The Use of Epoxy Patch Grafts for the Repair of Experimentally-Created Diaphragmatic Defects in Dogs

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ABSTRACT. Canine pericardium which had been treated with polyepoxy compounds (Denacol® EX-313) was used as a patch graft for the correction of experimentally-created diaphragmatic defects in five dogs belonging to the same litter. Clinical, macroscopic and histological examinations were conducted every month up to five months after suturing of the patch graft. Clinical examination of the patch graft showed no apparent abnormalities. Macroscopic examination conducted during autopsy showed that the patch graft maintained adequate elasticity for five months after suturing, the surface of the patch graft was covered with a thin membrane and neovascularization was observed. Histological examination showed that the surface of the patch graft was covered with a thin membrane. Inflammatory tissue reactions were observed at one month, but gradually decreased from the second month onwards. In addition, the patch graft had excellent tissue affinity. — Key words: canine pericardium, diaphragmatic hernia, patch graft.


Diaphragmatic hernia is a condition in which the abdominal viscera enter into the thoracic cavity through a tear or rupture in the diaphragm. Traumatic diaphragmatic hernia is common in dogs and cats [4]. In most of these cases, surgical correction by direct suturing of the ruptured portion holds through. However, in some cases for example, when direct suturing is not possible due to the largeness of the defect a long time has elapsed before surgery, there is very little tissue left for suturing, or there are congenital abnormalities of the diaphragm, etc., the closing of the diaphragmatic defect through the use of a patch graft becomes necessary.

At present, the materials being used clinically for patch grafts include mesh made of synthetic materials such as teflon [12], polypropylene [4], silicon rubber sheet [4], and internal and external oblique muscle fascia [5, 8], femoral muscle fascia [12] and omentum [1]. In general, biological materials for implantable artificial organs were crosslinked with glutaraldehyde (GA) [3]. The crosslinking with GA makes the materials insoluble, less biodegradable, and less antigenic, carries the disadvantage of making the materials more stubborn. We introduced a new hydrophilic crosslinking reagent, polyepoxy compounds (Denacol® EX-313), as a replacement for GA. Grafts crosslinked with Denacol® EX-313 (Nagase Chemical Ltd., Osaka, Japan) can keep the natural tissue compliance and is stronger than the GA crosslinked graft, thus providing excellent suturability and compliance match [6].

With the aim of producing a better patch graft, Denacol® EX-313 solution was used for the treatment of canine pericardium taken from dogs of the same kind, and the treated canine pericardium was used as patch grafts for the repair of experimentally-created diaphragmatic defects.

Five young healthy mix-breed dogs belonging to the same litter were used in this study. The dogs were 6–7 months old and weighed 6.6–8.3 kg at the time of the surgery. Canine pericardium of other dogs, treated with Denacol® EX-313, was preserved in 70% alcohol solution (Table 1) [7]. Immediately before use, the alcohol was dried off and the patch graft was immersed in a physiological saline solution. The patch graft was then used for the repair of experimentally-created diaphragmatic defects.

Premedication with 0.05 mg/kg of atropine sulfate (Tanabe Pharmaceutical Co., Ltd., Osaka, Japan) and 0.5 mg/kg of acepromazine maleate (Fermenta Animal Health Co., Kansas, U.S.A.) was followed by the induction of anesthesia using 15 mg/kg of thiopental sodium (Ravonal®, Tanabe Pharmaceutical Co., Ltd., Osaka, Japan). After intubation, controlled respiration was induced through intermittent administration of muscle relaxants such as 0.2

Table 1. Bridging treatment and preservation using Denacol® EX-313 solution

1. Canine pericardium was collected and preserved in 50% ethanol solution up to 3 weeks.
2. Immersion in 1% protamine solution for 1 hr.
3. Bridging in 4% prepared Denacol® EX-313 solution for 48 hr (diluted and prepared in pH 9.5 carbonate-bicarbonate buffer.
4. Enough ethanol is added to produce a solution of 5% ethanol.
5. The graft was washed in a water purifier and dechlorinated for 24 hr.
6. The graft was immersed in a gradually releasing 1% heparin solution for 1 hr.
7. The graft was washed again as in step 4.
8. The graft was preserved in 70% ethanol solution and refrigerate.
mg/kg of succinyl choline chloride (Succin®, Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan) and 0.1 mg/kg of pancuronium bromide (Mioblock®, Sanyko Co., Ltd., Tokyo, Japan) and maintenance anesthesia was carried out using 0.1% ketamine micro-mini drip administration technique. For surgery, the animals were positioned and restrained in right lateral recumbency and incisions were made on the left side of the body from the 8th to the 10th intercostal spaces. Experimental diaphragmatic defects were made by cutting out a round portion of the diaphragm, using a 50 mm diameter round frame. A moderately large trimmed previously-treated canine pericardial patch graft was sutured to the diaphragmatic defect, with the cardiac side of pericardium facing the thoracic cavity. The material used for suturing the patch graft was a 3-0 braided silk (Nesco Suture®: Nippon Shoji Co., Ltd., Osaka, Japan). Physical examination, blood tests, plain thoracic radiographic examination, macroscopic examination and histological examination were conducted at 1, 2, 3, 4 and 5 months after patch graft suturing. Animals were sacrificed by administration of an overdosed pentobarbital sodium (Nembutal®, Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) intravenously.

Physical examination of all dogs revealed no apparent postoperative complications. In addition, blood analysis and plain thoracic radiographic examination showed no abnormalities.

**Macroscopic examination:** There were no changes observed between the patch graft at the time of suturing and at 1–5 months after surgery; elasticity was also similar. In addition, the surface of the patch graft was covered with a thin membrane, and neovascularization was observed (Fig. 1). However, there were some adhesions seen between the sutured area and the lungs, liver and omentum.

**Histological examination:** At one month after the patch graft suturing, the surface of the patch graft was already covered with a thin membrane; there was moderate infiltration of connective tissue under the thin membrane and cellular infiltration of inflammatory cells consisting of fibroblasts, macrophages and lymphocytes, was observed (Fig. 2). The tissue under the thin membrane on the thoracic side was generally thinner than on the abdominal side. In addition, tissue reactions in the form of cellular infiltration under the thin membrane were more marked on the thoracic side than in the abdominal side, especially under the sutured area. At two months after patch graft suturing, although there were some tissue reactions seen around the sutured area, the inflammatory cells were relatively few and increased infiltration of connective tissue was observed. At three months after patch graft suturing, increased neovascularization was observed in both thoracic and abdominal sides and dense infiltration of connective tissue was also observed. In addition, inflammatory cellular infiltration and tissue reactions at the margins of the patch graft sutures had become less obvious. Examination of patch graft suture at four months after surgery showed similar characteristics as those at three months; however, there were adhesions between the lungs and the thoracic

side of the patch graft sutured area and between the liver and abdominal side of the patch graft sutured area. At five months after surgery, the tissue under the thin membrane had become a complete layer and cessation of inflammatory tissue reactions was observed (Fig. 3).

These results indicate that the Denacol® EX-313 treated canine pericardial patch graft used in treating experimentally-created diaphragmatic defects produced extremely favorable affinity to body tissues. These results agree with those of other studies [9], including one in which experimentally transplanted bovine venous bioprosthesis [7] made from Denacol® EX-313 was used. For surgical correction of diaphragmatic hernia in dogs using prosthesis, the following considerations should be taken into account: 1) appropriate surgical repairing technique; 2) durability of
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Fig. 3. Histological findings of patch graft five months after suturing (No. 5, HE stain, x 250). Inflammatory cells covering the membrane and tissue reactions decreased in both the thoracic and abdominal surfaces of the patch graft. T: thoracic cavity, P: patch graft, O: omentum, A: abdominal cavity.

the material; 3) tissue compatibility; 4) ease of suturing; 5) preservation method of material [11]; and 6) cost.

Additionally, it is important to select a material which has less probability of being rejected, less tissue reactions and no harmful effects to other organs. This study confirmed that materials treated with Denacol® EX-313 provided more than two times the extension capability and pulling strength than glutaraldehyde, another bridging material [10].

In this study, observation of sutured patch graft five months after surgery showed that there were little changes in elasticity, marked decreases in tissue reactions, and satisfactory tissue affinity after suturing. Aside from treatment with Denacol® EX-313, slow-releasing heparin treatment [9] of patch graft was also used to prevent adhesions between the patch graft and other organs (Table 1). Although adhesion was not observed at the patch graft, there were some adhesions seen in the sutured area. The adhesions are believed not to have been due to the patch graft, but to the braided silk suture material which produces strong tissue reactions in interrupted sutures. In addition, the greater adhesions observed in the abdominal side as compared to the thoracic side may be due to the smooth surface of the pericardial graft facing to the thoracic side and the lesser amount of movement of the abdominal viscera (e.g., liver) compared to the lungs of the thoracic cavity.

These results suggest that canine pericardial grafts made using Denacol® EX-313 are comparatively satisfactory material for the closure of diaphragmatic defects and have potential clinical applications.

REFERENCES