Elastomeric surgical sealant for hemostasis of cardiovascular anastomosis under full heparinization

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Received 22 April 2007; received in revised form 18 June 2007; accepted 25 June 2007; Available online 31 August 2007

Abstract

Purpose: We developed a novel surgical sealant, a viscous diisocyanated prepolymer, applicable to arterial hemostasis. The purpose of this study is to evaluate hemostatic effect of this surgical sealant under heparinized conditions. Methods: The effectiveness of this sealant was verified by applying it to the end-to-end anastomosis of canine carotid arteries. Five mongrel dogs were used. After a complete heparinization, the carotid arteries were clamped, divided, and end-to-end anastomoses were performed with four simple interrupted sutures. The sealant was coated on the anastomosis. After 5 min the clamps were removed and the hemostatic effect was evaluated. Three dogs were immediately subjected to macroscopic evaluation. Two dogs were subjected to angiography after 3 months and 16 months, respectively. Results: No bleeding occurred in any of the anastomoses immediately after the removal of the clamp. Macroscopic finding revealed no leakage of the sealant into the lumen. Carotid angiography revealed patent anastomoses without stenosis. Conclusion: A novel surgical sealant exhibited rapid and potent hemostatic effect on a moisturized tissue under full heparinization.

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Keywords: Vascular anastomosis; Carotid artery; Surgical sealant

1. Introduction

Complete and highly reliable hemostasis of anastomoses is essentially required for cardiovascular surgery. Several tissue sealants have been clinically used [1—7]. Regardless of the type of such sealants, a major common limitation is that they do not work well on highly moisturized tissues and particularly for fibrin glue under heparinized conditions. The sealant developed here is a fluorinated alkyl diisocyanate-endcapped poly(ethylene glycol-co-propylene glycol). The chemical structure of the sealant is shown in Fig. 1 [8]. The advantages of this surgical sealant are its relatively rapid curing characteristics in the presence of water, tight contact adhesion to topological tissues owing to its high water-uptake characteristic, and high elastomeric property of the cured sealant. Due to its elastomeric property, the sealant pulsates synchronously with arteries, and alleviates compliance mismatch with arteries, which may minimize stress concentration at the site of pulsating arterial anastomoses.

In this study, we examine the hemostatic effect of this tissue sealant on well-moisturized tissues of canine common carotid arteries under heparinized conditions and discuss the potential applicability of this adhesive in cardiovascular anastomosis.

2. Material and methods

2.1. Animal care

All animals received humane care in compliance with the ‘Principles of Laboratory Animal Care’ (the National society for Medical Research, USA), the ‘Guide for the Care and Use of Laboratory Animals’ (National Institutes of Health, USA; Publication No. 56–23, revised 1985), the Guidelines for Animal Experiments in the Faculty of Medicine, Kyushu University, and Law (No.105) and Notification (No.6) of the Japanese Government. The experiments were reviewed by the Committee of Ethics in Animal Experiments in the faculty of Medicine, Kyushu University.
2.2. Surgical sealant

The sealant was prepared by one-pot reaction of fluorinated hexamethylene disocyanate with copolymer of poly(ethylene glycol) (PEG) and poly(propylene glycol) (PPG) (weight ratio: 80 wt.% of PEG, 20 wt.% of PPG, molecular weight: approximately $4 \times 10^3$ g/mol; Fig. 1). The sealant synthesized is a viscous liquid of diisocyanate-endcapped prepolymer. Liquid PEG–PPG copolymer segment has very high water-uptake character, whereas fluorinated isocyanate group reacts with water to form cross-linking and polymerization. Although diisocyanates, such as tolylene diisocyanate, are known carcinogenic substances, current fluorinated hexamethylene disocyanate has no carcinogenic or mutagenic potential [9]. The design concept, detailed synthesis procedure, curing rate, elastomeric properties, tissue adhesivity, and workability were described previously [8, 9].

2.3. Animal preparation and vessel anastomosis

Five dogs weighing 15—17 kg were anesthetized with sodium thiamylal (30 mg/kg body weight, intravenous), followed by atropine sulfate (0.04 mg/kg body weight, intramuscular), and then intubated with an endotracheal tube. Vecuronium bromide (0.1 mg/kg body weight) was administered intravenously for muscle relaxation. Anesthesia was maintained with inhaled isoflurane (1—2%). The left and right carotid arteries (vessel diameters approximately 3—4 mm) were exposed in three dogs. Remaining two dogs were subjected to carotid angiography 3 months and 16 months after the surgery, respectively. In these dogs, only the left carotid artery was prepared for the anastomosis. During the operation, systolic blood pressure, measured with a femoral arterial catheter, was maintained above 140 mmHg. Heparin (1 mg/kg) was infused from the venous line before arteriotomy. Activated clotting time (ACT) of more than 1000 s was maintained throughout the experiment (normal ACT value is less than 150 s).

The arteries were clamped and divided. An end-to-end anastomosis was performed with four simple interrupted sutures using a 5-0 polypropylene suture material. Before applying the sealant, the distal clamp was transiently released and checked whether bleeding from the anastomosis occurs (Video 1). After reapplying the clamp, we sprayed the site of anastomosis with 5 ml of saline to keep the anastomosis in a moist condition. Then, the sealant (0.5 ml) was applied to the suture line using a 1 ml syringe. After 5 min, both the distal and proximal clamps were removed. Three dogs (six anastomoses) were sacrificed immediately after the operation. The anastomoses were perfused with 4% formalin for 30 min at 80 mmHg. The anastomoses were sectioned longitudinally to inspect whether the adhesive had protruded into the lumen. In two dogs (two anastomoses), the anastomoses were visualized with angiography at 3 months and 16 months after the operation. After performing angiography, the anastomoses were fixed with formalin as described above, and the specimens were stained with hematoxylin–eosin for histological examination.

3. Results

Anastomoses of common carotid arteries were performed under non-coagulable state with full heparinization (ACT: $125 \pm 15$ s and more than 1000 s before and after heparinization, respectively) and under physiological blood pressures (systolic and diastolic blood pressures; $162 \pm 13$ mmHg and $85 \pm 10$ mmHg before clamping and $176 \pm 18$ mmHg and $88 \pm 12$ mmHg after sealant application, respectively). Before the application of the sealant, all anastomoses bled when the distal clamp was transiently released (Table 1; Video 1). When the sealant was coated on the moisturized common carotid artery after the anastomosis, the viscous liquid sealant stayed at the coated site without spreading or sagging, and became a sponge-like elastomer with time (Fig. 2). When the clamps were removed 5 min after coating, no bleeding at any anastomoses was observed (Video 2). No additional sealant application was required. Pulsation at anastomoses was maintained. Fig. 3 shows a longitudinal macroscopic section of the anastomoses, which was obtained shortly after the sealant was applied.
applied to the anastomosis. No leakage of the sealant into the lumen was observed. On angiograms at 3 months and 16 months after the operation, the anastomoses were patent and no stenosis or pseudoaneurysm was observed (Fig. 4A and B). The applied sealant remained on the tissue 16 months after the operation. The luminal surface of the anastomosis was covered with a monolayer of regenerated cells, possibly endothelial cells. No intimal hyperplasia was detected. In the arterial wall, neither necrosis nor tumor growth was observed (Fig. 5A and B).

4. Discussion

In this study, we demonstrated the high efficacy of hemostatic effect of the developed sealant in moisturized and heparinized arterial anastomosis. As for workability,
mechanism is not linked with blood coagulation cascade reaction, complete hemostasis could be achieved even when the patient is on cardiopulmonary bypass before neutralization of heparin. This feature will be a great advantage for those patients with severe coagulopathy suffering from suture hole bleeding, such as those who undergo complicated aortic surgery for acute aortic dissection.

(3) Reduced compliance mismatching: Another beneficial feature of this surgical sealant is its highly elastomeric property of cured sealant. In addition to the inherent elasticity of polyurethane, the microporous structure of sponge enhances its elastomeric property. The elastomeric property of the sealant may minimize compliance mismatch and stress concentration at the anastomosis, thereby preventing turbulent flow at the anastomosis. It has been known that compliance mismatch between a vascular prosthesis and a native artery causes turbulent flow at the anastomosis. A similar phenomenon might occur at the site of anastomosis if a noncompliant sealant such as cyanoacrylate [7] was used. A turbulent flow at the junction or anastomosis may lead to intimal hyperplasia, resulting in graft stenosis or obstruction. In the present study, angiography performed 3 months and 16 months after the surgery showed neither stenosis nor occlusion of the carotid artery.

To date, several surgical sealants are available for clinical use. Biological sealants such as fibrin glue, albumin-based Bioglu, and gelatin-based GRF glue have inherent concerns regarding viral transmission and hypersensitivity, depending on the preparation and source. The latter two protein-based glues require formalin or glutaraldehyde for cross-linking or curing, the cured glue is hard and brittle and therefore compliance mismatch results in the spontaneous delamination from applied tissue. Some degree of elasticity, however, is incorporated into a newly designed glue (octyl-type) [10]. Regardless of biological or synthetic sealant, reliable adhesivity is not expected on highly moisturized tissue. On the other hand, the present liquid sealant is highly water-absorbable and curing proceeds on reaction with water, both of which enforce high adhesivity derived from high contact with topological tissue surface and good compliance matching due to durable elastomer (not hydrogel).

In this experimental model, we used only four stitches of simple interrupted sutures to test the sealant under severe and unpractical conditions. The sealant, as will be applied clinically, should be used to increase safety margin of the anastomosis performed with standard techniques. Although the sealant is water-absorbable, excessive moisture on tissue may impair sealing performance.

We could not make a control group without applying the sealant, because the animals would have bled to death. It was thus not practical to test the possibility of spontaneous hemostasis with local pressure application with sponges. In spite of not making a control group, we transiently removed the clamp before applying the sealant and confirmed massive bleeding from the anastomosis (Video 1). We considered from of two long-history sealants (fibrin and cyanoacrylate glues).

The distinct difference from other sealants is that only this sealant is water-absorbable and water-reactive, and produces an elastomer on tissues with high topological contact.

A totally synthetic surgical sealant, CoSeal, is a two-component solution of PEGs, each of which is derivatized with different types of reactive groups at their terminus. These aqueous solutions are mixed together and applied to form a hydrogel, thus helping to stop leaks in blood vessels. The potential toxic byproduct is produced by the reaction between two endcapped reactive groups. Although cyanoacrylate glue (methyl-type) is characteristic of very rapid curing, the cured glue is hard and brittle and therefore compliance mismatch results in the spontaneous delamination from applied tissue. Some degree of elasticity, however, is incorporated into a newly designed glue (octyl-type) [10].

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Table 2

<table>
<thead>
<tr>
<th>Appearance</th>
<th>Present sealant</th>
<th>Fibrin sealant</th>
<th>Cyanoacrylate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured</td>
<td>Moderately viscous liquid</td>
<td>Two individual solutions</td>
<td>Very fluid liquid</td>
</tr>
<tr>
<td>Adhesive strength</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial water absorption rate (g/g/min)</td>
<td>0.21</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Equilibrium water uptake (g/g)</td>
<td>1.8</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Gelation time (min)</td>
<td>1–3</td>
<td>NA</td>
<td>Less than 2</td>
</tr>
<tr>
<td>Elasticity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet 100% modulus (kgf/cm²)</td>
<td>5.5</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Wet stretching ratio (%)</td>
<td>855</td>
<td>NA</td>
<td>0</td>
</tr>
</tbody>
</table>

NA: not available.

a The amount of PBS uptake per 1 g of adhesive at the first 1 min of immersion.

b The amount of PBS uptake per 1 g of adhesive during 30-min immersion at 25 °C.

c The gel time of the equal weight mixture of adhesive and water, determined under agitation by a glass rod.

d Two callogen films, subjected to pre-wetting with water, were adhered under the load of 100 g/cm² for 5 min at 98% moisture atmosphere, and then adhesive strength was determined at the loading rate of 300 mm/min.

e The maximum stretching in PBS.
these observations that significant differences in terms of the amount of bleeding would be apparent with or without applying the sealant.

An obvious limitation of this study is the small number of experiments especially for long-term angiographical and pathological follow-up. More detail and follow-up period study on angiography and pathological examinations is now ongoing using several experimental models, which will be reported in the near future.

The sealant had undergone safety and toxicity test with small animal experiments complying with the regulation of Pharmaceutical and Medical Devices Agency of Japan. The sealant has been approved by Pharmaceutical and Medical Devices Agency of Japan to start multi-institutional clinical trails in patients undergoing thoracic aorta replacement. The clinical application of this sealant is expected to reduce morbidity in the most demanding operations in the field of cardiovascular surgery.

Acknowledgements

The authors thank Ms Megumi Katayama for her technical assistance.

References


Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejcts.2007.06.046.