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Comparative Study of Lyophilized Human Dura mater and Lyophilized Bovine Pericardium as Dural Substitutes in Neurosurgery

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Summary

In a prospective, controlled randomized study either lyophilized bovine pericardium or lyophilized human dura mater have been used as a patch for the closure of the dura in 102 patients. The aim of this investigation was to compare both materials in terms of immunogenic response of the patients. The rate of post-operative complications was comparably low in both groups (wound infection in 1/51 patients each). In regard of workability, thicknesss of the material and flexibility the pericardium patches were judged to be by far superior. Neither signs of a cellular nor of an intesified humoral response could be detected in patients who received the pericardium implants. Thus, lyophilized bovine pericardium seems to be a superior alternative for the surgical repair of dural defects.

Keywords: Dura repair; lyophilized dura mater; heterologous lyophilized pericardium.

Introduction

Lyophilized human dura mater has been implanted for more than 30 years as an alternative to autologous material to obliterate defects of the cranial or spinal dura mater[2, 3, 13, 16, 20, 21]. In addition, it has been used successfully for surgery of joints, the thoracic and the abdominal wall and the urinary tract[7, 10, 12, 17]. In order to meet the increasing demand of alternative material, a collagen implant consisting of *lyophilized* bovine pericardium has been developed. *Tanned* bovine pericardium has been used so far for artificial cardiac valves and as a patch material in cardiovascular surgery[4–6, 8, 9, 22].

The aim of this study was to compare lyophilized human dura mater (HDM) and *lyophilized* bovine pericardium (BP) in terms of intraoperative handling, wound healing and clinical complications as well as to investigate the immunogeneic properties of BP in neurosurgical patients.

Material and Methods

A prospective controlled randomized study was carried out from November 1986 to June 1987 in the Department of Neurosurgery at the Justus-Liebig-Universität Giessen. 102 Patients were included for implantation of either HDM (n = 51, Lyodura®, B. Braun, Melsungen, FRG) or BP (n = 51, Lyoplant®, B. Braun, Melsungen, FRG) where a dural implant was necessary. Informed consent was obtained from every patient prior to surgery. Patients who could not be asked because of their state of consciousness (e.g. severe head trauma, coma) had to be excluded as well as those patients suffering from an inflammatory disease. The surgical technique of the implantation was similar in both groups: after cutting the transplant to the required size it was fixed with some sutures at the dural rim of the defect. Closure was performed with a running suture using a round needle and non-resorbable material. Fibrin tissue glue was used as sealant. Every surgeon was asked to judge the handling of the transplant he had used in terms of rehydration, thickness, stiffness, workability, cutting characteristics, suturability and water tightness. The clinical course of convalescence, the concomitant pharmacotherapy and any complications were documented. A blood count, the sedimentation rate, serum electrolytes, GOT, GPT, AP and gamma-GT were determiend prior to surgery as well as on the fourth post-operative day and at discharge of the patient from our hospital.

In addition, blood samples were drawn before surgery and at least three months thereafter to detect humoral immunogeneic reactions. This was done by an enzyme-linked immunosorbent assay (ELISA) against collagen antibodies (Dr. E. Scholl, Melsungen). If the mean pre-operative extinction of all patients was exceeded by three times that of the standard deviation at the time of the post-operative check-up, the patient was classified as having developed antibodies against collagen.

The comparability of the HDM and the BP group was checked on the basis of the parameters of sex, age, height and body weight (t-test, Fischer's test). The results of the ELISA were statistically analyzed using Fischer's test.

Histology

On re-operation, mainly because of recurrence of tumour, biopsies of the grafted pericardium could be taken in 9 of these patients

from 4 days to 24 months after the original use. The specimen have been worked up histologically.

Results

There was no statistically significant difference between the HDM and the BP group in terms of age (mean age: HDM 45.8 years/BP 48.2 years), height (mean height: HDM 168.5 cm/BP 166.8 cm), weight (mean weight: HDM 73.1 kg/BP 73.2 kg) and sex (male/female: HDM 27/24, BP 21/30).

The distribution of the diagnoses were similar in either group (Table 1). Patients who received BP had slightly more concomitant non-neurosurgical diseases (Table 2).

According to blood count, sedimentation rate, electrolytes and transaminaes, there was no statistically significant difference detectable between both groups: either with regard to the preoperative or the postoperative value or to the differences thereof.

The dural defects which had to be closed ranged between 1 × 1 and 6 × 8 cm (mean size of the implants 14 cm^2). The mean duration of the operations (5 hours) was similar in both groups. The surgeons who operated upon the patients of this study were distributed similarly in both groups – they differed of course in regard to their experience according to the demand of each operation.

Fibrin glue was used in 47% of the cases in the HDM group and in 50% in the BP group (no significant difference). 12% of the HDM patients received antibiotics in comparison to 6% of the BP patients, 79% and 86% respectively were treated with steroids (no significant difference).

Table 1. *Diagnoses* (by histological examination)

	BP	HDM
Glioma	19	18
Ependymoma	3	1
Plexuspapilloma	0	1
Neurinoma	3	1
Meningioma spinal/cerebral	10	10
Angioblastoma/gliosarcoma	0	3
Craniopharyngeoma	0	4
Pituitary adenoma	3	3
Epidermoid	1	0
Angioma	2	2
Cystic TU	0	2
Metastasis	7	4
CSF fistula	1	1
Others	2	1

Table 2. *Concomittant Non-neurosurgical Diseases*

	BP	HDM
High blood pressure	3	1
Adiposity	5	3
Diabetes mellitus	2	1
Cardiac arrhythmia	2	0
Major surgery for other diseases	3	0
Seizures	5	2
Malignant tumour (extracerebral)	1	4
Left heart failure	1	0
Silicosis	1	0
Fractures	1	1
Disorders of the hypothalamo-pituitary axis	2	2
Others	1	4
n	27	18

Intraoperative Handling (Values in % of all Ratings)

According to the description of the surgeons, the workability of the transplant was superior in 98% of the BP group. Lyophilized dura mater was judged to be too stiff in 96% and to be of unconvenient thickness in 20% (vs. 0% in BP). Rehydration was described as too slow in 88% of HDM patches (vs. 0% BP). In terms of these five criteria, BP was rated statistically significantly superior to HDM. As far as cutting characteristics, suturability and water-tightness are concerned, both materials have been judged to be equally good.

The rate of intraoperative complications was 4% in both groups, none was related to the transplant:

haemorrhage from the basilar artery (BP), frontal dura completely torn during craniotomy (BP), intraoperative swelling of the occipital lobe (HDM) and minor air embolism (HDM). Post-operative complications occured in 6 patients of the BP group and in 3 of the HDM group (Table 3): there was one infection

Table 3. *Post-operative Complications*

	BP	HDM
Infection	1	1
Haemorrhage	1	0
Septic complication	1	0
Brain oedema	2	1
Thrombosis	1	1
n	6	3

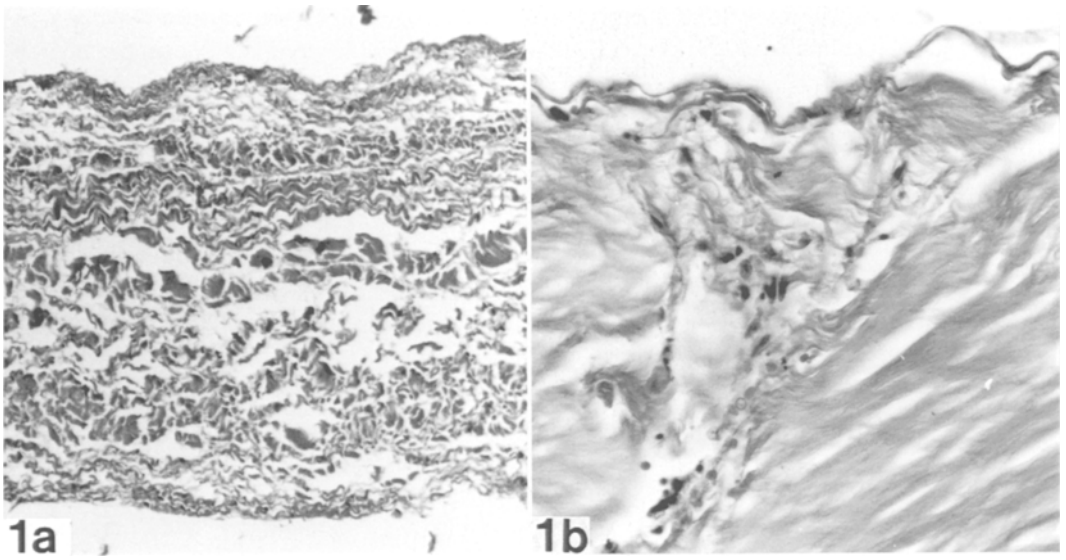

Fig. 1 a. Native pericardium before implantation. The specimen consist of only layer with a three-dimensional course of the slightly curled collagenous fibers. (× 70)
Fig. 1 b. Pericardium, 4 days after implantation. Mobile cells of the connective tissue (fibroblasts, histiocytes) have started to migrate into the implant through natural pores. (× 260)

in either group (a subgaleal empyema in a BP patient and a meningitis in a HDM patient).

The time of hospitalization was not related to either material (HDM 10.04 ± 4.6 days/BP 10.5 ± 5.9 days). Out of 102 patients, 78 serum samples could be obtained pre-operatively in order to determine antibodies against collagen. In 6 cases we found collagen antibodies already *prior* to surgery. We got control sera 3 months post-operatively from only 27 patients. Five of them revealed formation of antibodies in the ELISA according to the criteria mentioned above (1 HDM–4 BP). All of them had normal values before surgery was performed. The difference between both groups was statistically not significant (a = 0.187 in Fischer's test), however, the number of cases available for evaluation is rather small.

Histology

Before implantation, the lyophilized bovine pericardium consists of one layer with a three-dimensional course of slightly curled collagenous fibers and open interstitial spaces (Fig. 1 a). Four days after implantation, mainly fibroblasts and histiocytes started to migrate into the implant. These cells used the natural pores of the graft as an entrance and moved along the interstitial spaces (Fig. 1 b). After three months, an implant was found to be reduced in thickness by approximately one half, due to the loss of the thick collagenous bundles. Polarization microscopy revealed newly formed delicate collagenous fibrils which firmly connect the remnants of the graft with the external and internal neomembranes (Fig. 2). A biopsy of the implanted pericardium, taken 11 months after surgery, consisted of a portion with well preserved collagenous

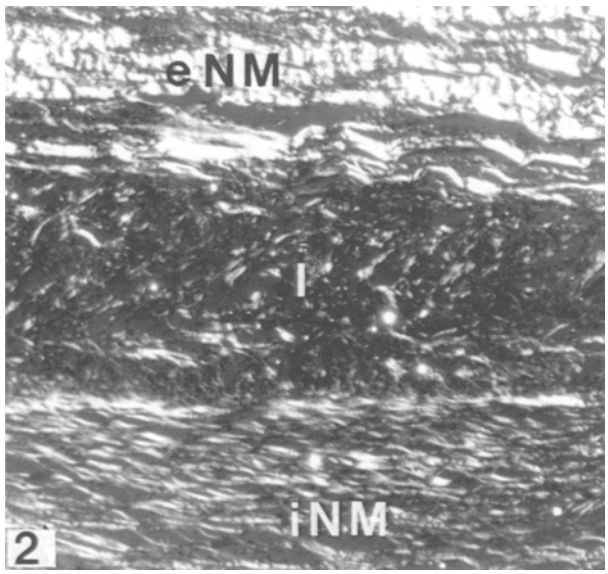

Fig. 2. Pericardium, 3 months p.i. The implant (*I*) is reduced in thickness by one half. Most of the thick strands of collagen are absorbed and replaced by delicate collagenous fibrils which, in addition, connect the implant with the inner (*iNM*) and the external neomembrane (*eNM*). (Polarized light, × 70)

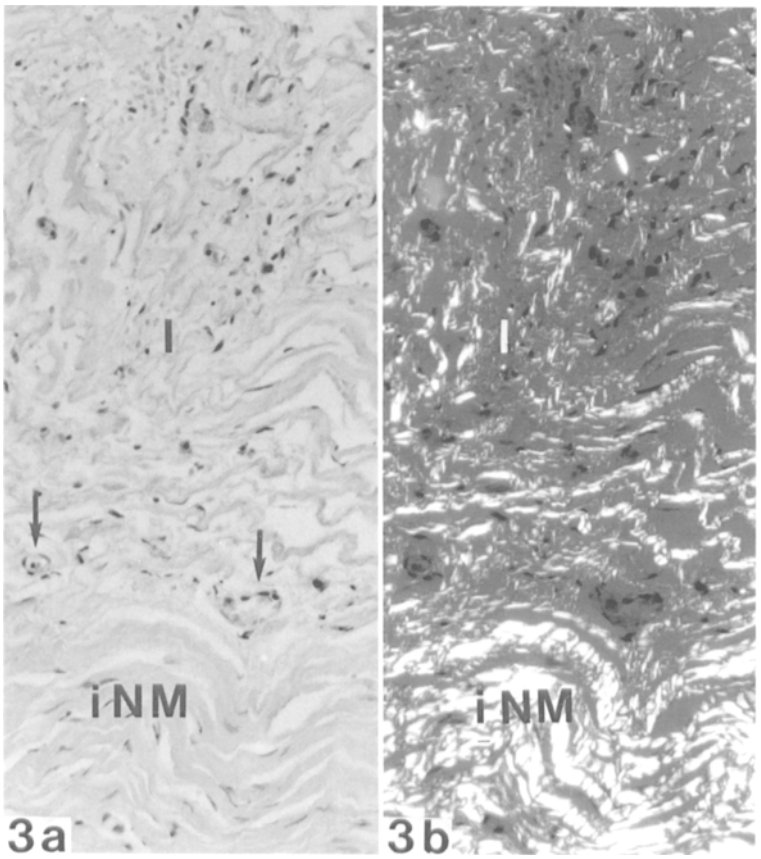

Fig. 3. Pericardium 11 months p.i. a) Under the conventional light microscope the implant (*I*) is firmly attached to the inner neomembrane (*iNM*). Several capillaries (arrows) are present at the implant/neomembrane border. The implant is abundantly populated with fibroblasts and histiocytes (× 175). b) Same specimen, polarized light (× 175)

fibers which was entirely revitalized (Fig. 3 a). The continuous transition of collagenous fibers between the implant and the inner neomembrane can be demonstrated under the polarization microscope. The fibers in both the implant and the neomembrane appear aligned in the direction of the natural trajectories of the dura mater (Fig. 3 b). Within the course of time the graft is progressively absorbed and replaced by the recipients-own connective tissue. After 24 months only a small layer of the implant was present, reduced to approximately one tenth of its original thickness (Fig. 4 a). The excellent incorporation into the host membranes is shown by polarization microscopy (Fig. 4 b). In the zone of transition between remnants of the original graft and its remodelled portion the original fibers disappear. They are replaced first by delicate fibers and then by dense connective tissue which is remodelled in the outer region of the replaced implant. The transitional zone is well vascularized (Fig. 4 c).

Discussion

Numerous efforts have been made to find an artificial material for the closure of dural defects in order to avoid the necessity of using autologous fascia or muscle. Autologous material has many advantages in terms of uncomplicated wound healing and lack of immunogeneity. However, its use requires another surgical procedure in these patients with the additional risk of impaired wound healing at the donor site and other related complications (e.g. muscle hernia). Thus, the ideal xenograft has to be immunologically neutral, of high flexibility and resitance against stretching and tension, water tight, causing minimal scar formation and to be available in large quantities. Lyophilized human dura mater meets many of these criteria to quite an extent. However, lyophilized bovine pericardium, which is a mesh of collagen fibers after removal of easily degradable proteins, can be supplied in large quantities. Its intraoperative properties, especially in terms of workability, flexibility, thickness and rehydration, was judged in our study to be superior in comparison to HDM. We did not observe any spontaneous haematoma at the site of the implant. The postoperative complications we had were not related to the dural substitutes in either group. There was only one wound infection among the HDM and the BP patients each.

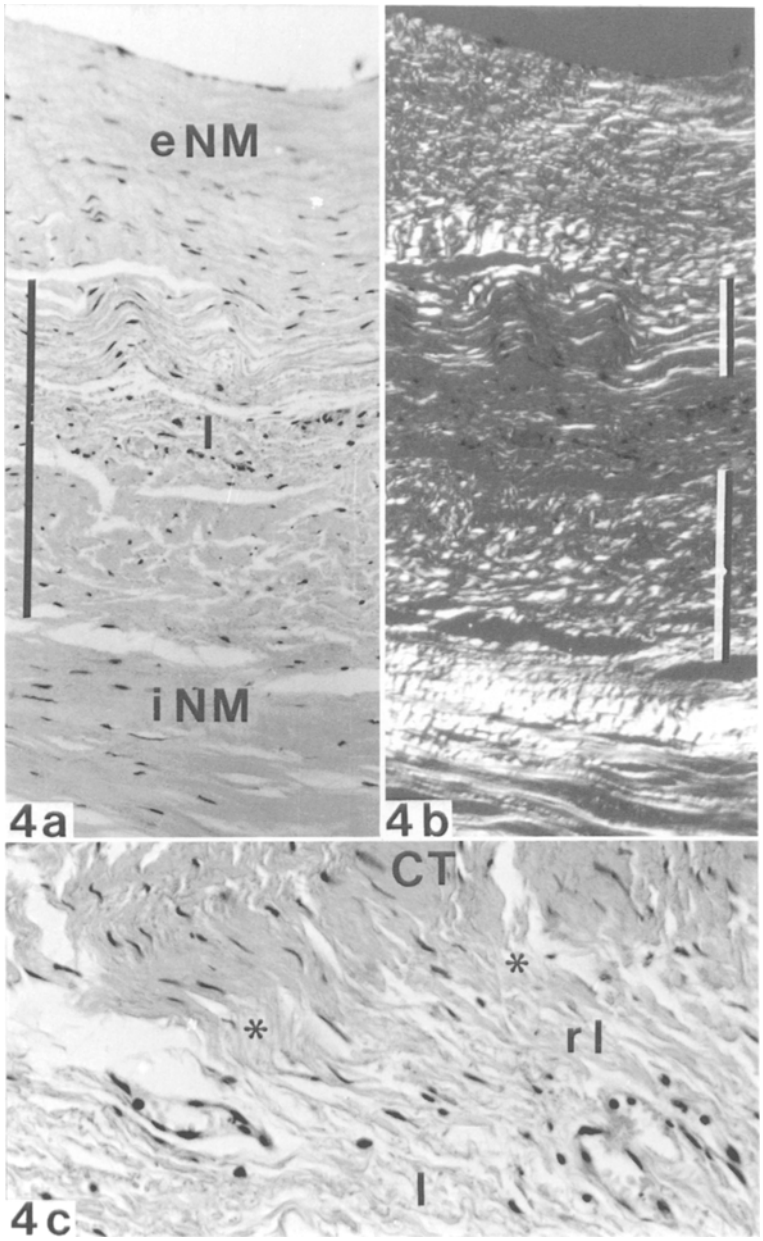

Fig. 4. Pericardium 24 months p.i. a) The graft (bar) is barely distinguishable from the covering neomembranes (*eNM, iNM*). It is reduced in size and largely remodeled. Remnants of the original implanted tissue are marked with *I* (× 75). b) With the polarization microscope the integration of the neomembranes with the remodeled portions of the implant (short bars) is evident (× 175). c) Detail of the zone of transition between remnants of the original implant (*I*) and its remodeled portion (*rI*). The original fibers disappear. They are replaced first by delicate fibers (asterisk) and then by dense connective tissue (*CT*). The transitional zone is well vascularized. (× 260)

The prosity of the material, which remains completely water-proof, accounts not only for the very good handling characteristics, but allows fibroblasts to invade the graft, to revitalize it and to replace it in the end. Conventionally preserved dura mater implants usually show unsatisfactory healing properties. The densely packed collagenous fibers of this connective tissue substitute prevent the migration of cells to revitalize the graft[15]. Subsequent calcification of the graft is a frequent consequence. In contrast, the surface of the BP is porous, thus allowing the cells of the neomembranes to anchor to the implant. Since during the course of time all implants are entirely replaced by the recipient's tissue (guide rail function according to Axhausen[1]), it can be suggested that the migrated cells cannot maintain the integrity of the collagenous fibers. This, however, holds also true for autologous non-preserved tissue grafts, for instance fascia lata[9]. If absorption processes are activated, the result of subsequent restructuring depends on the functional stress exerted on the implant (homostatic principle according to Longmire[14]) and on the type of cells in the wound bed. In the craniocerebral position the BP grafts were substituted by connective tissue which closely resem-

bled autochthonous dura. It should be explicitedly stressed that after replacement of dura mater by a (theoretically xenogeneic) BP implant, signs of *cellular* immune reactions were absent. We could not detect signs of *humoral* rejection of the grafts to a statistically significant greater extent in the BP group either. This is explained by the fact that collagen type I, the main constituent of both dura and pericardium, is not only weakly immunogenic[18] but also rather substrate-specific than species-specific and practically insoluble. These features reduce the possibility of antigenic recognition[11].

Although more experience, especially concerning the repair of CSF fistulas or reconstruction of the frontal base, is a need, we can conclude that *lyophilized bovine pericardium* seems to be a superior alternative to lyophilized human dura mater for the repair of dural defects.

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