

Prevention of Pericardial Adhesions with N-O Carboxymethylchitosan in the Rabbit Model

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ABSTRACT The presence of mediastinal adhesions significantly increases the morbidity and mortality of reoperative cardiac surgical procedures. Previous investigations have reported on the therapeutic utility of topical hydrogels in reducing the formation of postsurgical adhesions. The goal of the present study is to evaluate the ability of N-O carboxymethylchitosan (a glycosaminoglycan hydrogel derivative) to reduce the formation of postsurgical pericardial adhesions in a large-animal model. Sixteen adult New Zealand white rabbits were randomly assigned to one of two treatment groups. Group 1 subjects ($n = 8$) had N-O carboxymethylchitosan directly applied to the heart and retrosternal surfaces after sternotomy was performed, while subjects in group 2 ($n = 8$) had saline applied to these areas. After a period of 14 days the animals were sacrificed under anesthesia, and independent observers, blinded to treatment, graded the formation of pericardial adhesions. The severity of adhesion formation was significantly less in the group treated with N-O carboxymethylchitosan ($p < .01$). This study demonstrates that N-O carboxymethylchitosan markedly decreases the formation of poststernotomy adhesions in a large-animal model without untoward cardiac side effects. This hydrogel derivative may prove to be of great therapeutic value when used prophylactically in the setting of cardiac surgery.

KEYWORDS cardiac surgery, hydrogels, pericardial adhesion formation

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Surgical trauma is the most common cause of adhesion and scar formation. The presence of adhesions significantly increases the morbidity and mortality of reoperative cardiac surgery [1-7]. Several investigations have reported on the ability of topically applied hydrogel

compounds to reduce the formation of post surgical adhesions [8–12]. Although the exact mechanism by which these compounds prevent adhesions is unknown, it appears that they may act as an extracellular matrix substitute [13]. Components of this matrix, such as glycosaminoglycans, interact with matrix proteins in a specific and highly structured manner and play a major role in cellular migration and activation [14–19].

N-O carboxymethylchitosan (NOCC) is a glycosaminoglycan hydrogel derivative, with properties similar to the extracellular matrix [13]. This study examines the therapeutic ability of NOCC to reduce the formation of postsurgical pericardial adhesions. Using a surgical sternotomy pericardial adhesion model in the rabbit [20], we show that the topical application of NOCC markedly diminishes postoperative adhesion formation.

MATERIALS AND METHODS

Animals

Subjects were 16 adult male or female New Zealand white rabbits (*Oryctolagus cuniculus*), obtained from the Millbrook Farm Breeding Labs (Amherst, MA). Rabbits were individually housed with ad libitum access to water and high-fiber rabbit chow (PMI Feed, Inc., Richmond, IN).

Materials

NOCC was provided by Chitogenics, Inc. (Cedar Knolls, NJ). A 2% NOCC solution was prepared by dissolving 2 NOCC in 100 mL phosphate-buffered saline solution (sodium phosphate 10 mmol/L, NaCl 0.15 mol/L, pH 7.4) and then heating the solution in an autoclave. An NOCC gel was produced by cross-linking the 1% NOCC solution with dialdehyde and allowing the mixture to remain at room temperature for greater than 7 days. This procedure produces a viscous gel that could be applied with a syringe.

Procedure

After an overnight fast, each rabbit received buprenorphine (0.03 mg/kg subcutaneously) 45 to 120 min

prior to surgery for perioperative analgesia. Induction of anesthesia was accomplished with ketamine hydrochloride (35 mg/kg) and xylocaine (5 mg/kg) administered intramuscularly. The rabbits were endotracheally intubated and mechanically ventilated throughout the procedure. Anesthesia was maintained with halothane (0.5–1.5%) and 50% nitrous oxide. Rabbits received 50 mL lactated Ringer's solution intravenously during the procedure.

The subjects were assigned to one of two study groups by random number allocation. The first group ($n = 8$) had NOCC applied to the heart and retrosternal surfaces after sternotomy was performed, while subjects in group 2 ($n = 8$) had saline applied to these areas as a control.

In a fashion described by Okuyama, a 4- to 5-cm median sternotomy incision was performed, beginning at the xyphoid process and continuing rostrally [20]. After the sternum was bisected, a self-retaining sternal retractor was placed and the pericardium was opened, exposing the retrosternal surface of the heart. The anterior and lateral surfaces of the heart were abraded with surgical sponges and desiccated using oxygen at a flow rate of 5 L/min. This procedure was repeated three times. Rabbits receiving NOCC treatment had a total of 4–5 mL NOCC gel spread directly over the retrosternal surface of the heart and on the retrosternum. Following application of the gel, 2–3 mL of 2% NOCC solution was spread over the surrounding tissue. Control rabbits had an equal amount of 0.9 normal saline instead of NOCC spread over the same areas that NOCC was applied to. The incision was closed in two layers and the pericardium was left open. Closure of the sternum and muscle layers was accomplished with an interrupted horizontal mattress suture using 2-0 Vicryl (Ethicon, Somerville, NJ) The skin was closed with a 2-0 Vicryl suture in a subcuticular pattern.

All subjects had continuous two-lead electrocardiograph monitoring during surgery and recovery from anesthesia. Buprenorphine (0.03 mg/kg subcutaneously) was administered to all subjects for postoperative discomfort as needed. Fifty milliliters of lactated Ringer's solution was administered subcutaneously for supportive care in some rabbits on postoperative day 1. All subjects were observed twice

daily for the first 4 days after surgery and then daily thereafter until the 14th postoperative day.

On day 14 postprocedure, each rabbit was sedated with acepromazine maleate (1 mg/kg sq) followed by euthanasia with sodium pentobarbital (200 mg/kg intravenously). The sternum was carefully reflected rostrally to score for the presence of adhesions as previously described by Krause et al. [21].

Adhesions were graded by two independent observers who were blinded to treatment group, with the use of a grading scale (0 = no adhesions; 1 = mild transparent adhesions; 2 = multiple adhesions that easily separate; 3 = multiple thick adhesions).

Statistical Analysis

Data are presented as means and standard deviations, analyzed by Duncan's multiple range test with a one-way analysis of variance; differences were considered significant at $p < .05$.

RESULTS

As assessed on postoperative day 14, the control animals showed consistent and dramatic thoracic and mediastinal adhesions and fibrosis. Adhesions between the epicardium and undersurface of the sternum were severe and could not be taken down digitally. Animals receiving the topical application of NOCC, in contrast, showed very mild adhesions (see Figure 1). Pericardial adhesion scores for the two groups are summarized in Table 1. The severity of adhesion formation was significantly less in the group treated with NOCC (0.9 ± 0.6 for the NOCC treated group versus 2.75 ± 0.3 for the control group, $p < .01$).

DISCUSSION

In this standard animal adhesion model [6–10], NOCC markedly reduced adhesion formation and

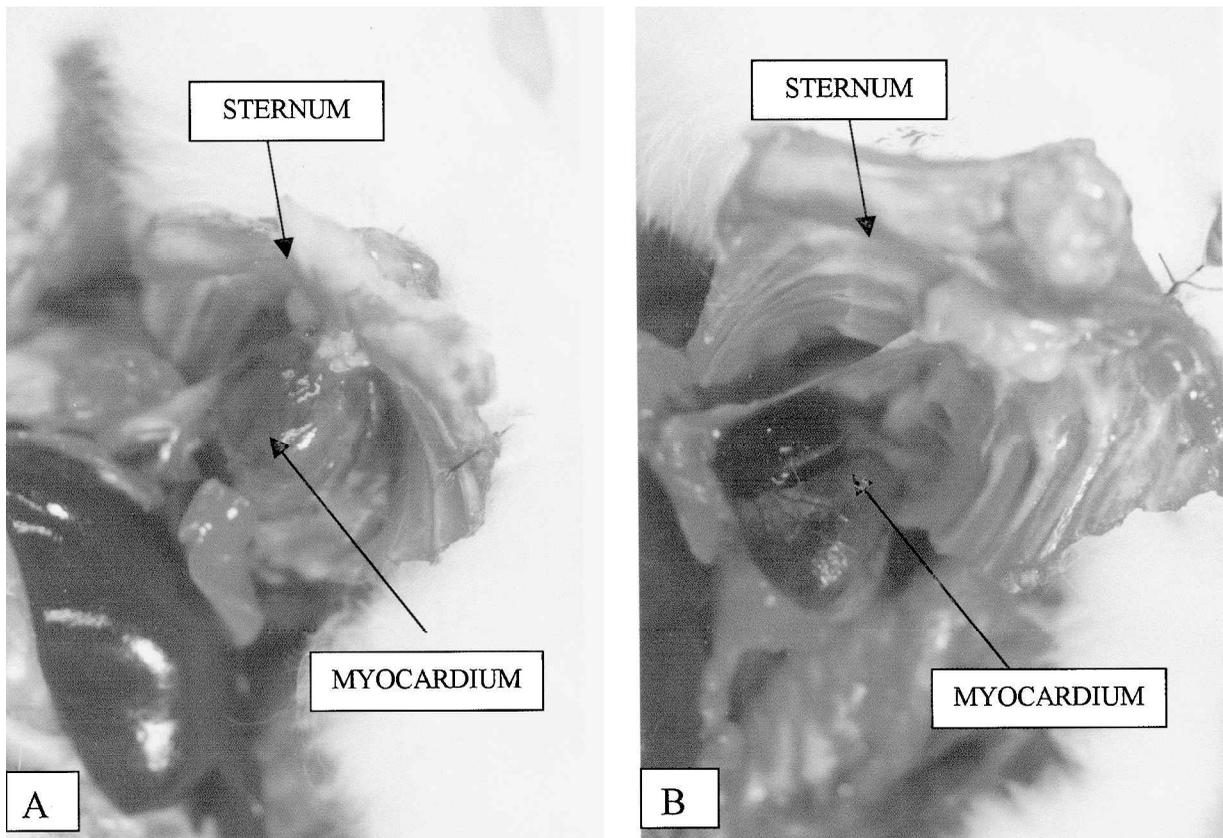


FIGURE 1 Myocardium that has been revealed by reflecting the sternum rostrally. (A) Significant adhesion formation in a control subject that was treated with saline only. Note that the myocardium is completely adherent to the retrosternal surface. (B) Absence of adhesion formation in a subject treated with NOCC.

TABLE 1 Pericardial adhesion evaluation scores

Subject	Saline		NOCC	
	Observer A	Observer B	Observer A	Observer B
	1	2	1	2
2	1	2	3	2
3	1	0	2	3
4	1	1	3	3
5	1	1	3	3
6	1	1	2	3
7	0	0	3	3
8	0	1	3	3
Mean \pm SD	0.9 \pm 0.6		2.7 \pm 0.3	

prevented scar formation. NOCC application on the surface of the beating heart did not cause any arrhythmias or ischemic changes in the immediate perioperative period. NOCC was easily applied to all traumatized surfaces. In addition, there were no signs of cardiac tamponade resulting from gel in the pericardial cavity. The pericardium was left open at the end of the procedure to better simulate the condition in open heart surgery. There are published studies concerning the efficacy of NOCC [21–23], but this is the first study utilizing a large-animal, beating heart, poststernotomy model.

A number of devices and techniques have been developed to avoid surgical scar and adhesion formation [1–7], including topical compounds that primarily function on the traumatized surface [8–12]. These compounds include hydrogels that are water soluble and biocompatible, with properties similar to those of the extracellular matrix [13]. NOCC has properties similar to hyaluronic acid, an extracellular matrix glycosaminoglycan that has been shown to play a regulatory role in wound healing and cell differentiation [15]. Thus, the function of NOCC in preventing wound fibrosis may be similar to that of hyaluronic acid.

The early inflammatory events during tissue injury are characterized by an acute exudation of cells (mainly neutrophils and macrophages) into the traumatized tissue. This response results in the efflux of extracellular matrix proteins, which provide a scaffold on which fibroblasts adhere. Between 4 and 6 days after injury, there is deposition of collagen that signals the beginning of fibrosis and ultimately leads to adhesion formation [20]. The movement of

neutrophils, macrophages, and fibroblasts through the extracellular matrix is intrinsic to many tissue processes including wound healing. The extracellular matrix plays a key role in regulating cell function and differentiation during this process.

NOCC is a new hydrogel derivative of chitin, the second most abundant polymer in nature [24]. Chitin can be deacetylated to yield the positively charged compound chitosan, and the addition of carboxymethyl groups to chitosan's nitrogen and oxygen centers renders the polymer negatively charged and water soluble [25]. NOCC is hydrophilic, lubricious, and viscoelastic. The cross-linked NOCC form produces a uniform gel that can be coated on traumatized surfaces. NOCC remains on the denuded mesothelial surfaces until it is degraded, and the resulting small-chain polysaccharides are then absorbed [25].

The mechanism of NOCC's activity on the traumatized surfaces is unclear. NOCC's function may be multifactorial. It has been shown that properties of the polymer surface, such as charge, influence their ability to absorb proteins [13, 25]. For example, hydrophobic hydrogels absorb large amounts of the extracellular matrix glycoprotein fibronectin. Adhesions are formed when collagen becomes bound to the collagen-binding site of fibronectin. Because NOCC is hydrophilic and negatively charged, it is likely to have a low affinity with fibronectin [13]. Thus, NOCC may act to prevent the hydrophilic interactions between the extracellular matrix molecules necessary for adhesion formation.

It is possible that NOCC may mediate the inflammatory response to tissue injury. Although *in vitro* assays for neutrophils and fibroblasts have been performed [22], *in vivo* studies will be requisite to delineate NOCC's role in inflammatory response regulation. Finally, NOCC may simply act as a resorbable barrier that maintains tissue separation.

In conclusion, the chitin derivative N-O-carboxymethylchitosan markedly decreases fibrosis and adhesion formation in a cardiac surgery animal model, without associated untoward cardiac side effects. This material may prove to be of great therapeutic utility in reducing the morbidity and mortality associated with reoperative cardiac surgery.

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