A physiological sealant for cerebrospinal fluid leaks

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A combination of concentrated platelets, thrombin, and fibrinogen was used to adhere a pericranial graft to surgically produced cerebrospinal fluid (CSF) fistulas in dogs. This sealant successfully stopped leakage of CSF in all fistulas produced in both acute and chronic preparations. All control animals leaked CSF acutely. In chronic control animals the CSF leaks sealed spontaneously but the grafts were not well incorporated. Histological examination of the grafts and underlying brain showed no injury to the brain or meningeal vessel from exposure to the platelet glue. Good fibrous union of the grafts to the dura was confirmed.

KEY WORDS • cerebrospinal fluid • fistula • platelets • sealant

REPAIR of cerebrospinal fluid (CSF) fistulas in the area of the sella and the sphenoid is technically difficult and often unsuccessful. There are significant rates of recurrence of leakage after both standard suture repair and free muscle and fascial grafts. Attempts to use organically synthesized glues, such as the cyanoacrylates, were at first thought promising, but have been found to induce an intense tissue reaction to the point of necrosis. They remain experimental drugs. We are proposing the use of a physiologically derived tissue adhesive in the surgical repair of CSF leaks; all the elements of this adhesive (platelets, fibrinogen, and thrombin) are biodegradable. We tested this physiological adhesive for its ability to seal surgically produced CSF leaks in dogs.

Materials and Methods
Preparation of Adhesive

To prepare the platelet concentrate for the adhesive, 100 cc of dog blood was drawn into acid citrate dextrose with ethylene-diamine-tetraacetic acid (EDTA). The blood was centrifuged at 220 G (times gravity), and the platelet-rich plasma taken off with a siliconized pipette. The platelets were then buttoned by centrifuging at 500 G for 12 minutes and resuspended in isotonic phosphate buffer pH 7.4. The final platelet concentration was 0.5 to 2 × 10^7/cu mm.

Human fibrinogen was purified according to the method of Blömback and Blömback; the fibrinogen concentration was 45 mg/ml. We used bovine thrombin* concentrated at 1000 μ/ml. An isotonic 1.4 mM calcium solution was prepared. For chronic experiments, the platelet preparation and thrombin were prepared in a sterile manner. The CaCl₂ was millipore-filtered.

Surgical Procedure

Large mongrel dogs of both sexes were anesthetized with intravenous pentobarbital, intubated, and allowed to breathe spontaneously. An indwelling catheter was in-

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*Bovine thrombin was obtained from Parke, Davis and Company, Detroit, Michigan 48232.
TABLE 1

Results of dural grafting in acute and chronic preparations

<table>
<thead>
<tr>
<th>Experimental Preparation</th>
<th>No. of Trials</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute experiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>graft + platelets</td>
<td>6</td>
<td>none leaked</td>
</tr>
<tr>
<td>graft alone</td>
<td>5</td>
<td>all leaked</td>
</tr>
<tr>
<td>chronic experiments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>graft + platelets</td>
<td>3</td>
<td>none leaked; good graft incorporation and coverage of brain</td>
</tr>
<tr>
<td>graft + platelets + cerebral edema</td>
<td>3</td>
<td>none leaked, but two had cerebral herniation through dural defect</td>
</tr>
<tr>
<td>graft alone</td>
<td>2</td>
<td>none leaked; grafts slipped; brain covered by thickened arachnoid</td>
</tr>
</tbody>
</table>

Acute Experiments

One to 2 hours after the closure of the dural defect, we tested for the presence of a leak by injecting fluorescein into the CSF and observing the graft site under a Woods light. A No. 22 spinal needle was placed into the cisterna magna and 0.25 ml of fluorescein dye was diluted with 3 cc of CSF and slowly injected. In one animal we placed an epidural pressure transducer, and increased the CSF pressure to 50 mm Hg by injection of physiological saline into the cisterna magna. This was done to test the strength of the dural graft. At the end of the experiment, the dura, graft, and underlying brain were removed for histological examination. The animals were then sacrificed.

In this group of nine animals, five had adhesive applied to a total of six dural defects. Four animals had a total of five graft-covered dural defects with no adhesive applied. One animal in each group had bilateral dural defects created.

Physiological sealant for CSF leaks

Chronic Experiments

The animals in the chronic group underwent the same surgical procedure as those in the acute group under aseptic technique. When the graft had been applied, however, the frontal sinus was closed using standard surgical procedure. We reopened the sinus 2 to 4 weeks later and tested the graft for a leak using fluorescein as indicated above. Pathological specimens were then obtained and the animals sacrificed. We applied the adhesive in six of the eight animals in the chronic group. Three of these animals had cerebral swelling induced by blunt trauma applied with a flat spatula to the frontal lobe behind the graft. No animals developed infection at the site of surgery.

Histological Techniques

The specimens were fixed in 10% buffered formalin. Routine hematoxylin and eosin stains were performed on all sections. Nissl and elastic van Gieson stains were done on sections of brain and meninges.

Results

The results of the trials are given in Table 1. In the acute experiments, all five dural grafts applied without the adhesive leaked CSF at 1 to 2 hours, as evidenced by the prompt appearance of fluorescein at the graft site. What appeared to be small spontaneous CSF leaks were noted in three dogs, both in the treated and control groups. These appeared as a slight fluorescein staining of the mucous membranes anteriorly and inferiorly. They were easily distinguished from the surgically prepared CSF fistulas, which produced a marked fluorescein staining. Of the six grafts applied with the adhesive, none leaked. In one of these animals, the CSF pressure was increased to 50 mm Hg without the appearance of a leak. The difference between the treated and the untreated group is significant at the p = 0.01 level.

Sections of the brain and meninges showed only some blood in the subarachnoid space associated with the surgical manipulations. There was no evidence of neuronal damage (Fig. 1). Meningeal vessels appeared normal and did not show evidence of damage secondary to the exposure to platelets (Fig. 2).

In the chronic animals examined 2 to 4 weeks after the procedure, there were no CSF leaks in either the experimental or the control groups. In two of three experimental animals that had cerebral swelling induced by trauma,
This combination succeeded in acutely and chronically sealing a graft applied to a surgically induced dural defect in dogs. Although the adhesive was able to withstand a CSF pressure of 50 mm Hg acutely, it could not contain edematous, herniating brain in two of three animals. A leak developed acutely in all control animals. In chronic control animals, the graft slipped off but the arachnoid seemed to close over the brain and seal off the leak. Of particular note is the fact that the platelet-fibrin combination did not induce tissue reaction and caused no demonstrable vascular injury. The platelet-fibrin plug is the physiological sealant of injuries to arteries. It appears also to have properties suitable for acutely sealing off CSF leaks in areas not accessible to standard surgical techniques.

Acknowledgments

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References


Discussion

Closure of CSF leaks is technically difficult in surgically inaccessible areas such as the sella and the sphenoid. This is of particular concern when transsphenoidal hypophysectomies are performed. We have evaluated a physiological biodegradable adhesive made up of platelets, fibrinogen, and thrombin.

Fig. 3. Photomicrograph of healed dural graft after 1 month. There is firm fibrous union of the graft above to the dura (longitudinal fibers). H & E, × 300.

Frontal lobe herniation occurred through the dural defect despite the adhesive. Microscopic examination of the dura (Fig. 3) showed firm fibrous union of the graft to the dura in four of the six animals treated with adhesive. In the two control animals and in two of the animals subjected to blunt trauma, the graft had slipped off partially and the brain was covered by thickened arachnoid.
Physiological sealant for CSF leaks


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