Mitomycin C in preventing spinal epidural fibrosis in a laminectomy model in rats

JIN-YUL LEE, M.D., WERNER STENZEL, M.D., HEINRICH EBEL, M.D.,
CHRISTOPH WEDEKIND, M.D., RALF-INGO ERNESTUS, M.D., AND NORFRID KLUG, M.D.

Department of Neurosurgery and Institute of Neuropathology, University of Cologne, Germany

**Object.** Extensive epidural fibrosis after lumbar surgery may be the underlying cause in most cases of failed–back surgery syndrome. Various materials have been used to prevent epidural fibrosis, but only moderate success has been shown.

Mitomycin C, an alkylosing antibiotic substance isolated from *Streptomyces caesipes*, potentially suppresses fibroblast proliferation after surgery. In this study, the authors investigated the effect of mitomycin C by local application on spinal epidural fibrosis in a rat laminectomy model.

**Methods.** Five Wistar rats underwent laminectomy at cervical, thoracic, and lumbar levels. Based on data obtained from ophthalmological studies, mitomycin C was applied to the laminectomy sites in various concentrations (0.01, 0.05, and 0.1 mg/ml). One laminectomy site in each rat was left untreated and thus served as a control. Evoked potentials were measured pre- and postoperatively, and all rats underwent clinical evaluation. Mobility status and evidence of neurological deficit were recorded. Twelve weeks later, the rats were killed, and the spinal column, including surrounding muscle tissue, was removed en bloc, decalcified, and fixed in formaldehyde. Epidural fibrosis was evaluated histologically.

In all mitomycin C-treated laminectomy sites, epidural scarring was significantly reduced compared with control sites. Remarkably, dural adhesions were absent in laminectomy defects treated with mitomycin C concentrations of 0.05 and 0.1 mg/ml. Moderate to marked epidural fibrosis with adhesion to the dura mater was noted at sites receiving 0.01 mg/ml of mitomycin C. All control sites showed dense epidural fibrosis with marked dura adherence.

**Conclusions.** In this experimental model, mitomycin C applied locally at a concentration of 0.1 mg/ml effectively reduced epidural fibrosis, completely avoided dural adherence, and induced no side effects.

**Key Words** • epidural fibrosis • laminectomy • mitomycin C • failed–back surgery syndrome

**Materials and Methods**

**Animal Model and Surgical Technique**

Five male Wistar rats, each weighing 400 to 450 g, were used in this study. In each, three laminectomies were performed at cervical, thoracic, and lumbar levels. Anesthesia was induced by intraperitoneal application of Nembutal (7.5 mg/kg body weight). Using an aseptic technique and an operating microscope, the dorsal skin and fascia were incised in the midline. After separation of the paraspinal muscles, the spinous processes were removed using a rongeur. A rectangular 5 × 2-mm laminectomy defect was created using a high-speed drill, leaving the dura clean and fully exposed. After hemostasis, mitomycin C in various concentrations of 0.01 (three sites), 0.05 (four sites), and 0.1 mg/ml (four sites) was applied for 5 minutes to the laminectomy defects with a piece of cotton wool. Afterward, the mitomycin C–soaked cotton wool was removed and the treatment of tracheal cicatrix after tracheal reconstruction.16,25,26,34

The present study was designed to evaluate the effect of mitomycin C on spinal epidural fibrosis in a rat laminectomy model. Additionally, it was our goal to determine its optimal concentration and its side effects in this model.
Mitomycin C to prevent epidural fibrosis

The laminectomy sites were irrigated immediately with saline solution to eliminate the surplus mitomycin C. The wound was closed in layers. One control laminectomy site in four rats was left untreated.

Preoperative and postoperative somatosensory and motor evoked potentials were measured and clinical evaluation was performed. Mobility status and evidence of neurological dysfunction were recorded.

Histopathological Evaluation

Histopathological evaluation was performed 12 weeks later. After the rats were killed by an overdose of pentobarbital, they underwent intracardial perfusion with phosphate-buffered saline (0.1 M pH 7.2) followed by 4% paraformaldehyde solution. The spinal column, including surrounding muscle tissue, was removed en bloc. After 4 days of decalcification, formalin-fixed paraffin-embedded sections were histopathologically analyzed. Each bloc was completely cut in 10-μm sections for optimal visualization of the laminectomy site. These sections were stained with H & E and elastica van Gieson. All specimens were histologically evaluated by a neuropathologist who was blinded to the treatment. Each specimen was examined for density and extent of the fibrosis with vascularity, inflammatory reaction, and the presence of dural adhesions. Based on the data reported by He, et al.,12 and Hinton, et al.,13 the sections were subjectively classified with regard to the extent of fibrosis and density along the dura mater: “light” scarring indicated loosely arranged fibrous tissue without dura adhesion; “moderate,” loose to dense connective tissue occupying less than two thirds of the laminectomy defect without or with partially dural adhesion; and “marked,” dense fibrous tissue completely filling out the laminectomy defect and firmly adhering to the dura.

Results

In all animals the postoperative course was uneventful. There was no case of neurological deficit, wound infection, or disturbance of wound healing. Somatosensory and motor evoked potentials were unremarkable pre- and postoperatively.

Histological analysis showed no arachnoidal adhesions in any case, but a significant difference was found between the treated and control defects in terms of the extent of postoperative epidural fibrosis and dural adhesion. In all laminectomy sites treated with 0.1 and 0.05 mg/ml of mitomycin C, dural adhesions were absent. The dura mater was found to be slightly thickened and separated from the epidural fibrous tissue through a thin, empty layer. The light, loosely arranged epidural scar tissue was minimally neovascularized in specimens treated with 0.1 mg/ml of mitomycin C. In each case in which 0.05 mg/ml of mitomycin C was applied, the epidural fibrous tissue was classified as moderate, especially laterally near the osseous structures bilaterally. Moderate to marked epidural fibrosis with widespread adhesions to the dura was noted in all cases of 0.01 mg/ml mitomycin C treatment. In no case did treatment with mitomycin C produce a specific inflammatory reaction of the dura mater or adjacent tissue. Furthermore, spinal neural tissue was unremarkable. All control sites showed dense epidural fibrosis with rich neovascularization, signs of chronic inflammation, and marked dural adherence to a various extent (Fig. 1).

Discussion

It has been widely recognized that the extensive epidural scar adhesions that develop after lumbar laminectomy and discectomy and involve nerve root entrapment, dural compression, and restriction of nerve root mobility may significantly contribute to unfavorable clinical outcome and recurring symptoms such as radicular and/or low-back pain.1,5,12,15,27,28,31,32 Although extensive epidural scar adhesions can be removed and the tethered nerve roots can be released through repeated operation, adhesions tend to recur after the secondary surgery, and the patient’s symptoms may even become worse than before the repeated operation.47,22,27

Based on the theory from LaRocca and Macnab,18 epidural fibrosis is caused by damaged posterior spinal muscles. Numerous biological and nonbiological materials have been implanted epidurally as a barrier between the exposed dura mater and surrounding muscles, and their scar-reducing potentials have been assessed.1–5,5,11,14,17–20,23,28,29,32,35 The authors of such studies performed within the last several decades have reported varying results.10,14,18,19

Recently, an antiadhesion barrier gel ADCON-L (Gliatec, Inc., Cleveland, OH) consisting of a carbohydrate polymer that prevents the invasion of fibroblasts, and a collagen-based gel, Gel Amidon Oxyde, have been demonstrated to decrease the epidural fibrosis in animal experiments.8,21,31,34 In a clinical study performed by Richter, et al.,30 however, they found no positive effect of treatment with ADCON-L in patients who underwent single-level lumbar microdiscectomy.

An epidural hematoma in the path of dissection also plays an important role in the formation of epidural fibrosis.18,32 In a preliminary study, we showed that Tachocomb, a local hemostytic agent consisting of a collagen patch covered with a fixed layer of the solid coagulation factors—fibrinogen and thrombin—and the fibrinolysis inhibitor aprotinin, was more effective in reducing the epidural fibrosis than other hemostytic materials. Complete prevention of scar tissue formation, however, was not achieved.20

Extending our previous result, we designed an experimental study using mitomycin C to prevent spinal epidural fibrosis in the rat laminectomy model. Mitomycin C has been readily used with remarkable success in ophthalmology for reducing the scar to promote the passage of the trabecula after glaucoma surgery and in the treatment of tracheal cicatrix for improving the patency of antrostomy after tracheal reconstruction.16,25,26,33 Mitomycin C is an alkylating antibiotic substrate isolated from S. caespitosus, which potentially suppresses fibroblast proliferation after surgery.9 This effect is attributed to the inhibition of RNA and protein synthesis as well as DNA replication by linking with guanidine in DNA.9 Because mitomycin C can be easily and rapidly absorbed into cells, it was applied in fluid form only once for few minutes during surgery with a piece of sponge. The period of mitomycin C therapy and its concentrations in glaucoma and laryngeal surgery, however, varied between 2 and 5 minutes, respectively, and 0.1 and 0.5 mg/ml, respectively. Serious complications associated with its application have been reported in glaucoma surgery, including epithelial defects, delayed wound healing, scleral blanching and melting, corneal perforation, necrotizing keratitis, and persistent hypotonic maculopathy.16,25,26,33

Based on ophthalmological data, we conducted this study to investigate, for the first time, the effect of mitomycin C in various concentrations to reduce epidural fibrosis after
laminectomy. In all cases, the postoperative course was uneventful without neurological deficits and disturbance in wound healing.

The histopathological analysis was performed after a 3-month period, which is generally needed for complete constitution of epidural fibrosis.32 Our analysis showed that there was less epidural scarring in the mitomycin C–treated laminectomy sites than in control sites. In all cases of 0.1-mg/ml concentrations of mitomycin C, there was a remarkable absence of dura adhesions, and the loosely arranged epidural fibrous tissue was clearly separated from the dura mater. In the laminectomy defects treated with mitomycin C at a concentration of 0.01 mg/ml, however, a widespread dural adhesion was observed. Furthermore, no side effect of mitomycin C on nervous tissue was noted (Fig. 1).

In summary, this study showed that mitomycin C applied local to the laminectomy defects is very effective in reducing epidural scar formation and preventing dural adhesion in adult rats, respecting the integrity of adjacent nerve tissue. The application of mitomycin C over the dura mater and around the nerve roots is uncomplicated. Because of the fluid state of mitomycin C, a compression of nerve tissue, such as that occurring with epidural implants, did not occur.24,29 Furthermore, any chronic inflammatory reaction of the dura mater was prevented.

Further studies are required to prove the excellent efficacy of mitomycin C and its definitive concentration for the reduction of epidural fibrosis and prevention of the dural adherence.

Conclusions

We have demonstrated that mitomycin C at a concentration of 0.1 mg/ml can effectively reduce epidural scar formation and prevent dural adhesion in vivo without causing side effects in an adult rat laminectomy model during the first 3 months after surgery.

Acknowledgments

We thank Mrs. Kokoscha and Mrs. Meyers for technical assistance.
Mitomycin C to prevent epidural fibrosis

References


Manuscript received April 28, 2003. Accepted in final form August 25, 2003. Address reprint requests to: Jin-Yul, Lee, M.D., Department of Neurosurgery, University of Cologne, Joseph-Stelzmann-Strasse 9, 50924 Köln, Germany. email: 912382@hanmail.net.