FULL PAPER *Surgery*

Experimental Study of Materials for Patch Graft on Right Ventricular Outflow Tract under Extracorporeal Circulation in Dogs—Comparison between Denacol^{R} EX-313-Treated Bovine Jugular Vein Graft and Expanded Polytetrafluoroethylene (EPTFE) Graft

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ABSTRACT. A comparison between a bovine jugular vein treated with a hydrophilic polyepoxy compound cross-linker (Denacol), and expanded polytetrafluoroethylene (EPTFE), an artificial material, as a patch graft for the reconstruction of the right ventricular outflow tract under extracorporeal circulation in dogs, as if they had pulmonic stenosis, was made. Hemodynamic and histological examinations were conducted two weeks after the transplantation. Hemodynamic problems were not observed in either the Denacol or EPTFE groups. Macroscopically, organization of new tissue on the graft surface was more marked in the EPTFE group than in the Denacol group, and newly-formed tissue was seen surrounding the border of the graft and burying it in the EPTFE group. In the Denacol group, microscopic findings revealed the presence of inflammatory cells and fibroblasts, and an invasion of the graft by collagen fibers and elastic fibers. In the EPTFE group, there was minimal cellular infiltration of the graft and a thick layer consisting of collagen fibers and fibroblasts was observed around the graft. These results indicated that two weeks after transplantation the graft was better assimilated and organized with blood vasculature in the patch graft in the Denacol group than in the EPTFE group.
KEY WORDS: canine, Denacol EX-313, EPTFE, patch graft, pulmonic stenosis.

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The occurrence of right ventricular outflow tract stenosis, one of the congenital cardiovascular abnormalities seen in dogs, is generally accompanied by various disorders such as pulmonic stenosis and tetralogy of Fallot. Correction of the condition commonly involves reconstruction of the narrow segment using various materials [3, 13, 14, 18, 19, 22]. However, only limited studies have been conducted regarding the use of these materials for transplantation [7, 9].

The present study investigated the hemodynamic and histological effects of using the hydrophilic polyepoxy compound cross-linker (Denacol)-treated graft and the expanded polytetrafluoroethylene (EPTFE) graft as transplantation materials for the reconstruction of right ventricular outflow tract stenosis with pulmonic stenosis under extracorporeal circulation in dogs. The evaluations were conducted two weeks postoperatively.

MATERIALS AND METHODS

Animals: Ten clinically healthy beagle dogs were examined physiologically and randomly distributed into two groups: An EPTFE group, (average weight 10.86 ± 0.55 kg, n=5) and a Denacol group, (average weight 10.69 ± 0.53 kg, n=5) with two males and three females in each group. The dogs were vaccinated with a mixed vaccine and were negative for microfilaria and Brucella organisms. General hematological examination, blood chemical examination, plain thoracic radiography, electrocardiography, and phonocardiography revealed no abnormal findings.

Preparation of the Denacol graft: The jugular veins of apparently healthy cattle were collected from a slaughter house. After trimming, they were placed in 1% protamine solution and cross-linked with 4% Denacol (Denacol^{R} EX-313, Nagase Chemical Ltd., Osaka, Japan) solution. The graft was then immersed in 1% heparin solution to produce an ionic bond with protamine, and preserved in 70% ethanol under cold storage. Before being use as a transplant, the graft was washed with physiological saline to remove the alcohol [7, 12].

Surgical anesthesia: The dogs were premedicated with atropine sulfate (0.04 mg/kg) and acepromazine maleate (0.4 mg/kg). After sufficient sedation was achieved, anesthesia was induced using thiamylal sodium (10 mg/kg). The dogs were intubated with an endotracheal tubes. 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 the 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 on the two groups of dogs under an extracorporeal circulation system using an artificial heart-lung machine [9, 21]. The Denacol grafts and the EPTFE (GORE-TEX^{R} ePTFE graft/

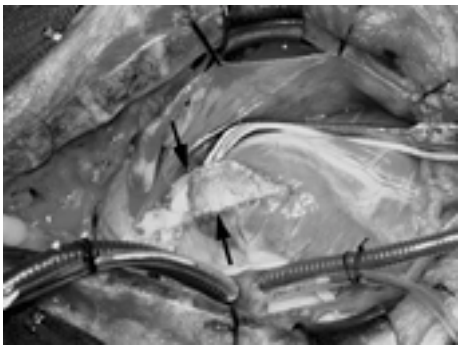

Fig. 1. A patch graft (Denacol) sewn over right ventricular outflow tract under extracorporeal circulation.

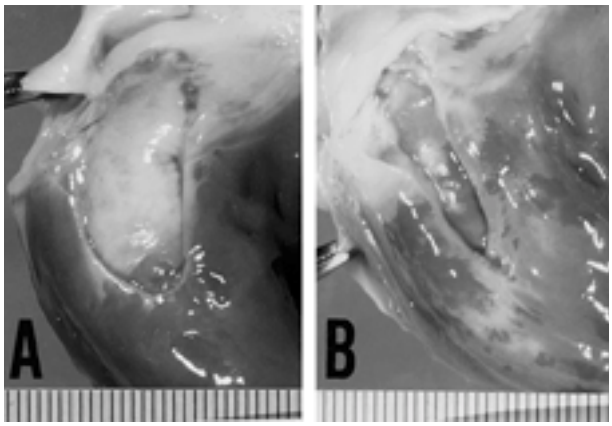

Fig. 2. View of the inner surface of the patch grafts (A: Denacol group No. 5, B: EPTFE group No. 5)

patch, W.L. Gore & Associates,Inc., Arizona, U.S.A) grafts were trimmed to produce a patch with a length of 40 mm and a width of 20 mm which were sutured at 2–3 mm intervals using 6–0 polypropylene. The Denacol graft was sutured in such a way that the inner surface of the bovine vena cava was in contact with the inner wall of the lumen of the right ventricular outflow tract. The operative site was then checked for hemorrhages and the thoracic cavity was closed (Fig. 1).

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

Clinical examinations: The following examinations were conducted two weeks postoperatively. A general hematological examination, blood chemistry, plain thoracic radiography, electrocardiography and phonocardiography were conducted in unanesthetized dogs. Pressure measurement and imaging of the right heart through a cardiac catheter and hemodynamic examinations were carried out with the dogs under general anesthesia.

Macroscopic and microscopic examinations : Tissue samples were collected from the euthanatized dogs, from the adhesion between patch graft and pericardium on the external surface of the heart and from thrombus adhesion inside the heart. The samples were fixed in formalin for histological examination. For histological examination, the samples were stained using hematoxylin-eosin, Masson-trichome and Elastica van Gieson stains.

Statistical analyses: The results of the experiments are expressed as mean ± standard deviation. A comparison of the means of the different parameters was conducted using the Student's *t*-test and statistical significance was established at the $p<0.05$ level.

RESULTS

Clinical examinations: Transplantation surgery in all dogs in both Denacol and EPTFE groups was carried out under identical conditions and all dogs showed remarkable recovery after the operation. In both groups, no abnormal findings were observed in hematological examination, blood chemical examination, plain thoracic radiography, electrocardiography and phonocardiography two weeks postoperatively. Examination of the right heart internal pressure revealed a maximum pressure gradient between right ventricular pressure and pulmonary arterial pressure of 1.8 ± 2.2 mmHg and 3.8 ± 2.3 mmHg in the Denacol and EPTFE groups, respectively; no significant difference between the two values was observed. Imaging of the right ventricular outflow tract revealed no apparent abnormalities in the region where the patch graft was transplanted.

Macroscopic findings: In all dogs, adhesions of thrombus on the inner surface of the graft wall were not observed during necropsy. In the Denacol group, the transplanted patch graft was clearly visible from inside the heart (Fig. 2A). In the EPTFE group, however, the surrounding tissues tended to coalesce and merge with the border of the graft and bury it, leaving only a small portion of the patch graft visible (Fig. 2B). The maximum width of the patch graft in the Denacol and EPTFE groups was 9.4 ± 2.7 mm and 3.5 ± 0.9 mm, respectively; the patch graft in the EPTFE group was significantly narrower than in the Denacol group ($p=0.0017$) and the newly-formed tissue tended to cover the graft surface and obscure the boundary of the graft by burying it (Fig. 3). In addition, the cutting thickness in the intermediate portion of the graft was significantly lower in the Denacol group (1.7 ± 0.6 mm) than in the EPTFE group (3.2 ± 0.8 mm) (Fig. 3, $p=0.014$).

Microscopic findings: In the Denacol group, the inside of the graft was invaded by inflammatory cells and fibroblasts and collagen fibers, especially the external surface of the graft. Also, invasion of the graft by elastic fibers and new blood vessels were observed in the Denacol group (Fig. 4A). In the EPTFE group, a layer consisting of inflammatory cells was observed on the graft surface. Thick collagen fibers and fibroblasts formed on the surface and at the cir-

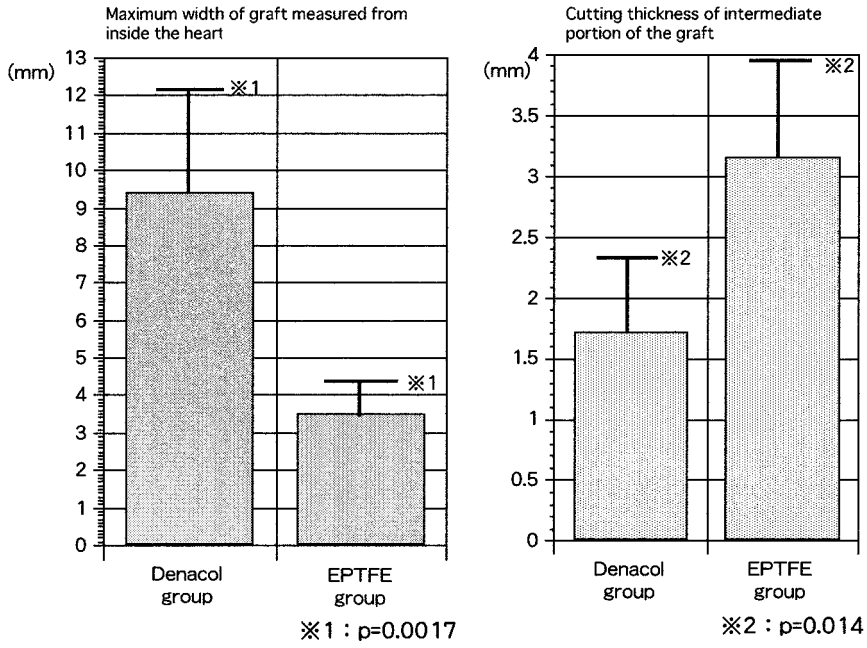

Fig. 3. Maximum width and cutting thickness of Denacol and EPTFE grafts.

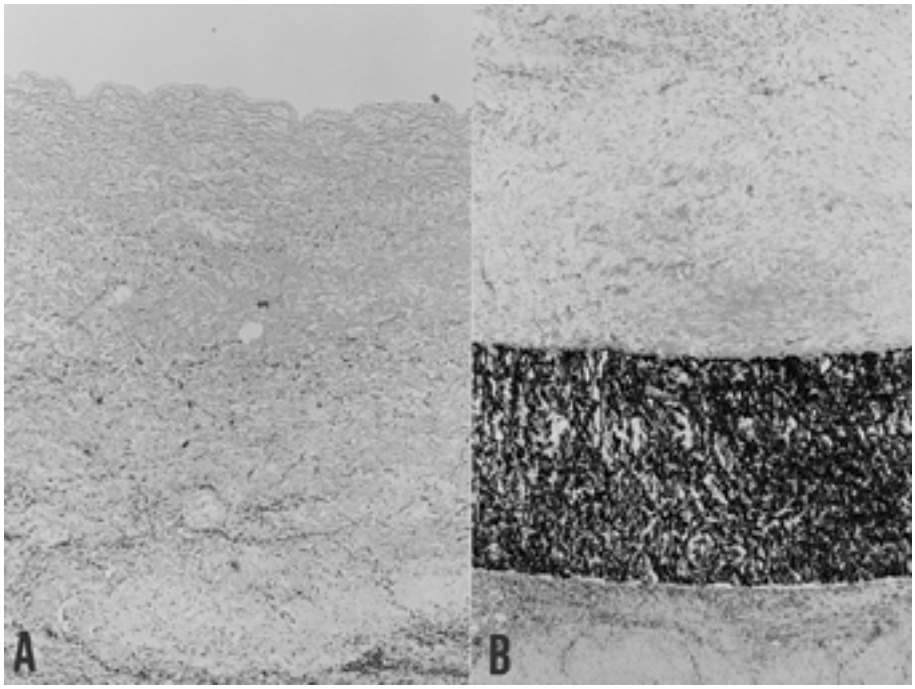

Fig. 4. Histological findings in patch grafts 14 days after implantation (A: Denacol group No.4. B: EPTFE group No. 5.). HE stain, × 73.

cumference of the graft in the EPTFE group (Fig. 4B). Compared to the Denacol group, the cellular infiltration and formation of capillary vessels were minimal in the inside of the graft in the EPTFE group, and the new tissues formed could easily be detached from the graft. Furthermore, although loose connective tissues were observed in the outermost portion of the graft in both groups, calcification and chondrometaplasia could not be seen.

DISCUSSION

Pulmonic stenosis is an abnormality which is commonly observed in various congenital cardiovascular abnormalities in the dog [15]. Various methods for surgical treatment of this condition have been reported [3–5, 13, 20, 22]. Among these methods, the use of a patch graft has proven to be a very useful method for correction of stenosis during right ventricular outflow tract reconstruction [13, 22]. Currently, the materials being used clinically for the replacement of blood vessels during cardiac surgery are synthetic cloths, autogenous tissues, and bioprosthesis, made up of homologous and heterogenous tissues which are non-antigenic [1, 2, 9, 16–18, 23]. The Denacol used in this experiment is more hydrophilic, has a greater cellular affinity and a lower cross-linking cytotoxic reaction [12], compared to classical glutaraldehyde (GA). In addition, an artery cross-linked with Denacol has a greater tensile strength, elongation rate and compliance compared to a GA-treated artery; a Denacol-treated artery has almost the same properties as a natural artery of the body [12].

In this experiment, comparison was made between patch grafts made up of either: 1) a bovine jugular vein cross-linked with Denacol, ionically-bonded with protamine using heparin in order to make it antithrombogenic; or 2) EPTFE, which is commonly used for patch grafts. The patch grafts of Denacol and EPTFE were used for the reconstruction of the right ventricular outflow tract with pulmonic stenosis, and the effectiveness of the graft was evaluated. Macroscopic findings of the grafts two weeks after transplantation showed more marked proliferation of new tissues in the graft surface of the EPTFE group than in the Denacol group. The new tissues tended to coalesce with the border of the graft and bury it in the EPTFE group. The excessive proliferation of new tissue on the surface of the graft and the coalescence with the border of the graft observed in the EPTFE group can lead to the postoperative complication of recurrence of outflow tract stenosis. These reactions in the surface and border of the graft in the EPTFE group were considered to have resulted from stress in the diastole around the graft. In the Denacol group, which had minimal loss of flexibility, there was no excessive proliferation of new tissues, since there was no change of the expansibility around the graft. Compared to the EPTFE group, the Denacol group showed minimal physical reaction to foreign bodies. The minimal formation of new tissue in the graft in this study was similar to that observed in other studies using Denacol [6, 12], and was different from the case in which the flexibility did not differ significantly with transplantation time. In previous experimental transplantation studies, comparison of Denacol-treated bioprosthesis and synthetic materials for replacement of diaphragmatic tissues showed that proliferation of new tissue was less remarkable in Denacol-treated grafts than in synthetic grafts [10]. In the Denacol grafts, there was minimal loss of flexibility during transplantation, spread of endothelial cells to the graft surface, minimal adhesion with other organs and very little invasion of elastic fibers [8, 11].

With regard to cutting thickness in the intermediate portion of the graft, the EPTFE group showed greater thickness than the Denacol group due to greater proliferation of new tissues in the EPTFE group than in the Denacol group. Although the artificial material in the EPTFE group could have produced the thicker structure, the Denacol group showed greater flexibility, a characteristic which is more important for this condition. The production of collagen fibers, fibroblasts and elastic fibers in the EPTFE group could be due to the stimulation of fibroblastic activity by the fibroblast growth factor present in the artificial high polymer material contained in EPTFE [24]. The effectiveness of the graft could be determined by its degree of incorporation in the tissues. In the EPTFE group, the graft could easily be detached from the new tissues. In the Denacol group, however, inflammatory cells coalesced with the graft and incorporated it with the surrounding tissues, forming a solid structure. This reaction is considered necessary during the initial phase of transplantation.

Thus, overall the present results two weeks after transplantation showed that the Denacol graft had greater compatibility with tissues than the EPTFE graft.

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