Preclinical Assessment of a Trileaflet Mechanical Valve in the Mitral Position in a Calf Model

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Background. **The bileaflet valve is currently the mechanical replacement valve of choice. Though durable, it does not closely mimic native valve hemodynamics and remains potentially thrombogenic.**

Methods. **Prototype trileaflet valves (T1 and T2) were implanted in the mitral position in calves. Group I calves received either a T1 valve (n = 12) or a control bileaflet valve (n = 5); Group II, either a T2 valve (n = 7) or a control bileaflet valve (n = 5). Valve function, perivalvular leakage, and transvalvular pressure gradients were evaluated. Also, long-term prototype leaflet wear was evaluated in vivo in one Group I calf (502 days) and two Group II calves (385 and 366 days). Calves were euthanized and necropsied at study termination, and major organs weighed and examined.**

Results. **Valve function was excellent and hematologic parameters remained normal in all calves that survived to study termination. Mean peak transvalvular pressure gradients were 10 ± 7 mm Hg for T1 valves, 6 ± 3 mm Hg for T2 valves, and 12 ± 4 mm Hg for bileaflet control valves. Clinically insignificant valvular regurgitation was observed in both prototypes. Explanted valves showed no thrombus-impaired leaflet motion, except in two T1-fitted calves and one T2-fitted calf. Major organs showed no evidence of clinically significant thromboembolic events. There were no other significant differences between the results of experimental and control groups.**

Conclusions. **Prototype trileaflet valves performed safely and effectively in the mitral position in calves, even without long-term anticoagulation. This warrants their evaluation as an equivalent alternative to bileaflet valves.**

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Annually, 96,000 Americans undergo operations to repair or replace heart valves [1]. Replacement options include biological and mechanical valves. While both options are viable, each has drawbacks. Biological valves typically have shorter useful lives because of their low durability and high rate of calcification, while mechanical valves cannot duplicate the natural valve's hemocompatibility and require lifelong anticoagulation [2–7]. Currently, the mechanical replacement valve of choice is the bileaflet valve.

We have tested a trileaflet valve comprised of pyrolytic carbon leaflets arranged in a trileaflet, central-flow configuration with reduced marginal flow and no central obstructions. In 1994, we reported on the initial in vivo evaluation of two early trileaflet prototypes (A2 and A3) in the mitral position in a calf model [8]. Both prototypes performed well in the two longest-living calves (219 and 281 days, respectively), neither of which received anticoagulation or antiplatelet therapy after postoperative day (POD) 30 [8]. This work has led directly to the iterative design and testing in vitro of trileaflet prototypes with leaflets made of clinical-grade pyrolytic carbon and rings made of titanium. We report here on the preclinical testing of these prototypes in the mitral position in a calf model in vivo.

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Material and Methods

In Vivo Studies

ANIMAL MODEL. A total of 26 Dexter and Corriente cross calves, each 4 to 6 months old and weighing 80 to 110 kg, were used for in vivo studies. Group I calves (n = 17) received either a T1 valve (n = 12) or a control bileaflet valve (either a Carbomedics Inc [Austin, TX] bileaflet valve [n = 4] or a TRI Technologies bileaflet valve [n = 1]). Group II calves (n = 9) received either a T2 valve (n = 7) or a control bileaflet valve (St. Jude Medical [St. Paul, MN] bileaflet valve [n = 2]). The T1 and T2 prototype valves are shown in Figure 1.

PREOPERATIVE CARE. Before surgery, baseline laboratory blood tests were performed, including analysis of blood chemistry, complete blood cell count (CBC), prothrombin time (PT), partial thromboplastin time (PTT), plasma free hemoglobin, fibrinogen, and lactic dehydrogenase

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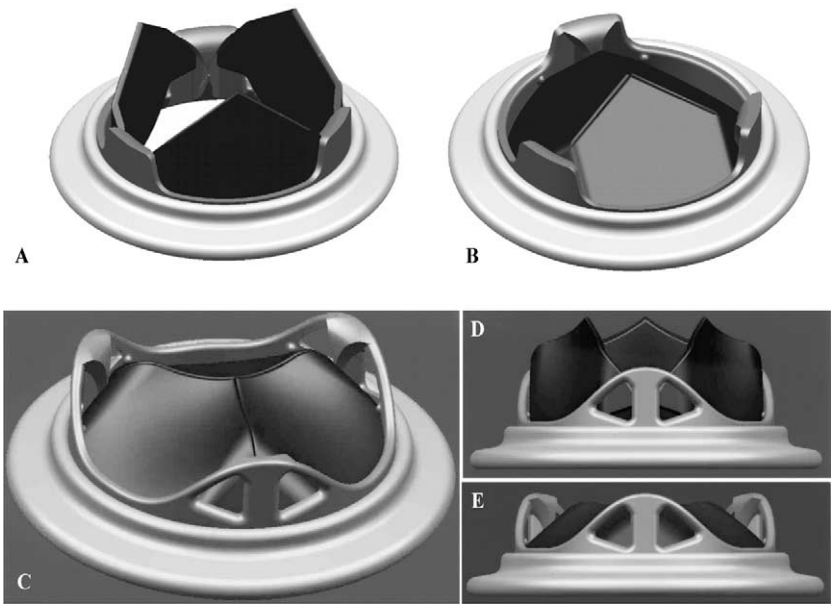

Fig 1. Trileaflet valve prototypes. T1 valve prototype, as seen from oblique view with leaflets open (A) and closed (B). T2 valve prototype with "windows," as seen from oblique view with leaflets closed (C), side view with leaflets open (D), and side view with leaflets closed (E).

(LDH). Other aspects of preoperative care have been previously described [8].

SURGICAL PREPARATION, OPERATIVE TECHNIQUE, AND INTRAOPERATIVE ECHOCARDIOGRAPHY. The surgical preparation and operative technique for mitral valve replacement (MVR) have been previously described [8]. All valves were 29 mm in diameter and were implanted in the mitral position and in the anatomical orientation after excision of the native leaflets. Immediately after MVR, transepicardial echocardiography was performed to verify proper valve function and absence of perivalvular leaks. Thereafter, calves were weaned from cardiopulmonary bypass (CPB), decannulated, and subjected to transepicardial echocardiography (TEE) using a transesophageal transducer and Sonos HP2000 echo machine. Data recorded included time velocity index (cm), time (msec), pressure ½ time (msec), peak velocity (cm/s), mean velocity (cm/s), peak gradient (mm Hg), mean gradient (mm Hg), and area (cm^2).

POSTOPERATIVE CARE. After surgery, calves were placed in custom-built stanchions for observation until chest tubes and arterial and intravenous (IV) catheter lines could be removed and the calves were deemed healthy and fully recovered from surgery.

In Group I, anticoagulation therapy consisted of continuous heparin infusion (500 to 1000 mg/d). PTT was monitored in order to maintain it at 1.5 to 2.0 times the baseline value. Calves remained in the intensive care unit for approximately 10 days to ensure proper healing and to allow administration of IV heparin anticoagulation therapy. After discontinuation of IV heparin therapy, the calves were returned to routine care.

In Group II, anticoagulation therapy consisted of continuous IV heparin infusion of 100 mg/d for 10 days. No conscious effort was made to regulate PTTs; these values usually remained very similar to baseline values throughout the 10-day regimen.

Neither group of calves received antiplatelet therapy at any time during the experiments.

LONG-TERM MANAGEMENT. During routine care, calves were evaluated daily for overall health, temperature, heart rate, respiration, and food consumption. At routine intervals, comprehensive laboratory blood tests were performed, including CBC, blood chemistry, and coagulation profiles.

STUDY TERMINATION. After 3 months (Group I) or 5 months (Group II), cardiac catheterization studies were performed before euthanizing the calves. Hemodynamics were assessed in terms of aortic pressure (AoP), left ventricular pressure (LVP), pulmonary capillary wedge pressure (PCWP), pulmonary artery pressure (PAP), and cardiac output (CO). Mitral valve regurgitation was assessed by left ventriculography in three contractile states: normal, hypercontractile, and hypocontractile. Hemodynamics were also assessed in these states. The hypercontractile state (defined as that state in which the derivative of pressure with respect to time [dP/dt] and the CO level were twice their baseline values) was induced by dobutamine drip infusion (20 to 100 mg/h). The hypocontractile state (defined as that state in which dP/dt and CO level were half the baseline values) was induced by esmolol drip infusion (1,000 to 2,000 mg/h). Mean valve transvalvular pressure gradients were calculated using the Gorlin technique [9]. After data collection, calves were given heparin (3 mg/kg) and then killed by IV administration of euthanasia solution (0.22 mL/kg).

NECROPSY. All calves (except for the two calves in Group I that were killed intraoperatively and at POD 1, respectively) underwent complete necropsy. Major organs, in-

cluding the brain (with rete mirabile), lungs, kidneys, adrenals, and heart, were examined grossly and photographed. Tissue specimens were taken for light microscopy analysis. Valves were removed and visually inspected, with light microscopy performed on the sewing ring area of each valve according to a detailed protocol. Both inflow and outflow tracts (atrial and ventricular sides, respectively) were evaluated in terms of depth, length, tissue type, presence of fibrinogenesis, and inflammation of the tissue covering the sewing ring and contacting the titanium valve ring. The leaflets of each experimental valve were assessed for freedom of motion and proper function. Any restriction or impingement of leaflet motion was noted in detail. Histologic host reactions were evaluated in terms of (a) depth and length (mm) of the neointimal growth on the anterior, lateral, and posterior quadrants of the atrial and ventricular surfaces; (b) fibrinogenesis (where 0 = none and 4 = excessive); and (c) inflammatory response (where 0 = none and 4 = excessive). The scores of these three aspects were averaged to obtain a final thrombogenicity score ranging from 0 (none) to 4 (excessive).

Analysis

Due to the small sample size, no statistical analyses were performed. Controls for Groups I (n = 5) and II (n = 2) were combined in analyses. The results were expressed as the mean ± standard deviation or percentage, as appropriate.

Results

In Vivo Experimental Results

MORTALITY AND MORBIDITY. In Group I, 7 of 12 experimental calves (58%) and 5 of 5 control calves (100%) survived to the study termination date (3 months) (Table 1). The remaining 5 calves (all experimental) were killed before the study termination date because they had early surgical or infectious complications. Complications included perivalvular leak diagnosed by transepicardial Doppler echocardiography and refractory post-CPB fibrillation (n = 1); improper closing of 1 or more leaflets due to a misplaced left arterial pressure (LAP) catheter that caused valve malfunction and prevented proper opening and closing of 1 or more leaflets (n = 1); pulmonary edema due to complete impingement of 2 leaflets by a misplaced suture that went undetected by Doppler echocardiography (n = 1); heart failure, later confirmed by necropsy, due to interference by a free-floating native leaflet (n = 1); and vegetative thrombus and leaflet malfunction, later confirmed by necropsy, due to vegetative endocarditis (n = 1). The calves with these complications were killed on POD 0, POD 1, POD 14, POD 25, and POD 56, respectively. One calf, fitted with a T1 valve, was kept alive after the study termination date (for a total of 502 days) in order to study valve leaflet wear in vivo. At explantation, the valve in this calf showed no overgrowth or abnormal thickening of the tissue covering the annulus and no tissue or thrombus on the atrial or ventricular aspects of the valve ring (Fig 2).

Table 1. Weight Gain and Study Duration In Vivo

Valve Type	Study Duration (days)	Weight Gain (% Initial Weight)	Daily Weight Gain (lb/day)
Group I experimental (n = 6)[a]			
T1	106	64	1.2
T1	97	144	3.1
T1	96	39	0.83
T1	89	34	0.81
T1	89	47	1.1
T1	87	67	1.3
Mean	94 ± 7.0	66 ± 41	1.4 ± 0.86
Group II experimental (n = 4)[b]			
T2	181	110	1.2
T2	167	97	1.3
T2	154	85	1.1
T2	150	56	0.84
Mean	163 ± 14	87 ± 23	1.1 ± 0.19
Groups I and II controls (n = 7)			
CMI	91	54	1.1
TRI	103	61	1.1
CMI	104	59	0.90
CMI	104	57	1.1
CMI	108	43	0.72
SJM	155	109	1.3
SJM	171	122	—
Mean	119 ± 31	72 ± 30	1.0 ± 0.19

[a] One additional calf in this group was kept alive for 502 days for long-term in vitro wear testing. The data for that calf are not included here. [b] Two additional calves in this group were kept alive for 385 and 360 days, respectively, for long-term in vitro wear testing. The data for these calves are not included here.

CMI = Carbomedics Inc; SJM = St. Jude Medical; TRI = TRI Technologies

In Group II, 6 of 7 experimental calves (86%) and 2 of 2 control calves (100%) survived to the study termination date of 5 months (Table 1). The remaining calf, which was fitted with a T2 valve, was killed before the study termination date (on POD 104) because it had systemic infection, as diagnosed by positive blood cultures. Two calves fitted with the T2 valve were kept alive after the study termination date (for a total of 385 and 366 days, respectively) in order to study valve leaflet wear in vivo. All T2 valves were fully functional and free from occlusive or problematic thrombus formation at explantation.

Table 1 shows the weight gain and study duration for the calves that survived beyond 2 months. In the table, the control calves in Group I (n = 5) and Group II (n = 2) are combined into a single group.

ECHOCARDIOGRAPHY AT IMPLANTATION. Echo results are summarized in Table 2. The results for the control animals (n = 5 in Group I and n = 2 in Group II) are combined in the

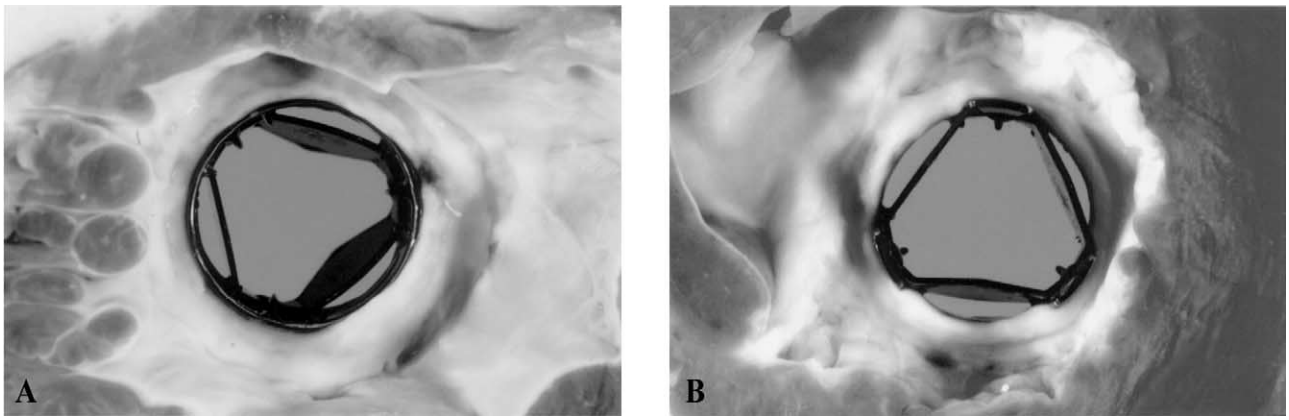

Fig 2. T1 trileaflet valve prototype used in a long-term (502-day) study of leaflet wear in vivo, as seen from (A) inflow (atrial) and (B) outflow (ventricular) sides.

first column of the table (n = 7). In Group I, intraoperative transepicardial echocardiography was performed immediately after valve replacement in all animals, but results are not reported for the 5 experimental calves that were killed prematurely before the planned study termination. In the remaining 12 cases from Group I (7 experimental and 5 control calves), perivalvular leaks were either minimal or nonexistent at the time of MVR, and valve function (regardless of valve type) appeared normal. In Group II, intraoperative transepicardial echocardiography showed good valve hemodynamic performance in all 9 cases (7 experimental and 2 control calves), indicating the consistency of our bovine model.

In Groups I and II, transvalvular pressure gradients of the trileaflet valves immediately after implantation compared favorably with those of the bileaflet controls. The mean peak gradient for T2 valves was less than for T1 valves or for all bileaflet controls combined (Table 2).

HEMODYNAMICS AT EXPLANTATION. At study termination, data on hemodynamics and valve function (as assessed by cardiac catheterization) were collected in three contractile states: normal, hypercontractile, and hypocontractile. In Group I, the mean transvalvular pressure gradients of T1 valves did not differ much from those of control bileaflet valves. As expected, in Group II, the mean transvalvular pressure gradients of both the T2 and control valves were considerably higher in the hypercontractile state: the T2 gradients were 10 ± 6 mm Hg, 21 ± 2 mm Hg, and 7 ± 2 mm Hg and the bileaflet valve gradients were 9 ± 2 mm Hg, 21 ± 2 mm Hg, and 8 ± 2 in the normal, hypercontractile, and hypocontractile states, respectively. This difference was expected because of the longer implantation period in the T2 series (5 months vs 3 months in the T1 series), resulting in greater weight gain, cardiac output, and stroke volumes in Group II calves.

Table 2. Intraoperative Echocardiographic Findings

	Bileaflet (Control) Valves (n = 7)	Trileaflet Valves	
		T1 (n = 7)	T2 (n = 7)
Mean peak gradient (mm Hg)	12 ± 4	10 ± 7	6 ± 3
Mean gradient (mm Hg)	6 ± 4	5 ± 4	3 ± 1
Area (cm^2)	3 ± 1	3 ± 1	3 ± 0.5
Time velocity index (cm)	28 ± 4	25 ± 9	21 ± 5

The amount of trileaflet valve regurgitation at explantation was estimated using Seller's grade. Significant mitral regurgitation (Seller's grade 3 to 3+) was defined as opacification of the left atrium after 2 beats following LV contrast injection [10, 11]. At heart rates ranging from 50 to 190 bpm and cardiac outputs ranging from 4 to 20 L/min, regurgitation in the normal, hypercontractile, and hypocontractile states, respectively, was less in Group II than in either Group I or the control group (Table 3).

HEMATOLOGY. Laboratory blood values were determined at three major time points in the study (baseline, midstudy, and study termination) for calves that survived to study termination. In all of these calves, WBC, RBC, hemoglobin, hematocrit, platelet count, LDH, and plasma free hemoglobin levels remained within normal limits. Except for platelet count, values at study termination were comparable to those at baseline. In all cases but the T2 series, platelet counts decreased significantly over the course of the study. In Group I, platelet counts at baseline versus study termination (3 months) were reduced for both bileaflet-fitted controls (920 ± 341 vs 458 ± 111) and trileaflet-fitted calves (953 ± 271 vs 541 ± 111). Likewise, in Group II, platelet counts at baseline versus study termination (5 months) were reduced for bileaflet-fitted controls (617 ± 431 vs 221 ± 187) and for trileaflet-fitted calves (577 ± 233 vs 490 ± 201), though the reduction was smaller for the latter.

NECROPSY. Necropsy revealed normal function of all trileaflet and control valves and normal endothelialization of the sewing ring in all calves that survived to the

Table 3. Hemodynamic and Necropsy Findings

Valve Type	Mortality (%)	Gradient (mm Hg)			Regurgitation (Hypocontractile State)[a]	Valvular Thrombosis Grade[b]	Renal Infarct Grade[c]
		Implantation		Explantation			
		Mean	Peak	Peak			
T1	5/12 (42%)	5 ± 4	10 ± 7	9 ± 2	1.6 ± 0.9	0.71 ± 0.76	0.57 ± 0.98
T2	1/7 (14%)	3 ± 4	6 ± 3	10 ± 6	0.9 ± 0.7	0.71 ± 0.76	0.43 ± 0.73
Control	0/7 (0%)	6 ± 4	12 ± 4	10 ± 3	1.9 ± 0.5	1.00 ± 0.82	0.29 ± 0.49

[a] Seller's grade: significant mitral regurgitation (3 to 3+) defined as opacification of the left atrium 2 beats after left ventricular contrast injection. [b] Thrombosis grade (valve ring): 0 = no visible thrombi; 1 = small thrombi <5 mm; 2 = moderate thrombi >5 mm. [c] Renal infarct grade: 0 = no infarction; 1 = 5% total surface area; 2 = >5% and <25% total surface area.

planned study termination date. All annuli (T1, T2, and control valves) were well healed, and the sewing rings were covered by dense fibrous connective tissue that, in some cases, overlapped the titanium ring.

All calves that survived to the planned study termination date were examined for the presence of thrombosis (eg, valvular thrombi and renal infarcts) (Table 3). In Group I, valvular thrombi were found just as often in T1-fitted calves as in bileaflet-fitted controls (57% [4/7] vs 60% [3/5]); renal infarcts were found more often in the T1-fitted calves (29% [2/7] vs 20% [1/5]). In Group II, valvular thrombi were found less often in T2-fitted calves than in bileaflet-fitted controls (57% [4/7] vs 100% [2/2]); the same was true for renal infarcts (29% [2/7] vs 50% [1/2]).

Thrombus-impaired leaflet motion was noted in 2 calves (30%) fitted with a T1 valve and 1 calf (14%) fitted with a T2 valve, but not in any control calves. One of the T1-fitted calves and the T2-fitted calf showed signs of endocarditis. None of the 3 calves with impaired leaflet motion at necropsy showed signs of clinical pathology or cardiac dysfunction ante mortem. Despite the complete absence of anticoagulation therapy after POD 10 in Group II calves, the histopathologic evidence of thromboembolic events in the rete mirabile, kidneys, or adrenal glands ranged from insignificant to nonexistent in both control and T2-fitted calves.

Comment

In terms of hemodynamics and thrombogenic potential, the trileaflet prototype valves we evaluated in the present study behaved similarly to bileaflet control valves (Table 3). Any surgical attrition in the study was due almost exclusively to the procedural learning curve. Thus, our preclinical testing of trileaflet valve prototypes in vivo indicates that they may be a safe and effective alternative to bileaflet valves.

We have conducted three studies of long-term leaflet wear in vivo, and the longest of these lasted 502 days in a T1-fitted calf. The calf in that study thrived, gaining more than 132 kg, and its mean transvalvular pressure gradient was less than 10 mm Hg despite a cardiac output of 15 to 20 L/min at the time of cardiac catheterization and euthanization. The explanted valve was free from thrombus or pannus overgrowth on the valve ring (Fig 2).

Intraoperative echo Doppler studies showed good hemodynamic performance of both trileaflet prototypes, indicating the stability and consistency of the bovine model. The mean transvalvular pressure gradients of the T1 and T2 prototypes immediately after implantation compared favorably with those of the bileaflet controls, and the peak gradient of T2 valves was notably lower at study term than that of bileaflet controls (Table 3).

Our in vivo cardiac catheterization studies showed that the T2 trileaflet prototype allowed less regurgitation than the bileaflet controls (Table 3). As expected, regurgitation for all valve types was mild to moderate and occurred most frequently during the hypocontractile state, a state that simulates heart failure (eg, low heart rate, low stroke volume, and low cardiac output). Valvular regurgitation in the other two states was deemed insignificant and normal for a mechanical heart valve. Except for one case of mild perivalvular mitral regurgitation confirmed at necropsy, any systolic mitral regurgitation seen on echocardiography or angiography was judged to be trivial and attributed to the expected presence of transleaflet systolic "washing jets." A small, brief puff of late diastolic transvalvular mitral regurgitation was noted on occasion after premature ventricular contraction, and in one case (during esmolol infusion), it occurred in association with depressed LV systolic function. We considered these few examples of hemodynamically insignificant "diastolic mitral regurgitation" to be secondary to transiently elevated left ventricular end diastolic pressure that was greater than left atrial pressure while the leaflets were still open during late diastole. In our opinion, the observed regurgitation was minimal and clinically insignificant in all valves tested. In summary, left ventriculography showed excellent valve function, regardless of valve type, in all long-term calf survivors and in all three states of contractility studied.

Both experimental trileaflet prototypes evaluated here were hematologically and biologically compatible. Blood chemistry and CBC values remained normal in all calves that survived to study termination. The subclinical decrease in platelet count that we observed in all calves (both bileaflet controls and trileaflet experimental animals) was to be expected, considering the affinity of bovine platelets for foreign surfaces regardless of the hemocompatibility of the blood interface or the laminar flow across the valve orifice [12]. Also, it is important to note that in no case did the decrease in platelet count precipitate any catastrophic activation of clotting cas-

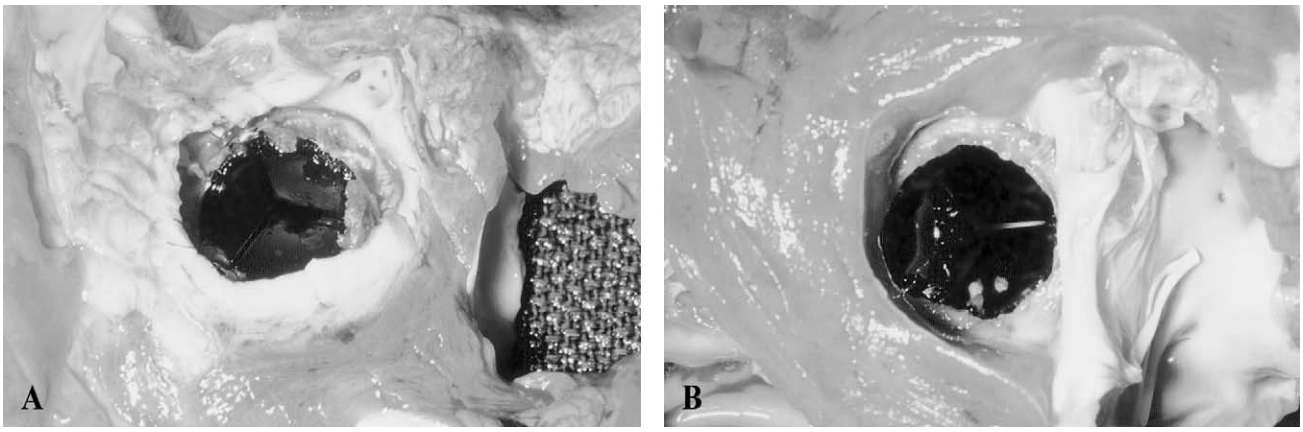

Fig 3. T2 trileaflet valve prototype with "windows" used in aortic position in vivo, as seen from (A) outflow (aortic) and (B) inflow (ventricular) sides. Acute thrombosis occurred 15 days after valve implantation.

cades, which would have led to valve thrombosis and malfunction.

In vitro studies have suggested that the trileaflet design not only minimizes marginal flow behind leaflets but also obstructs blood flow less, because all centrally situated structures are eliminated. Furthermore, the closing behavior mimics that of the natural aortic valve (which needs no back pressure or flow in order to close), thus helping minimize the cavitation potential.

The addition of windows at the commissures of the valve ring did not improve valve and kidney thrombus scores between the T1 and T2 prototypes (Table 3); nor was survival affected (since mortality in Group I was related to a surgical learning curve and not to thromboembolism) (Table 3). Overall, the windows seemed to have little effect, either beneficial or detrimental, in the present series of experiments with valves in the mitral position. However, during subsequent testing with smaller (21-mm-diameter) valves in the aortic position, the presence of the windows apparently led to increased turbulence and shear stresses in the window areas and subsequent thrombus formation (Fig 3). These detrimental effects were certainly increased by the combination of small valve size and the extreme hemodynamic conditions in the experimental calves (ie, cardiac outputs of up to 15 L/min). In response to these negative findings with the T2 valve in the aortic position, the windows were eliminated, and the resulting new version of the valve was subsequently retested in the mitral and aortic positions separately, with excellent results (manuscript submitted).

In developing a calf model for valve replacement and testing, our aim has been to achieve a more stringent in vivo testing ground for prosthetic valves. Historically, the large animal model most commonly used for mitral valve replacement and testing has been the sheep [13]. We believe our bovine model is superior to the ovine model for several reasons. As compared with the sheep, the juvenile calf is a growing, pannus-producing model that has a much higher cardiac output and a much stronger contractile force. Goodman [12] demonstrated that platelet functions in the bovine, as opposed to humans, are considerably attenuated in response to biomaterials. Peek and associates [14] reported that the coagulation system of sheep is less active than that of humans. Ledwozyw and associates [15] showed that factor VIII levels are significantly lower in sheep plasma than in human plasma. In hemocompatibility tests involving extracorporeal circulation, Mueller and associates [16] showed that calves have a greater tendency toward clot formation than do pigs, whose coagulation parameters are similar to those of humans. Mueller and associates also suggested that once a device is successfully tested in calves, it may be applied to humans without undue concern since humans are less vulnerable to thrombogenesis.

The preclinical results reported here indicate that the trileaflet valve we evaluated may, in its preliminary design and configuration, be equivalent to the bileaflet valve. The central flow patterns and optimal closing characteristics of our trileaflet valve, which contribute greatly to its "mechanical bioprosthetic" nature, may in clinical practice greatly reduce or even obviate the need for anticoagulation therapy. Our next steps will be to finish preclinical testing of the trileaflet valve in the aortic position, confirm the valve's safety and efficacy, and then proceed to clinical trials.

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