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Comparative studies of prosthetic materials in the left atrium of the dog

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Summary. To assess the healing process of various intracardiac prosthetic graft materials, we inserted autologous left atrium (control), autologous pericardium (AP), bovine pericardium (BP), polytetrafluoroethylene (PTEE) and woven Dacron (WD) patches into excised defects in the left atrial wall of 15 dogs. Two patches were implanted into each heart utilizing six patches for each material. Three months after graft placement, histological examination revealed chronic inflammation and fibrosis for all materials. Dense connective tissue surrounded the grafts in which fibrosis was most prominent. Cartilage formation occurred in 11 grafts: 5 BP, 4 PTFE, 1 control and 1 AP site. This change was not evident with WD. The extent of cartilage formation was greatest in BP. Bone formation occurred in 3 BP sites, 2 PTFE and 1 control site. Quantitative calcium concentrations were similar for all of the grafts without bone formation. Calcium concentrations at sites with bone formation averaged 15.13 mg/g $\pm$ 6.39 mg/g compared to 0.939 $\pm$ 0.419 for sites without bone formation ($p < 0.0001$). We conclude that while inflammation, fibrosis and connective tissue thickening occur with healing of all graft materials, cartilage and bone formation differ with respect to the material employed.

Key words: Intracardiac healing – Prosthetic materials – Left atrium

Introduction

Various processes related to the healing of prosthetic materials used in cardiac surgery have been described, and include calcification (Cohen et al. 1984; Ishihara et al. 1981; Sanders et al. 1980), infective endocarditis (McNamara and Latson 1982; McGoon 1982), thromboembolism (Duvoisin et al. 1967; Geha et al. 1982; Vidne et al. 1973), shrinkage of the material with obstruction to flow (Cabanoglu et al. 1984; Driscoll et al. 1977) and cartilage formation (Geha et al. 1979). Knowledge of differences in host reaction and in the biological healing process of the various biological and synthetic materials currently in use could provide information which may influence the choice of materials and perhaps reduce complications. In a previous report from this laboratory, several types of graft materials were placed into the right atrium of the dog and the subsequent healing process was described (Kadowaki et al. 1986). In the present study similar techniques were used to assess healing of the same graft materials used as left atrial patches.

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Methods

Fifteen adult mongrel dogs weighing 15 kg to 20 kg each were anesthetized with pentobarbital (30 mg/kg IV), intubated and mechanically ventilated. After paralysis with 1 cc Pavulon, a left thoracotomy was performed at the fourth intercostal space using sterile technique. The left atrium was exposed and two sites for patch placement were identified in each dog; one site (position A) was midway between the center of the atrium and the tip of the atrial appendage. The second site was midway between the center and the base of the atrium (position B). At each site a partial occlusion vascular clamp was placed and a circular portion of the atrial wall 1.5 cm in diameter was excised. Each site was then repaired with a patch of prosthetic material of the same dimensions, or the excised portion was restored to serve as an autologous control for the procedure. Graft materials which were studied included autologous pericardium, glutaraldehyde preserved bovine pericardium (Genetic Laboratories, Minneapolis, Minnesota, USA), polytetrafluoroethylene (PTFE or Gore-Tex; W.L. Gore and Associates, Inc., Flagstaff, Arizona, USA), and woven Dacron. Running 6–0 polypropylene was used to suture the patches in place.

Patch material was randomly assigned to position A or B and each of the five patch materials was used three times

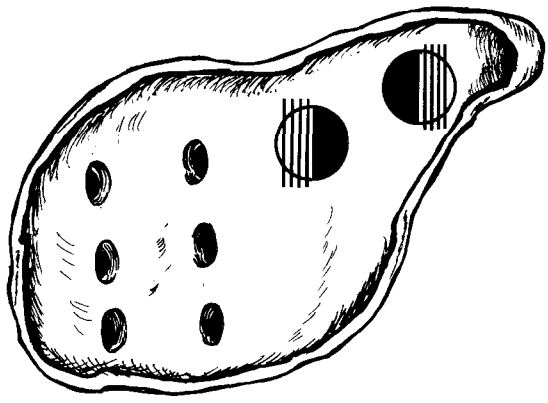

Fig. 1. Drawing illustrating placement of graft patches in the left atrium as viewed from the endocardial surface. Shaded area indicates material taken for calcium measurement and lines indicate orientation of interval samples taken for histological sections. The six pulmonary veins of the dog are depicted on the left side of the figure

at each position for a total of 30 patch sites. Bovine pericardium patches were rinsed three times, each time in fresh, sterile physiological saline. Following graft placement the pericardial incision was loosely approximated and the chest was closed. A chest tube was inserted and left in place for two to 3 h while the animal recovered. All animals were treated pre- and postoperatively with 100 mg Ampicillin IM. Dogs were cared for in the Carlson Animal Research Facility of the University of Chicago in accordance with the National Research Council's guide for the care and use of laboratory animals.

Three months following graft placement, each animal was anesthetized and underwent a median sternotomy. The atria were harvested after the heart was fixed in situ using a technique of controlled pressure perfusion-fixation with 5% glutaraldehyde solution (Glagov et al. 1963). This process consisted of cannulation of the innominate artery, clamping of the aorta and the superior and inferior vena cavae and infusion of fixative through the innominate artery into the aortic root at a constant pressure of 60 mmHg. Fixative was also poured over the surface of the heart. The heart was vented through an incision in the right atrium to allow decompression. After one litre of glutaraldehyde was perfused, the atria were carefully excised and immediately immersed in glutaraldehyde solution.

Each graft was cut in half. One half of each graft was removed and analysed for calcium content (Horwitz 1975). The other half was step-section and slices taken at 500 micrometer intervals were used for histological examination (Fig. 1). One to 12 sections were studied for each patch depending on the number of complete, symmetrical sections available for each site. The anterior half of 15 grafts was used for calcium determination and the posterior half for histology; for the other 15 grafts, selection of the samples was reversed. Each histological section was stained with haematoxylin and eosin and by the Gomori trichrome-aldehyde fuchsin method for connective tissue. Evidence of inflammation was based on evaluation of all of the interval sections for a given site. The 30 grafts were ranked according to the density of inflammatory cells present in the connective tissue surrounding the graft and within the graft material itself. The 30 grafts were then divided into four categories: 0 = no inflammatory cells, and values of 1 to 3 for minimum to maximum density of inflammatory cells, respectively. Fibrosis was noted and the thickness of the connective tissue reaction on both the endocardial and epicardial sides of the graft was measured on the central section at each site using a micrometer eyepiece. The endocardial and epicardial thicknesses were then added to yield a total value for fibrosis tissue reaction. Cartilage and bone formation were assessed in each section and the extent of cartilage formation was graded semi-quantitatively. Cartilage formation within the 30 grafts was ranked according to total area of cartilage formation of all serial sections and the grafts were again divided into four categories with 0 = no cartilage and values from 1 to 3 ranging from minimal to maximal cartilage formation. Calcium content was determined by flame atomic absorption spectrophotometry (Horwitz 1975) using an air-acetylene flame and results are reported as mg/g of dry tissue weight.

Frequency data were analyzed using the Chi square test for significance and comparisons of the semi-quantitative grades for inflammation and cartilage content were analyzed utilizing the Wilcoxon rank sum test. Calcium content and the fibrous tissue reaction were compared using the pooled Student's *t*-test. Connective tissue thickness between the epicardial and endocardial sides of each graft was compared with the paired Student's *t*-test. All possible combinations of the five materials were compared using these statistical methods.

Table 1. Results of histological examination and calcium quantitation

Material	Inflammation		Fibrosis				Calcium	Cartilage		Bone
	f	grade	f	thickness				f	grade	f
				endo	epi	total				
Control	4/6	1.2±0.40	5/6	0.76±0.10	0.56±0.88	1.3±0.18	3.85 ±3.5	1/6	0.50±0.50	1/6
Autologous pericardium	4/6	1.0±0.37	6/6	0.77±0.28	0.41±0.12	1.2±0.40	1.65 ±1.5	1/6	0.17±0.17	0/6
Bovine pericardium	5/6	2.0±0.52	6/6	1.1 ±0.26	0.88±0.11	2.0±0.36	10.8 ±6.8	5/6	1.5 ±0.43	3/6
PTFE	5/6	1.0±0.26	6/6	1.0 ±0.25	0.88±0.41	1.5±0.26	2.22 ±1.1	4/6	1.3 ±0.49	2/6
Woven dacron	6/6	1.2±0.17	6/6	0.89±0.21	0.61±0.13	1.5±0.17	0.243±0.98	0/6	0.0 ±0.0	0/6

Abbreviations: f = frequency; endo = endocardial side of graft; epi = epicardial side of graft. Fibrosis is reported in mm and calcium is reported in mg/g dry weight

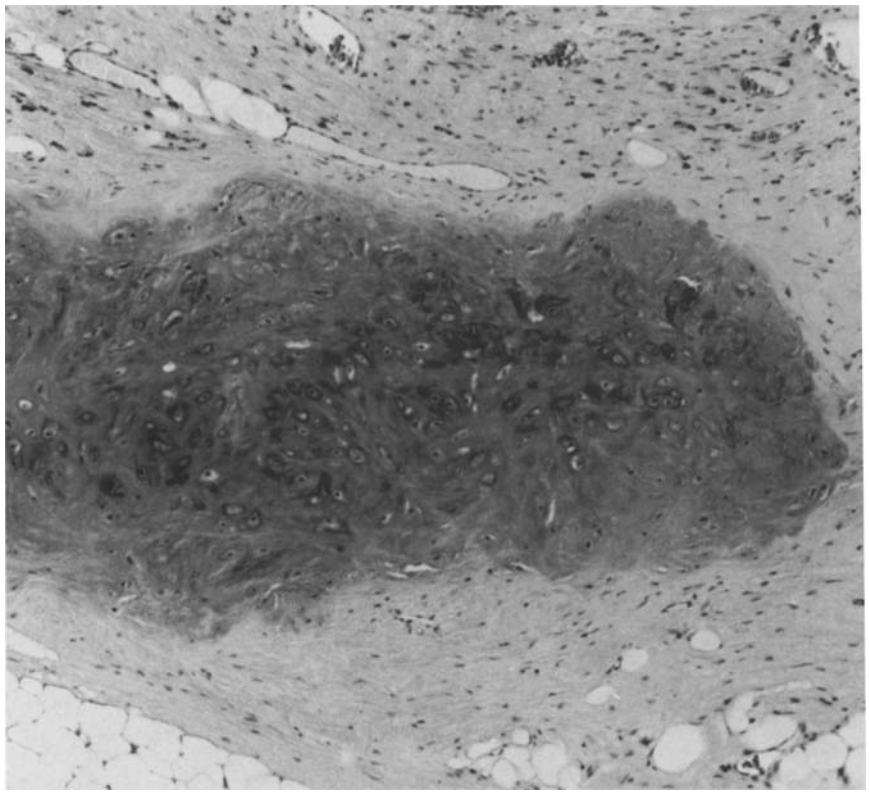

Fig. 2. Cartilage surrounded by dense connective tissue on the endocardial side of a PTFE graft. (H&E, magnification ×78)

Results

The results are tabulated in the Table 1 and are listed according to material as frequency (number of sites displaying a particular variable) or as mean ± SEM.

Inflammation occurred in 24 of 30 sites with a similar frequency and grade for all materials. Inflammatory cells consisted mainly of lymphocytes with occasional macrophages, giant cells, plasma cells and neutrophils.

Fibrosis surrounding the graft was present in 29 of 30 sites. The connective tissue reaction was thicker on the epicardial side for bovine pericardium as compared to autologous pericardium ($p = 0.0194$). Endocardial fibrosis for control grafts, however, was thicker than epicardial ($p = 0.0040$). For the other graft materials no differences were noted for connective tissue thickness when the endocardial and epicardial sides were compared.

Cartilage formation occurred with the greatest incidence and to the greatest extent with bovine pericardium and PTFE grafts and was less frequent and less extensive in control and autologous pericardium grafts. No cartilage was present in any of the six Dacron implants. Bovine pericardium grafts exhibited a significantly higher incidence of cartilage formation than controls ($p < 0.05$), autologous pericardium ($p < 0.05$) and Dacron ($p < 0.01$). PTFE also exhibited a significantly greater incidence of cartilaginous foci than Dacron ($p < 0.05$). The grade of cartilage formation was significantly greater with bovine pericardium than with autologous pericardium ($p < 0.05$) or Dacron ($p < 0.05$). Cartilage formation always occurred nearest the endocardial surface fo the grafts and in central portions of the grafts (Fig. 2). In two bovine pericardium and one PTFE sites cartilage also formed at the edge of the graft as well as in the adjacent atrial tissue (Fig. 3).

Bone formation (Fig. 4) occurred in bovine pericardium, PTFE and control grafts. The incidence of bone formation in bovine pericardium grafts was statistically different compared with autologous pericardium ($p < 0.05$) or Dacron ($p < 0.05$), neither of which displayed bone. Bone formation always occurred within a focus of cartilage. It was located at the center of the graft site in four instances and at the edge of the graft material in two instances.

Calcium content was greatest in bovine pericardium grafts and least in Dacron grafts with intermediate values in control, autologous pericardium and PTFE. Despite the difference in calcium con-

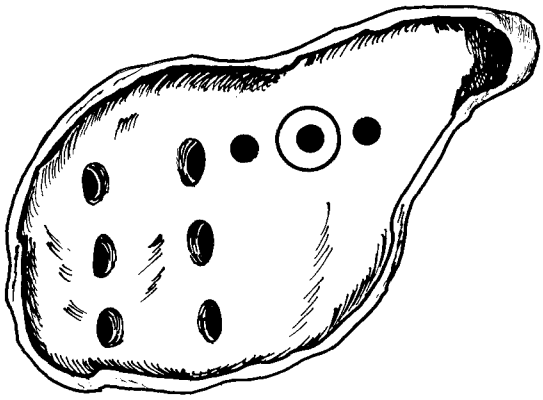

Fig. 3. Figure similar to Fig. 1 illustrating the location of cartilage at one of the two patch sites; open circle represents placement of the graft. Dark circles in the center and on either side of the open circle represent usual location of cartilage and bone formation in relation to atrium and patch

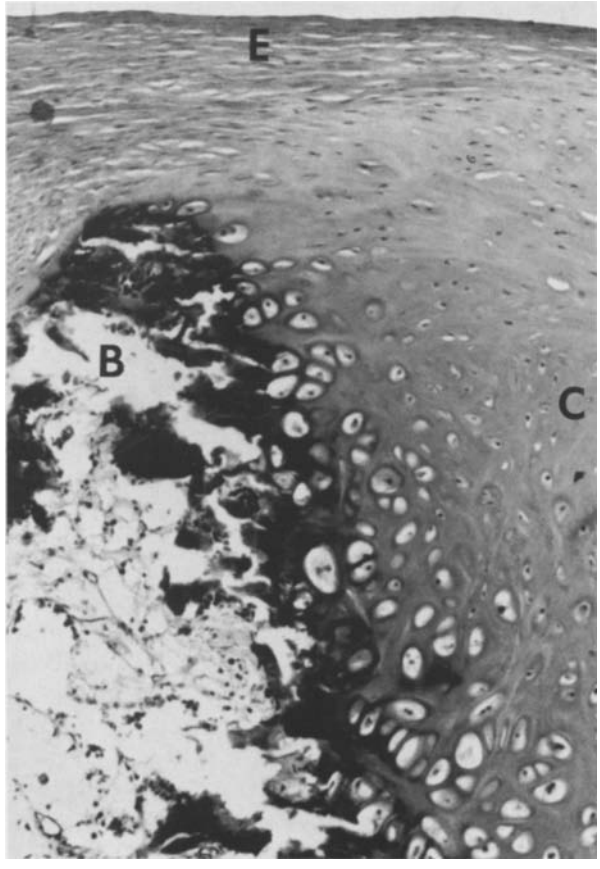

Fig. 4. Endocardial side (*E*) of a bovine graft with cartilage (*C*) on the right and trabecular bone (*B*) formation with adipose tissue and marrow elements in the center and the lower left. (H&E, magnification ×126)

tent between bovine pericardium and Dacron grafts, variability was marked and there was no statistically significant difference between these or any other groups.

Discussion

This histological study reveals that fibrotic thickening and chronic inflammation are characteristic features of the healing process of all five graft materials studied. Fibrosis in intracardiac healing has been documented previously (Kadowaki et al. 1986; Gavin et al. 1981; Stafford and McGoon 1973). Our findings show in addition that lymphocyte and plasma cell infiltration occurs in all types of grafts including autologous materials, suggesting that these cells are involved in an inflammatory process not necessarily provoked by foreign immunological factors.

Cartilage formation occurred in 37% of the grafts. This is considerably less than the 87% reported previously in right atrial grafts using the same materials and procedures ($p < 0.01$) (Kadowaki et al. 1986; Kadowaki et al. 1985). Shaw and Bassett (1967) and Hall (1969) have previously shown in vitro that cartilage formation from primitive, embryonic cells is induced by hypoxia. Thus the increased oxygen content of left atrial blood when compared with right atrial blood may account for the lower incidence of cartilage formation in the present study. These results, while confirming previous data showing differences in incidence of cartilage formation related to position of the graft (Arbustini et al. 1983), are not sufficient to draw firm conclusions regarding this finding. Other factors such as variations in chamber pressure or motion of the graft and/or atrium may also play a role in cartilage formation.

Calcium content ranged from 0.0577 mg/g to 40.17 mg/g dry weight but statistically significant differences between materials cound not be demonstrated due to the marked variability and the relatively small numbers of sites for each material. Studies of bioprosthetic valve leaflets have shown similarly wide ranges of calcium conent (Arbustini et al. 1983) and marked variability within sampling groups (Barnhart et al. 1982; Barnhart et al. 1982). Although bone formation was relatively uncommon, it was associated with elevated values of calcium content compared to non-ossified samples. The average calcium content for the sites showing bone formation was 15.13 ± 6.39 compared to 0.937 ± 0.419 for those without bone formation ($p < 0.0001$). Thus, increased calcium content and incidence of bone formation was closely related. No single explanation is available for the marked variability in calcium content of other specimens.

Both the site of implantation and the functional demands upon intracardiac prosthetic materials have been shown to be factors in the healing of

prosthetic materials (Cohen et al. 1984; Kadowaki et al. 1985; Gabbay et al. 1984). The present study suggests that Dacron has a low incidence of cartilage formation when used in left atrial grafts, while our previous study showed no advantage of Dacron over other materials when used in a right atrial position (Kadowaki et al. 1986). Thus, differences in the healing of graft materials are produced not only by the particular material but also by the location within the heart where the material is used.

Process such as fibrosis, which are common to virtually all graft materials, can also be utilized to the advantage of the surgeon. For example, Ebert has developed a two-staged repair of single ventricle in which he attributed the success of the procedure to the fibrotic reaction produced by the prosthetic material placed into the heat during the first stage (Ebert 1984). Further studies of these biological healing processes as they relate to position in the heart, differences in pressure, and differences in oxygen tension may lead to more rational use of the various prosthetic materials available to the surgeon.

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