The sealing effect of fibrin glue against alveolar air leakage evaluated up to 48 h; comparison between different methods of application

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Abstract

Objective: There is little experimental evidence to show how much positive airway pressure fibrin sealants can actually withstand, and in particular, how this effect changes over time. In the present study, we experimentally evaluated the sealing effect of fibrin glue against alveolar air leakage up to 48 h after application.

Methods: Beagles were used (n = 48). Under thoracotomy, approximately 5 × 10 mm defects (2 mm depth) were made on the lung surface. Fibrin glue sealants were applied to this defect in three ways. In rubbing and spray method, fibrinogen was rubbed, followed by spraying of both fibrinogen and thrombin solutions. In double layer method, fibrinogen was dripped, followed by thrombin. Collagen fleece, coated with fibrinogen and thrombin (TachoComb) was also tested. The minimum positive airway pressure which produced air leakage was measured for each sealed defect (seal breaking pressure, cmH2O) at 0, 3, 6, 12, 24, and 48 h after application (n = 6 at each time point).

Results: The seal-breaking pressure increased over time in all of the application methods. At 6 h, differences between methods were not significant but three defects in RS reached 70 cmH2O, the maximum pressure tested, compared with none in other two methods. At 12 h, the seal-breaking pressure was significantly higher in RS compared with the other two methods (rubbing and spray method vs TachoComb; 66 ± 3 vs 47 ± 17, P = 0.047, rubbing and spray method vs double layer method; 66 ± 3 vs 42 ± 18, P = 0.024). Beyond 24 h, sealing pressure reached close to 70 cmH2O in all the methods.

Conclusions: The results show that the sealing effect of fibrin glue is relatively unstable up to 12 h after its application. Rubbing and spray method may help the fibrin seal to reach its full strength faster compared with the other two methods.

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1. Introduction

Alveolar air leakage is a very common complication in lung surgery. Along with inadequate control of postoperative pain, persistence of air leakage was identified as the most common cause of delay in hospital discharge after thoracic surgery [1]. Many tissue sealants are being applied to prevent air leakage after surgery [2–8]. Among them, fibrin glue is a popular sealant with a variety of application methods [9,10]. However, there are also reports that indicate that the use of fibrin glue does not reduce the duration of chest-tube drainage or hospital stay [11–13]. This implies that, air leakage often restarts shortly after surgery despite the application of fibrin glue.

Intraoperatively, we test the efficacy of fibrin glue by applying positive airway pressure. But we usually do not apply pressure beyond 20–25 cmH2O, since it defeats the purpose to break the seal at this point. Clinically, it is not rare that air leakage becomes apparent, for example through the chest tube, shortly after surgery. While this may be air leakage from lesions that were missed during surgery, it is also true that airway pressure often spikes beyond the pressure tested, 25 cmH2O, as the patient recovers spontaneous breathing. The fibrin seal may be broken at this point. To our knowledge, there is little experimental evidence to show how much positive airway pressure fibrin sealants can actually withstand, and in particular, how this effect changes over time. In the present study, we experimentally evaluated the sealing effect of fibrin glue against alveolar air leakage up to 48 h after application. We also compared three different methods of application.

2. Materials and methods

2.1. Animals

Adult male beagles, 10-12 months of age, weighing 8–11 kg were used for this study (Toyota Trading Co., Kumamoto, Japan) (n = 36). Animals were housed individually and provided...
food and water ad libitum. All animal studies were approved by the School of Medicine, Keio University Institutional Animal Care and Use Committee. All animals received humane care in accordance with the Japanese Government Animal Protection and management law.

2.2. Fibrin glue application

The fibrin glue used in this study was Bolheal (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). We also compared, fibrinogen-based collagen fleece, TachoComb (ZLB Behring Co., USA). The mechanism of fibrin glue formation is well described [14]. The fibrin glue product consists of two components. Solution A is a protein concentrate consisting of fibrinogen, plasma fibronectin, factor XIII, and plasminogen, reconstituted in aprotinin solution. Solution B is thrombin reconstituted in calcium chloride solution. TachoComb is a collagen fleece coated with dry fibrinogen and thrombin on one side.

We applied fibrin glue in two different ways, rubbing and spray method and double layer method. In rubbing and spray method, solution A was dripped and gently rubbed onto the air leakage area. Then both solutions were sprayed simultaneously onto the rubbed surface as a mixed aerosol using Bolheal Spray Set (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan). In double-layer method, solution A was dripped onto the air leakage surface after which solution B was dripped. To apply TachoComb, the fibrinogen-coated side was first soaked in saline, and then was attached to the air leakage surface. The sheet was gently pressed with dry gauze for about 5 min so that the collagen fleece was attached to the lung surface with fibrin glue.

2.3. Experiment

The dogs were anesthetized with an intravenous injection of pentobarbital sodium (25-30 mg/kg). The dogs were placed in left decubitus position, intubated, and mechanically ventilated. Through a thoracotomy, defects were created on the right lung surface using scalpels, one on each of the three lobes (anterior, middle, and posterior). The defect size was created with the lung fully inflated at a positive airway pressure of approximately 20 cmH2O. The defect size was measured at 0, 3, 6, 12, 24, and 48 h after the application of the fibrin sealant (n = 6 at each time point). Except for time 0, thoracotomy was performed again under anesthesia. Air leakage pressure for each defect was evaluated separately by clamping the remaining two lobar bronchi with forceps. The maximum positive airway pressure applied was 70 cmH2O, since higher pressure induced air leakage from uninjured lung around the hilum. After the completion of seal-breaking pressure measurement at each time point, each animal was sacrificed with intracardiac injections of pentobarbital (1000 mg/body).

2.4. Histological examinations

A separate group of animals was used to obtain histological specimens because the fibrin seal is broken by the seal breaking pressure measurements. Two specimens for each method and time points were prepared (n = 12). The animals were sacrificed and the whole right lung was fixed in 10% neutral formaline. After fixation, each defect site was resected, embedded in paraffin, and processed in 3 μm sections for hematoxylin-eosin staining. Specimens were analyzed at Clinicopathological Division of Keio University Hospital in a blinded manner by M.M.

2.5. Statistical analyses

The results are presented as the mean±SD. Seal-breaking pressure per time point was compared between different methods using unpaired T-test. Differences within each method were tested using paired T-test. Significance was assumed at P<0.05.

3. Results

3.1. Seal breaking pressure measurements

The seal-breaking pressure increased over time in all the application methods (Fig. 1). At 0 h, seal-breaking pressure was significantly higher in rubbing and spray method compared with TachoComb (54±5 vs 36±6, P<0.001), and in TachoComb significantly higher compared with double layer method (36±6 vs 27±3, P=0.007). Seal breaking
pressure in rubbing and spray method declined significantly from 0 to 3 h (from 54 ± 5 to 38 ± 6, P < 0.001). At 3 h, seal-breaking pressure in double layer method tended to increase, and in TachoComb tended to decline compared with 0 h, but these changes were not significant. At 6 h, differences between methods were not significant but three defects in rubbing and spray method reached 70 cmH2O, the maximum pressure tested, compared with none in other two methods. At 12 h, the seal-breaking pressure was significantly higher in rubbing and spray method compared with the other two methods (rubbing and spray method vs TachoComb; 66 ± 3 vs 47 ± 17, P = 0.047, rubbing and spray method vs double layer method; 66 ± 3 vs 42 ± 18, P = 0.024). Beyond 24 h, sealing pressure reached close to 70 cmH2O in all the methods, with no significant differences between methods.

3.2. Histological examinations

The layer of fibrin covering the lung surface could be observed in all three methods by hematoxylin-eosin staining (Fig. 2). Up to 12 h, deeper penetration of fibrin into the injured lung parenchyma was seen in rubbing and spray method compared with the other two methods. This difference was not apparent between application methods beyond 24 h. Also, at 3 h, hemorrhage was more evident underneath the fibrin layer in rubbing and spray method compared with other two methods.

4. Discussion

Fibrin glue is derived from human, or in some products like bovine plasma, and hence, carry the same risks as blood transfusion. We have reported the possibility of viral transmission by clinical use of fibrin glue [16]. Despite these potential drawbacks, fibrin glue is widely used in order to reduce postoperative alveolar air leakage, but questions remain regarding its clinical efficacy [5,12,13,15]. The results of this study show that the sealing effect of fibrin glue is relatively unstable up to 12 h after its application. Clinically, this result suggests that coughing or positive pressure ventilation should be kept to a minimum for 12 h in order to fully exploit the sealing effect of fibrin glue.

The sealing effect of fibrin glue is affected by the concentration of fibrin, and how well it attaches to tissue. The concentration of fibrin depends primarily on how well the thrombin and fibrinogen solutions are mixed on application. The attachment of fibrin may be affected at least in part by its penetration into tissue. Rubbing and spray method is a method that we have recently devised. Our intention was to improve tissue penetration by rubbing fibrinogen into the lung parenchyma. We also utilized the effective mixing of the two solutions by aerosol to form a more even layer of fibrin in continuity with the penetrated fibrinogen, which is converted to fibrin by the spray. The present study suggests that rubbing and spray method may help the fibrin seal to reach its full strength faster compared with the other two methods. Histological findings, at least in part suggest that this may be due to the initial deeper penetration of fibrin into the lung parenchyma. We speculate that because of this, the attached surface area of fibrin was initially greater in rubbing and spray method compared with the other two methods. Presumably, this difference became insignificant with the formation of tissue-derived fibrin. We evaluated TachoComb and double layer method as the most widely used methods. Double layer method is the application method recommended by most manufacturers, and is therefore, presumably most often used. It is encouraging that both these methods provided satisfactory sealing effect beyond 24 h. Control experiments, in which no sealant was used, was not performed due to ethical reasons. In our preliminary studies, the alveolar leakage created in this experiment did not stop spontaneously, and respiratory distress was unavoidable even with the use of chest tubes. Regarding rubbing and spray method, there was haemorrhage underneath the fibrin layer at 3 h, which resolved at 6 h. Presumably this was caused by rubbing. This may in part explain the significant decrease in seal-breaking pressure in rubbing and spray method at 3 h. A less invasive way to infiltrate the fibrinogen solution, for instance the use of a soft sponge, is currently being studied.

References


