A collagen-based sealant to prevent in vivo reformation of epidural scar adhesions in an adult rat laminectomy model

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Object. The authors investigated the effect of a collagen-based sealant, Gel Amidon Oxydé (GAO), in preventing the reformation of epidural scar adhesions in an adult rat model of laminectomy.

Methods. Thirty-two adult Sprague–Dawley rats underwent a complete L5–6 laminectomy, after which the dura mater was exposed and the left adjacent L-4 and L-5 nerve roots were exposed. The surgical wound was then closed; 1 month later it was reopened. The epidural scar adhesions that developed were observed and carefully removed, leaving clean dura and nerve roots reexposed. In 16 experimental rats, GAO was placed onto the reexposed dura and around the nerve roots before it polymerized. No treatment was performed in 16 control rats. Postoperatively, all rats were healthy and without neurological deficit. The incisions healed within 1 week regardless of the treatment with the GAO. Three months after reoperation, magnetic resonance imaging revealed that important epidural adhesions were present in the control rats but not in the experimental rats. These findings were then confirmed by gross anatomical examination in which a white tissue layer was found over the dura without adhesions in the experimental animals, whereas significant epidural scar adhesions were demonstrated in the controls. Histological evaluation of the laminectomy site also showed that the peridural space in the experimental rats was larger than that in the controls.

Conclusions. The authors found that GAO may be a safe and effective antiscarring adhesion biomaterial in vivo. When placed into the laminectomy site, GAO may prove beneficial in preventing the formation and reformation of epidural scar adhesions in humans.

KEY WORDS • dura mater • laminectomy • membrane • nerve root • epidural scar adhesion

The formation of scar tissue is a necessary process for wound healing after injury or surgical intervention. Extensive epidural scar adhesions that often occur after lumbar spine surgery, however, have been implicated as a factor that contributes to continuing or recurrent radicular and/or low-back pain. These consequences are probably the result of extradural compression or tethering of the dura mater, resulting from the dense and thick scar tissues adherent to the surgically exposed dura mater and adjacent nerve roots. It has been suggested that fibrosis may be the underlying cause in as many as 24% of all failed–back surgery syndrome cases. Some patients who suffered greatly from fibrosis-induced postoperative symptoms have to undergo subsequent surgery to remove the extensive epidural adhesions and release the affected nerve roots. Although extensive epidural scar adhesions can be removed and the tethered nerve roots can be freed at the time of the reoperation, the adhesions will recur after the secondary surgery. In many cases the patient’s symptoms will be worse than before the reoperation. Therefore, it is necessary to develop a therapy that reliably prevents the reformation of the epidural adhesions after the secondary surgery.

For decades, many studies have been designed in which the surgeon places a synthetic or organic material, such as Silastic, synthetic membranes and foams, or free and pedicle fat grafts, into the laminectomy site as a barrier to prevent scar formation. Not one of these materials, however, has been widely accepted with consistent results. In previous studies, we showed that a collagen-based sealant, GAO, could effectively prevent in vivo epidural scar adhesions in adult rats after lumbar laminectomy. In this study, we sought to determine whether GAO can also prevent the reformation of the epidural scar adhesions after removing the developed fibrosis. It was designed to evaluate the effect of GAO on the prevention of epidural scar reformation in an adult rat lumbar laminectomy model. Such an experiment might have relevant clinical application for patients who undergo reoperation for failed–back surgery syndrome resulting from postlaminectomy fibrosis. The results were assessed by clini-
cal observation, MR imaging, gross anatomical examination, and histological analysis.

Materials and Methods

Thirty-two male adult Sprague–Dawley rats, weighing 350 to 400 g, were used in this study. All animals were handled according to French laws concerning animal experiments.

Surgical Procedure and Treatment

The surgical procedure was identical to that used in our previous study. Briefly, anesthesia was induced, and all rats underwent a complete L5–6 laminectomy, after which the underlying dura mater was exposed and the left adjacent L-4 and L-5 nerve roots were freed (Fig. 1). The surgical wound was closed in layers with No. 4-0 nylon sutures, and the rats were returned to their cages. The surgical wound was reopened 1 month after the first operation. The newly formed epidural scar adhesions were observed and carefully removed, leaving clean dura and nerve roots reexposed. Once the laminectomy site was well prepared and hemostasis obtained, the animals were assigned a number (1–32) and then randomly divided into two groups, one receiving and one foregoing GAO treatment.

In the 16 experimental rats, GAO was applied over the reexposed dura and around the nerve roots. Generally, 0.8 ml of GAO was used in each rat, which was an amount that could fill the laminectomy defect. Prior to the closure of the wound, polymerization of the deposited GAO occurred in approximately 2 or 3 minutes.

In the 16 control rats, no treatment was provided after removing the newly formed epidural scar adhesions.

After the surgical procedures and treatment, the wound was reclosed without further irrigation. The rats were then returned to their cages and observed for 3 months. All surgical procedures were performed in sterile conditions and were well tolerated by the animals. In the follow-up period, all observations and evaluations were performed by three independent observers in a double-blinded manner.

Clinical Observation

After surgical intervention and treatment, any evidence of infection or neurological deficits was recorded. Compared with the right nonoperated side, the left hindlimb function was recorded as 0° (complete paralysis), 1° (hindlimb muscles can be contracted but the limb cannot be mobilized), 2° (the hindlimb can be moved horizontally but cannot support the body), 3° (the hindlimb can support the body but with some difficulty), and 4° (normal hindlimb function).

Magnetic Resonance Imaging Evaluation

Magnetic resonance imaging has proven to be the most powerful diagnostic tool in the identification of scar tissue. In this study, MR imaging examinations were performed to evaluate the epidural scar adhesions in both experimental and control rats 3 months after the second surgery. Anesthesia was induced, and MR images were acquired using a 1.5-tesla whole-body MR imaging system (Signa, General Electric, Milwaukee, WI) with standard gradient coils. The anesthetized rats were in supine position so that the spinal segment to be examined was exposed, and the surrounding tissues, the dura, or the nerve roots were classified and described using the following: 0° (none), 1° (minimal), 2° (moderate), and 3° (significant). Blood loss during the dissection was also recorded using the same classification.

Histological Evaluation

Three months after the second surgical intervention, histological analysis was performed. After induction of anesthesia, the remaining rats were killed by an intracardiac perfusion with phosphate-buffered saline (0.1 M; pH 7.2), followed by 4% paraformaldehyde solution. The laminectomy area, including the vertebral column and surrounding tissues, was removed en bloc and then stored in 4% paraformaldehyde solution at 4°C. Each specimen was decalcified in 5% formic acid during a 3-week period and then embedded in paraffin. Two 5-μm longitudinal sections of each bloc segment were obtained from the posterior side of the spinal column by using a microtome to analyze adhesions between the dura of the exposed spinal cord and the surrounding muscles. For each remaining specimen, two complementary 5-μm transverse sections were also obtained (one at the level of the rostral part of the specimen, another at the level of the caudal part) to observe adhesions at the level of adjacent L-4 and L-5 nerve roots. Sections were stained with hematoxylin and eosin and analyzed under a light microscope. We searched for inflammatory cells, fibrosis, necrosis and associated tissue degeneration, residual GAO, reaction of the dura in front of the fibrosis, and epidural scar adhesions, as well as assessed the thickness of scar tissue. Results were classified and graded as the following: 0° (absent), 1° (discrete), 2° (moderate), 3° (marked), and 4° (severe). The distance between the last muscular fibers and the dura (peridural space) was noted and compared between the two groups.

Statistical Analysis

The chi-square test was used to analyze the results of gross anatomical examination. Significant difference was noted when the probability was less than 0.05.

Results

During the second surgery (1 month after the initial surgery), significant epidural scar adhesions were recorded in all rats with a classification of 2 or 3°. Meticulous microsurgical technique was performed to remove these scar tissues and reexpose the dura and nerve roots. Because the scar tissues were strongly adherent to the dura mater in some rats, dural injury was occasionally induced during the dissection. One control rat died immediately after the second surgery, most likely of complications re-
lated to the anesthesia. All the remaining rats were followed for 3 months.

Clinical Observation

After the initial and subsequent surgical interventions, the rats were healthy and ambulatory without evidence of neurological deficit. No superficial infection was found, and all incisions were healed within 1 week regardless of the treatment with GAO. Both the experimental and control rats could normally move their affected left hindlimb to an extent considered a functional classification of 4°.

Magnetic Resonance Imaging Evaluation

In experimental rats, MR images obtained at the laminectomy level revealed obvious interstitial space with hyposignal between the dura and the surrounding muscles (Fig. 2). Adhesions were not observed over the dura. Such an interstitial space was not observed in the control animals, in which the continuity between the surrounding muscles and the dura suggested that important epidural adhesions existed (Fig. 3). The L4–5 nerve roots were not always visible on MR images.

Gross Anatomical Examination

The initial surgical area was reexposed after 3 months following the second surgery and observed under the surgical microscope. The overall results are summarized in Table 1. Significant epidural scar adhesions were recorded in the control animals (Fig. 4A). These adhesions were formed directly between the dura and the surrounding muscles. Because of the dense and thick scar adhesions, reexposure of the dura mater and nerve roots was difficult. Removal of scar tissues may have led to dural injury. Grades of the epidural scar adhesions ranged from 1 to 3° (2.3 ± 0.7° [seven specimens]), as well as adhesions to L-4 and L-5 nerve roots (2.4 ± 0.5° [seven specimens]). During dissection, significant bleeding (Grade 2–3°) was also observed (2.6 ± 0.5° [seven specimens]).

In the experimental rats, no epidural scar adhesion (Grade 0°) was recorded between the dura and surrounding muscles (Fig. 4B). Adhesions to L-4 and L-5 nerve roots were graded from absent to minimal (Grade 0–1°; 0.3 ± 0.8 [six specimens]). Instead of the adhesions seen in control rats, a white tissue layer without evident blood vessels was found over the dura and nerve roots in all GAO-treated rats. No adhesion was recorded between this layer and the dura. The dura mater could be easily separated from surrounding tissues by removing this tissue layer. The dura appeared smooth and transparent, similar to its appearance at the previous operation. No cerebrospinal fluid leakage was noted in any of these animals. During dissection, little bleeding (Grade 0–2°) was seen (Grade 0.8 ± 0.7° [six specimens]).

There were significant differences in epidural scar adhesions (p = 0.004) and bleeding during the dissection (p = 0.042) between control and experimental groups at this time period. Results of statistical analysis are shown in Table 2.

Histological Evaluation

Histological evaluation was performed in seven control and eight experimental rats. After the rats were killed, four histological sections (two longitudinal and two transverse) were made in each rat. Only some of the sections contained the surgical zone. Because of technical difficulty and absence of intergroup homogeneity of section level, quantitation of epidural scar adhesions on histological sections was not considered accurate. We observed no inflammatory reaction at the laminectomy site either in experimental or in control rats. No GAO deposits were found in experimental animals. A larger peridural space was observed in experimental rats than in controls (Fig. 5). The protective layer that was observed by gross anatomical examination in GAO-treated rats appeared as a...
clear separating area between the dura and the surrounding muscles on histological sections.

**Discussion**

In this study, we investigated the preventative effect of GAO reformation of epidural scar adhesions in an adult rat laminectomy model. Based on the results we found that GAO inhibits the reformation of epidural scar adhesions compared with laminectomy sites not treated with GAO. This qualitative difference was particularly obvious on gross anatomical examination and by MR imaging of the laminectomy sites. As in our previous study, treatment with GAO did not affect the healing of skin, subcutaneous tissue, and muscle, but primarily prevented adhesion formation adjacent to the dura mater and the laminectomy site.

It has been widely recognized that the formation of dense and thick epidural scar following lumbar laminectomy and discectomy may significantly contribute to unfavorable clinical outcome and recurring symptoms. Numerous factors are involved in the pathogenesis of postoperative epidural scar adhesions. According to results of histological studies, epidural fat destruction, hematoma, and spine erector muscular fiber invasion seem to be the main factors leading to the formation of epidural scar tissues. Because the formation of such scar tissues cannot be prevented by administration of topical and systemic medications, surgical lysis in the past was required for the patients who experienced grave post-laminectomy symptoms resulting from the dense and thick epidural scar adhesions. Such surgical treatment, however, was unsuccessful because only the newly formed scar tissues were removed without eliminating the causative factors, and scar tissue reformed. For these reasons, a reliable material with the ability to eliminate or decrease these effects is desirable. We have shown that GAO may achieve this goal by effectively preventing epidural scar adhesions.

Regardless of the exact mechanisms of epidural scar adhesion, preventing or limiting fibroblast migration into the laminectomy defect seems to be a key feature affecting scar extension and adhesion. Gel Amidon Oxyd is a collagen gel that undergoes bioresorption. Applied in fluid form, it solidifies within 1 or 2 minutes, creating a physical barrier between tissues until it is degraded. This modification of the product property is related to the formation of chemical bonds between the two components—collagen and oxidized starch—thus creating internal crosslinks (polymerization). In an earlier study we showed that the fluid form of GAO can fill out the laminectomy defect and be spread over the dura and nerve roots easily and thoroughly. After polymerization, a reliable barrier is built up against the scar tissues facing the exposed dura. Such temporal GAO-created replacement of the destroyed epidural fat can also effectively prevent hematoma in the laminectomy defect, thereby reducing the potential factors contributing to the formation of epidural scar adhesions. The hemostatic effect is likely a consequence of GAO filling the entire laminectomy cavity and providing a gentle pressure to the

**TABLE 1**

<table>
<thead>
<tr>
<th>Graded Factor</th>
<th>Control</th>
<th>GAO-Treated</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>bleeding†</td>
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<td>2+</td>
</tr>
<tr>
<td>epidural scar adhesions‡</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td>adhesions to L4–5 nerve roots</td>
<td>3+</td>
<td>1+</td>
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</table>

* The plus sign (†) indicates that the recorded value is superior to one classification but still inferior to the next—that is, 1 < 1+ < 2.
† Bleeding during the dissection: 0 (none), 1 (minimal), 2 (moderate), and 3 (significant).
‡ Epidural scar adhesions between the dura and the surrounding muscles: 0 (none), 1+ (minimal), 2+ (moderate), and 3+ (significant).

**Fig. 4.** Photographs of gross anatomical examination showing strong epidural scar adhesions (arrows) in control rats (A) and no evidence in experimental rats (B). The reexposed dura (star) is indicated. A white tissue layer (arrowheads) covering the dura is visible in experimental rats. There is no evidence of adhesions between this layer and the dura.
surrounding injured tissues, by which the bleeding is stopped and the effusion of tissue fluid is limited. We have shown that these mechanisms are also effective on recurrent lesions. We addressed this by delivering GAO into the laminectomy defect and around the released nerve roots after the removal of the laminectomy-induced epidural scar adhesions. Analysis of the results showed that GAO can also form a physical barrier that effectively inhibits the reformation of epidural scar adhesions without side effects. In place of scar adhesion, GAO formed a thin white, nearly transparent connective tissue layer covering the dura and nerve roots in the laminectomy site. This connective layer, which progressively replaced the physical barrier formed by the bioresorbable GAO, persisted and prevented fibroblasts from making contact with the exposed dura and nerve roots. No adhesions were found in between this layer and the dura. This noncellular protective layer did not develop, further allowing reexposure of the dura mater without causing compression and tethering. Because GAO could be properly placed in the area that needed protection, healing of adjacent tissues was not affected.

**Conclusions**

This study demonstrated that GAO may be a safe and effective anti-scar adhesion biomaterial that can be applied in vivo without causing medically significant adverse effects. Because the 1st postoperative month is the decisive period in which the extent of scar formation is determined, the results obtained 3 months postoperatively suggest that GAO may prove beneficial in preventing the reformation of epidural scar adhesions in humans.

**Acknowledgments**

We are grateful to Dr. Philippe Gravagna (Imedex Biomateriaux) for kindly providing the GAO, Mr. Dominique Glutron (MR technologist, CIERM, Hospital of Bicêtre) for technical assistance, and Dr. Jean Michel Heard (Institut Pasteur, Paris, France) for critical review of the manuscript.

**References**


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**TABLE 2**

Results of the chi-square test

<table>
<thead>
<tr>
<th>Group</th>
<th>Bleeding</th>
<th>Scar Adhesions to L-4 &amp; L-5 Nerve Roots</th>
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</thead>
<tbody>
<tr>
<td>7 control rats</td>
<td>2.6</td>
<td>2.3</td>
</tr>
<tr>
<td>6 GAO-treated rats</td>
<td>0.8</td>
<td>0.3</td>
</tr>
<tr>
<td>p value</td>
<td>0.042</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Significant at p < 0.05.

**Fig. 5.** Photomicrographs showing a peridural space (arrows) between the operated nerve root (NR) and the surrounding muscles (M) in an GAO-treated rat (A) and a continuity (arrows) between the nerve root and the surrounding muscles in a control rat (B). H & E, original magnification × 10.

Manuscript received October 16, 2000. Accepted in final form February 19, 2002.
The work in this study was supported by a contract grant (no. 98E04) from Imedex, Biomateriaux (Trevoux, France).
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