FULL PAPER *Surgery*

A Long Term Comparison between Denacol® EX-313-Treated Bovine Jugular Vein Graft and Ultrafine Polyester Fiber Graft for Reconstruction of Right Ventricular Outflow Tract in Dogs

Hideki MATSUMOTO[1)], Shino SUGIYAMA[2)], Akira SHIBAZAKI[2)], Ryou TANAKA[2)], Kazuaki TAKASHIMA[1)], Yasuharu NOISHIKI[3)] and Yoshihisa YAMANE[2)]

[1)]Animal Clinical Research Foundation, 214–10 Yatsuya, Kurayoshi-shi, Tottori 682–0025, [2)]Department of Veterinary Surgery, Faculty of Agriculture, Tokyo University of Agriculture and Technology, 3–5–8 Saiwai-cho, Fuchu-shi, Tokyo 183–0054 and [3)]First Department of Surgery, Yokohama City University School of Medicine, 3–9 Fukuura, Kanazawa-ku, Yokohama-shi, Kanagawa 236–0004, Japan

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ABSTRACT. A Denacol EX-313 (Denacol)-treated bovine venous graft and an ultrafine polyester fiber (UFPF) graft were transplanted as patch graft into the right ventricular outflow tract under extracorporeal circulation in six dogs each experimentally. Hemodynamics in right heart and histological findings around the graft were compared between both groups over a period of one year after grafting. Pressure measurements and angiocardiography were performed through a cardiac catheter. Right ventricular pressure, pulmonary artery pessure, and right ventricle to pulmonary artery gradient were within normal limits in both groups at 1, 2, 3, 4, 6, and 12 months or more after grafting. No difference were seen between the values for the Denacol and the UFPF group. Histologically, the medial surface at the site of grafting was covered with vascular endothelial cells at one month after grafting in both groups. The density of the vascular endothelial cells increased with time after grafting, showing no clear difference between the two groups. Subendothelial layers comprised of collagen fibers, elastic fibers, and inflammatory cells decreased with time in both groups, but there was less cell infiltration in the Denacol group than in the UFPF group at all time points after grafting. In addition, the central cut thickness value of the graft tended to be thinner in the Denacol group than in the UFPF group at all observation time points after grafting. In the Denacol group, very slight metaplasia of cartilage was noted in a portion of the graft margin at six months or more after grafting, but no other abnormalities were observed. These results suggest that the Denacol-treated bovine venous graft has better grafting characteristics than the UFPF graft with easier intra-operative handlings and less tissue reactions after grafting.

KEY WORDS: canine, Denacol EX-313, patch graft, pulmonic stenosis, ultrafine polyester fiber.

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Patch grafts of pericardium, dacron, etc., have been used for reconstruction of the right ventricular outflow tract stenosis that is observed in pulmonic stenosis and tetralogy of Fallot [3, 5, 10], but because there have been problems, such as maneuverability and postoperative tissue reactions, it has been hoped that a new graft material would be developed. Polyepoxy compounds (Denacol® EX-313) is less toxic than the cross-linker glutaraldehyde that has been used in the past, and because it is hydrophilic, it is characterized by strong affinity for cells and has been used for treating natural graft material, such as bovine vein [4, 7, 14]. We transplanted Denacol® EX-313-treated bovine vein patch grafts into the pulmonary arteries of dogs, and observations over a short period of time revealed that they possessed properties superior to those of artificial material [6]. In the present experiment we observed hemodynamics and tissue findings over a period of one year after graft transplantation and made comparisons with artificial material.

MATERIALS AND METHODS

Animals: Twelve clinically healthy dogs were examined physiologically and randomly distributed into two groups: 1) Denacol group, (average weight 9.15 ± 3.14 kg, No. 1–6), four males and two females; and 2) UFPF group, (average weight 10.10 ± 0.86 kg, No.7–12), all males. The dogs were vaccinated with a mixed vaccine and were found to be negative for microfilaria. Complete blood count, blood chemistry, plain thoracic radiography, electrocardiography, and phonocardiography revealed no abnormal findings. The protocol used in this study was approved by The Tokyo University of Agriculture and Technology Institutional Laboratory Animal Care and Use Committee.

Preparation of the Denacol graft: The methods used to prepare the Denacol graft were previously described in detail [4, 6].

Anesthesia: The dogs were premedicated with atropine sulfate (0.04 mg/kg) and acepromazine maleate (0.4 mg/kg). After sufficient sedation had been achieved, anesthesia was induced using thiamylal sodium (10 mg/kg). The dogs were intubated with an endotracheal tube. Maintenance anesthesia was given through a combination of inhalation anesthesia using a mixture of oxygen and isoflurane and intravenous anesthesia using a 0.1% ketamine micro-mini drip administration technique. During thoracotomy, succinylcholine chloride (0.2 mg/kg) was administered intravenously, as necessary, under controlled respiration.

Operative technique: Reconstruction of the right ventricular outflow tract using a patch graft was performed in the two groups of dogs employing an extracorporeal circulation

system with an artificial heart-lung machine [12, 13]. The Denacol grafts and the UFPF (Toray Graft, Toray Co., Tokyo, Japan) grafts with a thickness of 0.5 mm were trimmed to produce a patch with a length of 40 mm and a width of 20 mm. These were sutured at 2–3 mm intervals using 5–0 polypropylene suture materials, after excising a portion of the pulmonary artery of the same size. The operative site was then checked for hemorrhages and the thoracic cavity was closed.

Postoperative management: The postoperative procedures consisted mainly of preventing infection by the use of antibiotics. No anticoagulant treatment was given. The dogs were evaluated clinically after surgery and then euthanized.

Clinical examinations: Complete blood count, blood chemistry, plain thoracic radiography, electrocardiography and phonocardiography were conducted 1, 2, 3, 4, 6, and 12 months or more, after grafting, for one anesthetized dog from each group. Dog No. 6 and No. 12 were sacrificed at 723 days and 375 days, respectively. Pressure measurements and angiocardiography, through a cardiac catheter, and hemodynamic examinations were carried out with the dogs under general anesthesia after the above-mentioned inspection had been completed.

Macroscopic and microscopic examinations: After every clinical examination, all dogs of both groups were euthanized. Extracardiac and intracardiac observation of the transplanted patch graft was conducted to ascertain conditions such as adhesion and thrombus formation, and to measure the width of the patch graft. After observation, a large sample was obtained including the patch graft suture area, and the thickness and the maximum width of the exposed graft surface were measured from inside the heart. A portion of the sample was immediately fixed in a 2.5% glutaraldehyde solution (pH 7.2–7.4) for examination by scanning electron microscopy (SEM) and the remainder was fixed in formalin for histological examination. For the latter, the samples were stained using hematoxylin-eosin, Masson-trichome and Elastica van Gieson stains.

RESULTS

Clinical examination: Transplantation surgery in all dogs in both the Denacol and UFPF groups was carried out under identical conditions and all animals showed remarkable recovery after the operation. No abnormal clinical findings were observed from 1 to 12 months or more after surgery.

Cardiac catheterization findings: Before necropsy, the internal pressure of the right heart was determined (Table 1). Hemodynamic parameters did not differ between the two groups and were within normal limits in both groups. Right ventricular angiography revealed no clear morphological changes in the region of the right ventricular outflow tract, into which the patch graft had been transplanted, in either group.

Macroscopic findings: Adhesion between the pericardium and the graft was noted in all animals of both groups. The cardiac cavity surface of the graft was slightly lustrous, smooth, white at one month after grafting in both groups. The internal surface of the graft was observed as a denser, and more lustrous, white tissue as the time after grafting increased (Table 2). These changes were more marked at 12 months or more after grafting (Fig. 1). The central cut thickness of the graft tended to be thinner in the Denacol group than in the UFPF at all observation time points after grafting (Table 3). The maximum width of the exposed graft measured from inside the heart tended to be larger in the Denacol group than in the UFPF group (Table 3).

Microscopic findings: At one month after grafting, some vascular endothelial cells were seen on the graft surface in both groups. Subendothelial layers comprised fibroblasts, inflammatory cells (lymphocytes, neutrophils, macrophages, giant cells, plasma cells), collagen fibers, and elastic fibers and were thinner in the Denacol group than in the UFPF group. Slight cell infiltration into the grafted membrane was noted in both groups. At 2, 3, and 4 months after grafting, inflammatory cells around the graft had disappeared almost completely in the Denacol group, but were still present in the UFPF group. Subendothelial layers comprised fibroblasts, collagen fibers, and elastic fibers around the graft and were thinner in the Denacol group than in the UFPF group (Fig. 2). At six months after grafting, the sur-

Table 1. Internal pressure of right heart in Denacol and UFPF group

Transplant periods	Denacol group				UFPF group			
	No.	RVP (mmHg)	PAP (mmHg)	RV-PA gradient (mmHg)	No.	RVP (mmHg)	PAP (mmHg)	RV-PA gradient (mmHg)
1 month	1	17/0	11/1	6	7	32/0	28/10	4
2 months	2	30/0	23/0	7	8	26/0	10/1	16
3 months	3	36/0	23/0	13	9	19/0	14/6	5
4 months	4	24/4	22/9	2	10	17/0	16/3	1
6 months	5	12/0	10/1	2	11	20/3	17/5	3
>12 months	6	32/6	28/14	4	12	28/0	19/5	9

• RVP: right ventricular pressure.
• PAP: pulmonary artery pressure.
• RV-PA gradient: right ventricle to pulmonary artery gradient.

Table 2. Comparison of macroscopic and microscopic findings in Denacol and UFPF group

	Denacol group						UFPF group					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9	No. 10	No. 11	No. 12
	1 M	2 M	3 M	4 M	6 M	>12 M	1 M	2 M	3 M	4 M	6 M	>12 M
(macroscopic findings)												
smoothness of inner surface	–	++	+	++	+++	+++	–	+	++	+++	+++	+++
thrombus	±	–	–	–	–	–	±	–	–	–	–	–
adhesion with pericardium	++	+	+	+	+	+	++	±	+	–	–	–
(microscopic findings)												
neo-endothelial cells	++	++	+++	+++	+++	+++	++	++	++	+++	+++	+++
sub-endothelial bleeding	++	+	±	–	–	–	++	+	±	–	–	–
neo-small blood vessels	+	++	++	++	++	++	++	++	++	++	++	++
inflammatory cells	+++	++	+	–	–	–	+++	+++	+++	++	+	++
cells infiltration into grafted membrane	+	++	+++	+++	+++	+++	±	+	++	++	++	++
collagen fibers surrounding graft	+	++	+++	+++	+++	+++	++	++	+++	+++	+++	+++
calcification	+*[1]	–	–	–	–	–	–	–	–	–	–	–
metaplasia of cartilage	–	–	–	–	+	±	–	–	–	–	–	–

*1 Slightly calcification was observed around some sutures.

Table 3. Cutting thickness of intermediate portion of the graft and maximum width of graft measured from inside the heart in Denacol and UFPF group

Transplant periods	Denacol group			UFPF group		
	No.	cutting thickness (mm)	maximum width (mm)	No.	cutting thickness (mm)	maximum width (mm)
1 month	1	2.0	6.0	7	2.5	5.0
2 months	2	1.5	5.0	8	2.0	9.0
3 months	3	1.5	8.0	9	2.0	8.0
4 months	4	1.0	9.0	10	2.0	4.0
6 months	5	1.0	14.0	11	2.0	11.0
>12 months	6	1.0	14.0	12	2.0	8.0

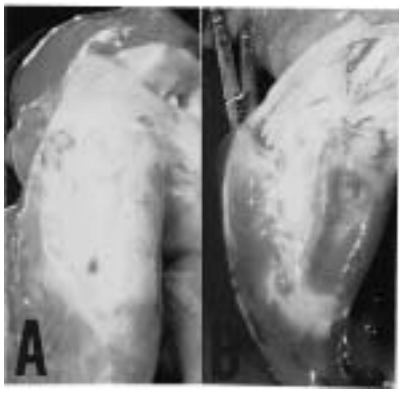

Fig. 1. View of the inner surface of the Denacol group and the UFPF group 12 months or more after grafting. (A: Denacol No.6, 723 days. B : UFPF No.12, 375 days)

face of the cardiac lumen was covered with dense vascular endothelial cells in both groups. There were few inflammatory cells in the Denacol group, but inflammatory cells was still observed around the graft in the UFPF group. Smooth muscle cells, fibroblasts, collagen fibers, and elastic fibers made thin layers just below the endothelium and these layers were thinner in the Denacol group than in the UFPF group. In the Denacol group, the original structure of elastic layers of the tunica media, which was derived from the venous patch graft, became unclear and angiogenesis became marked, showing replacement by new tissue (Fig. 3). At six months or more after grafting, the graft was assimilated by new collagen and elastic fibers, leaving the elastic layers of the tunic media derived from the venous graft alone in the Denacol group. There were few inflammatory cells, but very slight metaplasia of cartilage was confirmed at the margin of the graft membrane. On the other hand, in Dog No.5, inflammatory cells were still observed around the graft and the cell layer just below the vascular endothelium was still thick at the site of grafting in the UFPF group. However, reduced cell components were noted and cicatrisation was suggested at six months or more after grafting.

SEM findings: At one month after grafting, the graft surface was partly covered with endothelial cells in both groups. At two months after grafting, the endothelial cells were still sparse. However, the cells covered the graft surface extensively and almost completely, such that there was little area where the graft was exposed. At 12 months or more after grafting, there was little difference between the

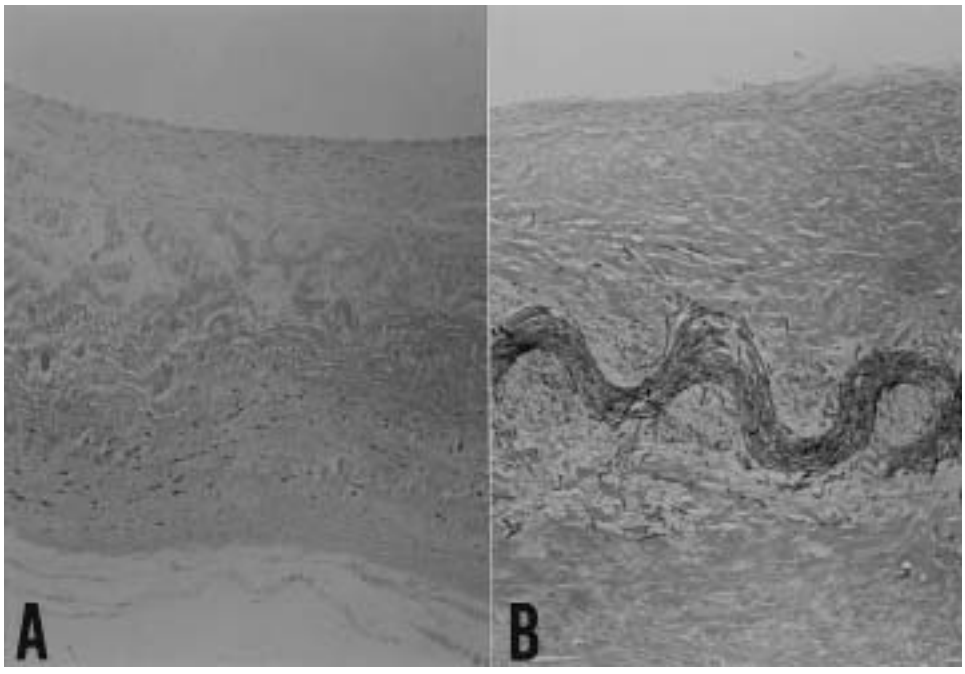

Fig. 2. Histological findings in patch grafts of intermediate portion 4 months after grafting. HE stain. × 73. (A: Denacol No. 4. B: UFPF No. 10)

two groups, and dense endothelial cells were observed. However, these cells were arranged in a more orderly fashion and the surface was smoother in the Denacol group than in the UFPF group (Fig. 4).

DISCUSSION

Since the Denacol-treated blood vessel used in the present study retains all the characteristics of natural blood vessels, its elasticity and distensability are much better than those of UFPF. Like the EPTFE, the UFPF requires no preclotting before use, and only slight laxity of marginal fibers after trimming was observed [8]. Compared to the UFPF, the trimming of the Denacol graft is much easier intra-operatively, and also, it does not dull the needle in the event of continuous suturing a graft. Thus, the Denacol graft is thought to be an excellent material in terms of handling.

In both groups, no clinical data suggesting hypotension, which would have caused in the patch graft transplant during grafting, was observed. Moreover, there was no evidence of hemodynamic abnormalities during grafting. From these observations, it appears that both grafts materials are available for the patch grafts.

To compare tissue reactions, the thickness of tissue around the graft was observed macroscopically and confirmed by measuring the central cut thickness of the graft at various times after grafting. The thickness was found to peak at one month after grafting and thereafter tended to decrease with time in both groups. Furthermore, the tissue around the graft was thinner in the Denacol group than in the UFPF group at all observation time points after grafting. Histologically, many inflammatory cells, fibroblasts, collagen fibers, and elastic fibers were noted around the graft at one month after grafting, and the layers made up of these cells were thinner in the Denacol group than in the UFPF group. This may be because of differences in the compliance, thrombus adhesion, the vital reaction to a graft, etc., between these two groups. Generally, discrepancies in compliance between a vessel and a graft are considered to be the main causes of constriction in a sutured site. The Denacol-treated blood vessel is much better than the UFPF in elasticity and distensibility. Therefore, the difference in compliance between a Denacol-treated graft and a UFPF graft may be the most imported factor in tissue reaction. In addition, as the Denacol graft had a sustained heparin release ability, the risk of thrombus adherence was reduced, fibrin deposition was inhibited and infiltration by fibroblasts and production of collagen fibers and other tissues were decreased. Noishiki *et al.* who succeeded in producing long-term patency using a thin artificial blood vessel described that an anti-thrombotic effect was conferred via binding of heparin molecules to protamine [7]. Furthermore, the reaction by fibroblast growth factor specifically observed when using the artificial molecular weight material such as UFPF [15] and the inflammation caused by the infection also contributed to the results. In the present study, histological examination of tissues around the graft showed more marked inflammatory cell infiltration in the UFPF group than in the Denacol group. There were few inflammatory cells at four months after grafting in the Denacol group, but such cells were still found at six months after grafting in the UFPF group. Therefore, the method of treatment or preservation

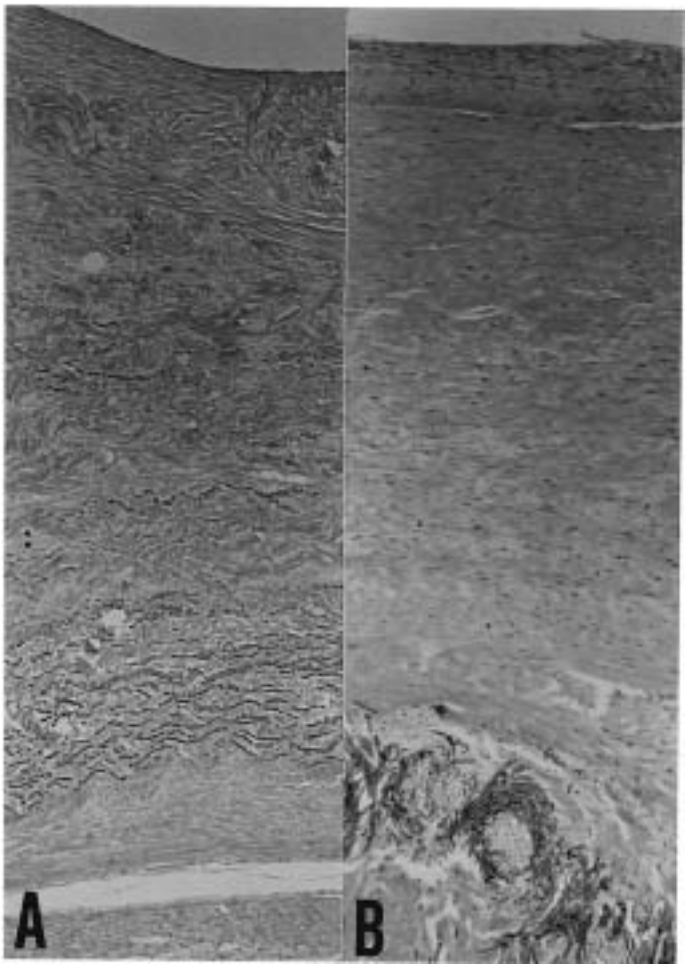

Fig. 3. Histological findings in patch graft of surrounding portion 6 months after grafting. HE stain. × 73. (A : Denacol No. 5. B: UFPF No. 11)

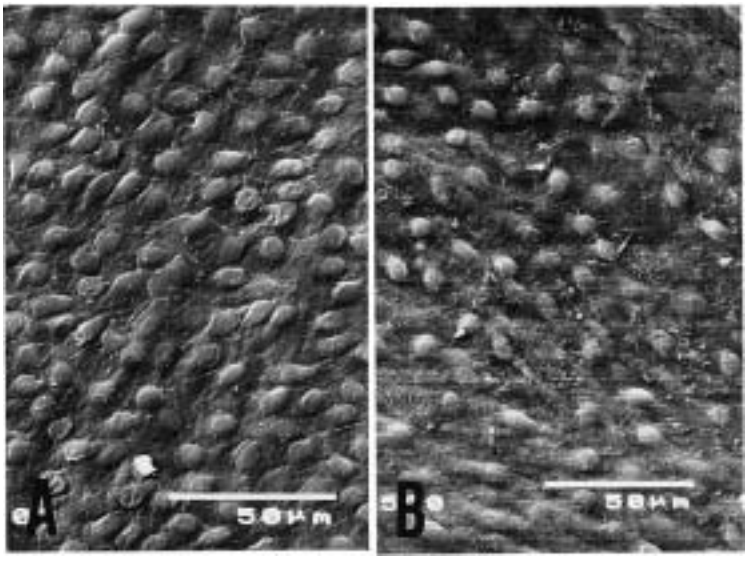

Fig. 4. Scanning electron microscopic view of inner surface of the Denacol group and UFPF group 12 months or more after grafting. (A: Denacol No. 6, 723 days. B: UFPF No. 12, 375 days)

of the Denacol-treated graft used in this study was considered to be safe, as Noishiki *et al.* reported [9].

Slight metaplasia of cartilage in a portion of the graft margin was found in Dogs No. 5 and No. 6 in the Denacol group. Ichikawa *et al.* reported no hemodynamic or histological abnormalities after transplantation of a Denacol-treated bovine carotid vein as a bypass graft to the right ventricular outflow tract were seen in eight dogs through 385 days observation period after grafting [2]. In the present study, no hemodynamic or morphological changes were detected even for a maximum observation period of 723 days. Therefore, the Denacol-treated graft and the UFPF graft were considered to be safe with respect to durability when applied clinically. However, the incidences of aneurysm and dilation are reportedly 36% and 21%, respectively, after grafting a glutaraldehyde-treated human umbilical cord vein graft [1]. The incidence of aneurysm was assumed to be 100% in human patients receiving peripheral vascular transplantation of such a biological graft [11]. It is necessary to carry out a more prolonged durability examination in the future.

Our results suggest that the Denacol graft is a material which can be applied clinically and possesses characteristics superior to those of the UFPF graft for extended reconstruction of the right ventricular outflow tract.

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