In Vivo Assessment of Novel Stentless Valve in the Mitral Position

Satoshi Kainuma, MD; Hitoshi Kasegawa, MD, PhD; Shigeru Miyagawa, MD, PhD; Hiroyuki Nishi, MD; Hitoshi Yaku, MD, PhD; Shuichiro Takanashi, MD, PhD; Kazuhiro Hashimoto, MD, PhD; Yukikatsu Okada, MD, PhD; Satoshi Nakatani, MD, PhD; Mitsuo Umezu, PhD; Takashi Daimon, PhD; Taichi Sakaguchi, MD, PhD; Koichi Toda, MD, PhD; Yoshiki Sawa, MD, PhD

on behalf of the Committee of the Japanese Society of Stentless Mitral Valve

Background: We conducted in vivo examinations of a newly designed stentless mitral valve (SMV), formed by suturing 2 leaflets with the “legs” serving as chorda tendinea, made from bovine pericardium, to a flexible ring.

Methods and Results: Seven pigs underwent implantation of the SMV constructed with a 23-mm (n=5) or 25-mm (n=2) Duran ring. Baseline echocardiography examinations were used to evaluate the annular anteroposterior diameter, and distance between the mitral annulus (MA) and papillary muscles (PMs) to determine SMV-leg length. After removing the native valve, the SMV-legs were fixed to the anterior and posterior PMs, followed by fixation of the ring to the native MA. Immediately after surgery, all animals presented none or trivial mitral regurgitation, with mean and peak trans-SMV pressure gradient values of 1.9±0.8 and 6.0±3.1 mmHg, respectively. The mean length of the SMV-leg was 19.4±3.9 mm, which correlated with the distance between anterior and posterior MA-PM (r=0.96 and 0.94, respectively, P<0.01 for both). The discrepancy between the anteroposterior diameter of the ring (outside diameter) and that of the native valve was 1.0±2.9 mm, which correlated with the trans-SMV pressure gradient (r=0.81, P=0.025).

Conclusions: In our preliminary study, the SMV demonstrated excellent diastolic inflow dynamics and closing function in vivo. Preoperative precise assessment of MV configuration may serve as a basis for selection of appropriate ring size and SMV-leg length. (Circ J 2015; 79: 553–559)

Key Words: Animal models; Echocardiography; Mitral valve

Valve replacement is a well-established modality for treatment of mitral valve (MV) disease. However, in young patients, including women of childbearing age, valve replacement still presents a problem because of the limited durability of bioprostheses, particularly when implanted in the mitral position and because of the difficulties associated with the long-term anticoagulation required for mechanical prostheses.

To improve the current situation, we need to find an optimal prosthesis resembling native MV anatomy and develop a new surgical treatment strategy for patients with indication. The stentless MV (SMV), newly designed by Hitoshi Kasegawa, embodies special characteristics in its design, as the large anterior leaflet has commissures formed without any suture line, and the folded structure of the 2 commissures streamlines the production process by avoiding additional sewing and may also help to avoid leakage after implantation. We previously reported excellent hydrodynamic performance of the SMV within the environment of a MV simulator capable of generating identical test conditions. However, no reports have been presented about its in vivo use and the implantation technique in the mitral position remains to be established. Therefore, we evaluated hemodynamic performance and valve function immediately after replacement with this novel SMV in an animal model.

Methods

We used 7 female pigs (Hiyoshi Farm Co Ltd, Kyoto, Japan) for a procedure utilizing the new SMV under standard cardio-
Valve Production

The SMV is designed to have annuloventricular continuity and provide similar performance to the native MV, representing a potential solution for the issues with conventional MV replacement. The present SMV has 2 major components: a large anterior leaflet with commissures that cover A1, A2, A3, AC, PC, P1, and P3 on the mitral map, and a small posterior leaflet. Using templates designed for suturing to a Duran flexible ring (Medtronic, Minneapolis, MN, USA), a large anterior and small pulmonary bypass. Preoperative echocardiography was performed to evaluate MV configuration, including distances between the mitral annulus (MA) and the anterior and posterior papillary muscle (PMs), as well as annular anteroposterior diameter (Figure 1). Postoperatively, in vivo hemodynamic performance and valve function were examined. We also analyzed the associations between SMV-leg length and distance from the MA to the PMs, and between SMV configuration (ie, discrepancy between anteroposterior diameter of flexible ring and that of the native valve) and trans-SMV pressure gradient.

All experimental procedures and protocols used in this investigation were reviewed and approved by an institutional animal care and use committee, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1996).

Figure 1. Baseline echocardiographic measurements of distances between mitral annulus and anterior (A, red dotted line) and posterior (B, red dotted line) papillary muscles (PM) and annular anteroposterior diameter (red solid line). LA, left atrium; LV, left ventricle; RV, right ventricle.

Figure 2. (A) Templates for suturing to a 23-mm Duran flexible ring. (B) Large anterior and small posterior leaflets were incised from a sheet of glutaraldehyde-treated bovine pericardium and carefully sewn to the corresponding flexible ring. (C) The length of the SMV-leg is determined as the distance between the bottom of the leaflet and the end of the leg. SMV, stentless mitral valve.
posterior leaflets were incised from a sheet of glutaraldehyde-treated bovine pericardium (Edwards Lifesciences, Irvine, CA, USA) and carefully sewn to the corresponding flexible ring, as previously described (Figure 2A, B). Each pair of “legs” (papillary flaps) was fixed temporarily at the marked point, using 7-0 polypropylene continuous suture. This procedure ensured good coaptation of the leaflets after valve implantation.

In this study, the size of the Duran flexible ring was randomly selected as either 23-mm (outside diameter, 22 mm) or 25-mm (outside diameter, 24 mm), and SMV production was performed prior to the procedure on a separate operating table. The sizes of the anterior and posterior leaflets were adjusted in relation to the flexible ring size (ie, when sutured to a 23- or 25-mm flexible ring, the SMV has an anterior leaflet with a height of 28 or 30 mm, respectively, and a posterior leaflet with a height of 14 or 15 mm, respectively). Data regarding the outside diameter of the ring were supplied by the manufacturer.

Echocardiographic Measurements and Analysis
Prior to establishing a cardiopulmonary bypass, baseline 2D epicardial echocardiography using an Artida (SSH-880CV) ultrasound system (Toshiba Medical Systems Corporation, Tochigi, Japan) was performed to evaluate annular anteroposterior diameter, and distances between the MA and anterior and posterior PMs.

After discontinuing cardiopulmonary bypass, epicardial echocardiography was repeated to evaluate valve function in terms of grade of mitral regurgitation (MR), mean and peak trans-SMV pressure gradient, and coaptation length of the SMV leaflets with stable hemodynamics. The severity of MR was graded semi-quantitatively from color-flow Doppler data. For this routine assessment, MR severity was characterized as none (0), trivial (1+), mild (2+), moderate (3+), or severe (4+) based on the length of regurgitation jet extending into the left atrium beyond the MV.

Implantation Technique of SMV
All operations were performed through a median sternotomy under routine cardiopulmonary bypass with systemic hypothermia. After the baseline echocardiographic assessment of MV configuration, standard cardiopulmonary bypass was established and myocardial preservation was achieved by intermittent cold cardioplegia. After cardiac arrest, the MV was exposed through either a right-sided left atriotomy or trans-septal superior approach. First, the native MV and relevant chordae were excised at their insertion points. The length of the SMV-legs, defined as the distance between the bottom of the leaflet and the end of a leg, was adjusted to match the distance between the MA and both anterior and posterior PMs (Figure 2C). SMV implantation was then commenced by fixation of the ends of the 2 leaflet legs to the septal side of the anterior and posterior PMs, respectively, using vertical mattress sutures of 4-0 polypropylene. Next, the SMV was sutured circumferentially to the native MA with 4-0 polypropylene in a continuous manner. After discontinuing cardiopulmonary bypass, repeat epicardial echocardiography was performed to assess valve function under stable hemodynamics. The animals were euthanized by intravenous injection of an overdose of pentobarbital sodium, and then the hearts were excised and opened for direct inspection.

Statistical Analysis
Continuous variables are expressed as the mean ± SD. Correlations between variables were tested with Pearson’s correlation coefficient (r). Statistical analyses were performed using the JMP 7.0 software package (SAS Institute, Cary, NC, USA).

Results
Preoperative Assessment of MV Configuration
SMV implantation was accomplished in 7 pigs with precise echocardiographic assessments performed under stable hemodynamics. At baseline, the mean distance between the MA and anterior and posterior PMs was 21.7 ± 2.5 mm (range, 17.2–24.4 mm) and 21.2 ± 2.6 mm (17.5–25.1 mm), respectively (Table 1). The mean MA anteroposterior diameter was 21.6 ± 2.1 mm (range, 17.5–24.0 mm), which substantially correlated with both the MA-anterior PM (r=0.78, P=0.035) and MA-posterior PM (r=0.71, P=0.073).

Hemodynamic Performance and SMV Function After Implantation
The size of the flexible Duran ring implanted was 23 mm in 5 and 25 mm in 2 pigs. After surgery, all animals presented none or trivial MR, with a mean leaflet coaptation length of 17.5 ± 2.2 mm (14.5–20.5 mm) (Table 2). The mean and peak trans-SMV pressure gradient values were 1.9 ± 0.8 mmHg (0.9–3.3 mmHg) and 6.5 ± 3.4 mmHg (3.3–12.3 mmHg), respectively, indicating no significant functional mitral stenosis.

Relationship Between SMV-Leg Length and Distance From MA to PM
The length of the SMV-legs was approximately adjusted to be

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Annular AP diameter (mm)</th>
<th>MA-anterior PM (mm)</th>
<th>MA-posterior PM (mm)</th>
<th>Duran ring size (mm)</th>
<th>Length discrepancy (mm)*</th>
<th>SMV-leg diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17.5</td>
<td>17.2</td>
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</tr>
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<td>22</td>
</tr>
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<td>25.1</td>
<td>23</td>
<td>−0.1</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>22.4</td>
<td>22.1</td>
<td>20.1</td>
<td>23</td>
<td>−0.4</td>
<td>19</td>
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<td>Mean±SD</td>
<td>21.6±2.1</td>
<td>21.7±2.5</td>
<td>21.2±2.6</td>
<td>–</td>
<td>1.0±2.9</td>
<td>19.4±3.9</td>
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</table>

*Length discrepancy calculated as anteroposterior diameter (outside diameter) of the ring minus that of the native valve. The outside diameter of the 23-mm and 25-mm Duran ring is 22 mm and 24 mm, respectively. AP, anteroposterior; MA, mitral annulus; PM, papillary muscle; SD, standard deviation; SMV, stentless mitral valve.
slightly shorter than the distance between the MA and anterior and posterior PMs. Consequently, the mean length of the legs was 19.4 ± 3.9 mm (13.0–24.0 mm), which correlated with the MA-anterior PM (r=0.96, P<0.01) and MA-posterior PM (r=0.94, P<0.01) (Table 2). Finely-balanced adjustment of SMV-leg length may contribute to forming a large surface for leaflet coaptation, without an excess amount of leaflet tissue.

Discrepancy Between Anteroposterior Diameter of the Flexible Ring and Native Valve, and Association With Trans-SMV Pressure Gradient

The discrepancy between the anteroposterior diameter of the flexible ring (outside diameter) and that of the native valve was 1.0 ± 2.9 mm (−2.0 to 6.5 mm), which positively correlated with the mean (r=0.77, P=0.040) and peak trans-SMV pressure gradients (r=0.81, P=0.025) values (Figure 3). In contrast, the discrepancy between the MA-anterior PM and SMV-leg length did not correlate with those values (r=0.66, P=0.12; r=0.70, P=0.08, respectively).

Postmortem Findings
Postmortem examination showed that the SMV was successfully attached to the native MA without interfering with the aortic valve in all animals (Figure 4).

Discussion

Although MV repair has several advantages compared with replacement, that procedure can result in the need for a subsequent MV operation for recurrent MV dysfunction,4–7 which is an important clinical consideration after MV repair. There was a trend toward better survival after re-repair than after replacement for the first repair failure; however, re-repair seems less likely to be indicated in those who present with valve-related failure, multiple mechanisms of valve dysfunction or those who have already received implantation of a small mitral ring, which leaves the surgeon with no other option but to replace the valve.5 In addition, there still exists MV pathology that is unlikely to be surgically repaired in the young, rheumatic population or with infectious endocarditis with marked destruction of the leaflet structure. Despite major advances in valve surgery, the optimal treatment strategy for patients with MV disease unsuitable for repair remains controversial. Currently available stented bioprosthetic valves are limited by such problems as calcification, structural failure, and hemodynamic disadvantages.2 Even though lifelong anticoagulation limits the use of mechanical prostheses, there is no other option but to implant a mechanical MV in young women or infants in whom the MA is too small to ac-

<table>
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<th>Case no.</th>
<th>MR grade</th>
<th>Coaptation length (mm)</th>
<th>Heart rate (beats/min)</th>
<th>Systemic AP (mmHg)</th>
<th>Mean trans-SMV pressure gradient (mmHg)</th>
<th>Peak trans-SMV pressure gradient (mmHg)</th>
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<tr>
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<tr>
<td>7</td>
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<td>19.5</td>
<td>95</td>
<td>82/49</td>
<td>0.9</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Mean±SD – 17.5±2.2 93±11 – 1.8±0.8 6.0±3.1

MR, mitral regurgitation; PG, pressure gradient. Other abbreviations as in Table 1.
commodate a conventional bioprosthesis. These drawbacks led us to develop a SMV, which offers the possibility of preserving the advantages of the native valve, such as a flexible annulus, with a large opening area and annuloventricular continuity, combined with sufficient coaptation.

In the present experimental study, we evaluated in vivo hemodynamic performance and valve function after MV replacement with the novel SMV, and found excellent diastolic inflow dynamics and closing function, as well as large leaflet coaptation, as shown by intraoperative echocardiography findings. These results are consistent with those of a previous study that investigated the performance of an SMV within the environment of a MV stimulator able to generate identical test conditions. Our experience has also shown that the present SMV implantation technique is reliable in short-term experiments.

The design of the novel SMV closely mimics the structure of the native MV, which has an anterior leaflet significantly larger than the posterior leaflet. Of note, the creation of a folded structure around the commissures, which is also analogous to the structure of the native valve, facilitates good leaflet coaptation and avoidance of MR after the operation. Ranganathan et al studied the morphology of human MVs obtained at autopsy, and found that the height of the anterior leaflet and that of the middle scallop of the posterior leaflet was approximately 22–24 mm and 12–14 mm, respectively, indicating that the novel SMV equipped with a 23- or 25-mm Duran ring will have slightly large leaflets as compared with a human native MV. These morphological aspects are considered to contribute to the excellent closing function of the SMV with large leaflet coaptation. Furthermore, its anatomical advantage may allow structural modifications of the SMV template (eg, reduction of leaflet volume to a certain extent) for better diastolic inflow dynamics. Future studies will focus on valve anatomy to determine the anatomic characteristics that lead to optimal systolic and diastolic SMV performance.

We noted that preoperative measurement of the perpendicular distance between the MA and PMs was useful to determine the length of the SMV-legs. It is likely that creation of an SMV-leg that is slightly shorter than the MA-PM distance will avoid excess leaflet tissue, thereby preventing impaired diastolic inflow dynamics. Our method may be justified by the results of a previous study by Komeda et al showing that the distance from the tip of the PM to the MA plane was constant, regardless of cardiac cycle and loading conditions. We consider that such evaluation is particularly important when performing SMV implantation in patients with MV pathologies, because that length is

**Figure 5.** Postoperative echocardiographic findings in case 1 (A, B) and case 2 (C, D). The pig in case 1 has a relatively redundant leaflet and slightly impaired valve opening during diastole at the mitral annulus level (A, green arrow). The pig in case 2 shows favorable diastolic inflow dynamics and closing function together with large leaflet coaptation.
not directly affected by any pathologic process involving the MV. Interestingly, the length between the MA and PMs observed in our animal model (approximately 21.2–21.7 mm) is likely comparable to that in patients with MV pathologies, shown to be a mean 22±5 mm, potentially allowing direct application in current implantation techniques in clinical settings. Although the optimal length of the SMV-leg, as well as optimal site of the PM to be attached (at the tip of the PM or lower down on its body), need further exploration, our findings offer some guidance, especially for determining the length of the SMV.

In the current study, the ring size of the SMV was not adjusted according to the anterior leaflet height or annular diameter of the native valve. Two pigs (cases 1 and 2) had a relatively small MA and underwent implantation with an SMV consisting of a 25-mm Duran ring, which created a considerable discrepancy between the anteroposterior diameter of the ring (ie, outside diameter) and that of the native valve of 6.5 and 3.4 mm, respectively. In case 1, but not case 2, postoperative echocardiography showed relatively redundant leaflet tissue and slightly impaired valve opening during diastole at the level of the MA (Figure 5). Notably, our findings demonstrated a positive correlation of the discrepancy between the anteroposterior diameter of the ring and that of the native valve with the postoperative trans-SMV pressure gradient, suggesting that using a large-sized flexible ring relative to the size of the native MA may adversely affect opening of the SMV. Although in those cases there was no significant functional mitral stenosis, attention should be paid to not creating a large discrepancy (possibly greater than 5 mm).

We consider there are 3 major advantages of SMV implantation in the mitral position. First, our SMV equipped with a 23- or 25-mm flexible ring can be implanted without causing an abnormal mitral gradient even if the MA is as small as 20 mm, suggesting the potential of this valve for patients with MAs too small to accommodate currently available mitral bioprostheses, or those who have already received implantation of a small mitral ring or prosthesis. Another advantage is that the SMV preserves annuloventricular continuity, leading to restoration of performance similar to that of a native MV. This anatomical benefit can have great physiological significance for patients with left ventricular dysfunction. Finally, the originally designed long legs of our novel SMV enabled creation of a tailor-made valve apparatus with flexibility for use in the animal studies, which led us to speculate that this SMV could be widely applied in clinical situations for patients with variable annulopapillary distances. Based on these favorable aspects, MV replacement utilizing the SMV may be an effective surgical alternative for treatment of ischemic or nonischemic MR secondary to left ventricular dysfunction. Because of its anatomical characteristics, the SMV also has potential to attenuate leaflet tethering of the MV, which is a primary mechanism of ischemic or nonischemic MR. Importantly, this SMV may also possess adaptability for further left ventricular remodeling, which is associated with recurrent MR after MV repair, though additional investigations are mandatory to confirm this issue.

It remains controversial regarding the appropriate materials used for construction of the SMV. Chemically treated autologous and xenogeneic (bovine and porcine) pericardial tissue has been extensively used for aortic and MV reconstructions. Autologous pericardium is preferred because it has several advantages including low cost, lack of donor-derived pathogens, and nonimmunogenic characteristics, although it has disadvantages such as limited availability and inflammatory changes that occur after pericardiotomy. A current biological substitute for autologous human pericardium is glutaraldehyde-fixed bovine pericardium, which was used in the present animal experiments. Glutaraldehyde fixation has been used to treat both homogeneic and xenogeneic pericardium to reduce immunogenicity, as well as to treat autologous pericardium in order to increase stiffness, making it easier to handle. The disadvantage of chemically treated pericardium is a lack of in vivo regeneration related to the presence of tissue cross-links. In addition, the toxicity of residual glutaraldehyde prevents cellular infiltration, repair, and growth. One possible solution would be to use acellular allogeneic pericardium that would be regenerated with autologous cells in vitro and in vivo, and that would grow with the patient and not be rejected, which would be especially advantageous for young patients. We hope that our study will stimulate further basic and clinical investigations of these issues.

Study Limitations

Although the present experimental study demonstrated excellent diastolic inflow dynamics of the novel SMV, even in a small MA, the short-term evaluation did not allow any valid conclusions. Long-term in vivo studies are absolutely necessary to assess long-term results with meticulous physiological (hemodynamic performance) and biological (durability) assessments of the transplanted SMV before more liberal use can be recommended. Because the transmitral gradient is a flow-dependent variable, that value should be interpreted with respect to hemodynamic factors, including ejection fraction and cardiac output. Lack of those hemodynamic data did not allow us to completely deny the possibility of underestimating the trans-SMV pressure gradient immediately after surgery. However, our data are consistent with the results of our previous study that evaluated the hydrodynamic function of the SMV using a mechanical simulator able to generate identical test conditions.

Conclusions

In our preliminary animal experiment, the SMV demonstrated excellent diastolic inflow dynamics and closing function, as well as large leaflet coaptation in vivo. Precise assessment of MV configuration may serve as a basis for selection of appropriate ring size and SMV-leg length, potentially serving as a useful basis for clinical application.

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Disclosures

The authors have no conflicts of interest to report. Conflicts of Interest: None to declare.

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References

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