Reduction in Postlaminectomy Epidural Adhesions in Sheep Using a Fibrin Sealant-Based Medicated Adhesion Barrier

Peter J. Richards,1,2 A. Simon Turner,3 Serge M. Gisler,1 Susan Kraft,3 Katja Nuss,4 Silke Mark,1,2 Howard B. Seim III,3 Jason Schense1

1 Kuros Biosurgery, Zürich, Switzerland
2 Centre for Applied Biotechnology and Molecular Medicine, Zürich University, Switzerland
3 Colorado State University, Fort Collins, Colorado
4 Musculoskeletal Research Unit, Zürich University, Switzerland

Received 22 May 2009; accepted 19 August 2009
Published online 19 November 2009 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.31533

Abstract: Epidural adhesion formation is believed to be a central governing factor in the prevalence of pain after spinal surgery and is regarded as being the primary instigator of neural tethering, leading to complications during revision surgery. In this study, we assess the effectiveness and safety of fibrin sealant supplemented with tributyrin, termed Medicated Adhesion Barrier (MAB), as an alternative means of reducing the incidence of posterior spinal epidural adhesion formation. Laminectomy defects in sheep were treated with MAB, fibrin sealant alone, ADCON1 Gel, or remained untreated. At 12 weeks postoperatively, the extent of fibrosis and epidural adhesion formation was evaluated using magnetic resonance imaging (MRI), peel-off testing, and histological examination. Initial in vitro analysis revealed that tributyrin was retained in fibrin gel in a time-dependent manner and was an effective inhibitor of fibroblast proliferation. Treatment of sheep with MAB significantly reduced both the prevalence (p < 0.05) and tenacity (p < 0.05) of epidural adhesions. The effectiveness of MAB in preventing epidural adhesions was found to be comparable with that of ADCON1 Gel. No adverse events were reported after the use of MAB. The MAB preparation seems to be an effective resorbable barrier for the prevention of epidural adhesions. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 92B: 439–446, 2010

Keywords: laminectomy; epidural adhesion; fibrin sealant; tributyrin; sheep

INTRODUCTION

Epidural adhesion formation is now widely accepted as being a major contributing factor to the onset of postsurgery radicular pain and lower extremity weakness after laminectomy1–7 and is believed to be directly responsible for the increased complication rates associated with spinal reintervention.8–12 Although the exact etiology of epidural adhesions has not been defined, it is apparent that several key events are intrinsic to the formation and development of postoperative fibrosis and subsequent epidural scar adhesion.13–15 A general feature seems to be the requirement of a direct contact between the exposed dura and invading fibroblasts, thereby allowing for the generation of localized dense fibrotic tissue and tethering of the thecal sac and nerve roots. With this in mind, numerous experimental and clinical studies have set out to prevent epidural adhesion formation by means of prophylactic intervention. Such treatment regimes have included modified surgical approaches,16,17 antiinflammatory agents,18,19 antibiotics,20,21 and a wide variety of biological and synthetic barriers including fat grafts,9,22 hyaluronan,23,24 collagen,9,25 gelatin foam,26 polylactide films,27,28 ADCON-L,8,29 and more recently, Oxiplex8/SP Gel3,30 There is also increasing evidence to suggest that fibrin sealant may also have antiadhesive properties.

The clinical use of fibrin sealant in neurosurgical procedures has been well documented. It has been extensively used for treatment of dural tears in craniotomy procedures and has been shown to be both effective and safe.31–33 Experimental studies suggest that fibrin sealant may also be useful in reducing the prevalence of epidural adhesions, at least within the time period directly after laminectomy surgery.34 This is further supported by the finding that Tissu-
colTM (Tisseel™) fibrin sealant not only allowed for effective dural sealing but also reduced scar formation at the surgery site in a porcine laminectomy model. Although these findings are encouraging, fibrin sealant alone is not sufficient to constitute an effective means of preventing long-term epidural adhesion formation and combination with other potential antiadhesive agents may be required.

Tributyrin is a prodrug of butyric acid and is primarily used as a flavoring agent and adjuvant in food products. It is also well regarded as being a potent pharmacological agent, inducing both growth inhibition and apoptosis in various tumor cell-lines in vitro. Furthermore, early clinical studies have shown tributyrin to be a safe and well-tolerated alternative to butyric acid in the treatment of patients with solid tumors. In this study, we exploited the antiproliferative effects of tributyrin to further enhance the adhesion barrier properties of fibrin sealant, thereby limiting the possibility of dural contact and subsequent adhesion formation.

MATERIALS AND METHODS

Test Material
The primary test material used in this study consisted of tributyrin in combination with Tissucol™ fibrin sealant, termed medicated adhesion barrier (MAB). Preparation of MAB consisted of first reconstituting the various constituents supplied with the TISSUCOL-KIT 2.0 as described in the manufacturer’s methods (Baxter, Vienna). Briefly, after preincubation at 37°C, lyophilized human-derived fibrinogen was dissolved in bovine aprotinin solution and transferred to a 2 mL syringe. The lyophilized human-derived thrombin component (500 IU/mL) was then reconstituted using the supplied calcium chloride solution and further diluted to a final concentration of 50 IU/mL using a solution consisting of tributyrin (0.6%) and sodium chloride. The final mixture was then repeatedly homogenized using a 23-G needle and finally aspirated into a 2 mL syringe. Both the fibrinogen and thrombin syringes were assembled into the Duploject system (Baxter, Vienna) according to the manufacturer’s instructions and applied directly to the laminectomy defect site using the applicator supplied.

Cell Proliferation Assay
The antiproliferative effect of tributyrin on mammalian cells in vitro was investigated using BJ human foreskin fibroblasts (ATCC, UK). Cells were grown in Dulbecco’s Modified Eagle Medium (DMEM) (Invitrogen) supplemented with 10% FCS, penicillin/streptomycin and l-glutamine and seeded at 5 × 10^3 cells/well in triplicates in 96-well culture plates and allowed to adhere for 16 h at 37°C in 5% CO₂. Fresh culture medium containing various concentrations of tributyrin was then added and cells incubated for a further 72 h. Cells were then washed and proliferation examined using Cell Proliferation Reagent WST-1 (Roche) according to the manufacturer’s instructions.

Release Assay
The release of tributyrin from the fibrin matrix was analyzed using a C¹⁴-labelled tributyrin tracer (American Radiolabeled Chemicals). A solution of calcium chloride containing thrombin (50 IU/mL), unlabelled tributyrin (2%), and C¹⁴-labelled tributyrin (0.5 µCi/mL) was homogenized using a 23-G needle and equal volumes added to fibrinogen in triplicate wells of a 48-well culture plate and left at room temperature for 2 h. After polymerization, 500 µL Hepes-based Ringer Solution (HRS) was added to the gels and plates incubated for up to 6 days at 37°C with regular changes of HRS. After tryptic digestion of the denuded fibrin gel, the remaining radioactivity within the digested gel and accumulated radioactivity in the HRS supernatants were determined using a Liquid Scintillation Counter equipped with QuantaSmart software (Packard).

Surgical Procedure
All animal research procedures performed in this study were approved by the Colorado State University Institutional Animal Care and Use Committee. A two-level laminectomy was carried out in skeletally mature female sheep (N = 27) as described previously. Briefly, after induction of general anesthesia, a 20 cm skin incision was made and the paraspinal muscles dissected off the spinous processes and laminae using electrocautery. The dorsal spinous processes were removed from T13 and L2 by drilling through the base, and later these structures were removed with rongeurs. Dorsal laminectomies of 3 cm × 1 cm extended between adjacent intercavitate spaces cranially and caudally and just medial to the articular facet joints bilaterally using a compressed air drill and burr. The wound was lavaged with normal physiological saline, and the articular facet joints were left intact. The test material was applied over the exposed dura site (Figure 1), and a waiting time of 3–5 minutes was allowed before closing the wound. The dense dorsal spinous fascia was reaposed using absorbable suture material, in a simple continuous pattern, whereas the subcutaneous tissue was closed with 2/0 absorbable suture material in a simple continuous pattern and skin with 2/0 monofilament nonabsorbable suture. Animals were euthanized after 12 weeks, and those treated with test material were directly compared with control groups consisting of untreated, fibrin sealant alone, or ADCON®-Gel (Wright Medical Technology Inc.) -treated sheep.

Magnetic Resonance Imaging
Imaging was carried out immediately after euthanasia of each animal by sagittal and transverse spin echo T1 and fast spin echo T2 using a 1.5 General Electric Medical Systems Signa HiSpeed LX MR instrument (GE Medical Systems Signa HiSpeed LX MR instrument (GE Medical...
The spine was imaged from T11 to L4 allowing visualization for one full vertebra cranial and caudal to the laminectomy site. The scoring system was developed using a modified version of a previous score system. Briefly, the percentage of dorsal epidural fibrosis was estimated per laminectomy site using two slices per defect. The spinal canal was divided into four equal quadrants, and the amount of epidural space filled by hypointense material (granulation tissue) was visually estimated.

Postmortem Gross Observations
The relative tenacity of spinal cord to adhere to the overlying fibrous tissue in the cranial laminectomy defects was evaluated semiquantitatively using a scoring system as described previously. Spines were removed en bloc and epaxial musculature dissected from the lateral and ventral aspects of the vertebrae. The transverse processes were removed and the vertebral bodies incised with a band saw along the length of the column just ventrally to the spinal canal, leaving a thin edge of bone covering the ventral spinal cord. The remaining bone was removed with bone rongeurs to expose the spinal cord within the spinal canal. The spinal cord was slowly elevated and dissected from the spinal canal in a ventral direction and adhesion of the dorsal spinal cord at the laminectomy sites evaluated by the surgeon blinded to the treatment. Tenacity of scarring was assigned a grade: 0, no adhesions; 1, thin membranous threads, easily detachable; 2, slight adhesion, requiring only minimal blunt dissection; 3, moderate adhesions requiring some sharp dissection; and 4, severe adhesions requiring extensive sharp dissection.

Histological Analysis
The caudal laminectomy defect sites were removed en bloc and fixed in 10% neutral buffered formalin. Histological sections were cut at several regions throughout the laminectomy defect site, stained with Hematoxylin and Eosin (H&E) and visualized using Image Pro Plus imaging system (Media Cybernetics, Silver Spring, MD). To assess the prevalence and severity of epidural adhesions, a blinded scoring system was developed using a modified version of a previously described method. Histological sections were assigned a grade: 0, no adhesions; 1, partial, loose adhesions only (<25% of dura affected); 2, moderate, loosely adhered (25–50% of dura affected); 3, moderate to severe, more dense adhesions (>50% but <100% of dura affected); and 4, severe, dense adhesions (100% of dura affected). In addition, quantitative histomorphometry was also used to measure the length of dura in contact with the overlying fibrous tissue (Leica IM1000 image Manager). The proportion of adhered dura was expressed as a percentage of the total dura length exposed within the laminectomy defect. A minimum of two sections per defect were evaluated, and only sections considered to be localized to the laminectomy defect site were included.

Statistical Analysis
All statistical analyses were carried out using SPSS16.0 software. Nonparametric analysis was carried out using the Kruskal-Wallis test and nondirectional Mann–Whitney U test. The Wilcoxon’s Signed Rank test was used to evaluate differences between groups of paired data. A p value <0.05 was considered statistically significant. Values are expressed as the mean ± standard error of the mean (SEM).

RESULTS

Antiproliferation
Tributyrin effectively reduced the proliferation of human fibroblasts at all concentrations tested after an incubation period of 72 h (Figure 2). The effects of tributyrin were shown to be dose dependent with the exception of tributyrin concentrations ranging from 66 to 265 μM where no significant differences in the percentage reduction of cell proliferation were observed. Cell proliferation was almost completely inhibited by tributyrin at the highest concentration tested (17 mM) where a 98% (±1.0) reduction was observed.

Retention of Tributyrin
The retention profile of tributyrin within fibrin matrix over a period of 6 days as determined using a C14-tributyrin tracer is shown in Figure 3. The MAB system comprising tributyrin within fibrin displayed delayed-release characteristics as evidenced by the fact that 42% (±2.45) of the C14-tributyrin tracer was still present within the fibrin gel after 24 h. Levels of C14-tributyrin within the gel continued to decline
throughout the study with 10.7 % (±1.26) and 3% (±0.43) C\textsuperscript{14}-tributyrin remaining after 2 and 3 days of incubation, respectively. By day 4, only 1.2% (±0.17) tributyrin remained in the fibrin gel, and by days 5 and 6, radioactivity levels remaining within the gel were less than 1%.

**Surgery**

All animals demonstrated good ambulatory function and remained healthy and active after application of the test item through to completion of the study. Macroscopic analysis of the laminectomy site 1 day after surgery in MAB-treated sheep revealed the fibrin to be well situated between the neural tissue and overlying hematoma and occupied the entire epidural space directly adjacent to the laminectomy defect (Figure 4).

**Radiography Findings**

Magnetic resonance imaging (MRI) analysis of both laminectomy levels was completed in all sheep and revealed noticeable fibrotic scarring at both the cranial and caudal surgical sites in the majority of animals (Table I). Overall, no significant differences in fibrosis were observed in the laminectomy defects between any of treatment groups. In addition, no significant differences in percentage fibrosis were observed between caudal and cranial defects within each treatment group.

**Observations at Gross Dissection**

Gross pathology revealed no unusual lesions in any of the laminectomy sites, suggesting all treatments were well tolerated locally. Peel-off scores were assigned to the spinal cords within each cranial laminectomy defect site to determine the level of epidural adhesion between the dura and overlying fibrotic tissue. In each case, the spinal cord was carefully elevated and graded in terms of how much dissection was required for its complete removal. High tenacity scores were consistently recorded in animals receiving no treatment (3.125 ± 0.4) or fibrin alone (3.33 ± 0.33) (Table II). Fibrin alone proved to be insufficient in reducing adhesion formation, when compared with untreated animals as demonstrated by the high tenacity score. Treatment with MAB resulted in a significant reduction in the severity

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Caudal Score (%)</th>
<th>Cranial Score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ($N = 6$)</td>
<td>85.83 ± 5.8</td>
<td>89.17 ± 6.88</td>
</tr>
<tr>
<td>Fibrin alone ($N = 5$)</td>
<td>91 ± 1.87</td>
<td>93.5 ± 3.67</td>
</tr>
<tr>
<td>MAB ($N = 5$)</td>
<td>92.5 ± 2.62</td>
<td>88.5 ± 4.3</td>
</tr>
<tr>
<td>ADCON\textsuperscript{G} Gel ($N = 6$)</td>
<td>85.83 ± 2.62</td>
<td>92.5 ± 3.53</td>
</tr>
</tbody>
</table>

\textsuperscript{a}p values between independent groups determined by Kruskal–Wallis test.

\textsuperscript{b}p values between dependent groups determined by Wilcoxon’s signed ranks test.
of adhesions compared with fibrin alone ($p = 0.037$). A marked reduction in adhesion tenacity was also observed in animals treated with ADCON$^R$ Gel, although significance was not attained ($p = 0.3$).

**Histology**

Transverse paraffin wax sections were prepared from various positions along the caudal laminectomy defect site and were assessed using a blinded scoring system. High adhesion scores were consistently recorded in untreated animals (3.47 ± 0.3) or animals treated with fibrin alone (3.25 ± 0.36) (Table II). There was a significant decrease in adhesion scores in animals treated with MAB (1.87 ± 0.46), when compared with both fibrin alone ($p = 0.03$) and untreated control animals ($p = 0.016$). Animals treated with ADCON$^R$ Gel also demonstrated a significant reduction in adhesion scores, when compared with untreated animals (2.34 ± 0.41; $p = 0.019$). Further analysis using quantitative histomorphometry revealed that both MAB and ADCON$^R$ Gel significantly reduced the prevalence of attached dura to overlying fibrous tissue by 47.5% ($p = 0.02$) and 47% ($p = 0.006$), respectively (Figure 5). A representative micrograph for the untreated control laminectomy site is shown in Figure 6. Total adhesion of the exposed dura to the overlying fibrous scar tissue is evident within the laminectomy defect. A representative micrograph of a defect treated with MAB is shown in Figure 7. The dura remains relatively free and unattached with only minor adhesive threads observed.

![Figure 5](https://example.com/figure5.png)  
*Figure 5. Histomorphometry analysis of epidural tethering to overlying fibrous tissue. A significant reduction in the prevalence of epidural adhesions was observed in both MAB- ($p = 0.02$) and ADCON$^R$ Gel- ($p = 0.006$) treated animals.*

![Figure 6](https://example.com/figure6.png)  
*Figure 6. Photomicrograph of a decalcified transverse histological section cut through an untreated control laminectomy site. Complete adherence of the dura to the overlying fibrous scar tissue is apparent (arrow). Histological score = 4. H & E stain (original magnification ×10). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]*

![Figure 7](https://example.com/figure7.png)  
*Figure 7. Photomicrograph of a decalcified transverse histological section cut through a laminectomy site treated with MAB. The dura remains free from any adhesions to the overlying fibrous scar tissue (arrow). Histological score = 1. H & E stain (original magnification ×10). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]*

**Table 2. Prevalence of Adhesions**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Tenacity Score$^a$ (Cranial)</th>
<th>Adhesion Score$^a$ (Caudal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated ($N = 8$)</td>
<td>3.125 ± 0.4</td>
<td>3.47 ± 0.3</td>
</tr>
<tr>
<td>Fibrin alone ($N = 6$)</td>
<td>3.33 ± 0.33</td>
<td>3.25 ± 0.36</td>
</tr>
<tr>
<td>MAB ($N = 7$)</td>
<td>2.14 ± 0.4$^b$</td>
<td>1.87 ± 0.46$^c$</td>
</tr>
<tr>
<td>ADCON$^R$ Gel ($N = 6$)</td>
<td>2.83 ± 0.31</td>
<td>2.34 ± 0.41$^c$</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM.

$^a$ $p$ values between independent groups determined by Mann–Whitney $U$ test.

$^b$ $p < 0.05$ when compared with fibrin alone.

$^c$ $p < 0.05$ when compared with untreated.
DISCUSSION

In this study, we demonstrate that fibrin sealant can be successfully used as an adhesion barrier after incorporation of tributyrin. This device, termed Medicated Adhesion Barrier (MAB), proved to be both safe and effective in reducing the prevalence of postoperative epidural adhesions in a sheep laminectomy model. Furthermore, MAB proved equally effective as ADCON-10 Gel in reducing histological adhesion scores and also had the added advantage of maintaining low tenacity scores, a property considered to be invaluable in reducing complications associated with surgical reintervention.42

There is now a growing body of evidence implicating epidural adhesion formation as a causative agent for postoperative pain after lumbar spinal surgery.1-7 Although the principal mechanism responsible for initiation of fibrosis and adhesion formation after laminectomy remains to be determined, various suggestions have been put forward including replacement of epidural fat by hematoma and invasion of the defect site by proliferating fibroblasts from the surrounding musculature.14 Therefore, the majority of antiadhesion studies have primarily focused on limiting the contact between the exposed dura and invading fibroblasts through the use of various barriers.3,9,22-30 However, the potential risks associated with some of these treatments and their inability to consistently reduce the incidence of postoperative epidural adhesions led us to investigate a safer and more reliable method of adhesion prevention using fibrin sealant.

Preliminary studies have demonstrated that fibrin sealant is effective in preventing early postlaminectomy scar formation in an experimental laminectomy model, although these effects were not evident at later stages of wound healing where epidural adhesion formation was comparable with untreated animals.34 Indeed, our study confirmed that use of fibrin sealant alone is ineffective in preventing long-term epidural adhesion formation. Therefore, in an attempt to further enhance the antiadhesive effects of fibrin sealant, we supplemented the fibrin gel with the butyric acid prodrug tributyrin.

Butyrate and butyric acid derivatives are well known to induce differentiation and apoptosis of neoplastic cells37,38,43 and have therefore gained acceptance as potential anticancer agents.39,40,44-46 In this study, we have demonstrated that tributyrin also has the capacity to downregulate the cellular activity of human neonatal fibroblasts. The incorporation of tributyrin within a fibrin gel provided for a delayed-release system thus allowing not only for the partial retention of tributyrin at the treatment site but also sufficient accessibility within the first days after administration. Therefore, the potential for tributyrin to actively prevent or impede the proliferation of invading fibroblasts was envisaged as being an acceptable means with which to enhance the antiadhesive barrier effects of the fibrin sealant. Indeed, incorporation of tributyrin into the fibrin matrix resulted in a significant decrease in the prevalence and tenacity of epidural adhesions, when compared with fibrin alone although levels of fibrotic scar tissue within the defect were not significantly altered. Similar discrepancies have previously been reported in clinical studies investigating the antiadhesive effects of a new synthetic gel, Oxiplus®/SP Gel, where MR images revealed no significant differences between treated and untreated patients although a clear reduction in postoperative pain score was evident after administration of the gel.3,47

In addition to preventing epidural scar formation, several other criteria need to be considered when evaluating the suitability of antiadhesive agents for use in spinal surgery. Compromised healing of dural tears and spontaneous CSF leaks are considered to be major concerns as indicated by the complications associated with ADCON-L®, where persistent CSF leakage was reported in several patients due to delayed healing of dural tears.48 The dural sealant ability of future generations of antiadhesive devices may therefore play a more central role in terms of evaluating product performance. This is already apparent as demonstrated by recent experimental laminectomy studies investigating the antiadhesive properties of both dural grafts and sealants.49,50 The previously reported dural sealant properties of fibrin gel during invasive spinal and cranial surgery31,33 may therefore afford MAB with the ability to both prevent adhesions and also repair damaged dura. Further studies are required to fully evaluate the sealing performance of MAB in the presence of dural tears.

In conclusion, a fibrin sealant containing the butyric acid prodrug tributyrin significantly reduces epidural adhesions histologically and adhesion tenacity as determined by peel off tests in a sheep model. Administration of MAB to laminectomy sites may therefore allow for a more satisfactory outcome after surgical intervention.

The authors thank Dr. Amy Lyons and Dr. Mike Karr for generating the histological sections used in this study.

REFERENCES


Journal of Biomedical Materials Research Part B: Applied Biomaterials


