Prevention of postoperative pericardial adhesions with a hyaluronic acid coating solution

Experimental safety and efficacy studies

Postoperative pericardial adhesions complicate reoperative cardiac procedures. Topical application of solutions containing hyaluronic acid have been shown to reduce adhesions after abdominal and orthopedic surgery. The mechanism by which hyaluronic acid solutions prevent adhesion formation is unknown but may be due to a cytoprotective effect on mesothelial surfaces, which would limit intraoperative injury. In this study, we tested the efficacy and safety of hyaluronic acid coating solutions for the prevention of postoperative intrapericardial adhesion formation. Eighteen mongrel dogs underwent median sternotomy and pericardiotomy followed by a standardized 2-hour protocol of forced warm air desiccation and abrasion of the pericardial and epicardial surfaces. Group 1 (n = 6) served as untreated control animals. Group 2 (n = 6) received topical administration of 0.4% hyaluronic acid in phosphate-buffered saline solution at the time of pericardiotomy, at 20-minute intervals during the desiccation/abrasion protocol, and at pericardial closure. The total test dose was less than 1% of the circulating blood volume. Group 3 (n = 6) served as a vehicle control, receiving phosphate-buffered saline solution as a topical agent in a fashion identical to that used in group 2. At resternotomy 8 weeks after the initial operation, the intrapericardial adhesions were graded on a 0 to 4 severity scale at seven different areas covering the ventricular, atrial, and great vessel surfaces. In both the untreated control (group 1, mean score 3.2 ± 0.4) and vehicle control (group 3, mean score 3.3 ± 0.2) animals, dense adhesions were encountered. In contrast, animals treated with the hyaluronic acid solution (group 2, mean score 0.8 ± 0.3) characteristically had no adhesions or filmy, transparent adhesions graded significantly less severe than either the untreated control (group 2 versus group 1, p < 0.001) or vehicle control (group 2 versus group 3, p < 0.001) animals. In separate experiments, six baboons were infused with 0.4% hyaluronic acid in phosphate-buffered saline solution in volumes equivalent to 2.5%, 5%, and 10% of the measured circulating blood volume. The 2.5% and 5% infusions had no effect on the parameters measured; infusion of the 10% volume produced transient hemodynamic, coagulation, and gas exchange abnormalities. Hyaluronic acid solutions are efficacious in the prevention of pericardial adhesions in this model, and they appear safe in doses five times the...
As reoperative cardiac procedures become more commonplace, research efforts toward the reduction or elimination of postoperative mediastinal and pericardial adhesions have intensified. Prior work has focused primarily on autogenous (fascia lata) and heterogenous (bovine, porcine, and equine pericardium) and synthetic (silicone, polytetrafluoroethylene) pericardial substitutes, providing a barrier to adhesion formation between the heart and overlying sternum. Unfortunately, inconsistent results have been reported with the use of these materials; in particular, problems of epicardial scarring and late calcification of the implants have occurred in both the experimental and clinical setting. These issues have led several investigators to discourage their continued use.7-10

Pharmacologic reduction of postoperative adhesion formation involving mesothelial surfaces has previously been described. Antiinflammatory agents have been used with some success,11, but concerns about wound healing have limited their general use. Topical use of fibrinolytic agents, particularly tissue plasminogen activator, has been studied12,13 although experimental use within the pericardium has been associated with excessive postoperative bleeding and swelling.14 A third pharmacologic approach involves the use of hydrophilic polymer solutions to "coat" mesothelial and other tissue surfaces, thus preventing postoperative adhesion formation. Efficacy with this method, using solutions containing agents such as dextran15 and polyvinylpyrrolidone,16,17 has been suggested in both the peritoneal and pericardial cavities. The exact mechanism by which these substances prevent adhesions is unknown but may be related to mechanical protection of serosal surfaces,16 thus limiting intraoperative injury. Alternatively, these solutions may facilitate mesothelial fibrinolysis.18

One particularly promising polymer is hyaluronic acid, a component of the extracellular matrix in mammals that has been conserved throughout evolution. It is ubiquitous within the human body and is found as a normal constituent of pericardial fluid. Dilute solutions of hyaluronic acid, in addition to being nonantigenic, are extremely slippery, even at very low concentrations. Hyaluronic acid solutions have been shown to reduce postoperative adhesion formation after abdominal19 and orthopedic operations.20

We hypothesized that topical use of a hyaluronic acid coating solution might reduce the intrapericardial adhesion formation seen at reoperation. To examine this question, we used an experimental model of severe pericardial adhesions, comparing hyaluronic acid–treated animals with appropriate control animals. In addition, separate studies were carried out to assess the safety of this agent; hemodynamic, hematologic, coagulation, and gas-exchange parameters after large parenteral doses were evaluated.

Methods

The protocols described herein were reviewed and approved by the Subcommittee on Animal Care, Massachusetts General Hospital (efficacy study) and Boston University School of Medicine (safety study). All animals received humane care in compliance with the "Principles of Laboratory Animal Care," formulated by the National Society for Medical Research, and the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health (NIH Publication No. 80-23, revised in 1985).

Efficacy study

Experimental groups. Eighteen mongrel dogs, weighing between 20 and 25 kg (mean 23.2 kg), were used in this study. The animals were divided equally into three experimental groups. Group 1 (n = 6) consisted of animals that underwent the experimental adhesion protocol without topical application of an experimental coating solution. These animals constituted the untreated control group. Group 2 (n = 6) underwent the protocol with topical application of a 0.4% solution of hyaluronic acid in phosphate-buffered saline (PBS) solution as described later. Group 3 (n = 6) underwent the same protocol, with application of PBS solution alone as described later. These dogs constituted the vehicle control animals.

Experimental protocol. All animals were fasted overnight before the surgical procedure. Anesthesia was induced with intravenous thiopental sodium (Pentothal; 10 mg/kg) followed by endotracheal intubation and maintenance of anesthesia with 0.5% to 2% halothane in oxygen with a volume ventilator. Cefazolin (1 gm) was given intravenously immediately before the operation. Lactated Ringer’s solution was administered intravenously to replace losses incurred during fasting plus maintenance at 3 ml/kg per hour.

With the use of aseptic technique, a median sternotomy was performed followed by midline pericardiotomy. The pericardium was suspended open with ligatures, so that the heart and pericardial space were widely exposed. To simulate dissection used in coronary bypass operations, we dissected free the fat pad on the anterior surface of the aorta and removed it. The heart and parietal pericardium were then subjected to a 2-minute period of forced warm air desiccation and mechanical abrasion.

*Hyaluronic acid solution was kindly supplied by the Genzyme Corp., Cambridge, Mass.
with a coarse gauze sponge. All epicardial and pericardial surfaces, in addition to the anterior surfaces of the great vessels, were similarly treated. The desiccation/ablation sequence was repeated every 20 minutes for 2 hours. The pericardium in all animals then was closed with 4-0 polyglactin acid suture material (Vicryl, Ethicon, Inc., Somerville, N.J.) followed by sternotomy and skin closure. The pericardial space and both pleural spaces were drained to underwater suction, with the drains removed at emergence from anesthesia. The animals were awakened and allowed to recover from anesthesia, with butorphanol (Stadol, 0.1 mg/kg intramuscular) given for postoperative pain relief.

In addition to the adhesion protocol, animals in groups 2 and 3 underwent topical application of either the dilute hyaluronic acid solution (group 2) or PBS solution alone (group 3) at the following times: at pericardiotomy, just before and after each desiccation/ablation episode, and at pericardial closure. At each application, 5 ml of solution was used, with aspiration of excess fluid (usually about 3 ml) from the pericardial well. This protocol resulted in a total dose of approximately 14 ml, or less than 1% of each animal’s total blood volume. All epicardial, pericardial, and great vessel surfaces were evenly coated.

Eight weeks after the initial procedure, resternotomy was performed. After midline pericardiotomy, six intrapericardial areas (each area named according to the involved epicardial surface) were evaluated with regard to adhesion formation: anterior, lateral, posterior, and inferior ventricular surfaces and left and right atrial surfaces. In addition, the intrapericardial great vessel surfaces were evaluated. The adhesions were graded on a scoring system of increasing severity by an observer blinded to the experimental groups: Grade 0 indicated that no adhesions were present; grade 1 adhesions were filmy, light, and transparent, with minimal fibrous stranding; grade 2 adhesions were continuous, but avascular, and could be taken down by blunt dissection; grade 3 adhesions were more significant, with some vascularity, and required sharp dissection; and grade 4 adhesions were dense, marked by obliteration of tissue planes. The animals were then killed with a pentobarbital overdose.

### Statistical analysis
The collected data produced seven adhesion “scores” for each animal, yielding 42 scores for each experimental group. All data was collected as whole numbers except where indicated. A mean adhesion score was generated on a scoring system of increasing severity by an observer blinded to the experimental groups: Grade 0 indicated that no adhesions were present; grade 1 adhesions were filmy, light, and transparent, with minimal fibrous stranding; grade 2 adhesions were continuous, but avascular, and could be taken down by blunt dissection; grade 3 adhesions were more significant, with some vascularity, and required sharp dissection; and grade 4 adhesions were dense, marked by obliteration of tissue planes. The animals were then killed with a pentobarbital overdose.

### Systemic safety study
This study was designed to assess the effect of a large parenteral infusion of 0.4% hyaluronic acid in PBS solution, equivalent to 2.5%, 5%, or 10% of the animal's measured blood volume. Hemodynamic, gas exchange, hematologic, and coagulation parameters were evaluated as described later. A separate infusion of PBS solution alone, equivalent to 10% of the blood volume in each animal, served as a control. Healthy male baboons (n = 6), weighing between 27 and 36 kg (mean 30.2 kg), were used in this study.

### Experimental protocol
Approximately 1 week before the study, each baboon’s red cell volume was measured with 51Cr-labeled autologous red blood cells, and the plasma volume was measured with 125I-labeled albumin. From these data, volumes of infusion solution equivalent to 2.5%, 5%, and 10% of the circulating blood volume in each animal were determined. Each animal served as its own control and was thus studied on four occasions: once after infusion of PBS solution in a dose equal to 10% of the blood volume (control) and then after infusions of test material in doses equal to 2.5%, 5%, and 10% of the total blood volume. The order in which the control and test infusions were given was randomized.

On the initial study day for each infusion, the animals were anesthetized with intramuscular ketamine (4 mg/kg), repeated as needed to maintain anesthesia. The right femoral artery was cannulated for mean arterial pressure measurement. A flow-directed pulmonary arterial thermodilution catheter was placed via the right internal jugular vein for measurement of central venous pressure, mean pulmonary arterial pressure, mean pulmonary arterial wedge pressure, and cardiac output. After a steady state was achieved, baseline samples were taken, followed by intravenous infusion of either the test or control material over a 15-minute period. Sampling was done before infusion and 0.5, 1, 4, and 6 hours and 1, 2, 3, 7, 14, 21, and 28 days after infusion.

### Hematocrit value, hemoglobin value, white blood cell count, and platelet count
were measured with an automated cell counter (model JT, Coulter Corp., Hialeah, Fla.). Whole blood viscosity was measured with a porous bed viscometer.21 Blood pH, oxygen tension, carbon dioxide tension, and methemoglo-

### Table I. Distribution of adhesion scores

<table>
<thead>
<tr>
<th>Score</th>
<th>Untreated controls</th>
<th>HA solution*</th>
<th>Vehicle controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atria</td>
<td>Anterior</td>
<td>Posterior</td>
<td>Lateral</td>
</tr>
<tr>
<td></td>
<td>3.5 ± 0.5</td>
<td>2.8 ± 0.4</td>
<td>3.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.8</td>
<td>0.7 ± 0.5</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>4.0 ± 0</td>
<td>3.2 ± 0.4</td>
<td>3.2 ± 0.4</td>
</tr>
</tbody>
</table>

**HA.** Hyaluronic acid. Data shown are mean ± standard deviation (n = 6 for each group).

### Table II. Summary of adhesion scores

<table>
<thead>
<tr>
<th>Score</th>
<th>Untreated controls</th>
<th>HA solution*</th>
<th>Vehicle controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>15</td>
<td>0</td>
</tr>
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<tr>
<td>4</td>
<td>14</td>
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<td>13</td>
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</tbody>
</table>

**HA.** Hyaluronic acid. Data represent raw collected scores, with 7 scores per animal, 42 scores per group.

*Group 2 versus groups 1 and 3, p < 0.001, Kruskal-Wallis test, Mann-Whitney rank sum test.
bin were measured with an automated blood gas analyzer (Nov-

aStat Profile 4, Nova Biomedical, Waltham, Mass.). Bleeding
time was measured by making a standard incision with a Sim-
plate II bleeding time device (Organon Technika, Oklahoma
City, Okla.). Prothrombin time, partial thromboplastin time,
thrombin time, and fibrinogen were measured with an auto-
mated clotting machine (Coag-A-Mate, Organon Technika).
Serum urea nitrogen and creatinine, total protein, albumin, lac-
tic dehydrogenase, alanine aminotransferase, and aspartate
aminotransferase levels were measured with an automated
chemistry analyzer (Beckman Instruments, Inc., Brea, Calif.).

Statistical analysis. Data were examined by one-way anal-
ysis of variance with repeated measures and Student-Newman-
Keuls test. Statistical significance was achieved at $p < 0.05$.

Results

Efficacy study. Table I shows the distribution of
adhesion scores observed. In all three groups, adhesion
formation was relatively uniform at all evaluated areas.
The scores recorded for groups 1 and 3, the controls,
indicate the severity of the experimental adhesions
achieved with this model.

Figs. 1 and 2 reveal typical findings seen at resternot-
omy. In both the untreated and vehicle controls, dense
adhesions were frequently encountered, so that intraperi-
cardial dissection was difficult. The adhesions were often
vascular, with virtual obliteration of tissue planes. Con-
comitant with dense adhesion formation was severe epi-
cardial scarring that obscured the epicardial vessels. In
contrast, hyaluronic acid–treated animals had either no
adhesions or light, filmy adhesions (grades 0 and 1) and
little epicardial reaction. The highest adhesion score
observed in the test group was grade 2; this adhesion was
localized along the anterior pericardial suture line and
over the anterior surface of the aorta from which the aor-
tic fat pad had been removed during the original opera-
tion.

Table II summarizes the frequency of adhesion scores
observed during the efficacy study. Mean adhesion scores
were calculated for each group, shown graphically in Fig.
3. A statistically significant difference was observed in
adhesion formation between the hyaluronic acid–treated

Fig. 1. Adhesion type. Top, Photograph of a vehicle control
shows vascular, solid adhesions necessitating sharp dissection.
Bottom, The transparent, filmy adhesions of hyaluronic acid–
treated animals, if present, were relatively easy to dissect.

Fig. 2. Epicardial scarring. Top, In the untreated control, sig-
nificant epicardial scarring was associated with adhesion for-
mation. In contrast, hyaluronic acid–treated animals (bottom)
had minimal epicardial reaction with clear visualization of cor-

nary anatomy.
Fig. 3. Overall mean adhesion score for each group, shown as mean ± standard deviation. Statistical analysis was done with Kruskal-Wallis and Mann-Whitney tests. HA, Hyaluronic acid.

Group 1: p < 0.001
Group 2: p < 0.001

Group 1
Untreated Control
Group 2
HA Solution
Group 3
Vehicle Control

Discussion

In this study we demonstrate that topical use of a dilute hyaluronic acid solution, as described, significantly reduces postoperative intrapericardial adhesion formation in an experimental canine model. Further, infusion of the hyaluronic acid coating solution up to a dose equivalent to 5% of circulating blood volume (at least five times greater than the dose shown to be efficacious in preventing adhesions) produces no changes in measured hemodynamic, gas exchange, hematologic, and coagulation parameters. Infusion of a volume equal to 10% of the intravascular blood volume results in transient hemodynamic, hematologic and coagulation abnormalities.

Mesothelial adhesion formation is thought to be initiated by injury to the serosal surface caused by ischemia, trauma, or infection. Within the pericardium, the presence of both blood and serosal injury have been identified as necessary for adhesion development. An ultrastructural study by Leak and associates, examining the temporal changes in an experimental model of pericarditis with subsequent adhesion formation, described the adhesion formation sequence after pericardial injury: (1) increased microvascular permeability within the first 24 hours, resulting in the accumulation of fluid, inflammatory cells, and fibrin within the pericardial space; (2) des-
The formation of postoperative pericardial adhesions in the clinical setting is often accompanied by a severe epicardial reaction, which can obscure coronary vessels and anatomic landmarks at reoperation. Extensive epicardial scarring has also been reported with various heterologous and synthetic pericardial substitutes. In this study, the degree of epicardial reaction observed was congruent with the amount of adhesion formation (Fig. 2), with the hyaluronic acid–treated animals having little or no scarring of the epicardial surface.

A particularly hazardous zone of adhesions associated with reoperative cardiac surgery is the area between the sternum and anterior ventricular surface, complicating sternal reentry. Prior attempts to limit adhesion formation after cardiac procedures focused primarily in this area, with a barrier (pericardial substitute) being used to prevent adherence of the right ventricular free wall to the overlying bone. A criticism of this study might be that this issue was not addressed. Because of concerns involving graft compromise and postoperative tamponade, the native pericardium is frequently left open after cardiac operations; use of an open pericardium technique in dogs produces inadequate adhesion formation because of the chest dimensions of the animal and relative lack of an anterior mediastinum. Thus, to achieve a reproducible model of adhesion formation, our experimental protocol included pericardial closure, and the study exclusively examined intrapericardial adhesions. With a closed pericardial model, we had little difficulty at resternotomy. Future studies with this material will need to examine the prevention of retrosternal adhesions, possibly with a pri-mate model.

In addition to its antiadhesion effects, hyaluronic acid solutions have been used clinically in ophthalmologic, orthopedic, and oral/maxillofacial surgery because of the unique viscoelastic properties of the material. Because of the high viscosity, administered hyaluronate solutions are retained in the anterior chamber of the eye and serve to protect fragile corneal endothelial surfaces during intraocular lens implantation. Injected into the joint space, hyaluronic acid solutions act as lubricants to provide pain relief in those with osteoarthritis and certain temporomandibular joint disorders. Interestingly, topical hyaluronic acid solutions have also been shown to be beneficial in the healing of tympanic membrane perforations.

Use of an intrapericardial hyaluronic acid coating solution during cardiac surgical procedures would invariably lead to the introduction of the material into the intravascular space, either by direct entry (e.g., arteriotomy, ventriculotomy) or, more likely, through aspiration into the cardiopulmonary bypass circuit. Hyaluronic acid is a normal constituent of serum and is rapidly catabolized within the intravascular space, with a half-life of only a few minutes. This short half-life may explain why infusion of the 0.4% hyaluronic acid solution equivalent to 2.5% or 5% of the circulating blood volume did not have a demonstrable effect on the various parameters measured. The clearance of hyaluronic acid can be described by Michaelis-Menten kinetics, and infusion of the 10% volume may briefly exceed the maximal metabolic rate, resulting in a transient increase in blood viscosity. Although whole blood viscosity is usually dependent on...
the prevailing hematocrit value, changes in viscosity in the setting of a stable hematocrit value (and arterial oxygen content) can cause independent changes in cardiac output and systemic vascular resistance.36 In addition, similar to whole blood, solutions containing hyaluronic acid act in a non-Newtonian fashion with viscosity being highly dependent on the shear rate.29 This could theoretically cause problems in the microcirculation, where the increased viscosity associated with low shear rates could induce stasis and sludging within vessels.37 However, evidence of this effect was not seen in this study, with at least indirect measurements of microcirculatory function (renal and liver function indices) remaining unchanged by the hyaluronic acid infusion.

The transient increase in bleeding time seen with the 10% volume infusion is not well explained. Evidence of platelet dysfunction in the setting of high levels of hyaluronic acid in the serum has been reported previously in patients with Wilms tumor.38 The mechanism of this platelet dysfunction is unknown but may be due to an interaction between hyaluronic acid and the platelet membrane glycoproteins. In this study, this effect was seen only at doses greater than ten times the dose effective at reducing intrapericardial adhesions.

In summary, the topical use of solutions containing hyaluronic acid reduces the amount and severity of postoperative pericardial adhesion formation in this canine model. In separate experiments in a primate model, these solutions appear to be safe and nontoxic in amounts up to five times the effective antiadhesion dose. The encouraging results of this study suggest that topical hyaluronic acid solutions hold promise in the prevention of postoperative pericardial adhesions.

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