In daily neurosurgical practice it is very important to seal injured or incised dura mater. Extradural or subcutaneous cerebrospinal fluid leakage is common after cranial or spinal surgery and leads to poor wound healing, wound infection, encephalitis, meningitis, chronic subdural hematoma, and pseudomeningocele formation. Cerebrospinal fluid leakage after supratentorial surgery is rare. The main underlying mechanism of leakage is thought to be lower intradural hydrostatic pressure at the surgical site due to the patient’s position during surgery, which is almost always supine. Infratentorial or spinal surgery is performed under higher cerebrospinal fluid pressures and so the incidence of leakage after such interventions is higher. Various methods and substances have been developed to prevent leakage after cranial and spinal surgery. The importance and the incidence of subclinical cerebrospinal fluid leakage is still a debate.

The use of GM-CSF in experimental animal models and preclinical studies has revealed that it facilitates wound healing. Granulocyte-macrophage colony–stimulating factor is a multipotent cytokine with a molecular weight of 15 to 35 kD and acts primarily to stimulate proliferation and differentiation of hematopoietic progenitor cells. Chronic skin ulcers of various underlying causes have been treated with GM-CSF. In the present study we evaluate the effects of GM-CSF on dural healing after experimentally induced cerebrospinal fluid leakage. Dural closure and healing were evaluated histologically.

Materials and Methods

Rat Model

Thirty-two adult male Wistar rats weighing between 250 and 300 g were used in these experiments. The animals were kept under a 12-hour light/12-hour dark cycle and allowed free access to food and tap water. All experimental procedures were approved by our Institutional Review Board and performed in accordance with the local guidelines in research to minimize animal discomfort.

Anesthesia and Surgical Procedure

Anesthesia was induced via intramuscular administration of 5 mg/kg ketamine hydrochloride (Ketalar, 5% solution, Levent) and 10 mg/kg xylazine (Rompun, 2% solution, Bayer). The rats were num-

The effects of topical granulocyte-macrophage colony–stimulating factor on dural healing in rats after induced cerebrospinal fluid leakage

GÖKHAN KURT, M.D.,1 ALP ÖZGÜN BÖRÇEK, M.D.,1 BERKER CEMIL, M.D.,1 NEŞE LORTLAR ÜÇKANŞ, M.D.,2 FIKRET DOĞULU, M.D.,1 AND M. KEMALİ BAYKANER, M.D.3

Departments of 1Neurosurgery and 2Histology, and 3Division of Pediatric Neurosurgery, Gazi University Faculty of Medicine, Ankara, Turkey

Object. Dural defects must be repaired to protect the central nervous system from contamination. Although there are various experimental and commercial substances available for this purpose, the ultimate method of watertight dural closure has yet to be discovered. In this study, the authors investigate the effects of topically applied recombinant mouse granulocyte-macrophage colony–stimulating factor (GM-CSF) on dural healing in a rat model of dural injury and cerebrospinal fluid leakage.

Methods. In this experimental model, a dural defect at the level of the L1–2 vertebrae was created in 32 Wistar rats. Sixteen animals were treated with locally applied recombinant mouse GM-CSF postoperatively, and 16 animals received normal saline. The effects of GM-CSF on dural healing, cerebrospinal fluid leakage, and wound healing were assessed 2 and 4 weeks postoperatively. Dural healing was evaluated histologically.

Results. Dural healing was increased in rats treated with GM-CSF compared with rats in the control group. This difference was statistically significant (p < 0.05).

Conclusions. Cerebrospinal fluid leakage may impede healing of dural defects. Topically applied GM-CSF seems to aid in dural healing. (DOI: 10.3171/SPI-07/10/419)
hered with ear tags and their midbacks were shaved and cleaned with 10% pVP/iodine. Using aseptic technique and a surgical microscope, a midline incision was made along the spinous processes of the lumbar area. After dissecting the fascia and the paraspinal muscles, the spinous processes of the L-1 and L-2 vertebrae were removed. A laminectomy was performed with a high-speed drill. The dura mater was opened longitudinally for 5 mm with a No. 15 scalpel, and cerebrospinal fluid leaks were observed with an operating microscope.

The 32 rats were randomly assigned to each of two groups. In the 16 rats in the control group (Group I), 3 ml of normal saline was applied to the dural defects. The experimental group (Group II) received 50 µg of recombinant mouse GM-CSF (Biosource) applied topically to the dural incision. The wounds were closed in layers, except for the dura mater, after the operation. All rats underwent postoperative clinical evaluation, and mobility status and evidence of neurological deficits were recorded.

**Histological Examination**

Subsets of eight rats from each group underwent histological examination of the durotomy site at 2 weeks (designated Groups IA and IA) and 4 weeks postoperatively (designated Groups IB and IIB). For this evaluation the rats were killed with an overdose of pentobarbital administered intraperitoneally. Afterwards, the spinal column corresponding to the laminectomy–durotomy site, including the surrounding muscle tissue, was removed en bloc. The specimens were fixed in 10% buffered formalin solution for 1 week and then placed in decalcifying solution until complete decalcification had occurred. One-mm-thick sections obtained from the laminectomy–durotomy site were then obtained, and each section was embedded in paraffin. Serial sections 5-µm-thick were cut with a microtome and stained with H & E and Masson Trichrome. All specimens were evaluated by the same histologist, who was blinded to the groups. The grading system used for evaluation was modified from the criteria set forth by Lasa et al.5 (Table 1). The cell types present, granulation, collagen deposition, and vascularization characteristics were recorded to determine the extent of the healing process at the durotomy site.

**Statistical Analysis**

The chi-square test was used to determine the differences between the groups. A probability value less than 0.05 was accepted as statistically significant.

**Results**

None of the rats died or developed neurological deficits during the experiment. There was no observable cerebrospinal fluid collection or leakage from the wounds, and none of the animals developed wound infections.

In axial sections, cell types, extent of granulation, status of collagen deposition, and vascularization status were evaluated. Increasing histological grade meant increasing amounts of granulation tissue and collagen deposition and maturation. As the histological grade increased, the polymorphonuclear leukocyte infiltration decreased and fibroblast accumulation increased. Therefore, specimens determined to have Grade 3 changes at the surgical site were considered better than closed dural lesions. A summary of the histopathological grades of the animals in each group is given in Table 2. Grade 3 healing properties were observed only at the durotomy sites of the animals killed 4 weeks after the application of GM-CSF (Group IIB; Fig. 1A). The other groups did not demonstrate Grade 3 healing properties. However, only control group animals (killed at either week of the experiment) demonstrated Grade 0 healing properties (Fig. 1B). There was no difference in extent of dural healing between the control groups killed 2 (Group IA) and 4 (Group IB) weeks after the durotomy (p=0.619). Animals killed at the end of the second week (Groups IA and IA) and animals in the groups killed at the end of the fourth week (Groups IB and IIB) demonstrated statistically significant differences related to dural healing (p=0.007 and p=0.001, respectively). Additionally, comparison of the groups treated with GM-CSF revealed a statistically significant difference between the animals killed at Week 2 and at Week 4 (Groups IIA and IIB; p=0.029).

**Discussion**

Most procedures in daily neurosurgical practice require dural opening to gain access to the diseases of the central nervous system, and unintended dural tears are not rare. Cerebrospinal fluid leakage after neurosurgical intervention has been found to increase complications and even death rates in some cases. Additionally, these leaks protract the recovery period, causing major economic consequences such as prolonged hospital stays due to infection and difficulty in determining the exact site of the leakage with current imaging tools.7 Therefore, the prevention of cerebrospinal fluid fistulas is of utmost importance.

Whether made deliberately or not, dural defects should be closed in a watertight fashion when possible so that the dura mater can maintain its basic function—protection of the central nervous system. Various materials and techniques have been developed for this purpose, from simple watertight suturing to the use of synthetic materials such as N-butylcyanoacrylate and fibrin glue; however, determination of the best dural sealant requires further study.

Soon after hematological stimulants were discovered, it was recognized that these agents have beneficial effects on wound healing. This is the result of the proliferative and complex cellular effects of these agents and is also true of GM-CSF. The healing characteristics of GM-CSF have been evaluated. Increasing histological grade meant increasing amounts of granulation tissue and collagen deposition and maturation. As the histological grade increased, the polymorphonuclear infiltrate decreased and fibroblast accumulation increased. Therefore, specimens determined to have Grade 3 changes at the surgical site were considered better than closed dural lesions. A summary of the histopathological grades of the animals in each group is given in Table 2. Grade 3 healing properties were observed only at the durotomy sites of the animals killed 4 weeks after the application of GM-CSF (Group IIB; Fig. 1A). The other groups did not demonstrate Grade 3 healing properties. However, only control group animals (killed at either week of the experiment) demonstrated Grade 0 healing properties (Fig. 1B). There was no difference in extent of dural healing between the control groups killed 2 (Group IA) and 4 (Group IB) weeks after the durotomy (p=0.619). Animals killed at the end of the second week (Groups IA and IA) and animals in the groups killed at the end of the fourth week (Groups IB and IIB) demonstrated statistically significant differences related to dural healing (p=0.007 and p=0.001, respectively). Additionally, comparison of the groups treated with GM-CSF revealed a statistically significant difference between the animals killed at Week 2 and at Week 4 (Groups IIA and IIB; p=0.029).

**TABLE 1**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of granulation at the surgical site. No or some inflammatory cells. No capillaries or collagen fibers.</td>
</tr>
<tr>
<td>1</td>
<td>Minimal granulation tissue at surgical site. Inflammatory cells &amp; a few fibroblasts. Some collagen fibers &amp; capillaries.</td>
</tr>
<tr>
<td>2</td>
<td>Severe granulation tissue at the surgical site. Dense fibroblasts &amp; collagen fibers. Capillary network present.</td>
</tr>
<tr>
<td>3</td>
<td>Chronic period. Wound healing has ended. Some fibroblasts. Organized collagen fibers &amp; dense capillary network.</td>
</tr>
</tbody>
</table>

* Adapted from Lasa et al., 1993.

**TABLE 2**

<table>
<thead>
<tr>
<th>Group</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA (control group, wk 2)</td>
<td>5</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IIA (GM-CSF group, wk 2)</td>
<td>—</td>
<td>3</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>IB (control group, wk 4)</td>
<td>3</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>IIB (GM-CSF group, wk 4)</td>
<td>—</td>
<td>—</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

**J. Neurosurg: Spine / Volume 7 / October, 2007**
Dural healing and GM-CSF

Fig. 1. Photomicrographs of rat tissue samples obtained 4 weeks after the laminectomy–duratomy in the experimental (A) and control groups (B). A: Grade 3 wound healing with dense collagen formation (arrowhead) over the spinal cord (asterisk) is demonstrated at the laminectomy site. B: Grade 0 wound healing with no obvious collagen formation over the laminectomy–duratomy site (arrowheads) or the spinal cord (asterisk) is demonstrated. Masson Trichrome stain, original magnification × 2.5.

been shown previously. Chronic venous ulcers, problem-

atic wounds, Behçet disease, Kaposis sarcoma, and melano-

ma have been found to benefit from intra- or perilesional ap-

plication of GM-CSF according to various dose regimens. The main route of action for this drug is through the

Further stud-

g, is one of the most common for GM-CSF in

other experimental studies in the literature.

The quality of the dural healing remains time-dependent, like the heal-

ing of any ordinary wound. The dose regimen chosen in this

study, 50 μg, is one of the most common for GM-CSF in

other experimental studies in the literature. Further stud-

ies are needed to determine the minimum effective dose

under different time durations. Although the number of ani-

mals in this study is not enough to make general assump-

tions, it seems that GM-CSF may have positive effects on

healing in dural lacerations.

Conclusions

In the present study we demonstrate that local applica-

tion of GM-CSF to the site of cerebrospinal fluid leakage

may facilitate dural healing and prevent complications.

Further studies with larger groups of animals are required.

References

2. Boente P, Sampaio C, Brandão MA, Moreira ED, Badaro R, Jones TC: Local peri-lesional therapy with rhGM-CSF for Kas-

pó’s sarcoma. Lancet 341:1154, 1993
3. Bussolino F, Ziche M, Wang JM, Alessi D, Morbidelli L, Cre-

8. Erdem E, Dinc S, Erdem D, Ustün H, Caydere M, Alagöl H: Effects of intraperitoneal chemotherapy and GM-CSF on anas-

9. Hoffman RA: Cerebrospinal fluid leak following acoustic neu-

roma removal. Laryngoscope 104:40–58, 1994
10. Jachkowicz E, Zabernigg A, Gatterer C: Reombinant human gran-

ulocyte-macrophage colony-stimulating factor applied locally in


locyte-macrophage colony-stimulating factor in chronic leg ul-

G. Kurt et al.


Accepted June 27, 2007.