Prevention of postoperative adhesions with the chitin derivative N-O-carboxymethylchitosan

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The ideal barrier agent for the prevention of surgical adhesions has remained elusive. We have examined the ability of a new hydrogel N-O-carboxymethylchitosan, a derivative of chitin with properties similar to the extracellular matrix, to prevent adhesions when applied topically to traumatized mesothelial surfaces. In two rodent adhesion models (pericardial and peritoneal), the application of N-O-carboxymethylchitosan significantly prevented or minimized the formation of scar and fibrosis. According to a scoring system from 0 to 3 (0 = no adhesions and 3 = severe dense adhesions), control groups in each model consistently produced severe dense adhesions (2.9 ± 0.2, 2.7 ± 0.3). All treated groups consistently scored less than 1.0, indicating minimal or no fibrosis. The differences between the control and treated groups were statistically significant ($p < 0.05$). Thus, the application of N-O-carboxymethylchitosan to traumatized mesothelial surfaces may have significant potential in the prevention of postoperative adhesion formation. (WOUND REP REG 1996;4:53-57)

Surgical trauma is the most common cause of adhesion and scar formation. Adhesions increase the morbidity and mortality of reoperative surgery severalfold.[1-7] Multiple barrier products (topical hydrogels) have been investigated for minimizing the occurrence of postoperative 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 the extracellular matrix including glycosaminoglycans interact with matrix proteins in a specific and highly structured manner and play a major role in cellular migration and activation.[14-19]

We have examined the potential of N-O-carboxymethylchitosan (NOCC), a glycosaminoglycan hydrogel derivative of chitin with properties similar to the extracellular matrix,[13] to prevent surgical induced adhesion formation in the rat. Using both a pericardial foreign body model and an abdominal cecal abrasion model, we show that the topical application of NOCC markedly diminishes postoperative adhesion formation.

NOCC	N-O-carboxymethylchitosan

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MATERIALS AND METHODS

NOCC was provided by Chitogenics Inc. (Cedar Knolls, N.J.). A 2% NOCC solution was prepared by dissolving 2 gm NOCC in 100 ml of phosphate-buffered saline solution (sodium phosphate 10 mmol/L, NaCl 0.15 mol/L, pH 7.4) by heating in an autoclave. A NOCC gel was produced by polymerizing 100 ml of the 2% NOCC solution with 1.2 ml of 4% glyoxal and allowing the mixture to remain at room temperature overnight. This procedure produces a viscous gel which can be manipulated with a syringe.

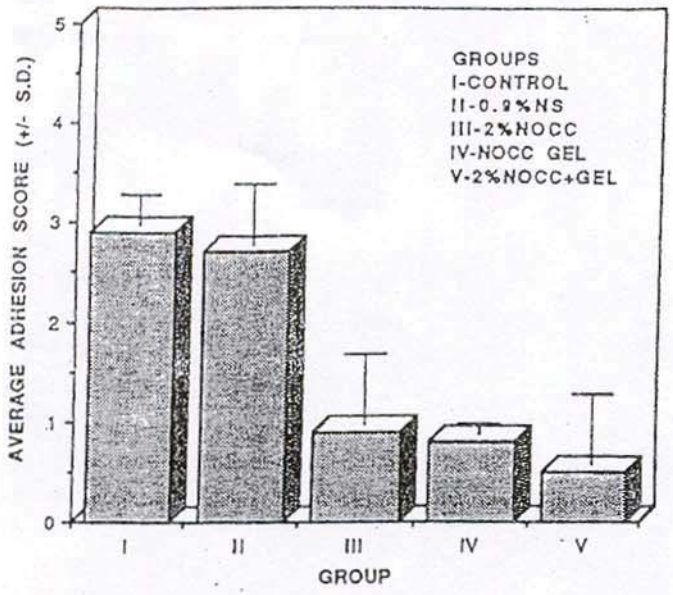

Figure 1 Effect of NOCC on the formation of pericardial adhesions. Adhesions were induced with 100 mg/ml magnesium silicate, with or without inclusion of indicated concentrations of NOCC solution or gel, and scored after 7 days. Results represent the mean ± standard deviation from three experiments of six animals per experimental group for a total *n* of 18 animals per group.

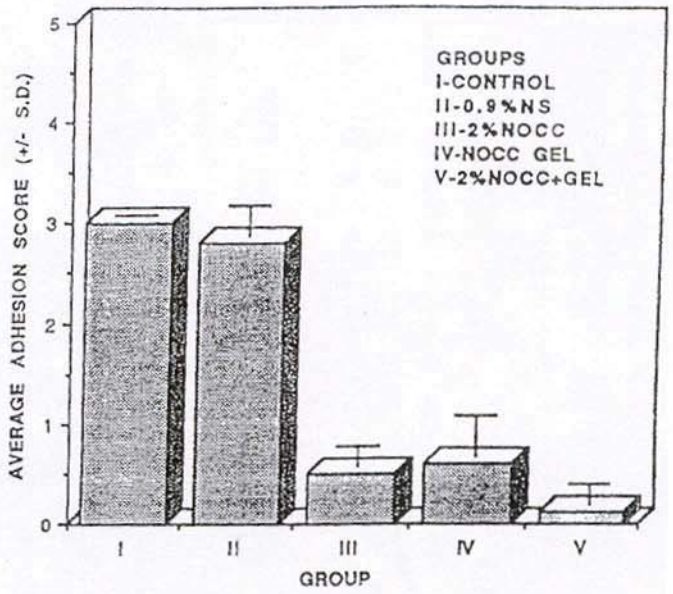

Figure 2 Effect of NOCC on the formation of cecal adhesions with NOCC. Adhesions were induced by cecal abrasion, with or without inclusion of the indicated concentration of NOCC solution or gel, and scored after 7 days. Results represent the mean ± standard deviation from three experiments of six animals per experimental group for a total *n* of 18 animals per group.

Animal protocol

Male Sprague-Dawley rats (approximately 250 gm; Taconic Farms, Inc., Germantown, N.Y.) were assigned to one of five experimental groups consisting of six animals each, with each experimental set repeated a minimum of three times. The data shown in Figure 1 and 2 represent all three experiments combined for a total *n* of 18 animals per treatment group. Animals were anesthetized with an intraperitoneal administration of sodium pentobarbital, and pericardial or peritoneal adhesions were induced as described later. Animals in group I received either talc slurry alone (pericardium) or abrasion alone (peritoneum). Group II was similar to group I with the topical application of 1 ml of normal saline solution. Group III received the topical application of a 2% NOCC solution (1 ml pericardium or 3 ml peritoneum). Group IV received 2% NOCC gel (1 ml pericardium or 3 ml peritoneum). Group V received a combination of gel and solution (a total of 2 ml in pericardium or 5 ml in peritoneum). All animals were killed for analysis after 7 days. No side effects were observed as a result of trauma in any group of animals. Animal protocols were approved by the UMDNJ Institutional Animal Care and Use Committee.

Pericardial adhesion model

Animals were anesthetized, the lower midline thorax was shaved and the skin was disinfected with 70% ethyl alcohol. A 1 cm skin incision was then made in the midline of the lower sternal area. The pericardial region was entered between the intercostal space and, 1 ml of 10% medical grade talc (10 mg/ml magnesium silicate; Isomedex, Inc.) slurry was applied topically over the heart with a syringe. This procedure was followed by topical application of either saline solution or NOCC. The wound was closed in two layers with 4-0 silk, and animals were returned to their animal care facility.

Cecal abrasion model

For this procedure, the lower abdomen was shaved, and the skin was disinfected with 70% ethyl alcohol. A 2 cm lower midline incision was made, and the abdomen was entered. The cecum was isolated, and a dry 4 × 4 gauze (12 Ply-USP Type VII, Kendall, Johnson & Johnson Medical, Inc. Arlington, Tex.) was used to abrade the surface (2 × 1 cm area) until punctate hemorrhages were visible as previously described.[11] This procedure was followed by the topical application of the study material. The wound was closed in two layers with 4-0 silk.

Adhesion measurement

Pericardial adhesions were assessed on anesthetized animals through a transverse plane of the upper abdomen. By elevating the lower costal border, the dia-

phragm was incised near its costal attachments to expose the heart without violating the substernal space. The abdominal model was similarly assessed after a transverse incision was made in the upper abdomen, then extended inferiorly. This produced a flap that exposed the cecum without violating the original wound incision and preperitoneal space above the cecum. Adhesions were graded by four independent, single blinded observers with the use of a grading scale from 0 to 3 (0 = no adhesions; 1 = mild transparent adhesions; 2 = multiple adhesions that easily separate; 3 = multiple thick dense adhesions).

Statistical analysis

Data are presented as mean ± standard deviation, analyzed by Duncan's Multiple Range Test with a one-way analyses of variance. Differences were considered significant at $p < 0.05$.

RESULTS

The ability of NOCC to prevent pericardial adhesions was assessed with the use of two distinct experimental protocols. Control animals receiving talc solution, either with or without a posttreatment saline solution injection, showed consistent and dramatic thoracic adhesions and fibrosis (Figure 1). Adhesions between pericardial and thoracic wall were frequent and severe, without indications of compromised cardiac function. Animals receiving topical NOCC application, in contrast, showed dramatically reduced frequency of adhesions, and, where present, the level of severity was diminished considerably (Figure 1). This included animals that received either a 2% NOCC solution (experimental group III), NOCC gel (group IV), or a combination of solution plus gel (group V). The difference in relative estimated severity between the NOCC-treated and control group was statistically significant ($p < 0.05$). In group V, the topical application of both NOCC solution and gel appeared to inhibit adhesion formation greater than when NOCC solution or gel was used alone, although this was not statistically significant.

We also examined the effects of NOCC in a cecal abrasion model in which adhesions are induced by mechanical stress. With the use of this model, adhesions were less dramatic than in the talc-induced pericardial model. However, control animals consistently showed intracecal adhesion scars and often had adhesion plaques between cecum and the adjacent organs such as liver. Relative to the controls, NOCC-treated animals had a dramatic reduction in adhesion formation (Figure 2). Again, animals treated with the combination of NOCC solution plus gel showed almost no adhesions but rather showed a smooth glistening mesothelial surface.

DISCUSSION

In this study we have shown that NOCC markedly decreased postoperative adhesions in two distinct rodent models of adhesion formation. In both pericardial and peritoneal models, NOCC caused a threefold reduction in the level of adhesions observed. 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, which 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 injured tissue with injury to the mesothelial lining.[20] The submesothelial tissue is associated with a marked inflammatory vascular response. 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, and the extracellular matrix plays a key role in regulating cell function and differentiation during this process.

It has been shown that hyaluronic acid induces interleukin-1 production in rabbit macrophages.[21] Interleukin-1 promotes collagenase and proteoglycan synthesis and depresses the synthesis of collagen, the major constituent of adhesions.[20] Hyaluronic acid also inhibits cell migration in collagen gels in vitro.[19] Additionally, hyaluronic acid reduces the adhesion of inflammatory cells to various substrata. This effect may be related to the polymer's surface charge or viscosity.[13,16] Clinically, hyaluronic acid has been shown to reduce adhesion formation in a canine model of peri-

cardial adhesions, although the mechanism of action remains unknown.[12]

NOCC is a new hydrogel derivative of chitin, the second most abundant polymer in nature,[22] with properties similar to hyaluronic acid. Chitin can be deacetylated to yield the positively charged compound chitosan, and the addition of carboxymethyl groups to chitosan's nitrogen centers renders the polymer negatively charged and water soluble.[23] 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 before being degraded, and the resulting small chain polysaccharides are then absorbed.[23]

The mechanism of NOCC's activity on the traumatized surfaces is unclear. NOCC is a hydrophilic glycosaminoglycan with a physical similarity to the extracellular matrix. As such, NOCC's function may be multifactorial. It has been shown that properties of the polymer surface, such as charge, influence their ability to adsorb proteins.[13,23] For example, hydrophobic hydrogels absorb large amounts of the extracellular matrix glycoprotein fibronectin. The collagen binding domain of fibronectin provides a scaffold during wound healing on which collagen binds to form adhesions. 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.

There may also be a role for the specific molecular conformation of the hydrogel. It is generally accepted that hydrophilic amino or hydroxyl groups are activators of complement activation, whereas the hydrophilic carboxyl groups are inhibitors.[13] Complement activation plays a significant role in tissue injury and adhesion formation, and the hydrophilic carboxymethyl groups of NOCC may block this activation. Finally, the influence of hydrogels on lymphocytes has also been shown. Chitin has been shown to inhibit the respiratory burst in neutrophils, in contrast to hyaluronic acid which enhanced neutrophil activation.[24] The reactive oxygen intermediates of neutrophil activation have been implicated in the tissue destructive events during inflammation.[25] In addition, the ability of hydrogels to induce macrophage activation is related to the occurrence of functional chemical groups, the carboxyl moiety having the least effect on macrophage activation.[13]

In conclusion, this study shows that the anionic compound NOCC, a hydrogel derivative of chitin, prevents adhesions in both a standard cecal abrasion model and a talc foreign body model. It is unclear, however, at what level NOCC effects the pathogenesis of adhesions. NOCC, as a glycosaminoglycan with properties similar to the extracellular matrix, may interact with the inflammatory exudate at multiple levels. Further research is necessary to determine its precise role of action at both the cellular and extracellular matrix level.

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