Reduction of epidural fibrosis after laminectomy in rabbits by omental free graft

N. Brkljaca Bottegaro, J. Kos, B. Pirkic, O. Smolec, Z. Grabarevic, M. Hohsteter, J. Selanec, Z. Vrbanac

Faculty of Veterinary Medicine, University of Zagreb, Zagreb, Croatia

ABSTRACT: Epidural fibrosis is an extradural scar tissue formed after a laminectomy procedure. It is associated with persistent pain after spinal surgery and an increased risk of complications during revision surgery. The aim of this study was to determine the preventive effects of local application of an omental free graft in minimising spinal epidural fibrosis in a rabbit laminectomy model. Twenty two rabbits were randomly divided in two groups, a control group of seven and an experimental group of 15 animals. A dorsal laminectomy at levels L_1 to L_3 was performed on each rabbit of both groups. Prior to the laminectomy procedure, the animals from the experimental group were submitted to a laparotomy in order to obtain the free omental graft. The graft was then applied to the same animal at the dural deffect. All rabbits were euthanised six weeks after surgery and spine segments L_1 to L_3 were removed. Histological sections were evaluated for fibrosis intensity at the laminectomy level, the adhesion degree between *dura mater* and fibrous tissue and the presence of the foreign body reaction. A statistically significant correlation was established for the foreign body reaction presence and belonging to the group, which can be explained by the omental effects on inflammation reduction and healing promotion. The degree of adhesion between the *dura mater* and fibrous tissue and the intensity of the fibrous tissue at the laminectomy level were lower in the experimental group although the differences were not statistically significant. The use of free omental grafts is thus a promising technique in epidural fibrosis prevention.

Keywords: spinal surgery; extradural fibrous tissue; omentum; rabbit model

Epidural fibrosis describes extradural fibrous tissue formation after a laminectomy procedure. Even today, the most quoted etiopathogenic theory is the one proposed by LaRocca and MacNab in 1974. They suggested that interspinal haemorrhage after a laminectomy constitutes a scaffold for the migration of fibroblasts from the periosteum and paraspinal muscles. Subsequently, granulation tissue forms and deposits collagen fibres. These collagen fibres finally mature into a dense fibrotic scar recognized as the "postlaminectomy membrane". Epidural scar formation causes tractions on the *dura mater* and nerve roots, which results in lower back pain. Furthermore, it makes revision surgery more time-consuming and increases the risk of complications (Welch et al. 2002). Though extensive epidural scar adhesions can be removed and nerve roots can be freed, the adhesions will recur after secondary surgery (Liu et al. 2002). Therefore, most authors consider preventing epidural fibrosis formation to be the best therapy (Griffet et al. 1992; Henderson et al. 1993; Golan et al. 1995; Sandoval and Hernandez-Vaquero 2008). Besides meticulous surgery and careful haemostasis as a preventive intraoperative measure, biomaterial implantation is of great importance as a mechanical barrier between *dura mater* and overlying tissue. It acts as a barrier for fibroblast migration that consequently reduces collagen deposition. Several materials have been tested as barriers with variable or limited success in preventing or lessening fibrosis formation. These have included fat grafts (Brant et al. 1983; Langenskiold and Valle 1985; Van Akkerveeken et al. 1986; Kanamori et al. 2001), polilactic acid mem-

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branes (Lee and Alexander 1984), methyl metacrilate (Kuivila et al. 1988), vicryl mesh (Nussbaum et al. 1990), fibrin glue (Vaquero et al. 1993), silastic membranes and politetrafluoroethylene barriers (DiFazio et al. 1995), dextrane 70 (Ceviz et al. 1997), mitomycin-C (Dogulu et al. 2003), hyaluronan (Akeson et al. 2005), oxidised regenerated cellulose (Temel et al. 2006), monoclonal antibodies against intercellular adhesion molecule (Sabuncuoglu et al. 2007), 10-hydroxycamptothectine (Sun et al. 2008), amniotic membranes (Tao and Fan 2009), chitin (Keskin and Esen 2010), methylene blue (Farrokhi et al. 2011) and liposome-encapsulated hydroxycamptothectin (Yang et al. 2011).

The omentum is a highly vascular organ with a rich source of angiogenic factors that promote blood vessel growth. The potent lymphatic system of the omentum can absorb large amounts of oedema fluids and remove metabolic wastes and toxic substances (Alagumuthu et al. 2006). Omentum is also rich in growth factors and stem cells that promote healing and tissue regeneration (Litbarg et al. 2007). Therefore, our hypothesis was that free omental graft would be a suitable interposition membrane which could reduce postoperative epidural fibrosis and consequent scar adhesion after laminectomy procedures. In the present study, we examined the effect of local application of free omental grafts on reducing epidural fibrosis in a rabbit laminectomy model.

MATERIAL AND METHODS

Animal population

Twenty two healthy male cross-bred rabbits, weighing 1950 to 2300 g, aged four months were used in this study. They were randomly divided in two groups: a control group of seven and an experimental group of 15 animals. All procedures were approved by the institutional animal care and use committees of the Faculty of Veterinary Medicine, University of Zagreb and the Croatian Ministry of Science, Education and Sport.

Surgical procedures

Anaesthesia was induced by a combination of midazolam (Dormicum®, Roche, Switzerland) 1.5 mg/kg *i.m.*, phentanil (Fentanyl®, Janssen Pharmaceutica N.V., Beerse, Belgium) 0.06 mg/kg *i.m.* and medetomidine hydrochloride (Domitor®, Orion Pharma, Espoo, Finland) 0.2 mg/kg *i.m.*, followed by maintenance with a mixture of isoflurane (Forane®, Abott Laboratories, SAD) and O_2 administered *via* a facemask. Prior to the laminectomy procedure the experimental group animals underwent laparotomy surgery in order to obtain the omental grafts of 5 × 10 mm. Each animal was then prepared for a dorsal laminectomy procedure. The surgical field was clipped and prepared with clorexidine soap and solution. The area was then draped in an aseptic manner. A midline skin incision was performed from the first lumbal vertebra (L_1) to the third lumbal vertebra (L_3) carried sharply down to the lumbosacral fascia, which was incised sharply to expose the spinous processes. The paraspinal musculature was subperiostally detached to expose the second lumbal vertebra. A total laminectomy was performed at the second lumbal vertebra (L_2) vertebra in each animal by removing the spinous process with a Stille-Luer rongeur and the excision of the laminae with a pneumatic burr and fine neurosurgical punches. The laminectomy osseous defect was approximately 4 × 8 mm in size. The *ligamentum flavum* and epidural fat were removed and *dura mater* was exposed. The *dura mater* of the experimental group rabbits was covered with the free omental graft. In the control group of rabbits the *dura mater* remained uncovered. The wound was closed in three layers.

Histological evaluation

All animals were euthanised six weeks after surgery. The spine segment from L_1 to L_3 was removed *en bloc* and fixed in 4% buffered formalin solution for five days. The specimen was than placed in decalcifying solution until decalcification was complete. The laminectomy site was then identified, its sections embedded in paraffin, and serial sections (3–5 μm) cut with a microtome and stained with haematoxylin and eosin (HE) for examination. The histological sections were evaluated microscopically by two professional pathologists, who were blind to the groups. In the histopathological evaluation, fibrosis intensity at the laminectomy level, adhesion degree between the *dura mater* and fibrous tissue and the level of foreign body reaction were investigated. The fibrosis intensity at the laminectomy level was scored as follows: 0 – no

reaction seen; 1 – mild reaction; 2 – moderate reaction; 3 – severe reaction. The scoring system used for the adhesion degree between the *dura mater* and fibrous tissue was: 0 – (0–5% fibrosis adherent to the *dura*), 1 – (5–35% fibrosis adherent to the *dura*), 2 – (35–70% fibrosis adherent to the *dura*), 3 – (greater than 70% of *dura* at injury site densely adhered). The level of the foreign body reaction was graded as 0 – no reaction seen; 1 – moderate reaction; 2 – severe reaction.

Statistical analysis

Statistical analysis was performed using the Statistica program, version 6.0. Multiple regression analysis and comparison of percentage share between control and experimental groups were used for data analysis. $P < 0.05$ was deemed statistically significant.

RESULTS

Clinical observation

At the time of sacrifice all animals were active and ambulatory and no neurological deficits were seen. There were no signs of infection and all wounds were well healed within 10 days in both animal groups.

Histological findings

There were signs of fibrotic tissue in all the specimens at the laminectomy level. No bone regeneration was demonstrated in any group. The fibrotic tissue appeared more prominent in the control group with the fibrous tissue densely populated with fibroblasts and frequently attached to the *dura*

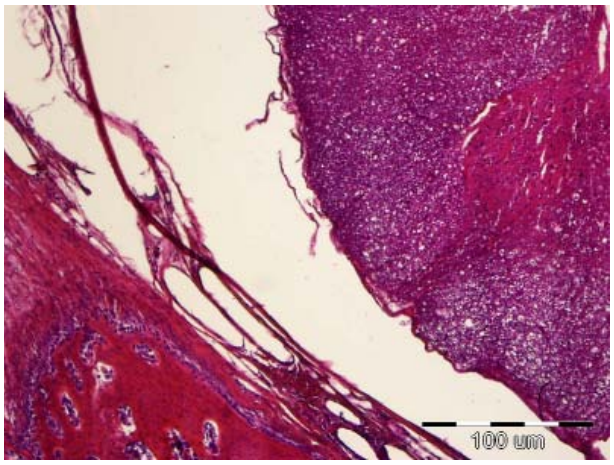

Figure 1. Adhesions between the *dura mater* and fibrous tissue; control group (HE)

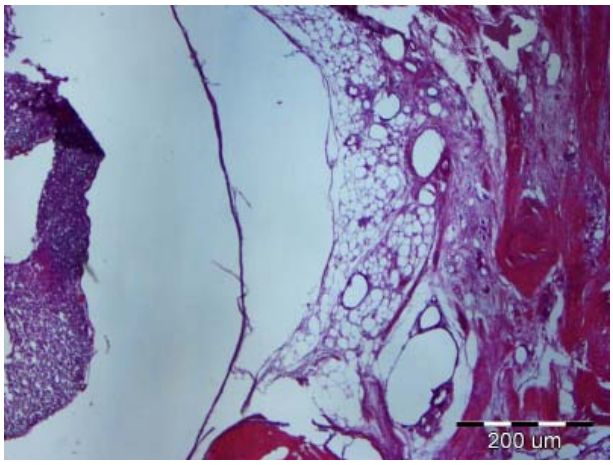

Figure 2. *Dura mater* free of adhesions; experimental group (HE)

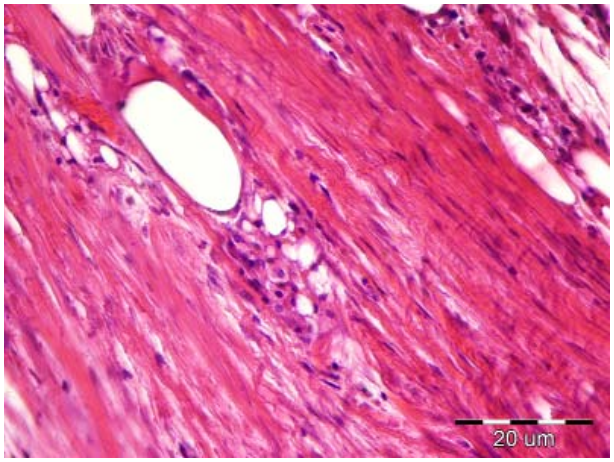

Figure 3. Dense fibrous tissue; control group (HE)

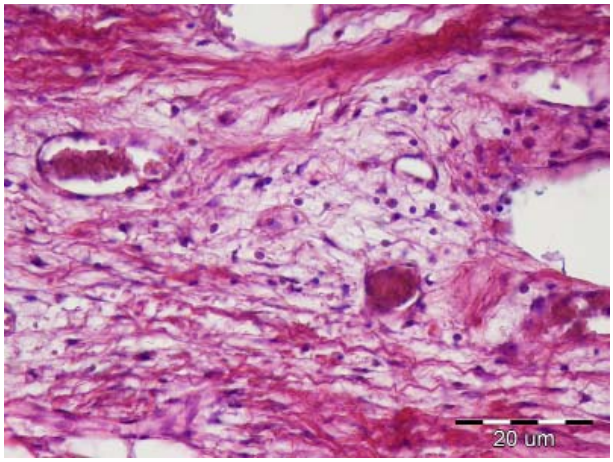

Figure 4. Low density fibrous tissue; experimental group (HE)

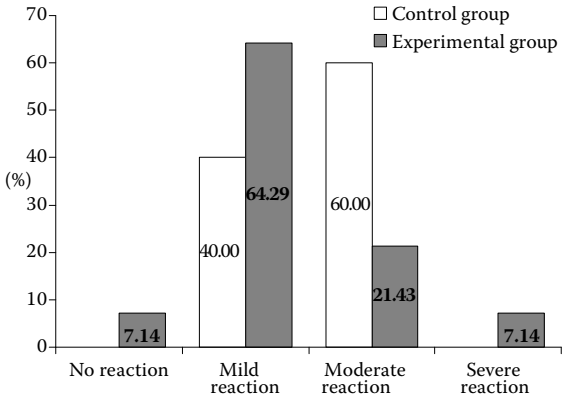

Figure 5. Comparison of fibrosis intensity at the laminectomy level

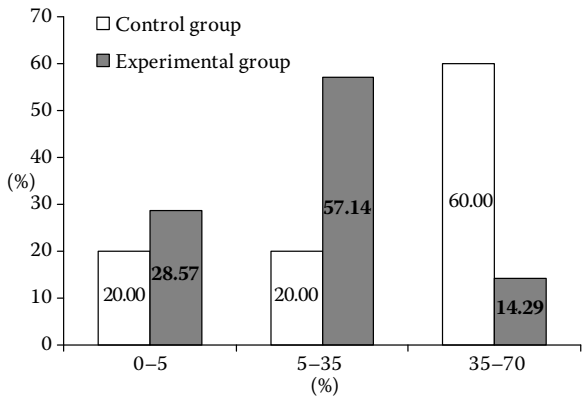

Figure 6. Comparison of adhesion degree percentage between *dura mater* and fibrous tissue

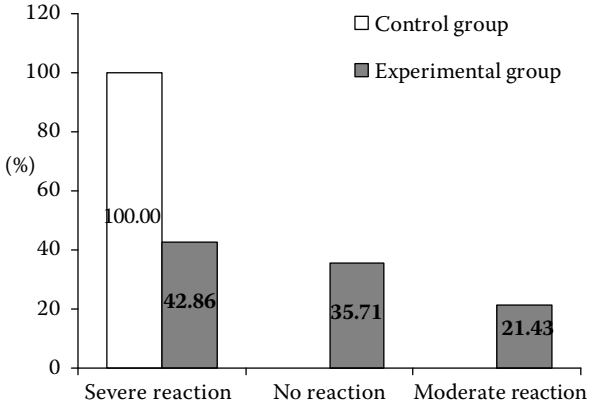

Figure 7. Comparison of the levels of foreign body reactions

mater. In contrast, the experimental sites revealed less pronounced fibrosis and less scar tissue attached to the *dura*. The representative histological images for each group are shown in Figures 1–4.

The correlation coefficients of multiple regression analysis and their significance for all parameters in correlation with the groups are shown in Table 1.

Parameter comparison between the control and experimental group are presented in Figures 5–7.

DISCUSSION

Epidural fibrosis is one of the most common problems associated with spinal surgery. In this condition scar tissue adheres to the *dura mater* or nerve roots are formed in order to repair the local defect of the vertebral lamina created by the laminectomy. However, this scar formation may be compressive and restrict the mobility of the nerve root which often leads to an unfavourable clinical outcome (North et al. 1991; Songer et al. 1995; Ross et al. 1996). Concurrently epidural fibrosis increases the hazards of revision spine surgery and contributes to the occurrence of the "failed back surgery syndrome" (Robertson 1996; Ross et al. 1996; Chan and Peng 2011).

In studies evaluating the prevention of epidural fibrosis, many treatment strategies have been de-

Table 1. Correlation coefficients of multiple regression analysis and their significance for all parameters in correlation with group determination

	Fibrosis intensity at the laminectomy level	Adhesion degree between *dura mater* and fibrous tissue	The level of foreign body reaction
Multiple R	0.207880	0.329502	0.462528
Multiple R^2	0.043214	0.108571	0.213932
Adjusted R^2	–0.013067	0.056134	0.167693
F(13.31)	0.767824	2.070513	4.626631
P	0.393105	0.168329	**0.046154**
s.e.	0.688440	0.724134	0.701140

scribed with variable or limited success in preventing scar formation and reducing epidural adhesion without compromising the wound healing process. These have included the interposition of a physical barrier to limit the migration of fibroblasts from the musculature to the exposed *dura mater* in the early healing phase (Tatsui et al. 2006). For this purpose several biological and synthetic materials have been used. One of the most commonly used materials is a free fat graft (Kanamori et al. 2001). Several authors have noted promising results with its use (Yonk-Hing et al. 1980; Henderson et al. 1993). However, the use of the free fat graft is not without complications, such as seroma formation, infection in the donor area, scar dimpling, limited laminectomy area coverage, and the migration of the graft, which have been implicated as causes of cauda equina syndrome in several cases (Cabezudo et al. 1985; Yamagami et al. 1993; Imran and Halim 2005).

The omental graft is frequently used in neurosurgery due to its high ability to provoke revascularisation and thus allow the underlying and adjacent brain to receive increased blood flow, oxygen, omental neurotransmiters and neurotrophic factors (Alagumuthu et al. 2006). Moreover, pedicled or free omental grafts can be transposed to the lumbar subarachnoid space to resorb cerebrospinal fluid (Goldsmith 1994; Normington et al. 1996).

In the present study, omental free graft was selected as a barrier because of its biocompatibility and easy manipulation. We have chosen the application of a free omental graft because the use of a walking graft is more complicated and it is proven that free omental grafts maintain their volume and properties (Cuice et al. 2003). Reports in the literature (Langenskiold and Kiviluoto 1976; Temel et al. 2006) agree that significant bone regeneration will occur about nine weeks postoperatively in rabbit models. Therefore, this study limited the evaluation period of epidural fibrosis to only six weeks to eliminate the confounding variability in of bone growth.

The effect of the free omental graft was histologicaly evaluated through three parameters. The fibrosis density at the laminectomy level represented the degree of fibroblast proliferation and therefore scar tissue formation. The degree of adhesion between the *dura mater* and fibrous tissue was used to estimate the capability of forming a mechanical barrier between the *dura mater* and underlying tissue. The presence or absence of foreign body response was an indicator of the free omental graft efficacy in preventing inflammatory reactions. It consists in preventing the penetration of inflammatory cells and prostaglandine release, which has a direct effect on fibroblast hyperplasia and scar tissue formation.

In this study a decrease in the fibrosis density at the laminectomy level and in the degree of adhesion between the *dura mater* and fibrous tissue were found. This can be explained by the omentum's haemostatic features, in particular with the property of increasing protrombin activation and the faster conversion of fibrinogen into fibrin (Logmans et al. 1996). This leads to the faster formation of smaller clots, which would consequently become a mold for the epidural fibrotic tissue. We noticed that the level of foreign body reaction was significantly lower with the application of the free omental graft. We assume it was due to the known omental anti-inflammatory properties (Alagumuthu et al. 2006), as well as its positive effect on regeneration and healing (Litbarg et al. 2007).

We have reported that epidural fibrosis can be developed experimentally in the rabbit model after lumbar laminectomy. The results of this study show free omental grafts to be safe and well tolerated in the rabbit model and to significantly reduce foreign body reactions and also reduce both the formation and direct contact of the fibrotic tissue with the underlying *dura mater*.

The goal of laminectomy is to reduce the increased pressure in the spine caused by oedema formation. Free omental graft application at the laminectomy site absorbs blood and oedemal fluid (Goldsmith 2009), thus participating not only in preventing epidural fibrosis but also potentially in the healing of the spinal cord.

In conclusion, our study suggests that free omental grafts are effective anti-scar adhesion materials, which can decrease adhesion tenacity and scar size at the site of epidurals. Our findings also indicate the potential of applying free omental grafts in clinical practice in order to minimise postoperative complications.

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Corresponding Author:

Name: Nika Brkljaca Bottegaro, University of Zagreb, Faculty of Veterinary Medicine, Surgery, Orthopedic and Ophtalmology Clinic, Heinzelova 55, 10 000 Zagreb, Croatia

Tel. +38 598 898 401, E-mail: nikabb@vef.hr