7. Mizuguchi K, Damm GA, Bozeman RJ, Akkerman JW, Aber GS, Svejkovsky PA, Bacak JW, Orime Y, Takatani S, Nosé Y, Noon GP, DeBakey ME. Development of the Baylor/NASA axial flow ventricular assist device: in vitro performance and systematic hemolysis test results. Artif Organs. 1994;18:32–43.
8. Nojiri C, Kijima T, Maekawa J, Horiuchi K, Kido T, Sugiyama T, Mori T, Sugiura N, Asada T, Umemura W, Ozaki T, Suzuki M, Akamatsu T, Westaby S, Katsumata T, Saito S. Development status of Terumo implantable left ventricular assist system. Artif Organs. 2001;25:411–3.
9. Yamane T, Maruyama O, Nishida M, Kosaka R, Sugiyama D, Miyamoto Y, Kawamura H, Kato T, Sano T, Okubo T, Sankai Y, Shigeta O, Tsutsui T. Hemocompatibility of a hydrodynamic levitation centrifugal blood pump. J Artif Organs. 2007;10(2): 71–6. Epub 2007 Jun 20.
10. Umezu M, Yamazaki K, Yamazaki S, Iwasaki K, Miyakoshi T, Kitano T, Tokuno T. Japanese-made Implantable Centrifugal Type Ventricular Assist System (LVAS): EVAHEART. Biocybern Biomed Eng. 2007;27:111–9.
11. National Institute of Advanced Industrial Science and Technology. R&D Working Group report for active implants: innovative artificial heart systems. 2006. http://www.aist.go.jp/aist_j/aistinfo/report/entrust/iryoukiki/2006/techrep_artificalheart_ft2006.pdf. Accessed 25 April 2012 (in Japanese).
12. Yokoyama Y, Kawaguchi O, Shinshi T, Steinseifer U, Takatani S. A new pulse duplicator with a passive fill ventricle for analysis of cardiac dynamics. J Artif Organs. 2010;13:189–96.
13. Pantalos GM, Koenig SC, Gillars KJ, Giridharan GA, Ewert DL. Characterization of an adult mock circulation for testing cardiac support devices. ASAIO J. 2004;50(1):37–46.
14. Gibber M, Wu ZJ, Chang WB, Bianchi G, Hu J, Garcia J, Jarvik R, Griffith BP. In vivo experience of the child-size pediatric Jarvik 2000 heart: update. ASAIO J. 2010;56(4):369–76.
15. Litwak P, Butler KC, Thomas DC, Taylor LP, Macha M, Yamazaki K, Konishi H, Kormos RL, Griffith BP, Borovetz HS. Development and initial testing of a pediatric centrifugal blood pump. Ann Thorac Surg. 1996;61:448–51.
16. Weiss WJ. Pulsatile pediatric ventricular assist devices. ASAIO J. 2005;51(5):540–5.
17. Kitao T, Ando Y, Yoshikawa M, Kobayashi M, Kimura T, Ohsawa H, Machida S, Yokoyama N, Sakota D, Konno T, Ishihara K, Takatani S. In vivo evaluation of the "TinyPump" as a pediatric left ventricular assist device. Artif Organs. 2011;35: 543–53.
18. Timms D, Hayne M, McNeil K, Galbraith A. A complete mock circulation loop for the evaluation of left, right, and biventricular assist devices. Artif Organs. 2005;29:564–72.
19. Fleming S, Thompson M, Stevens R, Heneghan C, Plüddemann A, Maconochie I, Tarassenko L, Mant D. Normal ranges of heart rate and respiratory rate in children from birth to 18 years of age: a systematic review of observational studies. Lancet. 2011;377: 1011–8.
20. Park MK, Menard SW, Schoolfield J. Oscillometric blood pressure standards for children. Pediatr Cardiol. 2005;26(5):601–7.
21. Ministry of Economy, Trade and Industry. R&D Guidelines for active implants 2007: innovative artificial heart systems. 2007. http://www.meti.go.jp/policy/mono_info_service/service/iryou_fukushi/downloadfiles/200705-2.pdf. Accessed 25 April 2012 (in Japanese).
22. Kiani A, Gilani Shakibi J. Normal value of left ventricular end-systolic elastance in infants and children. IJMS. 2003;28:169–72.
23. Smith JJ, Kampine JP. Circulatory physiology: the essentials. Lippincott Williams and Wilkins, Baltimore; 1984.

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ORIGINAL ARTICLE

In vivo evaluation of an in-body, tissue-engineered, completely autologous valved conduit (biovalve type VI) as an aortic valve in a goat model

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Abstract Using simple, safe, and economical in-body tissue engineering, autologous valved conduits (biovalves) with the sinus of Valsalva and without any artificial support materials were developed in animal recipients' bodies. In this study, the feasibility of the biovalve as an aortic valve was evaluated in a goat model. Biovalves were prepared by 2-month embedding of the molds, assembled using two types of specially designed plastic rods, in the dorsal subcutaneous spaces of goats. One rod had three projections, resembling the protrusions of the sinus of Valsalva. Completely autologous connective tissue biovalves (type VI) with three leaflets in the inner side of the conduit with the sinus of Valsalva were obtained after removing the molds from both terminals of the harvested implants with complete encapsulation. The biovalve leaflets had appropriate strength and elastic characteristics similar to those of native aortic valves; thus, a robust conduit was formed. Tight valvular coaptation and a sufficient open orifice area were observed in vitro. Biovalves ($n = 3$) were implanted in the specially designed apico-aortic bypass for 2 months as a pilot study. Postoperative echocardiography showed smooth movement of the leaflets with little regurgitation under systemic circulation (2.6 ± 1.1 l/min). α-SMA–positive cells appeared significantly with rich angiogenesis in the conduit and expanded toward the leaflet tip. At the sinus portions, marked elastic fibers were formed. The luminal surface was covered with thin pseudointima without thrombus formation. Completely autologous biovalves with robust and elastic characteristics satisfied the higher requirements of the systemic circulation in goats for 2 months with the potential for valvular tissue regeneration.

Keywords In vivo tissue engineering · Heart valve · Autologous tissue · Aortic valve · Systemic circulation

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Introduction

Prosthetic valve replacement is a common treatment for severe valvular heart disease. There is a tendency to select bioprosthetic valves over mechanical valves. A major advantage of using bioprosthetic devices is that administration of anticoagulants such as warfarin is not required; however, disadvantages include time-related structural deterioration and pathogenicity (e.g., mad cow disease) [1].

Bioprosthetic devices are usually heterologous (made of bovine pericardia or porcine valves) and require chemical treatment to reduce immunogenicity, which could accelerate their degeneration, including calcification and/or time-related functional failure. Advances in biotechnology and tissue engineering may provide promising solutions to overcome the limitations of current heart valve substitutes [2–4]. Recently, autologous bioprostheses with enhanced maturation characteristics such as anticoagulation, self-repair, tissue regeneration, and growth adaptability have been developed using in vitro tissue engineering technology. Some investigators have successfully implanted in vitro tissue-engineered heart valves (TEHV) in animals and humans by using either decellularized natural tissues [3] or biodegradable synthetic polymers as scaffolds [2]. More recently, allogeneic TEHVs were successfully used for aortic root replacement in lambs [5].

To develop autologous prosthetic tissues that can endure high pressures, we have been focusing on the use of in-body tissue architecture technology [6–9], which is a novel concept in regenerative medicine based on the phenomenon of tissue encapsulation of foreign materials in living bodies. This technology involves the use of living bodies as a reactor and is simple, safe, and cost-effective. Since 2007, we have used in-body tissue architecture technology to develop a series of autologous trileaflet heart valves, called biovalves [10–12]. Last year, a type V biovalve, made of tissues completely autologous with the sinus of Valsalva, was shown to successfully function as an allogeneic conduit valve in the pulmonary valve position for up to 3 months in beagle models [13]. Recently, we have developed a type VI biovalve that did not need pretreatment before implantation by altering the design concept of the mold used to prepare the biovalves so that the trileaflet was obtained in the open form (unpublished data). In this study, the possibility of the type VI biovalve as an aortic valve was evaluated by implantation at the apico-aortic bypass in a goat model as a pilot study.

Methods

Preparation of biovalves

All animals received care according to the Principles of Laboratory Animal Care (formulated by the National Institutes of Health, publication no. 56-23, received 1985), and the research protocol (no. 22-2-4) was approved by the ethics committee of the National Cerebral and Cardiovascular Center.

Three goats (aged 1–2 years; body weight 40–50 kg) were used in this study. A specially designed concave acrylate rod (diameter 16 mm; length 35 mm; Fig. 1a) and convex silicone rod (diameter 16 mm; length 37 mm; Fig. 1a) were assembled with a small 0.5-mm aperture to prepare a cylindrical mold for the biovalve organization (Fig. 1b). The mold was designed to separate the leaflets from each other in the open form. The concave-shaped rod had three removable projections that resembled the three protrusions of the sinus of Valsalva. We placed eight molds into the dorsal subcutaneous pouches of each of the three goats (a total of 24 biovalves) under anesthesia that was induced with 10 mg/kg of ketamine and maintained with 1–3 % isoflurane. After 2 months had elapsed (Fig. 1c), we harvested the implants, which were completely encapsulated with robust connective tissue (Fig. 1d). After treatment with 0.6 % glutaraldehyde for 10 min, the rods were removed from both ends of the developed tubular tissue; furthermore, the type VI biovalves with three protrusions resembling the sinus of Valsalva were well formed. Three membranous leaflets from the inside of the conduit were obtained separately (Fig. 1d). Three out of 24 biovalves were used for transplantation, and the rest were used for evaluation of valve motion characteristics ($n = 6$), mechanical properties ($n = 9$), and histological examination ($n = 6$).

In vitro valve motion

The motion of the biovalve leaflets was recorded using a video camera at a frequency of 20 frames per second and was analyzed in conjunction with the circuit flow pattern by using a modified Windkessel pulsatile flow circuit model used in our previous study [14] (working fluid 0.9 % saline; pulsatile rate 62 bpm; flow rate 627 ml/min). The Reynolds and Womersley numbers were 956–1195 and 10.3–11.7, respectively, and were calculated under heart rates ranging from 70 to 90. We used the cardiac output averaged bypass flow, which is generally 80 ml/min per body weight (kg). The upper pressure was set at 120–150 mmHg and lower pressure at 50–80 mmHg as the systolic and diastolic aortic pressure.

Mechanical properties

Biovalves and native aortic valves ($n = 3$) were used as samples. The native aortic valve was simultaneously obtained from each goat when the 2-month implanted biovalve was harvested. The mechanical properties of the leaflets and conduits were measured using a tensile tester. The samples were cut in a circumferential direction and opened. Tissue specimens, 7 × 7 mm, were tested in humid conditions under a tissue extension rate of 3 mm/min. The ultimate tensile strength and elongation at breaking, indicative of tissue strength and tissue extensibility, respectively, were obtained from the stress-strain curves. The modulus, indicative of tissue stiffness, was calculated as the slope of the linear part of the stress-strain curves.

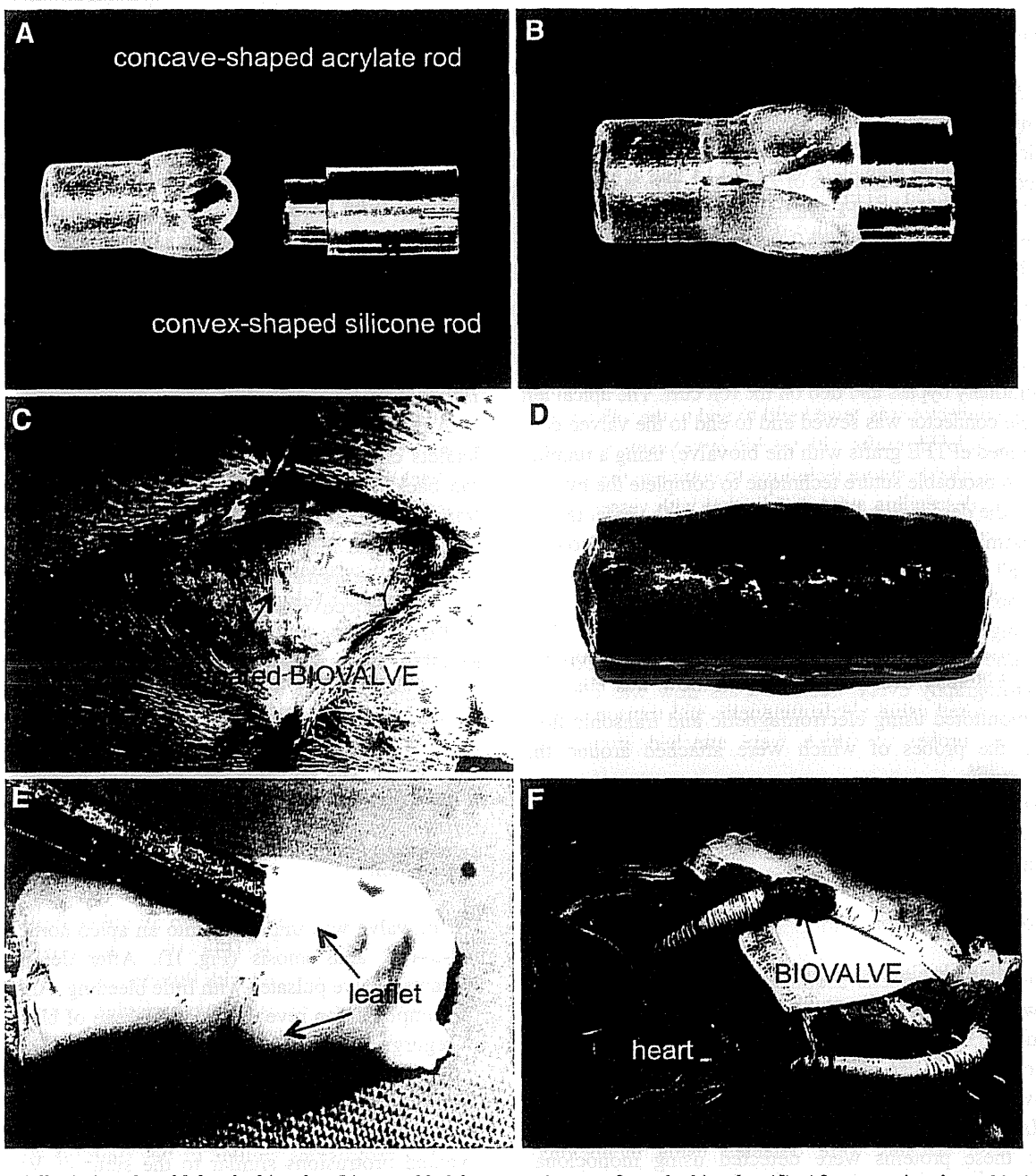

Fig. 1 Specially designed mold for the biovalve (**b**) assembled from a concave-shaped acrylate rod and a convex-shaped silicone rod (**a**) with a small 0.5-mm aperture for the formation of the leaflets. The concave-shaped rod contains three removable projections resembling the protrusions of the sinus of Valsalva. The molds were embedded for 2 months in a dorsal subcutaneous pouch in a goat. **c** After 8 weeks of implantation, the mold was encapsulated by connective tissue to form the biovalve (**d**). After removing the molds from each end of the implant, three thin leaflets were observed in the luminal side of the conduit. **e** Macroscopic observation of the formed leaflets after inversion of the biovalve conduit. **f** The biovalve was implanted between the apical portion of the left ventricle and descending aorta with interposition of ePTFE grafts without the use of cardiopulmonary bypass

In vivo evaluation of biovalves in the systemic circulation

To evaluate the biovalve under systemic circulation, we conducted an apico-aortic bypass with interposition of vascular grafts in the goat. Anesthesia was induced with 10 mg/kg of ketamine and maintained with 1–3 % isoflurane. The heart was exposed through a left thoracotomy at the fifth subcostal region. A valved conduit was composed of 14-mm ringed expanded polytetrafluoroethylene (ePTFE) grafts (GORE-TEX, W.L. Gore & Associates, Inc., Newark, DE). After treatment with a 1 % saline solution of water-soluble argatroban, a biovalve was sewed end to end to the ringed ePTFE grafts at both ends using a running 5-0 non-absorbable

suturing technique. The distal end of the valved conduit (ringed ePTFE grafts with the biovalve) was sewed end to side to the descending aorta using a partial occluding clamp and a running 4-0 non-absorbable suturing technique. An apical left ventricle connector was composed of a custom-made stainless steel conduit (outer diameters of 20 and 14 mm at either end) and a 14-mm ringed ePTFE graft. A felt cuff was sewed to the left ventricular (LV) apex with 2-0 polyester sutures with a felt strip. After injection of heparin sodium (200 U/kg), the LV apex was cored with a 19-mm custom-made ventricular coring device. Then, the apical left ventricle connector was inserted through the felt cuff into the LV apex without cardiopulmonary bypass and tied on the felt cuff. The apical left ventricle connector was sewed end to end to the valved conduit (ringed ePTFE grafts with the biovalve) using a running 4-0 non-absorbable suture technique to complete the bypass. Finally, the descending aorta was ligated with vessel tape at the proximal portion of the anastomosis of the valved conduit so that all of the blood flow to the abdominal aorta was supplied from the apico-aortic bypass.

An angiography was performed after implantation. The valve function was evaluated using transthoracic Doppler echocardiography every week. Bypass flow was continuously monitored using electromagnetic and transonic flow meters, the probes of which were attached around the ePTFE grafts.

Postoperative systemic anticoagulation for the ePTFE grafts was maintained with oral administration of warfarin sodium and aspirin.

Histological evaluation

The biovalve specimens acquired after implantation were fixed with 10 % formalin, embedded in paraffin, sliced into longitudinal sections, and finally stained with hematoxylin-eosin or Elastica van Gieson. In addition, a few sections of biovalve were also stained for α-smooth muscle actin (α-SMA) and factor VIII by immunohistochemical techniques; these proteins were detected using monoclonal antibodies (Dako Japan, Kyoto, Japan).

Statistics

Quantitative data were represented as mean ± standard deviation.

Results

Preparation and properties of biovalves

The assembled molds (Fig. 1b) embedded in the subcutaneous pouches of a goat for 2 months showed complete encapsulation with the connective tissue and marked neovascularization (Fig. 1c). The implants were easily harvested because only very fragile, irregular, and redundant tissues connected the developed biovalves and surrounding subcutaneous tissues, which could be dissected easily (Fig. 1d). The convex and concave rods were smoothly removed from each end of the implant because there was no adhesion between the molds and biovalves. The conduit had three protrusions, which were formed because of the shape of the concave substrate, resembling the sinus of Valsalva. A membranous tissue in the shape of a trileaflet was formed at the aperture of the combined rods, as intended by its design (Fig. 1e).

Analysis of the video data showed that the biovalve leaflets closed rapidly and tightly in synchronization with the backward flow in the diastolic phase (Fig. 2). In the transition phase of the flow direction, the valve opened smoothly and fully without flapping or hitting the conduit wall. The regurgitation ratio and orifice ratio were 12.0 and 82.7 %, respectively.

The strength and modulus of the leaflet part of the biovalve were 830 ± 270 and 1083 ± 289 kPa, which were almost equal to the native values, whereas in the conduit part, the strength and modulus of the biovalve were about five times larger than those of the native aorta (Fig. 3a, c). The biovalve had excellent suppleness similar to the native valve (Fig. 3b).

Application

The biovalve was implanted into an apico-aortic bypass by end-to-end anastomosis (Fig. 1f). After declamping, the implanted valve pulsated with little bleeding. An angiograph after implantation revealed good passage of blood flow and no regurgitation at the level of the biovalve (Fig. 4).

The valve function was evaluated using transthoracic Doppler echocardiography every week. Up to 2 months after implantation, echocardiographic examination revealed protrusions similar to the sinus of Valsalva and rapid opening (Fig. 5a) and closing (Fig. 5b) of the leaflets, and Doppler echocardiography (Fig. 5c) did not yield substantial evidence of stenosis and regurgitation.

The bypass flow by the electromagnetic flow meter was 2.6 ± 1.1 l/min throughout the experiment. A graph of flow waveform almost 2 months after implantation was presented in Fig. 6. The waveform has been maintained compared to pre-implanted pulsatile flow waveforms in Fig. 2.

In the 2 months after implantation, the goat was euthanized, and the biovalve implants were harvested. Macroscopic observation revealed that the shape and size of the leaflets were well maintained compared to those of native heart valve leaflets.

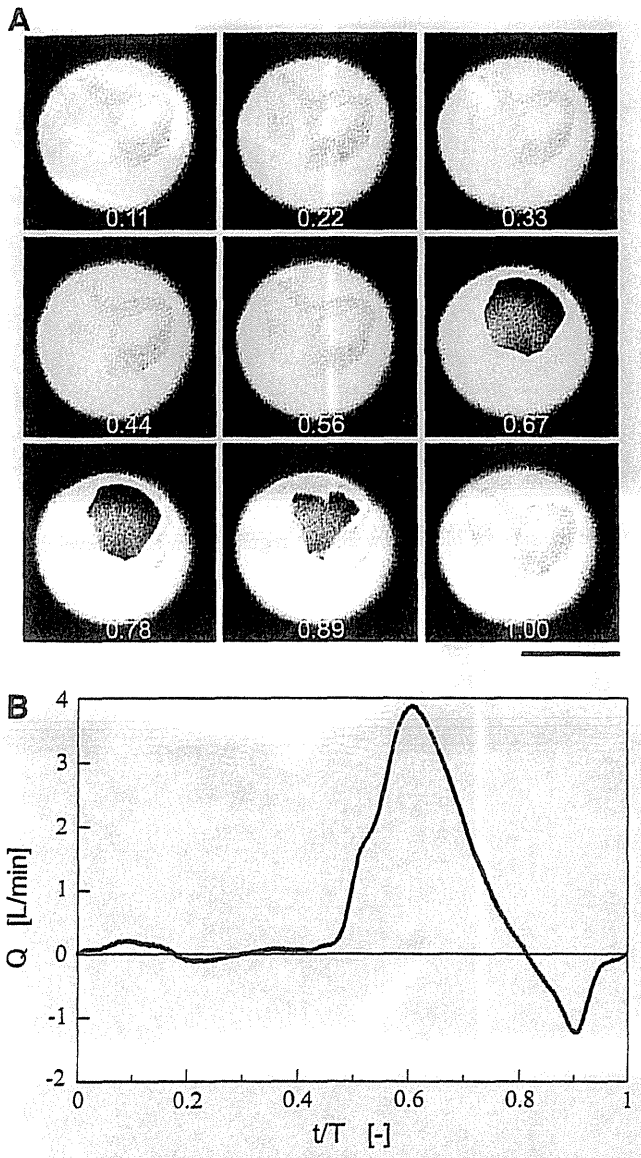

Fig. 2 **a** Valve movement under pulsatile conditions in one cycle ($T = 0.97$ s). The pulsatile rate was 62 bpm, and the average flow rate was 627 ml/min. The numbers in the photos are time/T. *Bar* 10 mm. **b** Pulsatile flow waveforms in one cycle

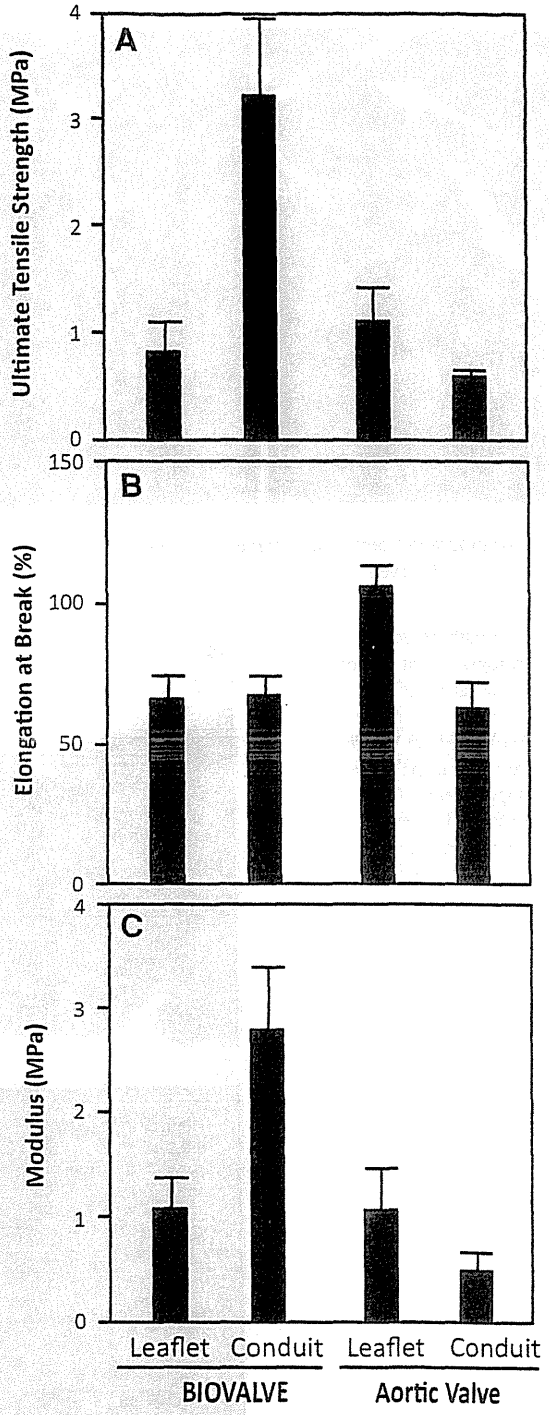

Fig. 3 Comparison of mechanical properties between biovalves and goat aortic valves. **a** The ultimate tensile strength, indicative of tissue strength, and **b** elongation at the break, indicative of tissue extensibility, were obtained from the stress-strain curves. **c** The modulus, indicative of tissue stiffness, was calculated as the slope of the linear part of the stress-strain curves. The error bars represent mean ± standard deviation ($n = 9$)

Histological evaluation

The whole body of the biovalve before implantation, including the valve leaflets and conduit, was mainly composed of collagen-rich tissue with fibroblasts (Fig. 7a). There were few elastic fiber and vascular cells. After implantation, wall thickness at the conduit and sinus of Valsalva significantly increased without any stenosis of the conduit (Fig. 5b), whereas leaflet thinness was well maintained (Fig. 7b). The conduit possessed a large amount of neovascularization (Fig. 7c). At the sinus, a thick elastic fiber formed although the main extracellular component was still collagen (Fig. 7d). Predominant cell types observed at the medial layer of the entire conduit walls included α-SMA positive, smooth muscle cells, and

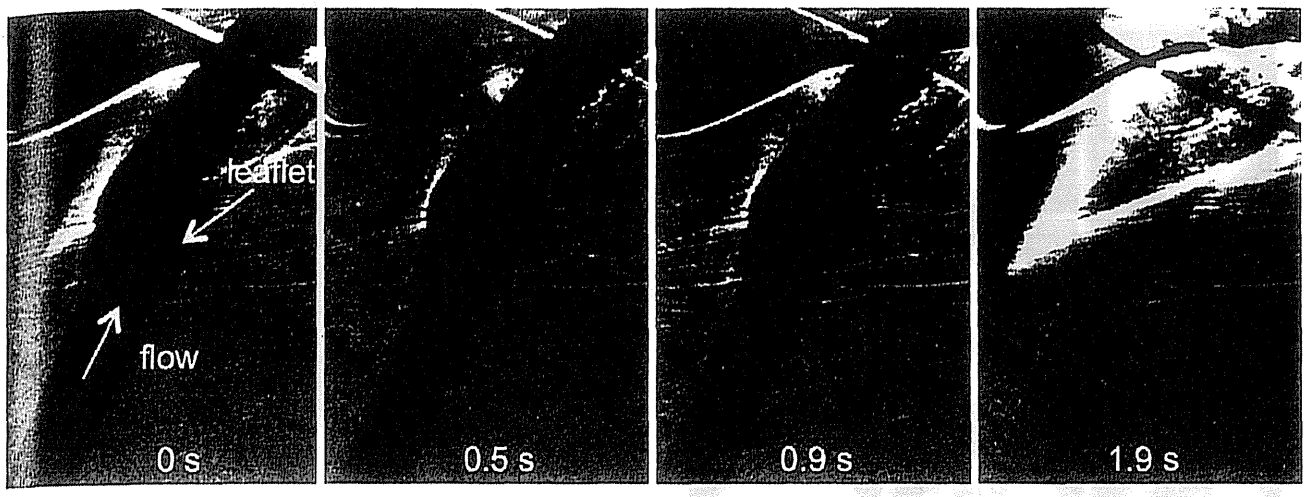

Fig. 4 Angiography performed immediately after biovalve implantation. Good passage of blood flow and no regurgitation at the level of the biovalve were observed

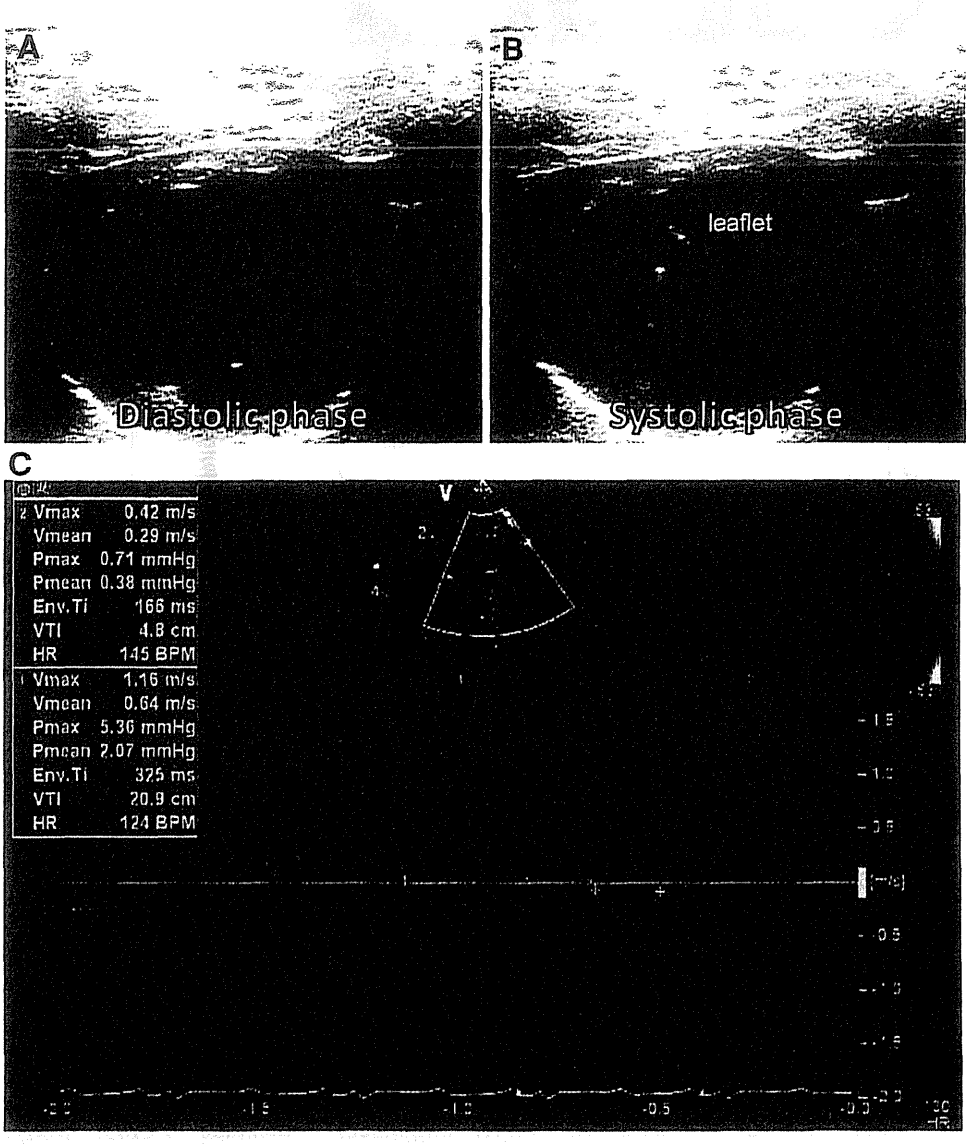

Fig. 5 Transthoracic echocardiography at 2 months after implantation of the biovalve indicated smooth leaflet movement at the opened (**a**) and closed (**b**) positions. The color Doppler echo (**c**) showed good forward flow at the systolic phase and trivial regurgitation at the diastolic phase

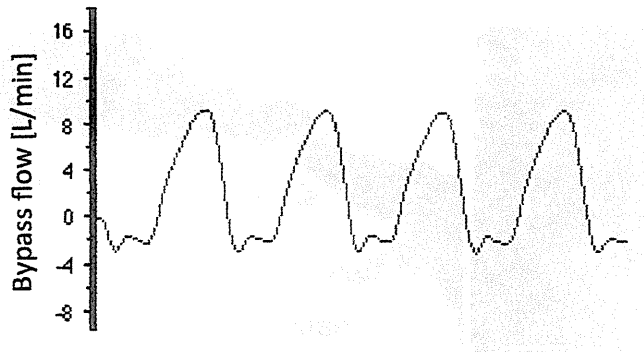

Fig. 6 A graph of flow waveform by the electromagnetic flow meter almost 2 months after implantation. The valve function has been almost maintained for 2 months

myofibroblasts (Fig. 7e–g) migrating toward the tip of the leaflet (Fig. 7h). Although the luminal surface was not covered with endothelial cells yet, no thrombus formation was observed on the smooth pseudointima (Fig. 7e, f).

Discussion

Biovalves were implanted into the systemic circulation as a pilot study with a limited experiment number and follow-up time in a goat model, although the sheep model is accepted by the FDA or other agencies as the most favorable model with regard to biodegradation/calcification. However, this is the first study reporting successful implantation of completely autologous tissue-valved conduits with no artificial support materials to the systemic circulation as aortic valves.

Due to the enormous number of patients suffering from aortic valve diseases, constructing aortic TEHVs has been the major study focus of many research groups. However, one of the predominant limitations for the development of an aortic TEHV is the use of an appropriate animal model for testing in the physiologically systemic circulation. Even in large animals, the anatomical implantation of the aortic valve conduit requires complex procedures such as cardiopulmonary bypass (CPB) and coronary arterial reconstruction. Therefore, in many previous studies, the prosthetic valves were implanted in the descending aorta [15, 16], where the pressure and flow pattern were completely different from the outflow of the left ventricle. Since implanted valves do not usually close tightly enough in this condition, valvular degeneration is often promoted. For this reason, our in vivo evaluation of biovalves in the systemic circulation with an apico-aortic bypass is worth reporting. Others have implanted the aortic valve in the pulmonary position, thus excluding the influence of systemic pressure on the implanted grafts from the beginning [17]. We have already reported successful implantation of the biovalves to the pulmonary valve position under CPB in a beagle model [13]. In a low-pressure condition, the biovalves functioned as pulmonary valves for 3 months with valvular tissue reconstruction. In this study, a potential of the biovalve as an aortic TEHV has been further increased.

The evaluation of valve function in vitro was performed under the pulsatile condition of 62 bpm with a flow rate of 627 ml/min. The condition was set for the implantation to goats weighing of about 40–50 kg, in which the average cardiac outflow is about 3.2–4.0 l/min (measured bypass flow: 2.6 ± 1.1 l/min) at a heart rate of 70–90 bpm. In the pulsatile flow circuit model, saline solution was selected as a working fluid for prevention of injury in biovalves made of natural tissues. The kinetic viscosity of blood is 4.44×10^{-6} m^2/s, which is about four times more than that of saline solution (1.00×10^{-6} m^2/s). Therefore, the flow condition in the circuit between the two different fluids, which is defined by the Reynolds number and Womersley number, needed to be adjusted for the evaluation of valve function using saline solution. The pulsatile rate and the flow rate calculated by the two numbers were 17.5–22.5 bpm and 800–1,000 ml/min (650 ± 275 l/min at the bypass), respectively.

Our biovalves were able to withstand systemic pressures without suffering structural or functional deterioration for as long as 2 months. The robust and elastic properties were obtained by slight treatment with diluted glutaraldehyde, which has been used clinically in porcine aortic valve or aortic valve repair. By designing a novel mold that reorganized biovalve construction, we developed the type VI biovalve, which demonstrates nearly perfect valve function. The key difference between the type V and VI molds is the aperture shape for leaflet formation. By preparing the valve leaflets in the open form, less regurgitation and an increase in the orifice ratio were observed upon in vitro examination. Furthermore, little regurgitation and no significant stenosis were confirmed during the observation period.

Since the biovalve was isolated from the heart and vascular tissues by ePTFE grafts in this model, α-SMA-positive cells that appeared in the conduit wall might have migrated from the surrounding connective tissue or could have originated from the fibroblasts existing in the biovalve wall since they were implanted. Further study is necessary to investigate their origin.

Regarding biovalve preparation for implantation, we followed the method of treatment with glutaraldehyde described by Duran et al. [18–22] for an aortic valve repair using an autologous pericardium leaflet. They explained that treated pericardium is used to increase the height of native aortic valve leaflets and commissures resulting in an increase in the coaptation zone. Glutaraldehyde treatment can provide more resistance against retraction and

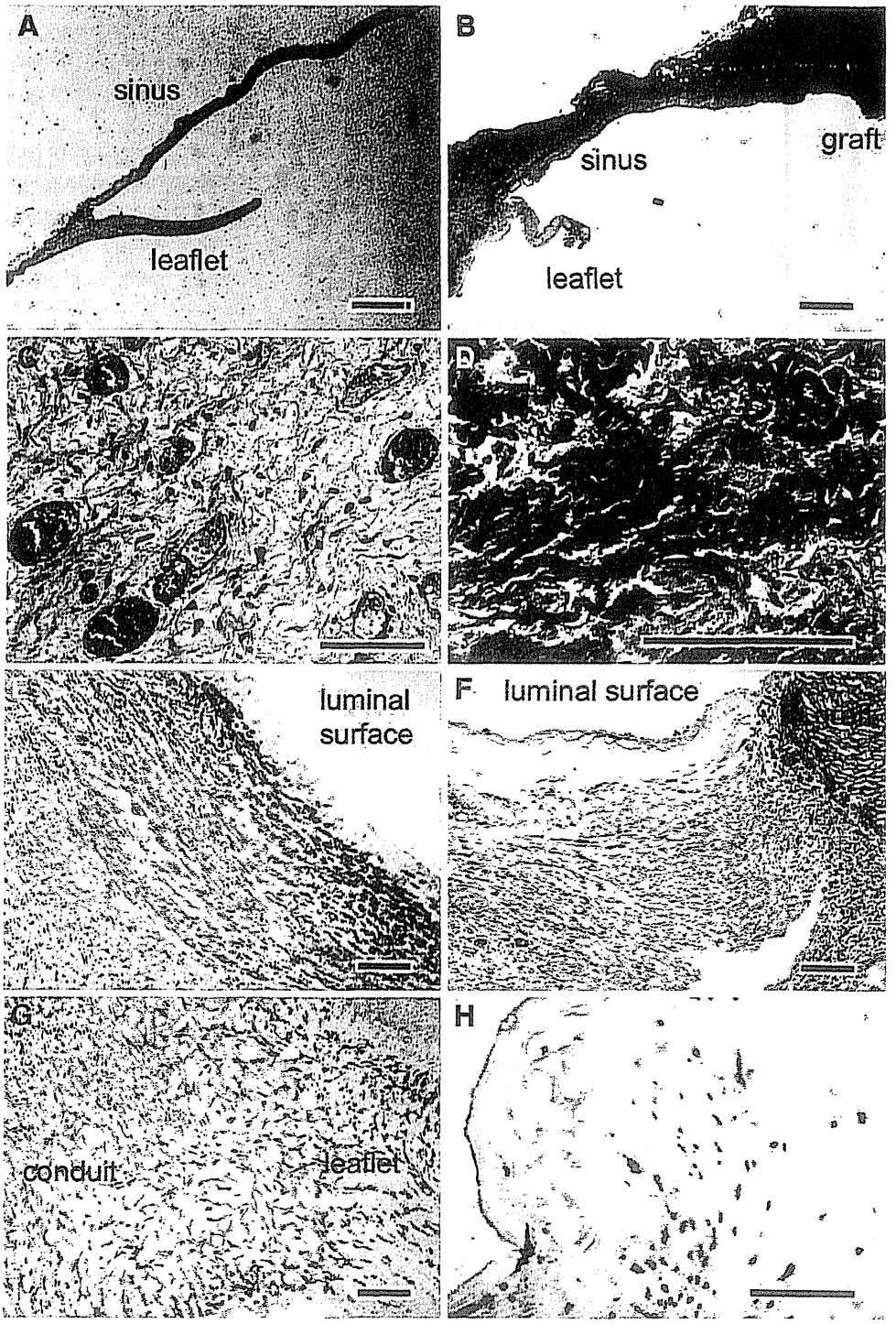

Fig. 7 Longitudinal sections of biovalve tissue before (**a**) and after (**b**) implantation (hematoxylin and eosin stain, *bar* 2 mm). **c** Neovascularization at the implanted biovalve conduit (hematoxylin and eosin stain, *bar* 100 μm). **d** Elastica van Gieson staining revealed a *black-colored* thick elastic fiber in the sinus region (*bar* 100 μm). Immunohistological staining for α-SMA revealed that predominant smooth muscle cells or myofibroblasts were observed at the sinus region (**e**), the proximal anastomosis region (**f**), and base (**g**) and tip (**h**) of the leaflet (*bar* 100 μm)

degeneration and maintain the intrinsic tissue pliability of the pericardium. However, treatment with glutaraldehyde may destroy native cells and cause denaturation of connective tissue even though the immersion is at a low dose and for a short time. Further examination is needed to determine the optimal method for biovalve preparation.

At 2 months after implantation, there were few endothelial cells at the luminal surface, whereas no thrombus formation was observed. Implantation of the biovalve at the aortic root or directly anastomosed to the native aorta was expected to result in the rapid migration of both endothelial and mesenchymal cells from the anastomotic sites, leading to quick tissue organization and maturation. This was observed in the anatomically implanted beagle model. Further implantation experiments with longer observational periods are ongoing.

Conclusion

Completely autologous type VI biovalves with robust and elastic characteristics appropriate for aortic valve replacement were developed by in-body tissue architecture technology using an improved mold design. Slight glutaraldehyde treatment enabled the type VI biovalve to achieve desirable idealistic mechanical properties for aortic valve replacement with respect to both valvular function and surgical handling. The biovalves withstood systemic pressure without unexpected dilatation or aneurysm formation in the conduit portion and degeneration or sclerosis of the valvular leaflet, which could be responsible for aortic valve regurgitation or stenosis within 2 months of implantation. Rapid tissue maturation with elastic fiber formation and the predominant appearance of α-SMA-positive cells in the completely autologous tissue without synthetic support materials might induce the growth potential of the valves.

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References

1. Cosgrove DM, Lytle BW, Taylor PC, Camacho MT, Stewart RW, McCarthy PM, Miller DP, Piedmonte MR, Loop FD. The Carpentier-Edwards pericardial aortic valve. Ten-year results. J Thorac Cardiovasc Surg. 1995;110:651–62.
2. Shin'oka T, Ma PX, Shum-Tim D, Breuer CK, Cusick RA, Zund G, Langer R, Vacanti JP, Mayer JE. Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model. Circulation. 1996;94:164–8.
3. Dohmen PM, Ozaki S, Nitsch R, Yperman J, Flameng W, Konertz W. A tissue engineered heart valve implanted in a juvenile sheep model. Med Sci Monit. 2003;9:97–104.
4. Vesely I. Heart valve tissue engineering. Circ Res. 2005;97:743–55.
5. Baraki H, Tudorache I, Braun M, Höffler K, Görler A, Lichtenberg A, Bara C, Calistru A, Brandes G, Hewicker-Trautwein M, Hilfiker A, Haverich A, Cebotari S. Orthotopic replacement of the aortic valve with decellularized allograft in a sheep model. Biomaterials. 2009;30:6240–6.
6. Nakayama Y, Ishibashi-Ueda H, Takamizawa K. In vivo tissue-engineered small-caliber arterial graft prosthesis consisting of autologous tissue (biotube). Cell Transplant. 2004;13:439–49.
7. Watanabe T, Kanda K, Ishibashi-Ueda H, Yaku H, Nakayama Y. Development of biotube vascular grafts incorporating cuffs for easy implantation. J Artif Organs. 2007;10:10–5.
8. Sakai O, Kanda K, Ishibashi-Ueda H, Takamizawa K, Ametani A, Yaku H, Nakayama Y. Development of the wing-attached rod for acceleration of "Biotube" vascular grafts fabrication. J Biomed Mater Res B Appl Biomater. 2007;83:240–7.
9. Watanabe T, Kanda K, Ishibashi-Ueda H, Yaku H, Nakayama Y. Autologous small-caliber "Biotube" vascular grafts with argatroban loading: a histomorphological examination after implantation to rabbits. J Biomed Mater Res B Appl Biomater. 2010;92:236–42.
10. Hayashida K, Kanda K, Yaku H, Ando J, Nakayama Y. Development of an in vivo tissue-engineered, autologous heart valve (the biovalve): preparation of a prototype model. J Thorac Cardiovasc Surg. 2007;134:152–9.
11. Hayashida K, Kanda K, Oie T, Okamoto Y, Ishibashi-Ueda H, Onoyama M, Tajikawa T, Ohba K, Yaku H, Nakayama Y. Architecture of an in vivo-tissue engineered autologous conduit "Biovalve". J Biomed Mater Res B Appl Biomater. 2008;86:1–8.
12. Nakayama Y, Yamanami M, Yahata Y, Tajikawa T, Ohba K, Watanabe T, Kanda K, Yaku H. Preparation of a completely autologous trileaflet valve-shaped construct by in-body tissue architecture technology. J Biomed Mater Res B Appl Biomater. 2009;91:813–8.
13. Yamanami M, Yahata Y, Uechi M, Fujiwara M, Ishibashi-Ueda H, Kanda K, Watanabe T, Tajikawa T, Ohba K, Yaku H, Nakayama Y. Development of a completely autologous valved conduit with the sinus of valsalva using in-body tissue architecture technology: a pilot study in pulmonary valve replacement in a beagle model. Circulation. 2010;122:S100–6.
14. Nakayama Y, Yahata Y, Yamanami M, Tajikawa T, Ohba K, Kanda K, Yaku H. A completely autologous valved conduit prepared in the open form of trileaflet (type VI biovalve): mold design and valve function in vitro. J Biomed Mater Res Part B Appl Biomater. 2011;99B:135–41.
15. Conconi MT, Rocco F, Spinazzi R, Tommasini M, Valfrè C, Busetto R, Polesel E, Albertin G, Dei Tos A, Iacopetti I, Cecchetto A, Zussa C, Grigioni M, Parnigotto PP, Nussdorfer GG. Biological fate of tissue-engineered porcine valvular conduits xenotransplanted in the sheep thoracic aorta. Int J Mol Med. 2004;14:1043–8.
16. Gulbins H, Pritisanac A, Pieper K, Goldemund A, Meiser BM, Reichart B, Daebritz S. Successful endothelialization of porcine glutaraldehyde-fixed aortic valves in a heterotopic sheep model. Ann Thorac Surg. 2006;81:1472–9.
17. Van Nooten G, Somers P, Cornelissen M, Bouchez S, Gasthuys F, Cox E, Sparks L, Narine K. Acellilar porcine and kangaroo aortic valve scaffolds show more intense immune-mediated calcification than cross-linked Toronto SPV valves in the sheep model. Interact Cardiovasc Thorac Surg. 2006;5:544–9.
18. Duran CMG, Alonso J, Gaite L, Alonso C, Cagigas JC, Marce L, Fleitas MG, Revuelta JM. Long-term results of conservative repair of rheumatic aortic valve insufficiency. Eur J Cardiothorac Surg. 1988;2:217–23.
19. Duran CM, Gometza B, Kuma N, Gallo R, Bjonastad K. From aortic cusp extension to valve replacement with stentless pericardium. Ann Thorac Surg. 1995;60:S428–32.
20. Duran CMG, Gometza B, Kuma N. Aortic valve replacement with freehand autologous pericardium. J Thorac Cardiovasc Surg. 1995;110:511–6.
21. Duran C, Gometza B, Kuma N, Gallo R, Bjonastad K. Treated bovine and autologous pericardium: surgical technique. J Cardiac Surg. 1995;10:1–9.
22. Ozaki S, Kawase I, Yamashita H, Uchida S, Nozawa Y, Matsuyama T, Takatoh M, Hagiwara S. Aortic valve reconstruction using self-developed aortic valve plasty system in aortic valve disease. Interact Cardiovasc Thorac Surg. 2011;12:550–3.

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ORIGINAL ARTICLE

Early clinical outcomes of new pediatric extracorporeal life support system (Endumo® 2000) in neonates and infants

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Abstract We investigated early clinical outcomes of a new extracorporeal life support (ECLS) system (Endumo® 2000, Heiwa Bussan, Tokyo, Japan), which consists of a ROTAFLOW centrifugal pump, a BIOCUBE oxygenator with plasma-leakage-tight polymer fibers, and a biocompatible coating (T-NCVC® coating), in pediatric patients <1 year old. From 2008 to 2011, 31 patients required ECLS. Except for 1 patient who was instituted with a transitional ECLS device, a conventional ECLS system (pediatric Emersave®, TERUMO, Saitama, Japan) was initiated in 14 patients before December 2009 (6 boys, 63.4 ± 87.1 days old, 3.1 ± 1.0 kg), and the Endumo® 2000 was initiated in 16 patients after December 2009 (8 boys, 43.9 ± 78.5 days old, 3.2 ± 0.7 kg). Primary reasons for the institution of ECLS were intraoperative low output syndrome in 11 patients, post-cardiotomy cardiopulmonary collapse in 9 patients, and other reasons in 10 patients. The median support period was 21.7 ± 20.7 days and the total number of circuit exchanges was 83. The median first circuit durability was significantly longer in the Endumo group [8.0 days (range 5.9–13.2) vs. 4.4 days (1.9–8.3)] ($p = 0.020$). Significant cranial hemorrhage occurred in only 1 patient, who received the Emersave system. The success rate for weaning from ECLS was 14.3 % in the Emersave group and 56.3 % in the Endumo group. Univariate analysis showed that usage of the Endumo® 2000 was a predictor for successful weaning from the ECLS ($p = 0.017$) as well as survival at discharge ($p = 0.032$). The Endumo® 2000 system provided safe and effective cardiopulmonary support without complications.

Keywords Extracorporeal life support · Membrane oxygenation · Endumo · Pediatrics

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Introduction

Extracorporeal life support (ECLS) is the principal treatment for cardiopulmonary collapse in pediatric populations; however, the survival rate at hospital discharge is still dismal [1–14]. When using the ECLS, it is essential to avoid complications in any organs (mainly intracranial hemorrhage) until recovery of the cardiac and/or respiratory function. However, the management of anti-coagulation is more difficult in small patients <1 year old (especially in neonates and early infants) as compared to other populations, due to the immaturity of their blood coagulation–fibrinolytic systems, which sometimes forces the unexpected termination of the ECLS [12, 14].

During long-term support, the ECLS circuit must be exchanged due to oxygenator deterioration caused by clot formation and/or plasma leakage, thrombus formation in the circuit itself, or decreased centrifugal pump flow. Even when the circuit is primed with blood and albumin, however, circuit exchange dilutes endogenous adrenaline, coagulation factors, and drug agents, so frequent circuit

exchange disrupts the effective recovery of cardiopulmonary function, which may decrease the success rate for the weaning of patients with clinically borderline cardiopulmonary function from ECLS.

To overcome these issues, improvements must be made to the ECLS device that will allow the dose of heparin administration for systemic anti-coagulation and the frequency of circuit exchange to be reduced.

The National Cardiovascular Center Research Institute have created a new quick-loading and heparin-coated ECLS system (Endumo® 2000, Heiwa Bussan, Tokyo, Japan), which consists of a ROTAFLOW® centrifugal pump (Maquet, Rastatt, Germany) and an ECLS circuit coated using a newly developed heparin coating technique (T-NCVC® coating; National Cardiovascular Center, Osaka, Japan, and Toyobo, Osaka, Japan) as well as an oxygen membrane (BIOCUBE 2000®, Nipro, Osaka, Japan). In the study described in the present paper, the early clinical outcomes associated with the use of the Endumo® 2000 system in pediatric patients <1 year old were reviewed, focusing in particular on its safety and durability.

Materials and methods

Patients

The National Cerebral and Cardiovascular Center Institutional Review Board approved this retrospective study and waived the need to obtain patient consent. From January 2008 to December 2011, 31 patients <1 year old required ECLS at this study's research center. Primary indications for ECLS institution were intraoperative low output syndrome in 11 patients, post-cardiotomy cardiopulmonary collapse in 10 patients, neonatal fulminant myocarditis related cardiogenic shock in 3 patients, and other indications in 7 patients. Until December 2009, 14 consecutive patients (6 boys, median age 17 days old, median body weight 3012 g) required ECLS support with the pediatric Emersave® system (TERUMO, Saitama, Japan). From December 2009, 16 consecutive patients (8 boys, median age 9.5 days old, median body weight 2937 g) required ECLS support with the Endumo® 2000 system (Endumo group). One patient who was instituted with a transitional ECLS device from Emersave® to Endumo® 2000 in November 2009 was excluded from this study. There was no significant difference in patient characteristics between the groups (Table 1).

During the study period, ECLS was not instituted as a bridge to ventricular assist system implantation because there was no appropriate device for younger populations. Also, Japanese organ transplantation registration changed to allow the harvesting of organs from pediatric donors from July 2011 onwards; however, pediatric–pediatric heart transplantation was performed in only 1 patient during this study period [15] because of a severe shortage of pediatric

Table 1 Patient characteristics

	Emersave® group	Endumo® group	*p* value
N	14	16	
Male:female (*n*)	6:8	8:8	0.70
Age at institution [days, median (range)]	17 (0–287)	9.5 (0–306)	0.64
Body weight [g, median (range)]	3012 (1299–5600)	2936 (2233–5200)	0.79
Main diagnosis (*n*)			
Atrial isomerism		1	
Atrioventricular septal defect		1	
Coronary artery fistula		1	
Critical aortic stenosis		1	
Double inlet left ventricle		1	
Ebstein's anomaly		2	
HLHS and variant	8	4	0.07
IAA complex		1	
MS, hypo LV, DORV	1		
Neonatal fulminant myocarditis		3	0.09
PA/IVS	1		
TAPVC, PVO	1		
Tetralogy of Fallot	1	1	
Truncus arteriosus communis	2		
Cause of institution [*n*, (%)]			
Preoperative respiratory failure	1		
Preoperative cardiogenic shock		3	
Intraoperative LOS	7	4	0.20
Intraoperative PH		3	
Postoperative cardiogenic shock	4	5	
Postoperative PH	2	1	
ECLS access (*n*)			
Neck	1	3	
Chest	13	13	
Additional systemic atrial vent (*n*)	0	1	
Concomitant ECUM (*n*)	4	2	
Use of PD (*n*)	10	15	

HLHS hypoplastic left heart syndrome, *IAA* interrupted aortic arch, *MS* mitral stenosis, *DORV* double outlet right ventricle, *PA/IVS* pulmonary atresia with intact ventricular septum, *TAPVC* total anomalous pulmonary venous connection, *PVO* pulmonary venous obstruction, *LOS* low output syndrome, *PH* pulmonary hypertension, *ECUM* extracorporeal ultrafiltration method, *PD* peritoneal dialysis

donors. Therefore, all of the patients awaited recovery of their cardiopulmonary function during ECLS support.

ECLS institution

Arterial and venous cannulae were placed directly in the aorta and systemic atrium with the chest left open in patients who underwent open heart surgery, and if the patient underwent repair of the aortic arch (such as Norwood palliation), the arterial cannula was connected to the ePTFE graft, which was anastomosed to the innominate artery for systemic and cerebral perfusion during the operation. In patients who developed nonsurgical cardiogenic shock, both cannulae were placed in the internal jugular vein and the carotid artery for venoarterial ECLS.

For sudden cardiopulmonary collapse, a compact and simple ECLS system (99kun) was used with a priming volume of 99 ml and a priming time of 3 min or less for the emergent initiation of ECLS without continuous heparin administration, before conversion to a long-term-use ECLS system. The 99kun consisted of a centrifugal pump (Mix flow pump®, JMS, Hiroshima, Japan), a hollow-fiber membrane oxygenator (Oxia IC®, JMS, Hiroshima, Japan), and a PVC tube (TOYOBO, Osaka, Japan) coated with the T-NCVC coating, without a heat exchanger. The full length of the circuit tube was 85 cm.

Patient management during ECLS

All patients were deeply sedated with muscular relaxants. Daily cranial echography was performed for early detection of cranial hemorrhage. Inotropic support (usually dopamine of 3 mg/kg/min) was continued to avoid afterload mismatch and subsequent pulmonary edema. The mean blood pressure was maintained at 40 mmHg, and systemic vasodilators were aggressively administered for better peripheral perfusion. A mechanical ventilator was usually set at the resting breathing mode: respiratory rate of 10 times/min, pressure control of 12 cm H_2O, fraction of inspired oxygen of 0.5, and positive end-expiratory pressure of 4 cm H_2O. In patients with bronchial or pulmonic hemorrhage, the positive end-expiratory pressure was set at 6–8 cm H_2O. Diuretics were routinely administered to remove body edema completely, and peritoneal dialysis was occasionally used as a renal replacement therapy.

Continuous heparin was administered as an anti-coagulation therapy, and maintained to achieve an activated clotting time of between 180 and 200 s in patients with Emersave support, and between 160 and 200 s in patients with Endumo support. At the same time, platelet and fresh frozen plasma was aggressively transfused to keep platelet counts higher than 100,000/mm^3 and the prothrombin time–international normalized ratio at <2.0.

ECLS circuit exchange was conducted for the following reasons: A (device-related complications): (1) deterioration in the gas exchange of the oxygenator (thromboembolism, plasma leakage, wet lung), (2) decreased centrifugal pump flow, and (3) clot formation in the bypass circuit; B (biological reaction): (1) hemolysis. Circuit exchange was performed under intravenous anesthesia without increasing inotrope support or volume load. The circuit could always be changed within 1.5 min of the arrest in ECLS support.

Study methods and statistical analysis

Patients' medical records were retrospectively reviewed to (1) evaluate the first circuit durability and critical intracranial hemorrhaging, (2) compare early clinical outcomes between the Endumo group and Emersave group, and (3) to perform predictor analysis of successful weaning from the ECLS system and survival at discharge.

The first circuit durability was defined as the period from the institution of the first circuit to its exchange, so only patients who required a first circuit exchange were enrolled [Emersave group: $n = 10$ (71.4 %), Endumo group: $n = 5$ (31.3 %)]. Patients who did not require a first circuit exchange due to their death or successful weaning from the ECLS system were excluded.

Critical intracranial hemorrhage was defined as an irreversible intracranial hemorrhage despite a satisfactory recovery of cardiac function. Successful weaning from the ECLS was defined as a patient who was weaned from the ECLS and did not require re-initiation during the same hospital stay. Notably, a decision by the patient's family to terminate is rare in Japan, and financial issues are not a reason for declining the ECLS support, because the national health insurance covers almost all medical costs during hospitalization.

Pre- and post-operative data were expressed as the mean ± standard deviation or median (range). Data were analyzed using SPSS (SPSS Inc., Chicago, IL, USA), and differences were considered statistically significant when the p value was <0.05.

Results

Circuit durability and occurrence of critical intracranial hemorrhaging

The median total ECLS support period was 5.5 days (range 0.3–65.0) in the Emersave group and 7.0 days (range 0.8–29.7) in the Endumo group. The median first circuit durability was significantly longer in the Endumo group [8.0 days (range 5.9–13.2)] than in the Emersave group [4.4 days (1.9–8.3)] ($p = 0.020$) (Table 2). Significant

Table 2 Comparison of early clinical outcomes in the Endumo group to those in the Emersave group

Parameter	Emersave group	Endumo group	*p* value
N	14	16	
Total ECLS support period (days, median [range])	5.5 (0.3–65.0)	7.0 (0.8–29.7)	
1st circuit durability (days, median [range])	4.4 (1.9–8.3)	8.0 (5.9–13.2)	0.020
Fatal cranial hemorrhage (n)	1	0	
Thrombus formation (n)			
In left ventricle	0	1	
Above aortic valve	0	1	
Survival (*n*, %)			
At 30 days from ECLS institution	7 (50.0)	10 (62.5)	0.49
At 3 months from ECLS institution	2 (14.3)	9 (56.3)	0.017
At discharge	2 (14.3)	8 (50.0)	0.038
Weaning from ECLS (*n*, %)	2 (14.3)	9 (56.3)	0.017

ECLS extracorporeal life support

irreversible intracranial hemorrhage occurred in only 1 patient in the Emersave group. On the other hand, 2 patients with neonatal fulminant myocarditis developed thrombus formation in the left ventricular cavity or in the aortic sinus during ECLS with the Endumo system, both of which were successfully dissolved by argatroban hydrate administration.

Comparison of early clinical outcomes between the Emersave group and Endumo group

As compared with the Emersave group, the Endumo group showed a better survival rate at 3 months after the institution of ECLS and at discharge (Table 2). The probability of successful weaning from the ECLS system was 14.3 % (2/14) in the Emersave group and 56.3 % (9/16) in the Endumo group ($p = 0.017$).

Predictor for survival at discharge and successful discontinuation from ECLS

Univariate analysis showed that usage of Endumo® 2000 was the only significant predictor for both successful weaning from the ECLS system ($p = 0.017$) (Table 3) and survival at discharge ($p = 0.038$) (Table 4). A diagnosis of no HLHS or its variant was the other predictor for survival at discharge (Table 4).

Table 3 Predictor for successful weaning from the ECLS system

	Weaning	Expired	*p* value
Usage of Endumo (*n*)	9/11	7/19	0.017
BW (kg)	3.5 ± 8.2	2.8 ± 8.2	0.052
Without HLHS or variant (*n*)	9/11	9/19	0.063
Biventricular physiology (*n*)	5/11	6/19	0.14
Concomitant ECUM (*n*)	1/11	5/19	0.26
Post-cardiotomy (*n*)	3/11	9/19	0.28
Age (days old)	65 ± 101	46 ± 71	0.55

BW body weight, *HLHS* hypoplastic left heart syndrome, *ECUM* extracorporeal ultrafiltration method

Table 4 Predictor for survival at discharge

	Survived	Expired	*p* value
Without HLHS or variant (*n*)	9/10	9/20	0.018
Usage of Endumo (*n*)	8/10	8/20	0.038
BW (kg)	3.5 ± 8.5	2.8 ± 8.0	0.054
Biventricular physiology (*n*)	6/10	5/20	0.061
Concomitant ECUM (*n*)	1/10	5/20	0.35
Post-cardiotomy (*n*)	3/10	9/20	0.45
Age (days old)	68 ± 105	45 ± 69	0.54

BW body weight, *HLHS* hypoplastic left heart syndrome, *ECUM* extracorporeal ultrafiltration method

Discussion

This study showed the safety and clinical efficacy of the Endumo® 2000 system as compared to the conventional Emersave system in patients <1 year old. The usage of the Endumo system provided longer support without the need for frequent circuit exchange. Critical cranial hemorrhage was not seen in the Endumo group. The usage of Endumo was the predictor for successful weaning from the ECLS system and for survival at discharge.

During ECLS support in small patients, hemorrhagic tendencies or hemodynamic instability occur after the circuit exchange, partly due to dilution of blood viscosity, endogenous catecholamine, and coagulation factors. Therefore, frequent circuit exchange obviously delays or interrupts the recovery of cardiopulmonary function, particularly in clinically borderline patients. Longer circuit durability of the Endumo® 2000 system reduces the frequency of circuit exchange, and may help to improve overall outcomes.

Whereas several reports have shown that a longer duration of ECLS is a significant risk factor for survival at discharge [1–3], there were some cases who survived long after ECLS [6]. A few days usage of ECLS seems to be sufficient to facilitate recovery from cardiopulmonary dysfunction as a part of multi-organ failure caused by significant capillary leakage syndrome after prolonged

aortic cross clamping or cardiopulmonary bypass. Indeed, 7 of the 11 successfully weaned patients needed ECLS for 4 days or less. On the other hand, the remaining 4 patients showed sufficient recovery after ≥1 week of ECLS; all of those patients were supported by the Endumo system. Although prolonged ECLS does not promise further myocardial recovery, this study shows that longer ECLS may save more patients if the circuit is rarely exchanged and hemorrhagic tendencies or coagulopathy are well controlled during support.

This study is the first to demonstrate the clinical safety and efficacy of the Endumo® 2000 system in neonates and infants. This ECLS circuit is coated using a newly developed heparin coating technique, which is characterized as an ionic complex of bonded heparin and aliphatic coupling reagents [16, 17]. Also, the microporous hollow-fiber membrane oxygenator with a dense layer at the blood- and gas-contacting surface was made of polymethylpentene [18, 19]. In animal experiments, at least 30 days of heparin-free use and more than 5 months of use with trivial heparin administration (<140 s of activated coagulation time) were successfully achieved without encountering any thromboembolic events [18, 19]. Though continuous heparin administration is still recommended clinically because the ROTAFLOW centrifugal pump is not coated with the T-NCVC coating, a shorter target activated clotting time may be possible.

The other factor that contributed to improving the outcome is thought to be the application of the ROTAFLOW centrifugal pump [20]. This device has a unique structure ("spiral housing"), which provides an ideal distribution of blood flow and minimizes hemolysis and thrombus formation. Also, the small priming volume (32 ml) and minimal surface area inhibits excessive blood–material interaction, which may play an optimal role in particular in neonates and early infants with shock and vascular hyperpermeability.

Thrombus formation in 2 patients with fulminant neonatal myocarditis who were supported by the Endumo system is a matter of concern. Although a severely reduced ventricular contraction was the major reason, more restrictive anticoagulation control may prevent this complication. It is a fact that thrombus formation in the Endumo system can be safely avoided by reducing heparin administration, but the anti-coagulation protocol should be revised for patients with an almost silent ventricle.

Study limitations

This was a retrospective study of a relatively small cohort, and the therapeutic outcome may have been affected by the differences in the patient characteristics in each group. Although there was no significant difference in diagnosis between both groups, the Emersave group included more single-ventricle patients, such as those with HLHS and its variant, whereas the Endumo group included 2 neonatal fulminant myocarditis patients. HLHS and its variant are widely known to be a risk factor for successful weaning from ECLS. On the other hand, neonatal fulminant myocarditis patients are likely to be weaned from ECLS after aggressive medical treatment. Although this study clearly demonstrated the safe and long-term use of the Endumo system without the need for frequent circuit exchange, it is recommended that a prospective randomized trial should be performed to confirm the clinical superiority of the Endumo system.

Conclusion

Although it is recommended that a prospective randomized trial should be performed to confirm the therapeutic superiority, the Endumo® 2000 system promises and long-term cardiopulmonary support without complications for patients waiting for cardiopulmonary recovery.

Conflict of interest None declared.

References

1. del Nido PJ. Extracorporeal membrane oxygenation for cardiac support in children. Ann Thorac Surg. 1996;61:336–9.
2. Duncan BW, Ibrahim AE, Hraska V, del Nido PJ, Laussen PC, Wessel DL, Mayer JE Jr, Bower LK, Jonas RA. Use of rapid-deployment extracorporeal membrane oxygenation for the resuscitation of pediatric patients with heart disease after cardiac arrest. J Thorac Cardiovasc Surg. 1998;116:305–11.
3. Aharon AS, Drinkwater DC Jr, Churchwell KB, Quisling SV, Reddy VS, Taylor M, Hix S, Christian KG, Pietsch JB, Deshpande JK, Kambam J, Graham TP, Chang PA. Extracorporeal membrane oxygenation in children after repair of congenital cardiac lesions. Ann Thorac Surg. 2001;72:2095–101.
4. Kolovos NS, Bratton SL, Moler FW, Bove EL, Ohye RG, Bartlett RH, Kulik TJ. Outcome of pediatric patients treated with extracorporeal life support after cardiac surgery. Ann Thorac Surg. 2003;76:1435–41.
5. Hoskote A, Bohn D, Gruenwald C, Edgell D, Cai S, Adatia I, Van Arsdell G. Extracorporeal life support after staged palliation of a functional single ventricle: subsequent morbidity and survival. J Thorac Cardiovasc Surg. 2006;131:1114–21.
6. Thourani VH, Kirshbom PM, Kanter KR, Simsic J, Kogon BE, Wagoner S, Dykes F, Fortenberry J, Forbess JM. Venoarterial extracorporeal membrane oxygenation (VA-ECMO) in pediatric cardiac support. Ann Thorac Surg. 2006;82:138–44.
7. Cooper DS, Jacobs JP, Moore L, Stock A, Gaynor JW, Chancy T, Parpard M, Griffin DA, Owens T, Checchia PA, Thiagarajan RR, Spray TL, Ravishankar C. Cardiac extracorporeal life support: state of the art in 2007. Cardiol Young. 2007;17(Suppl 2):104–15.
8. Alsoufi B, Al-Radi OO, Nazer RI, Gruenwald C, Foreman C, Williams WG, Coles JG, Caldarone CA, Bohn DG, Van Arsdell

GS. Survival outcomes after rescue extracorporeal cardiopulmonary resuscitation in pediatric patients with refractory cardiac arrest. J Thorac Cardiovasc Surg. 2007;134:952–9.
9. Chan T, Thiagarajan RR, Frank D, Bratton SL. Survival after extracorporeal cardiopulmonary resuscitation in infants and children with heart disease. J Thorac Cardiovasc Surg. 2008;136:984–92.
10. Alsoufi B, Al-Radi OO, Gruenwald C, Lean L, Williams WG, McCrindle BW, Caldarone CA, Van Arsdell GS. Extra-corporeal life support following cardiac surgery in children: analysis of risk factors and survival in a single institution. Eur J Cardiothorac Surg. 2009;35:1004–11.
11. Delmo Walter EM, Alexi-Meskishvili V, Huebler M, Loforte A, Stiller B, Weng Y, Berger F, Hetzer R. Extracorporeal membrane oxygenation for intraoperative cardiac support in children with congenital heart disease. Interact Cardiovasc Thorac Surg. 2010;10:753–8.
12. Copeland H, Nolan PE, Covington D, Gustafson M, Smith R, Copeland JG. A method for anticoagulation of children on mechanical circulatory support. Artif Organs. 2011;35:1018–23.
13. Mazzucotelli JP, Leprince P, Litzler PY, Vincentelli A, Le Guyader A, Kirsch M, Camilleri L, Flecher E, Reflection Group on Mechanical Circulatory Support (GRAM). Results of mechanical circulatory support in France. Eur J Cardiothorac Surg. 2011;40:e112–7.
14. McMullan DM, Emmert JA, Permut LC, Mazor RL, Jeffries HE, Parrish AR, Baden HP, Cohen GA. Minimizing bleeding associated with mechanical circulatory support following pediatric heart surgery. Eur J Cardiothorac Surg. 2011;39:392–7.
15. Ueno T, Fukushima N, Sakaguchi T, Ide H, Ozawa H, Saito S, Ichikawa H, Sawa Y. First pediatric heart transplantation from a pediatric donor heart in Japan. Circ J. 2012;76:752–4.
16. Sato M, Kashiwabara S, Tanaka H, Tatsumi E, Taenaka Y, Takano H. In vitro antithrombogenic evaluation of newly developed heparinized coating material. Jpn Artif Organs. 1999;28:502–8.
17. Nishinaka T, Tatsumi E, Katagiri N, Ohnishi H, Mizuno T, Shioya K, Tsukiya T, Homma A, Kashiwabara S, Tanaka H, Sato M, Taenaka Y. Up to 151 days of continuous animal perfusion with trivial heparin infusion by the application of a long-term durable antithrombogenic coating to a combination of a seal-less centrifugal pump and a diffusion membrane oxygenator. J Artif Organs. 2007;10:240–4.
18. Tatsumi E, Taenaka Y, Nakatani T, Akagi H, Sekii H, Yagura A, Sasaki E, Goto M, Nakamura H, Takano H. A VAD and novel high performance compact oxygenator for long-term ECMO with local anticoagulation. ASAIO Trans. 1990;36:M480–3.
19. Nishinaka T, Tatsumi E, Taenaka Y, Katagiri N, Ohnishi H, Shioya K, Fukuda T, Oshikawa M, Sato K, Tsukiya T, Homma A, Takewa Y, Takano H, Sato M, Kashiwabara S, Tanaka H, Sakai K, Matsuda T. At least thirty-four days of animal continuous perfusion by a newly developed extracorporeal membrane oxygenation system without systemic anticoagulants. Artif Organs. 2002;26:548–51.
20. Mender N, Podechtl F, Feil G, Hiltmann P, Sebending F. Seal-less centrifugal blood pump with magnetically suspended rotor: Rot-a-Flot. Artif Organs. 1995;19:620–4.

Insufflation of Hydrogen Gas Restrains the Inflammatory Response of Cardiopulmonary Bypass in a Rat Model

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Abstract: Systemic inflammatory responses in patients receiving cardiac surgery with the use of the cardiopulmonary bypass (CPB) significantly contribute to CPB-associated morbidity and mortality. We hypothesized that insufflated hydrogen gas (H_2) would provide systemic anti-inflammatory and anti-apoptotic effects during CPB, therefore reducing proinflammatory cytokine levels. In this study, we examined the protective effect of H_2 on a rat CPB model. Rats were divided into three groups: the sham operation (SHAM) group, received sternotomy only; the CPB group, which was initiated and maintained for 60 min; and the CPB + H_2 group in which H_2 was given via an oxygenator during CPB for 60 min. We collected blood samples before, 20 min, and 60 min after the initiation of CPB. We measured the serum cytokine levels of (tumor necrosis factor-α, interleukin-6, and interleukin-10) and biochemical markers (lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase). We also measured the wet-to-dry weight (W/D) ratio of the left lung 60 min after the initiation of CPB. In the CPB group, the cytokine and biochemical marker levels significantly increased 20 min after the CPB initiation and further increased 60 min after the CPB initiation as compared with the SHAM group. In the CPB + H_2 group, however, such increases were significantly suppressed at 60 min after the CPB initiation. Although the W/D ratio in the CPB group significantly increased as compared with that in the SHAM group, such an increase was also suppressed significantly in the CPB + H_2 group. We suggest that H_2 insufflation is a possible new potential therapy for counteracting CPB-induced systemic inflammation. **Key Words:** Cardiopulmonary bypass—Rat cardiopulmonary bypass model—Systemic inflammatory response—Hydrogen gas.

Extracorporeal life support devices, such as the cardiopulmonary bypass (CPB), preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs (1,2). However, cardiac surgery with the use of CPB is often accompanied by a systemic inflammatory response, contributing significantly to the morbidity and mortality during CPB (3–5).

Possible factors responsible for the inflammatory response are the blood contact with the surface of the extracorporeal circulation unit, endotoxemia, surgical trauma, ischemic reperfusion injury, and blood loss (6,7). The increase in cytokines, such as interleukins, necrosis factor, and bradykinin (8,9), aggravates the inflammatory response during CPB (10–12).

Recent studies have shown that drinking hydrogen enhanced water prolongs survival of cardiac allografts and may protect cardiac allografts from allograft vasculopathy in rats model (13). The inhalation of hydrogen gas (H_2) has been shown to reduce infarct size in the rat model of myocardial (14) and cerebral (15) infarction through antioxidant effects.

We hypothesized that insufflation of H_2 would attenuate the systemic inflammatory response with a

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reduction of inflammatory cytokine levels, providing protective effects against organ tissue damage during CPB. Therefore, in this study, we investigated the effect of H_2 insufflation on levels of serum cytokines, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-10 (IL-10), and biochemical markers lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in a rat CPB model. In addition, we measured wet-to-dry weight (W/D) ratio of the lung.

MATERIALS AND METHODS

Animal

The study was approved by the National Cerebral and Cardiovascular Center Research Institute Animal Care and Use Committee, and all procedures met the National Institutes of Health guidelines for animal care.

Sprague-Dawley rats (male 400–450 g) were housed three per cage under a 12-h light–dark cycle with food and water available ad libitum.

Anesthesia, surgical preparation, and CPB

The animals were anesthetized with pentobarbital sodium (50 mg/kg body weight intraperitoneal injection), placed in the supine position with electrocardiograph monitoring and rectal thermocouple in place. Then, a tracheostomy and intratracheal intubation was performed using a 14G cannula (Insyte BD Medical, Sandy, UT, USA), and rats were ventilated with a respirator (Model SN-480-7, Shinano Seisakusho Co., Ltd, Tokyo, Japan). Ventilation was volume controlled at a frequency of 60/min, a tidal volume of 8–10 mL/kg body weight, and a positive end expiratory pressure of 3 cm H_2O. Rectal temperature was maintained at 36°C throughout the experiment. Arterial blood pressure was monitored (Model 870, PowerLab system, AD Instruments, Castle Hill, Australia) via the femoral artery, which was cannulated with polyethylene tubing (SP-31 Natsume Seisakusho Co., Ltd, Tokyo, Japan). The left common carotid artery with a polyethylene tubing (SP-55 Natsume Seisakusho Co., Ltd) served as the arterial inflow cannula for the CPB circuit. Heparin sodium 500 IU/kg was administered after placement of this cannula. A 16G cannula (Insyte BD Medical) was advanced through the right external jugular vein into the right atrium and served as a conduit for venous outflow.

The CPB circuit consisted of a membranous oxygenator (Senko Medical Co., Ltd, Osaka, Japan), tubing line (Senko Medical Co., Ltd), and roller pump (Micro tube pump MP-3 Tokyo Rikakikai Co., Ltd, Tokyo, Japan) primed by 8 mL of Ringer's solution, 3 mL of mannitol, 3 mL of sodium bicarbonate, and 1 mL (1000 IU) of heparin.

Experimental design

The animals were divided into three groups: Sham operation (SHAM, negative control), CPB (positive control), and CPB + H_2 groups. The SHAM group ($n = 5$) received sternotomy only. In the CPB group ($n = 7$), CPB was initiated and maintained for 60 min. In the CPB + H_2 group ($n = 7$), hydrogen gas was added into the oxygenator during CPB at a concentration of 14 000 ppm (O_2 flow : H_2 flow = 1:1) for 60 min.

CPB was initiated and maintained at 60 mL/kg/min. Arterial pressure of carbon dioxide ($PaCO_2$) and arterial pressure of oxygen (PaO_2) were maintained at 35–45 mm Hg and 300–400 mm Hg, respectively. Blood samples were collected at three defined time points, before CPB (pre-CPB), 20 min after initiation of CPB and 60 min after initiation of CPB (end-CPB).

To evaluate the inflammatory responses (16), TNF-α, IL-6, and IL-10 were measured (ELISA kit, R&D Systems, Minneapolis, MN, USA). The biochemical markers for evaluating organ damage (17), LDH, AST, and ALT were measured (DRI-CHEM 7000, Fujifilm, Kanagawa, Japan).

Blood gases, pH, hemoglobin concentration, and electrolytes were also measured. Animals in which the hemoglobin level declined to less than 7 g/dL at any point were excluded from the study. All animals were sacrificed at the end of CPB by myocardial potassium injection and the left lung was harvested and divided into three parts. The superior third was used for the calculation of W/D ratio. The lung block was weighed before and after desiccation for 72 h in a drying oven at 70°C.

Statistics

All data are expressed as mean ± standard deviation. Comparison among groups was performed using analysis of variance. Fisher Protected Least Significant Difference post hoc test was used for subsequent comparison between groups at the same time. All statistical analyses were performed using StatView 5.0 (Abacus Concepts, Berkeley, CA, USA). Significance was set at $P < 0.05$.

RESULTS

Before CPB, the serum levels of inflammatory and biochemical markers were not statistically different

TABLE 1. *Hemodynamic variables, Hb and blood gas partial pressures before and during CPB*

	Group	Pre-CPB	CPB 20 min	CPB 60 min
MAP (mm Hg)	SHAM	119 ± 10	100 ± 13	107 ± 11
	CPB	115 ± 16	96 ± 18	73 ± 19*
	CPB + H_2	111 ± 18	92 ± 14	67 ± 11*
HR (beat/min)	SHAM	387 ± 39	374 ± 38	389 ± 26
	CPB	396 ± 29	379 ± 37	341 ± 55
	CPB + H_2	390 ± 34	365 ± 23	340 ± 23
PaO_2 (mm Hg)	SHAM	102 ± 11	101 ± 9	99 ± 10
	CPB	100 ± 3	383 ± 30*	362 ± 29*
	CPB + H_2	99 ± 9	370 ± 35*	351 ± 47*
$PaCO_2$ (mm Hg)	SHAM	38 ± 4	40 ± 5	36 ± 6
	CPB	41 ± 2	34 ± 6	35 ± 5
	CPB + H_2	41 ± 4	36 ± 3	38 ± 4
Hb (mg/dL)	SHAM	15.3 ± 2.1	15.2 ± 1.0	14.5 ± 0.9
	CPB	14.3 ± 1.3	9.9 ± 1.1*	9.4 ± 1.0*
	CPB + H_2	15.0 ± 1.7	9.8 ± 1.5*	9.5 ± 0.9*

Variables are expressed by mean ± standard deviation.
* $P < 0.05$ versus SHAM group at the same time.
MAP, mean arterial pressure.

among the SHAM, CPB, and CPB + H_2 groups. During CPB, systemic arterial blood pressure and heart rate were unaffected by H_2. Table 1 presents the changes in hemodynamic variables, hemoglobin (Hb) concentration and PaO_2 and $PaCO_2$ from both CPB and SHAM groups during experiments.

Serum inflammatory and biochemical markers remained unchanged during experiment periods in the SHAM group. In the CPB group, all the systemic inflammatory markers increased significantly, reaching a maximum (TNF-α: 1347 ± 199 pg/mL, IL-6: 1763 ± 297 pg/mL, IL-10: 1208 ± 228 pg/mL) at the end of CPB. However, in the CPB + H_2 group, the increase in the levels was significantly suppressed by 53–57% compared with the CPB group (Fig. 1a–c).

In the CPB group, the levels of biochemical markers significantly increased (LDH: 916 ± 263 U/L, AST: 128 ± 42 U/L, ALT: 60 ± 17 U/L) 20 min after the CPB initiation and increased further (LDH: 1210 ± 289 U/L, AST: 201 ± 30 U/L, ALT: 147 ± 43 U/L) 60 min after the CPB initiation as compared with the other groups. In the CPB + H_2 group, the elevated levels of biochemical markers were significantly suppressed by 55–65% 60 min after the CPB initiation as compared with the CPB group (Fig. 1d–f).

The CPB groups showed significantly higher W/D ratio than the SHAM group. However, the increase in W/D ratio was significantly suppressed in CPB + H_2 group (SHAM: 4.67 ± 0.19, CPB: 5.59 ± 0.18, CPB + H_2: 5.04 ± 0.21) (Fig. 2).

DISCUSSION

The present data showed that during CPB the serum cytokine levels (TNF-α, IL-6, and IL-10) and biochemical markers (LDH, ALT, and AST) were significantly elevated in the CPB group compared with the SHAM group, indicating that a systemic inflammatory response and organ damage occurred in our rat CPB model. During CPB, blood pressure and Hb were maintained around 80 mm Hg and 10 g/dL, respectively. From these data, our rat CPB model is considered to be equivalent to the established human CPB, which is often associated with systemic inflammation and organ damage (5,18,19).

Possible factors responsible for the inflammatory response during CPB are blood contact with the surface of the extracorporeal circulation unit, endotoxemia, surgical trauma, ischemic reperfusion injury, and blood loss (6,7). Many studies showed the walls of the CPB circuit activate white cells, platelets, and the complement system. Activated leukocytes release cytotoxic agents and reactive oxygen species (ROS) associated with the systemic inflammation and organ damage (20–22). The increase in cytokines, such as interleukins and necrosis factor (8,9), aggravates the inflammatory response (10–12). These complex interactions during CPB lead to further inflammation (10–12).

In this study, we used H_2 that selectively reduces the hydroxyl radical, the most cytotoxic of ROS, and effectively protected cells (15). H_2 is known to have advantages as a potential antioxidant: it rapidly

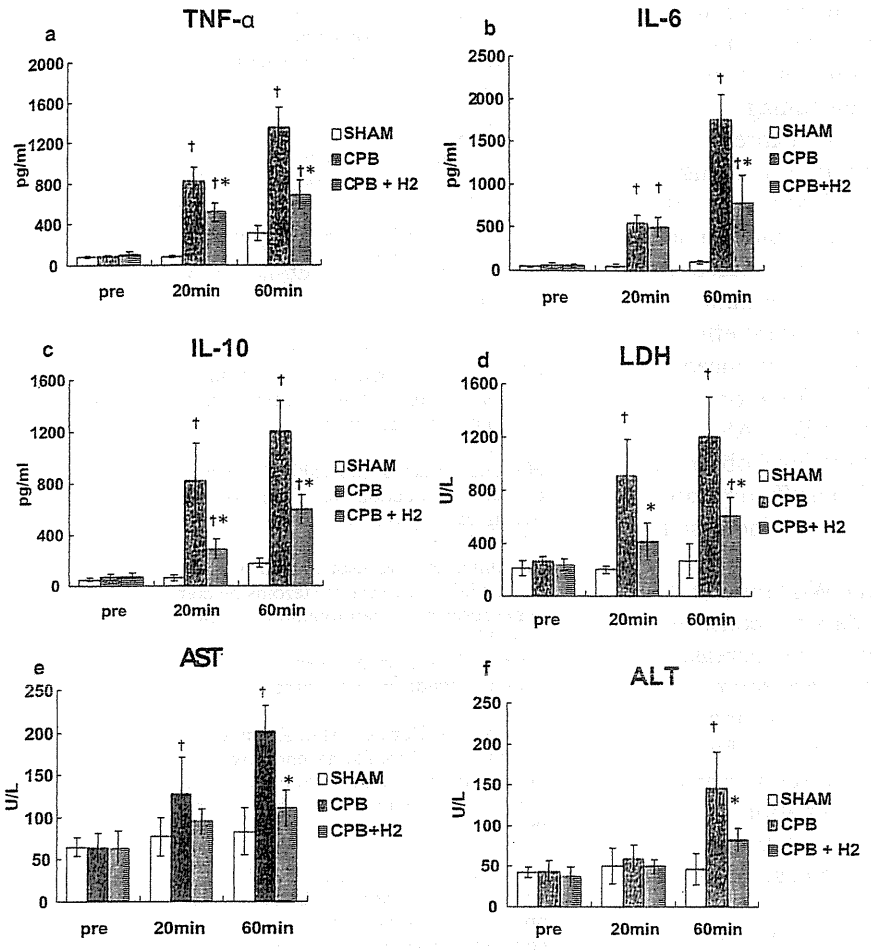

FIG. 1. Serum tumor necrosis factor (TNF)-α (a), interleukin (IL)-6 (b), interleukin (IL)-10 (c), lactate dehydrogenase (LDH) (d), aspartate aminotransferase (AST) (e), and alanine aminotransferase (ALT) (f). †$P < 0.05$ versus SHAM group, *$P < 0.05$ versus CPB group at the same time periods.

diffuses into tissues and cells and does not affect ROS that function in cell signaling, and thereby, has little adverse effects (15,23). We showed for the first time that H_2 insufflation significantly suppressed the elevated levels of serum cytokines (TNF-α, IL-6, and IL-10) and biochemical markers (LDH, AST, and ALT) during CPB. Possible mechanisms for the decrease in biochemical markers is that H_2 insufflation suppressed the cell damages due to the direct action of the hydroxyl radical (15,23). Because ROS is known to trigger a cytokine cascade initiated by TNF-α release (24), it is also possible that H_2 insufflation suppressed cytokine generation via the ROS-scavenging effect. Notably, a recent study suggested H_2 inhalation reduces infarct size by scavenging ROS in a rat model of myocardial ischemia-reperfusion injury (14). In addition, drinking hydrogen enhanced water protected cardiac and aortic allograft recipients from allograft vasculopathy purportedly via antioxidant and anti-inflammatory effects (13). Considering these previous findings and the present data together, we suggest that H_2 insufflation not only attenuates the direct cell-damaging effect of ROS, but also inhibits the proinflammatory cytokine generation, reducing biochemical markers reflecting organ damage in the rat CPB model.

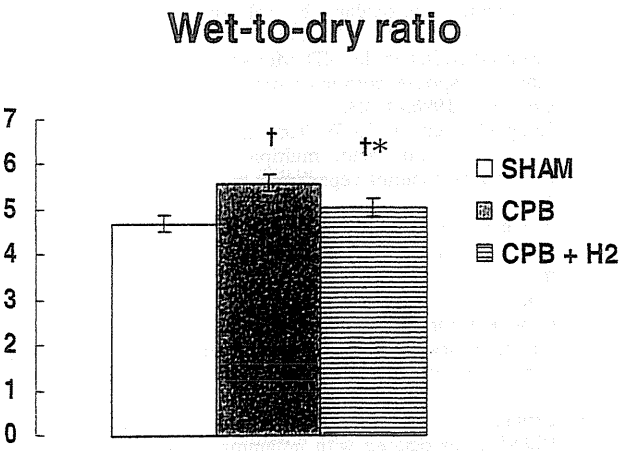

FIG. 2. Wet-to-dry ratio of the left lung. †$P < 0.05$ versus SHAM group, *$P < 0.05$ versus CPB group.