvular leakage was observed in one dog. Twelve months after MVR, mild transvalvular regurgitations were observed in two dogs, one of two dogs had paravalvular leakage six months after MVR; however, prosthetic valve regurgitations were not observed in the other dogs (Fig. 1) Diastolic atrioventricular pressure gradient was not significantly different at seven days, six months and twelve months after MVR (14.02 ± 1.49 mmHg, 16.69 ± 1.38 mmHg and 27.96 ± 5.18 mmHg, respectively), though the values tended to increase gradually. PHT was not significantly different at seven days, six months and twelve months after MVR (99.20 ± 19.36 msec, 115.40 ± 18.20 msec and 89.60 ± 9.77 msec, respectively). MVA was not significantly different at seven days, six months and twelve months after MVR (2.68 ± 0.60$cm^2$, 2.13 ± 0.38$cm^2$ and 2.55 ± 0.23$cm^2$, respectively).

The LVEdD values before MVR and seven days, six months and twelve months after MVR were 33.68 ± 1.06 mm and 28.48 ± 1.31 mm, 37.20 ± 2.40 mm and 39.06 ± 2.41 mm, respectively. Significant difference ($P<0.05$) was observed between values seven days after MVR and six months after MVR, and seven days after MVR and twelve months after MVR (Fig. 2-A). The LVEsD values before MVR and seven days, six months and twelve months after MVR were 20.92 ± 0.68 mm, 16.80 ± 1.77 mm, 23.60 ± 2.73 mm and 24.20 ± 2.10 mm, respectively; no significant differences were observed (Fig. 2-B). The LVPWd values before MVR and seven days, six months and twelve months after MVR were 6.56 ± 0.19 mm, 10.56 ± 0.83 mm, 6.40 ± 0.75 mm and 7.04 ± 0.32 mm, respectively; significant differences ($P<0.05$) were observed between values before MVR and seven days after MVR, and seven days after MVR and twelve months after MVR (Fig. 2-C). The LVPWs values before MVR and seven days, six months and twelve months after MVR were 10.52 ± 0.71 m, 13.72 ± 1.19 mm, 10.00 ± 1.00 mm and 11.04 ± 0.58 mm, respectively; no significant differences were observed among the groups (Fig. 2-D). The IVSd values before MVR and seven days, six months and twelve months after MVR were 7.28 ± 0.66 mm, 10.36 ± 0.37 mm, 7.00 ± 0.63 mm and 7.48 ± 0.70 mm, respectively; no significant differences were observed among the groups (Fig. 2-E). The IVSs values before MVR and seven days, six months and twelve months after MVR were 10.44 ± 0.37 mm, 14.08 ± 0.87 mm, 10.20 ± 0.97 mm and 11.26 ± 1.22 mm, respectively; significant difference ($P<0.05$) was observed only between values before MVR

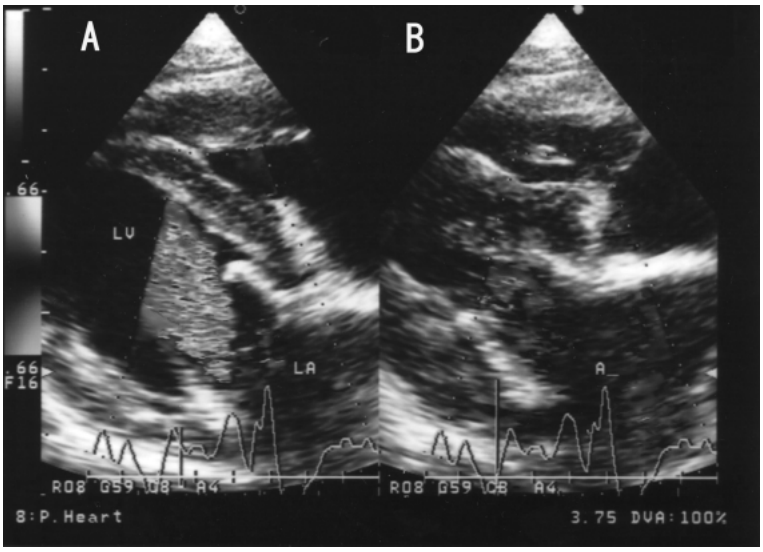


Fig. 1. Color Doppler of the porcine bioprosthetic valve flow of a dog 12 months after MVR (A. diastolic view; B. systolic view). MR and thrombogenesis were not observed.

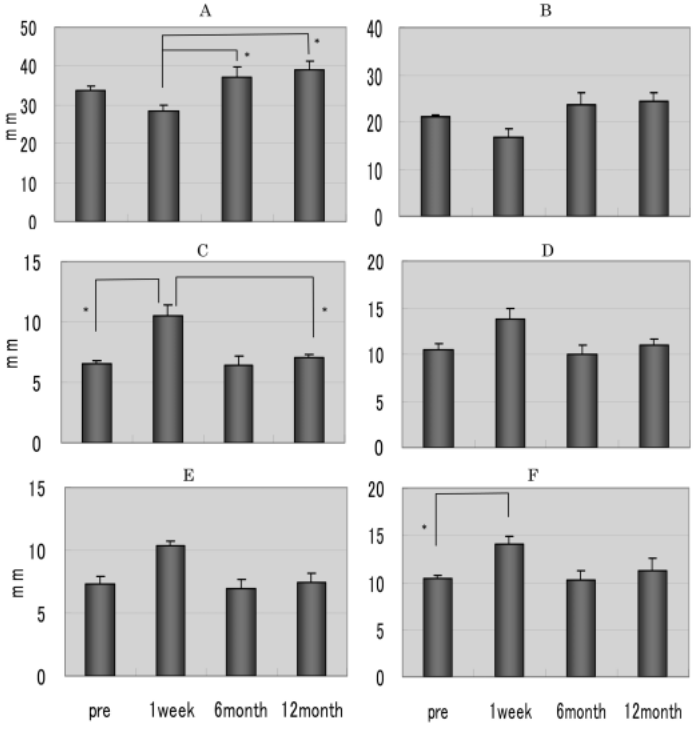


Fig. 2. M-mode echocardiography. (A) left ventricular end-diastolic diameter, (B) left ventricular end-systolic diameter, (C) left ventricular posterior wall thickness in diastole, (D) left ventricular posterior wall thickness in systole, (E) interventricular septum thickness in diastole, (F) interventricular septum thickness in systole. The data represent mean ± SE. *: significant difference ($P<0.05$).

and seven days after MVR (Fig. 2-F). The FS values before MVR and seven days, six months and twelve months after MVR were 36.94 ± 2.49%, 41.42 ± 4.08%, 36.60 ± 4.73% and 38.46 ± 1.70%, respectively; no significant difference was observed among the groups.

## DISCUSSION

Many types of xenograft bioprosthetic valves have been implanted in humans, including Hancock II bioprostheses (porcine bioprosthesis), Carpentier-Edwards bovine pericardial valves and Mosaic bioprosthesis (porcine bioprosthesis), and the long-term prognoses of these valves have been reported in human medicine [4, 8]. Glutaraldehyde is the most commonly used cross-linking reagent for biological tissue (bioprosthetic valves and vascular grafts) fixation. The cross-links reduce the antigenicity and biodegradability of the tissue, reduce its thrombogenicity and sterilize the tissue [23]. Bioprosthetic valves have a possibility of prosthetic valve failure due to calcification, pannus formation and tissue degeneration; several reagents have been investigated to inhibit them [5, 6, 18]. Polyepoxy compounds reduce calcification, degeneration and thrombosis of biological tissue and have lower cross-linking cytotoxic reaction [17, 22]. Previous studies reported the excellent durability of polyepoxy compound fixed bovine jugular vein graft for reconstruction of right ventricular outflow tract in dogs [15]. The canine aortic bioprosthetic valve was fixed in glutaraldehyde and an epoxy compound, and an apico-aortic valve conduit using the valve was found to be effective for surgical treatment of aortic stenosis in dogs [11]. The rate of thromboembolism (%/year) decreased significantly at each time interval (1 to 10, 11 to 90 and over 90 days) after operation for MVR (55%, 10% and 2.4%/year, respectively) in human medicine [10]. Short-term complications after MVR consists mainly of thrombosis that related to anticoagulation therapy, however, long-term complications are related to the durability and the antithrombogenicity of the bioprosthetic valve [20]. Therefore, as a first step, the performance of the newly developed porcine bioprosthetic valves were evaluated in a previous study, with resultant good results during short-term evaluation [21]. In this study, long-term performance was investigated and the durability and antithrombogenicity of the developed bioprosthetic valve were evaluated clinically.

In electrocardiography, though, R wave tended to increase in height gradually and the mean electrical axis indicated left axis deviation, there was no significant difference observed in values before MVR and after MVR. It indicated that the left ventricular over-loading pattern occurring could not be denied.

In echocardiography, thrombogenesis was not observed in the prosthetic valve for twelve months. Slight paravalvular leakage was observed in one dog at six months after MVR. Paravalvular leakage was caused by partial dehiscence of the valve or infection. Severe paravalvular leakage usually has symptoms of heart failure and is treated surgically [12]. However, the leakage observed in this study was mild without impairment of functional capacity. In this dog, the paravalvular leakage was no symptoms of heart failure or infection in six months after MVR. Twelve months after MVR, slight transvalvular regurgitation was observed in this dog and this might be an influence of paravalvular leakage. Mild transvalvular regurgitation was observed in another dog twelve months after MVR. Valvular regurgitation was reported in bioprosthetic valves [9, 25]. Carpentier-Edwards valves, valvular regurgitation was absent in about 37.5%, trivial in 45%, mild in 15%, and moderate in 2.5% [9]. In this study, a significant change in diastolic atrioventricular pressure gradient, auxocardia in X-ray were not observed. However, diastolic atrioventricular pressure gradient tended to increase gradually, and mild pulmonary edema was observed in two dogs at twelve months after MVR. Diastolic atrioventricular pressure gradient was greater than 16mmHg and PHT was greater than 180 msec, suggesting abnormal functioning of the bioprosthetic valves [2, 16]. Increase in prosthetic transvalvular gradient was reported in many types of bioprosthetic valves and presumably was caused by progressive calcification and/or pannus formation [1, 6, 14]. Therefore the valve used in this study might degenerate and increase in flexural rigidity. The degeneration of the valve might cause the increasing trend of atrioventricular pressure gradient and valvular regurgitation. The mild pulmonary edema might be caused by the influence of both.

On the other hand, PHT was not significantly different and was within the normal range as the bioprosthetic valve. MVA was calculated using PHT, and in normally functioning prosthetic valves, the MVA was 2.1 ± 0.7 $cm^2$ in Carpentier-Edwards porcine valves [25]. It was suggested that the MVA had been kept constant thought the valve degenerated in this study. Moreover, it was considered that the antithrombogenicity of the prosthetic valve had been maintained because the symptoms of thromboembolism and thrombogenesis of the prosthetic valve were not observed.

In M-mode echocardiography, it is suggested that these changes in size of the left ventricle were not due to the porcine bioprosthetic valve but to the operative stress caused by MVR, because significant changes were observed only seven days after MVR. Left ventricular fractional shortening (FS) is one of the most common measurements of left ventricular function [3, 13], and FS was in the normal range for twelve months in this study.

We concluded that the newly developed bioprosthetic valves had antithrombogenicity, thought the durability might not be enough in the long-term period. Based on our study, we believe the valves were one of the viable options for treatment of MR in dogs. We will try to perform MVR using the valve in an experimental chronic MR model before clinical cases of MR in dogs.

ACKNOWLEDGEMENT. The authors would like to thank the staff of the Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Tech-

nology, and Animal Clinical Research foundation for their support in this study.

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