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PREVENTION OF POSTOPERATIVE PERICARDIAL ADHESIONS WITH A HYALURONIC ACID COATING SOLUTION: EXPERIMENTAL SAFETY AND EFFICACY STUDIES

BY

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Postoperative pericardial adhesions complicate reoperative cardiac procedures. Topical applications of solutions containing hyaluronic acid (HA) have been shown to reduce adhesions following abdominal and orthopedic surgery. The mechanism by which HA solutions prevent adhesion formation is unknown, but may be due to a cryoprotective effect on mesothelial surfaces, thus limiting intraoperative injury. In this study, we tested the efficacy and safety of HA coating solutions for the prevention of postoperative

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intrapericardial adhesion formation.

Eighteen mongrel dogs underwent median sternotomy and pericardiotomy followed by a standardized two hour protocol of forced warm air desiccation and abrasion of the pericardial and epicardial surfaces. Group 1 (n = 6) served as untreated controls. Group 2 (n = 6) received topical administration of 0.4% HA in phosphate-buffered saline (PBS) at the time of pericardiotomy, at twenty 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 as a topical agent in a fashion identical to Group 2. At resternotomy eight weeks after the initial surgery, 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 HA 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 vs. Group 1, $p < 0.001$) or vehicle control (Group 2 vs. Group 3, $p < 0.001$) animals.

In separate experiments, six baboons were infused with 0.4% HA in PBS 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.

HA solutions are efficacious in the prevention of pericardial adhesions in this model, and appear safe in doses five times the amount needed to prevent adhesions. Further studies investigating the mechanism by which these solutions prevent adhesions, their optimal dose and method of application, and documentation of their safe use in humans are warranted.

ABSTRACT

Postoperative pericardial adhesions complicate reoperative cardiac procedures. Topical application of solutions containing hyaluronic acid (HA) have been shown to reduce adhesions following abdominal and orthopedic surgery. The mechanism by which HA solutions prevent adhesion formation is unknown, but may be due to a cytoprotective effect on mesothelial surfaces, thus limiting intraoperative injury. In this study, we tested the efficacy and safety of HA coating solutions for the prevention of postoperative intrapericardial adhesion formation.

Eighteen mongrel dogs underwent median sternotomy and pericardiotomy followed by a standardized two hour protocol of forced warm air desiccation and abrasion of the pericardial and epicardial surfaces. Group 1 (n = 6) served as untreated controls. Group 2 (n = 6) received topical administration of 0.4% HA in phosphate-buffered saline (PBS) at the time of pericardiotomy, at twenty 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 as a topical agent in a fashion identical to Group 2. At resternotomy eight weeks after the initial surgery, 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 HA 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 vs. Group 1, $p<0.001$) or vehicle control (Group 2 vs. Group 3, $p<0.001$) animals.

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MINI-ABSTRACT

In an experimental canine model, topical application of 0.4% hyaluronic acid in phosphate buffered saline significantly reduced postoperative pericardial adhesion formation compared to untreated and vehicle controls. In additional experiments, infusion of the test material in a volume equal to 5% of the circulating blood volume in baboons had no effect on selected hemodynamic, hematologic, gas exchange and coagulation parameters compared to controls.

INTRODUCTION

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) [1], heterogenous (bovine, porcine and equine pericardium) [2-4], and synthetic (silicone, PTFE) [5-7] 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 [8-10].

Pharmacologic reduction of postoperative adhesion formation involving mesothelial surfaces has previously been described. Anti-inflammatory 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 studied [12,13], 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 has been suggested in both the peritoneal and pericardial cavities, using solutions containing agents such as dextran [15] and polyvinylpyrrolidone [16,17]. 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 (HA), 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 lubricous, even at very low concentrations. HA solutions have been shown to reduce postoperative adhesion formation following abdominal [19] and orthopedic surgery [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, an experimental model of severe pericardial adhesions was utilized, comparing hyaluronic acid treated animals with appropriate controls. In addition, separate studies were carried out to assess the safety of this agent, evaluating

hemodynamic, hematologic, coagulation and gas exchange parameters following large parenteral doses.

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).

1. EFFICACY STUDY

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

Experimental Protocol. All animals were fasted overnight prior to the surgical procedure. Induction of anesthesia was accomplished with intravenous pentothal (10 mg/kg), followed by endotracheal intubation and maintenance of anesthesia with 0.5 to 2% halothane in oxygen using a volume ventilator. Cefazolin 1 gm intravenously was given immediately prior to surgery. Lactated Ringers was administered intravenously to replace losses incurred while fasting plus maintenance at 3 ml/kg/hr.

Using aseptic technique, a median sternotomy was performed followed by midline pericardiotomy. The pericardium was suspended open with ligatures, widely exposing the heart and pericardial space. To simulate dissection used in aortocoronary bypass surgery, the fat pad on the anterior surface of the aorta was dissected free and removed. The heart and parietal pericardium were then subjected to a two minute period of forced warm air desiccation and mechanical abrasion using a coarse gauze sponge. All

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* HA solution kindly supplied by the Genzyme Corp., Cambridge, Massachusetts, 02142

epicardial and pericardial surfaces, in addition to the anterior surfaces of the great vessels, were similarly treated. The desiccation/abrasion sequence was repeated every 20 minutes for 2 hours. All animals then underwent pericardial closure with 4-0 polyglactic acid suture material (Vicryl™, Ethicon Inc., Somerville, NJ), 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 above adhesion protocol, animals in Groups 2 and 3 underwent topical application of either the dilute HA solution (Group 2) or PBS alone (Group 3) at the following times: at pericardiotomy, just before and after each desiccation/abrasion 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 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. Following 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; grade 4 adhesions were dense, marked by obliteration of tissue planes. The animals were then euthanized 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 for each animal and subsequently for each experimental group, reported as mean ± standard deviation. The collected raw data were evaluated using the nonparametric Kruskal-Wallis test, with localization of intergroup differences using the Mann-Whitney rank sum test. Statistical significance was achieved at $p<0.05$.

2. SYSTEMIC SAFETY STUDY

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

Experimental Protocol. Approximately one week prior to study, each baboon's red cell volume was measured with ^{51}Cr labeled autologous red blood cells, and the plasma volume was measured using ^{121}I labeled albumin. Using this data, infusate volumes 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 following infusion of PBS in a dose equal to 10% of the blood volume (control), and 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 fifteen minute period. Sampling was done prior to infusion and 0.5, 1, 4, and 6 hours and 1, 2, 3, 7, 14, 21, and 28 days following infusion.

Hematocrit, hemoglobin, white blood cell count, and platelet count were measured using an automated cell counter (Model JT, Coulter Corp., Hialeah, FL). Whole blood viscosity was measured using a porous bed viscometer [21]. Blood pH, PO_2, PCO_2, and methemoglobin were measured using a automated blood gas analyzer (NovaStat Profile 4, Nova Biomedical, Waltham, MA). Bleeding time was measured by making a standard incision using a Simplate II bleeding time device (Organon Technika, Oklahoma City, OK). Prothrombin time, partial thromboplastin time, thrombin time and fibrinogen were measured using an automated clotting machine (Coag-A-Mate, Organon Technika). Serum urea nitrogen (BUN) and creatinine, total protein, albumin, lactic dehydrogenase (LDH), alanine aminotransferase (SGPT), and aspartate aminotransferase (SGOT) were measured using an automated chemistry analyzer (Beckman Instruments Inc, Brea, CA).

Statistical Analysis. Data were examined using one-way analysis of variance (ANOVA) with repeated measures and Student-Newman-Keuls test. Statistical significance was achieved at $p<0.05$.

RESULTS

1. EFFICACY STUDY

Table 1 shows the distribution of adhesion scores observed. In all 3 groups, there was relative uniformity of adhesion formation 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.

Figures 1 and 2 reveal typical findings seen at resternotomy. In both the untreated and vehicle controls, dense adhesions were frequently encountered, making the intrapericardial dissection difficult. The adhesions were often vascular, with virtual obliteration of tissue planes. Concomitant with dense adhesion formation was severe epicardial scarring, obscuring the epicardial vessels. In contrast, HA-treated animals had either no adhesions or light, filmy adhesions (Grades 0-1) and little epicardial reaction. The highest adhesion score observed in the test group was a grade 2; this was localized along the anterior pericardial suture line and over the anterior surface of the aorta where the aortic fat pad was removed during the original surgery.

Table 2 summarizes the frequency of adhesion scores observed during the efficacy study. Mean adhesion scores were calculated for each group, shown graphically in Figure 3. There was a statistically significant difference in adhesion formation between the HA-treated group (mean score 0.8 ± 0.3), the untreated controls (3.2 ± 0.4) and vehicle controls (3.3 ± 0.2). There was no significant difference in adhesion formation between the untreated controls and those controls treated with PBS vehicle alone.

2. SYSTEMIC SAFETY STUDY

2.5% and 5% Groups. The infusion of 0.4% HA in PBS in volumes equivalent to 2.5% and 5% of the baboon's measured blood volume had no significant effect on any of the measured parameters compared to the control infusion.

10% Group. Following infusion of a 0.4% HA solution in an amount equivalent to 10% of the circulating blood volume, there were no significant changes in central venous pressure, heart rate, or pulmonary arterial pressure. These animals did have a significant increase in mean arterial pressure in the first 30 minutes following infusion (+10% change), a finding not observed in the controls. Cardiac output decreased significantly (-13% change) in the first hour, which returned to baseline within 4 hours.

There was a corresponding increase in blood viscosity (+52% change), systemic vascular resistance (+26% change) and pulmonary vascular resistance (+34% change), gradually returning to pre-infusion levels by 4 hours. There were no significant changes in the arterial PO_2, PCO_2, or pH following the 10% volume infusion. The hematocrit, white cell count, and platelet count were not changed following infusion. However, the bleeding time significantly increased in the 10% group, to a level twice that of controls. This remained elevated for 72 hours post-infusion. The prothrombin time, partial thromboplastin time, thrombin time, fibrinogen levels were not significantly changed. The BUN, creatinine, total protein, albumin, SGOT, SGPT, and LDH were unchanged by the 10% infusion.

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 HA coating solution up to a dose equivalent to 5% of circulating blood volume (at least 5 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, either secondary to ischemia, trauma, or infection. Within the pericardium, the presence of both blood and serosal injury have been identified as necessary for adhesion development [22]. An ultrastructural study by Leak *et al.* [23], examining the temporal changes in an experimental model of pericarditis with subsequent adhesion formation, described the adhesion formation sequence following 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) desquamation of injured mesothelial cells by 72 hours, with attachment of inflammatory cells to denuded serosal surfaces and ongoing fibrin deposition; and 3) by one week, evidence of fibrinolysis and collagen deposition, with the collagen fibrils providing a matrix for subsequent vascular and lymphatic ingrowth. By two weeks, focal adhesions were observed, and by one month large areas of the pericardial space were obliterated by fibrosis.

Pericardial and peritoneal surfaces are known to possess fibrinolytic activity [24], which has been shown to be reduced following mesothelial injury [25]. Impaired fibrinolysis has been postulated as the

cause of postoperative adhesion formation; although originally attributed to a decrease in tissue plasminogen activator (t-PA), recent studies suggest inflamed mesothelial surfaces produce increased amounts of plasminogen activator inhibitor [26,27]. Topical t-PA solutions have been used successfully to prevent adhesion formation in a dose-dependent manner [28]; however, in addition to concerns over postoperative bleeding, at least one study has suggested topical use of t-PA might impair wound healing, as measured by wound hydroxyproline content [28].

A different approach to adhesion prevention employs the use of hydrophilic polymer solutions, which are used to coat the serosal surfaces and prevent intraoperative mesothelial injury. Preservation of mesothelium would, at least in theory, provide for retained fibrinolytic activity within the peritoneal or pericardial cavity. The relationship between viscous polymer solutions and fibrinolysis is largely unknown, although one study suggests these coating solutions may, through a barrier effect, increase the t-PA levels at the serosal surface [18]. HA molecules even in dilute solutions tend to entangle and mesh together, forming bridging networks which act sterically to impede the diffusion of other macromolecules [29]. The half-life of HA within the pericardial space is 3 to 4 days [30], beyond the onset of fibrinolysis after mesothelial injury. Furthermore, in contrast with other pharmacologic methods of adhesion prevention, increased concentrations of HA within tissues has been reported to be beneficial to wound healing [31].

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 (Figure 2), with the HA-treated animals having little or no scarring of the epicardial surface.

A particularly hazardous zone of adhesions associated with reoperative cardiac surgery are those between the sternum and anterior ventricular surface, complicating sternal reentry. Prior attempts to limit adhesion formation after open heart procedures focused primarily in this area, using a barrier (pericardial substitute) 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 *intra*pericardial adhesions. With a closed pericardial model, we experienced little difficulty at resternotomy. Future studies with this material will need to examine the prevention of retrosternal adhesions, possibly with a primate model.

In addition to its anti-adhesion effects, HA solutions have been used clinically in ophthalmologic, orthopedic, and oral/maxillofacial surgery due to 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 [32]. Injected into the joint space, HA solutions act as lubricants to provide pain relief in those with osteoarthroses [33] and certain temporomandibular joint disorders [34]. Interestingly, topical HA solutions have also been shown to be beneficial in the healing of tympanic membrane perforations [35].

Use of an intrapericardial HA 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. HA is a normal constituent of serum and is rapidly catabolized within the intravascular space, with a half-life of only a few minutes [29]. This may explain why infusion of the 0.4% HA 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 HA can be described by Michaelis-Menton kinetics [29], and infusion of the 10% volume may briefly exceed the maximal metabolic rate (V_{max}), resulting in a transient increase in blood viscosity. Although whole blood viscosity is usually dependent on the prevailing hematocrit, changes in viscosity in the setting of a stable hematocrit (and arterial O_2 content) can cause independent changes in cardiac output and systemic vascular resistance [36]. In addition, similar to whole blood, solutions containing HA 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 was not seen in this study, with at least indirect measurements of microcirculatory function (renal and liver function indices) remaining unchanged by the HA 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 HA serum levels 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 HA 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 reduce the amount and severity of postoperative pericardial adhesion formation in this canine model. In separate experiments using a primate model, these solutions appear to be safe and nontoxic in amounts up to five times the effective anti-adhesion dose. The encouraging results of this study suggest that topical HA solutions hold promise in the prevention of postoperative pericardial adhesions.

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Table 1: Distribution of Adhesion Scores

	Ventricular Surfaces				Atria		
	Anterior	Posterior	Lateral	Inferior	Right	Left	Great Vessels
Untreated Controls	3.5 ± 0.5	2.8 ± 0.4	3.2 ± 0.4	3.5 ± 0.9	3.5 ± 0.5	2.8 ± 0.4	3.8 ± 0.4
HA Solution	1.2 ± 0.8	0.7 ± 0.5	1.2 ± 0.4	0.8 ± 0.4	0	0.3 ± 0.5	1.6 ± 0.8
Vehicle Controls	4.0 ± 0	3.2 ± 0.4	3.2 ± 0.4	3.2 ± 0.4	3.3 ± 0.5	3.0 ± 0	3.2 ± 0.8

Data shown are mean ± standard deviation
n = 6 for each group

Table 2: Summary of Adhesion Scores

Score	Group 1 Untreated Controls	Group 2 HA Solution*	Group 3 Vehicle Controls
0	0	15	0
1	0	21	0
2	4	6	1
3	24	0	28
4	14	0	13

Data represent raw collected scores, with 7 scores per animal, 42 scores per group
* Group 2 vs. Groups 1 and 3, $p < 0.001$, Kruskal-Wallis test, Mann-Whitney rank sum test

Figure 1

Adhesion type. The upper photograph of a vehicle control shows vascular, solid adhesions requiring sharp dissection. The transparent, filmy adhesions of HA-treated animals, if present as in the lower photo, were relatively to dissect.

Figure 2

Epicardial scarring. In the untreated control (upper photograph), significant epicardial scarring was associated with adhesion formation. In contrast, the HA-treated animals (lower photograph) had minimal epicardial reaction with clear visualization of coronary anatomy.

Figure 3

Overall mean adhesion score for each group, shown as mean ± standard deviation. Statistical analysis using Kruskal-Wallis and Mann-Whitney tests.

Figure 1

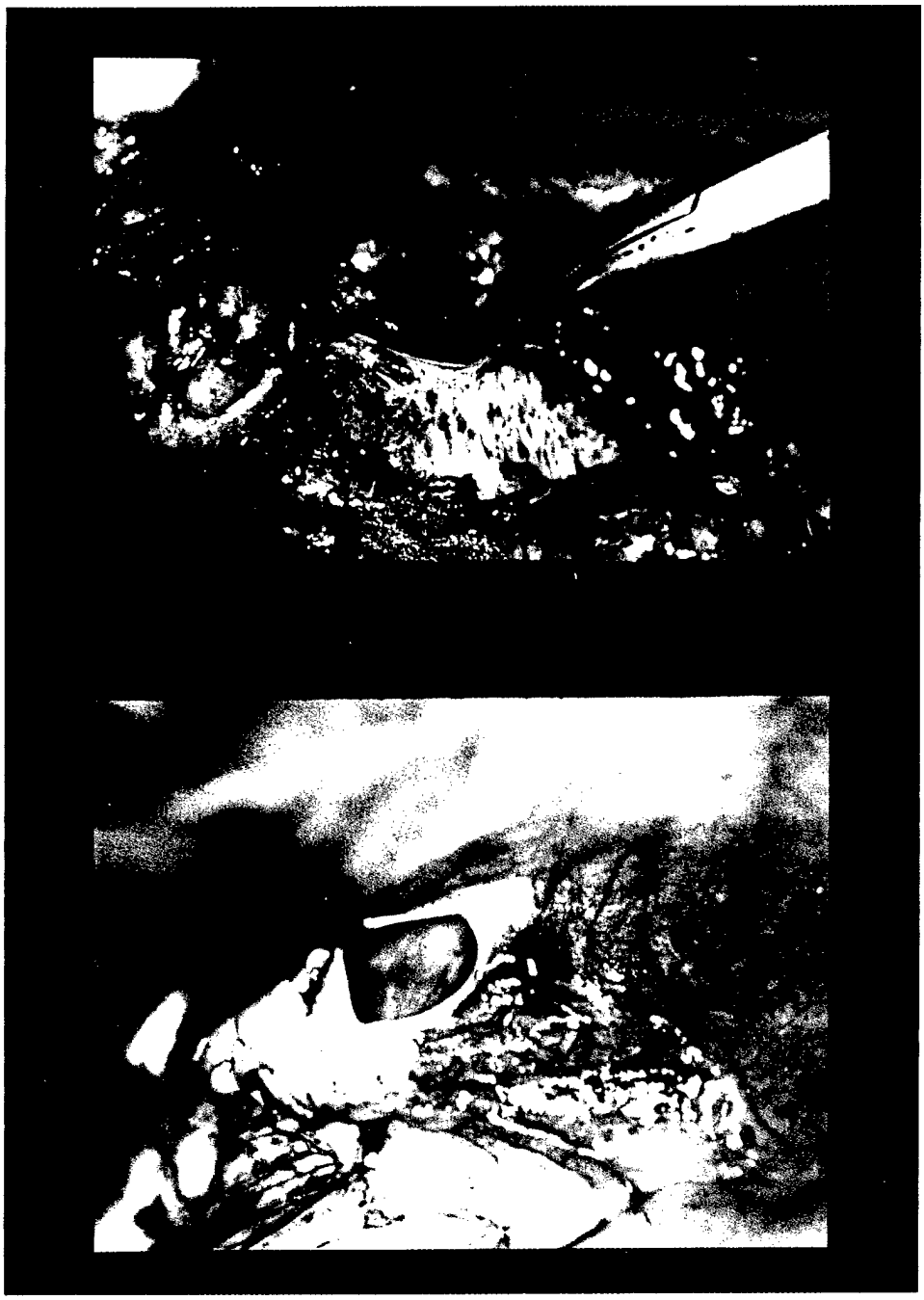

Figure 2

Figure 3