Enhancement of Withstanding Pressure of Fibrin Sealant by Modified Mixing Ratio of Fibrin Sealant Components for Skull Base Reconstruction

—Technical Note—

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Abstract

A method to enhance the withstanding pressure of fibrin sealant in gasket-seal closure to prevent cerebrospinal fluid (CSF) leakage after extended transsphenoidal surgery (ETSS) was investigated by adjusting the mixing ratio of the components. A plastic chamber (200 ml) was constructed with a lid made of hydroxyapatite with a hole 10 mm in diameter. The chamber could be pressurized via an opening in the side wall. The hole in the hydroxyapatite lid was covered with a Gore-Tex sheet, 15 mm in diameter. The margin of the sheet was free. Solutions A (fibrinogen 80 mg/ml) and B (thrombin 250 units/ml) of fibrin sealant were mixed in volume ratios of 1:1, 2:1, and 5:1, and applied to the Gore-Tex sheet, then water was introduced to cover the fibrin sealant. The pressure was measured at which air leakage occurred from the side of the Gore-Tex sheet. The pressure values for A/B ratios of 1:1, 2:1, and 5:1 were $117 \pm 23.8$ mmH$_2$O (mean $\pm$ standard error) ($n = 5$), $234 \pm 38.8$ mmH$_2$O ($n = 5$), and $345 \pm 36.4$ mmH$_2$O ($n = 5$), respectively, in the acute phase (5 minutes after application of fibrin sealant). Pressures were increased after 24 hours, and that for 5:1 was the highest ($373 \pm 40.4$ mmH$_2$O, $n = 5$). The use of devices such as syringes specially designed to mix solutions A and B in the ratio of 5:1 can easily enhance the preventive effect of fibrin sealant against CSF leakage in ETSS.

Key words: fibrin sealant, extended transsphenoidal surgery, cerebrospinal fluid leakage

Introduction

One of the most important tasks in skull base surgery, including extended transsphenoidal surgery (ETSS), is prevention of postoperative cerebrospinal fluid (CSF) leakage. Various methods, which use autologous tissues, biological agents, artificial agents, and other materials, are employed to prevent CSF leakage$^{1,2,5,9}$ and fibrin sealant is the most commonly used agent. In ETSS, fibrin sealant covers the reconstructed site and adheres to the surrounding tissue, particularly to the bony surface of the sphenoid sinus. Concerning the adhesion strength of fibrin sealant, experimental studies of the withstanding pressure using animal skin or dura mater have been conducted in the neurosurgical field.$^{3,7,10}$ However, there have been no reports of investigation into the adhesion strength of fibrin sealant to rigid tissue such as bone, which is less likely to exhibit a biological reaction in the acute phase. We previously developed a model based on the gasket-seal closure$^6$ that mimicked sellar reconstruction in ETSS, to examine the withstanding pressure of fibrin sealant.$^8$ As a result, we reported that fibrin sealant was stable against a pressure of approximately 200 mmH$_2$O during the acute phase, and approximately 400 mmH$_2$O after 24 hours. Fibrin sealant showed high adhesion strength to the bone surface model. These values correspond to actual clinical experience including CSF leakage due to sudden postoperative increase in intracranial pressure, and further improvements to enhance the strength should be considered. Higher concentrations of fibrinogen are reported to lead to higher adhesion strengths of fibrin sealant.$^4$ In this study, we investigated the possibility of enhancing the withstanding pressure of fibrin sealant by adjusting the mixing ratio of the sealant compo-
Methods

We prepared a plastic chamber 200 ml in volume. A lid made of hydroxyapatite with a hole 10 mm in diameter was attached to the opening of the lid, which was 38 mm in diameter. The chamber could be pressurized via an opening in the side wall. The 10 mm hole in the hydroxyapatite lid was covered with a Gore-Tex sheet, 15 mm in diameter (W. L. Gore & Associates, Co., Ltd., Tokyo). The margin of the sheet was free and not fixed (Fig. 1). Fibrin sealant (Bolheal; Kaketsuken, Kumamoto, Kumamoto) was applied to the Gore-Tex sheet to seal the plastic chamber. Fibrin sealant consists of solutions A and B; solution A contains fibrinogen (80 mg/ml), blood coagulation factor XIII (75 units/ml), and aprotinin (1000 KEI/ml), and solution B contains thrombin (250 units/ml) and calcium chloride (5.9 mg/ml). Solutions A and B were mixed at the following volumes: 2.5 ml of A and 2.5 ml of B (fibrinogen 40 mg/ml) (1:1 A/B), 2.5 ml of A and 1.25 ml of B (fibrinogen 53.3 mg/ml) (2:1 A/B), and 2.5 ml of A and 0.5 ml of B (fibrinogen 66.7 mg/ml) (5:1 A/B). Each solution was divided five times and alternately applied. Water was introduced into the chamber to cover the fibrin sealant after the enzymatic reaction had proceeded for 5 minutes. The inside of the plastic chamber was then slowly pressurized (50 ml/min) and the pressure was measured at which air leakage occurred from the side of the Gore-Tex sheet. The same experiments were performed at 24 hours after application of the fibrin sealant.

Results

Withstanding pressure increased with higher ratios of A/B in the acute phase (5 minutes after application of fibrin sealant) as follows: $117 \pm 23.8 \text{ mmH}_2\text{O}$ (mean ± standard error) ($n = 5$) at 1:1, $234 \pm 38.8 \text{ mmH}_2\text{O}$ ($n = 5$) at 2:1, and $345 \pm 36.4 \text{ mmH}_2\text{O}$ ($n = 5$) at 5:1; and in the chronic phase after 24 hours as follows: $157 \pm 20.6 \text{ mmH}_2\text{O}$ ($n = 5$) at 1:1, $262 \pm 33.9 \text{ mmH}_2\text{O}$ ($n = 5$) at 2:1, and $373 \pm 40.4 \text{ mmH}_2\text{O}$ ($n = 5$) at 5:1. The strength with A/B 5:1 was significantly higher than with 1:1 (Steel-Dwass multiple comparison test, $p = 0.023$) at both 5 minutes and 24 hours after application of fibrin sealant. The trend test (Jonckheere-Terpstra test) showed the trend of $1:1 < 2:1 < 5:1$ was clearly observed in both phases ($p < 0.001$). These findings indicated that mixing solution A with a relatively smaller volume of solution B, or a higher concentration of fibrinogen (66.7 mg/ml), results in higher withstanding pressure. The withstanding pressure at 24 hours after application of fibrin sealant was a little higher than that in acute phase (5 minutes), but not significantly. The 5:1 mixture was still strongest in the late phase (24 hours).

Discussion

This experimental study showed the clinically important finding that a mixing ratio of 5:1 of fibrin sealant solutions A and B significantly strengthens the withstanding pressure at 5 minutes and 24 hours after application of fibrin sealant. The section of “Dosage and Administration” in the instruction sheet of fibrin sealant describes that solutions A and B should be mixed in an equal ratio. However, mixture of solution A with solution B in the ratio of 5:1, with increased final concentration of fibrinogen (66.7 mg/ml), significantly enhanced the adhesion strength. The viscosity of the fibrinogen solution increases depending on its concentration. Fibrinogen solution with high viscosity is difficult to mix and cumbersome to handle. In our experiments, the mixture ratio of 5:1 was thought to be the limit for mixture in the clinical use. However, that report was based on use of the spray method to apply a uniform mixture of the two solutions. In ETSS, where the surgical field is small and limited, spray application is difficult. Therefore, we mixed solutions A and B five times each so that homogeneity in the mixture of the two solutions could be secured.

The withstanding pressure for the equal volume ratio was $117 \pm 23.8 \text{ mmH}_2\text{O}$ in the present experiment. Our previous experiment conducted with the gasket-seal closure showed a withstanding pressure of approximately 200 mmH$_2$O in the acute phase, using similar fibrin sealant agent (Beriplast P; CSL-Behring K.K., Tokyo). The experimental design was different. Instead of bone surface, the previous experiment used epoxy-glass laminate, but this time,
hydroxyapatite was applied. The latter is more suitable as a substitute for bone surface. The present data were considered to be convincing. Furthermore, solutions A and B mixed in the volume ratio of 5:1 significantly increased withstanding pressure to $345 \pm 36.4$ mmH$_2$O in the acute phase and $373 \pm 40.4$ mmH$_2$O in the late phase (24 hours), which could prove to be very valuable information.

In endoscopic or ETSS, various methods of sellar reconstruction are employed, and fibrin sealant is most commonly applied. In this study, we mimicked sellar reconstruction in ETSS. Focusing on adhesion of fibrin sealant to the bone tissue of the sphenoid sinus, which exhibits relatively little biological reaction in the early postoperative period, we made an experimental model that likewise has no biological reaction, where the dural defect was covered with a Gore-Tex sheet, and hydroxyapatite was employed as a substitute for bone, to get information on the actual relationship between fibrin and bone.

Bolheal was used as the fibrin agent in this experiment. Compared with the components of Beriplast P and Tisseel (Baxter Healthcare Corp., Westlake Village, California, USA), the most commonly used fibrin agent worldwide, Bolheal and Beriplast P contain 80 mg/ml of fibrinogen in solution A and Tisseel contains 90 mg/ml, whereas Bolheal has 250 units/ml of thrombin in solution B, Beriplast P has 300 units/ml, and Tisseel has 500 units/ml. Although we have not directly compared experimental results using these three fibrin sealants, the strength of the fibrin sealant appears to depend on the fibrinogen concentration in solution A and the thrombin concentration in solution B. In the mixing of the components, a large volume of solution B dilutes solution A, which will reduce the withstanding pressure. Our test results should correspond to those found with Beriplast P and Tisseel.

When fibrin sealant is applied, the Gore-Tex sheet tends to float, probably because of its hydrophobic properties. To prevent the Gore-Tex sheet from floating, we first applied a small volume of solution A around the Gore-Tex sheet, and then applied solution B, which increased the final concentration of fibrinogen at the surface in contact with hydroxyapatite, and strengthened the withstanding pressure. This procedure could prevent floating of Gore-Tex sheet. In actual ETSS, CSF leakage could be prevented using specially designed syringes to apply solutions A and B in the volume ratio of 5:1 (Fig. 2).

In postoperative situations, intracranial pressure may suddenly rise due to severe sneezing and coughing, etc. Therefore, higher withstanding pressure is better. The mixture 5:1 can easily enhance the withstanding pressure of fibrin sealant. In the future, we will evaluate the effects of changes in the component mixing ratio in fibrin glue sealant on the course of wound healing, and identify the optimal smaller volume of solution B for sealing of the defect of the sella.

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**Conflicts of Interest Disclosure**

The authors have no personal financial or institutional interest in any of the drugs, materials, or devices in the article. All authors who are members of The Japan Neurosurgical Society (JNS) have registered online Self-reported COI Disclosure Statement Forms through the website for JNS members.

**References**


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