Experimental study on effective application of fibrin glue

Hajime Kin, MD, PhD · Takayuki Nakajima, MD, PhD
Hitoshi Okabayashi, MD, PhD

Abstract

Purpose. Fibrin glue is effective for maintaining hemostasis after anastomosis and for filling needle holes after cardiothoracic and vascular surgery, but few experimental studies concerning methods of application to obtain more effective hemostasis have been reported.

Methods. Bolheal was used as the fibrin glue. Fibrinogen solution (A, 0.3 ml) and thrombin solution (B, 0.3 ml), components of fibrin glue, were applied to the needle holes by the following four methods: group 1 (n = 8), drip method; group 2 (n = 8), spray method; group 3 (n = 8), rub-and-spray method; group 4 (n = 8), rub-and-rub method. Additional studies were done in groups 3 and 4 to evaluate the hemostatic effect with different curing times and temperatures.

Results. The pressure at which the fibrin sealant ruptured were significantly higher in group 3 (109 ± 16 mmHg) and group 4 (113 ± 7) (for both groups: P < 0.05 vs. group 1 (22 ± 8) and group 2 (64 ± 10)). The pressure increased with prolongation of the curing time, and significant differences were noted between the pressures at ≥2 min and that at 30 s (both groups: P < 0.05 vs. 30 s). The curing temperature had no significant influence in the two groups. Microscopically, the glue effectively plugged the needle holes in groups 3 and 4.

Conclusion. Compared with the current drip and spray methods, more effective hemostasis was obtained by rubbing on the fibrin glue.

Key words Fibrin glue · Fibrin sealant · Hemostasis · Rubbing method · Cardiac surgery

Introduction

Fibrin sealant has been widely used in a number of settings, such as thoracic surgery, adult and pediatric cardiac surgery, and vascular surgery, with satisfactory results. A recent multicenter randomized trial demonstrated more rapid hemostosis, reduced blood loss, and a shorter time to hemostasis when fibrin sealant was applied to sites of bleeding from needle/suture holes in polytetrafluoroethylene (PTFE) grafts anastomosed to the femoral artery.

Recent experimental studies have assessed a novel technique that involves rubbing the anastomosis with fibrinogen and thrombin solutions. Recently, Itano reported a novel rub and soak technique, in which the lung surface is rubbed with fibrinogen solution and a sheet impregnated with thrombin solution is applied to seal pulmonary air leaks. We have been using a rub and rub technique, which involves rubbing the anastomosis separately with the fibrinogen and thrombin solutions. However, there are no data to confirm that any of these methods is more effective for achieving hemostasis than the conventional technique.

Therefore, we investigated the influence of fibrin glue on the hemostatic effect in relation to various application methods, curing times, and curing temperatures.
Materials and methods

Fibrin sealant

The fibrin sealant Bolheal (Chemo-Sero Therapeutic Research Institute, Kumamoto, Japan) was used in this study. This product is composed of solutions A and B. Solution A contains human fibrinogen (80 mg/ml), human clotting factor XIII (75 U/ml), and bovine aprotinin (1000 KIE). Solution B contains human thrombin (250 U/ml) and calcium hydrochloride (5.9 mg/ml). The blood components were obtained from volunteer blood donors in Japan after screening for hemoglobin antigen, hepatitis C virus antibody/deoxyribonucleic acid, anti-human immunodeficiency virus antibody, and anti-human T-cell lymphoma virus 1 antibody. To prevent viral contamination, fibrinogen and factor VIII were treated by heating them at 65°C for 144 h, and thrombin was treated by heating at 65°C for 96 h. Fibrinogen was subjected to nanofiltration with a Planova 35 N filter (pore size 35 nm; Asahi Chemical Industry, Tokyo, Japan); and factor VIII, thrombin, and bovine aprotinin were filtered with a Planova 15 N filter (pore size 15 nm).

Experimental groups

Solution A (fibrinogen 0.3 ml) and solution B (thrombin 0.3 ml) of the fibrin glue were applied to the needle holes by the following four methods: group 1 \((n=8)\), dripping method—i.e., dripping on solutions A and B in turn; group 2 \((n=8)\), spray method—i.e., spraying on solutions A and B simultaneously through a nozzle at a pressure of 0.75 mmHg; group 3 \((n=8)\), rub and spray method—i.e., rubbing solution A on the needle holes and then spraying on both solutions A and B through a nozzle; group 4 \((n=8)\), rub and rub method—i.e., rubbing solution A on the needle holes and then rubbing on solution B.

Comparison of the pressure resistance of fibrin glue (measurement of the rupture pressure)

To compare the pressure resistance of fibrin glue between the four methods of application, a PTFE graft (8 mm atrium Hybrid PTFE graft; Atrium Medical, Hudson, NH, USA) was used. Three needle holes were made in the PTFE graft with an 18-gauge needle. One end of the PTFE graft was connected to a syringe-type infusion pump and the other end to a manometer. Then 25% human serum albumin solution was sprayed onto the PTFE graft where the needle holes had been made to simulate contact with blood products in the clinical setting. Three minutes after application of the fibrin glue under 25°C condition, the circuit was filled with red saline solution and was gradually pressurized using the syringe-type infusion pump at a constant rate of 50 ml/h. The pressure at which the red solution leaked from any part of a hole was defined as the “rupture pressure” and was measured (mmHg).

Comparison of the strength of fibrin glue after different curing times

To evaluate the strength of the glue after different curing times, tests were done at 30 s and 1, 2, and 3 min in groups 3 and 4 \((n=8)\).

Comparison of the strength of fibrin glue at different curing temperatures

To evaluate the strength of the glue after curing at different temperatures, we conducted tests at three temperatures in groups 3 and 4. Temperatures maintained in saline and the experimental room were set at 15°, 25°, and 35°C. The curing time was set at 3 min \((n=8)\).

Histological examination

At 3 min after sealing with fibrin glue in each group, the graft was fixed in 10% neutral buffered formalin. After fixation, each needle hole and its surroundings was cut out and embedded in paraffin. Then, 4-μm sections were stained with hematoxylin and eosin (H&E).

Statistical analysis

Results are expressed as the mean ± standard error. All data were analyzed with the Sigma Stat 3.1 for Windows statistical software package (SPSS, Chicago, IL, USA). One-way analysis of variance (ANOVA) and ANOVA with repeated measures were used as was appropriate to analyze results for the four groups, with post-hoc between-group comparisons being done by the Student-Newman-Keuls test with correction for multiple comparisons. \(P < 0.05\) was considered significant.

Results

Experiment 1

The mean rupture pressures in group 3 \((109 ± 16)\) and group 4 \((113 ± 7)\) were higher than those in group 1 \((22 ± 8)\) and group 2 \((64 ± 10)\). There was no significant difference between groups 3 and 4 (Fig. 1).