Prevention of experimental postoperative peritoneal adhesions by N,O-carboxymethyl chitosan

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Background. *Postsurgical adhesion formation can result in significant morbidity and, to a lesser extent, death. The purpose of this experiment was to assess the ability of N,O-carboxymethyl chitosan (NOCC) to prevent postsurgical adhesion formation in vivo.*
Methods. *Randomized groups of Sprague-Dawley rats were studied under two abdominal surgery models, the uterine horn model and the small bowel laceration model, for the ability of NOCC to reduce the incidence and severity of adhesion formation. Adhesions in animals were assessed after death by a blinded observer 10 to 14 days after surgical manipulation.*
Results. *NOCC consistently reduced the size, strength, and number of adhesions in both rat models. NOCC was also found to be more effective than hyaluronic acid at inhibiting adhesion formation.*
Conclusions. *NOCC is a more effective antiadhesion agent than is the more expensive hyaluronic acid. Although the exact mechanism of NOCC's antiadhesion activity is as yet unclear, the novel chemical is of particular interest for clinical use. (Surgery 1996;120:866-70.)*

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FIBROUS ADHESIONS are a major cause of intestinal obstruction.[1] Moreover, they can result in ureteral obstruction, fistula formation, and chronic abdominal-pelvic pain and can interfere with fluid distribution in the peritoneum.[2] Peritoneal trauma, resulting from such diverse effects as drying, ischemia, thermal injury, infection, or the presence of a foreign body, has long been recognized as a stimulus for adhesion formation.[3] Many of these stimuli are unavoidable consequences of surgery. Because of difficulties in eliminating the trauma of surgical manipulation, recent efforts to lower the incidence of adhesion formation have been focused on inhibiting the body's response to these stimuli.

Hyaluronic acid (HA), a glycosaminoglycan component of extracellular matrix, has been shown to play a role in normal wound healing[4,5] and in inhibiting pericardial[6] and peritoneal[7,8] adhesion formation. However, HA has proved unattractive in a clinical setting because of its high cost and limited efficacy.[9,10]

N,O-carboxymethyl chitosan (NOCC) is a novel agent with structural similarities to HA.[11] NOCC is available in a variety of forms depending on the nature and extent of cross-linking. We sought to investigate the antiadhesion potential of a 2% NOCC solution and a more viscous cross-linked 1% NOCC gel in two clinically relevant rat models of abdominal surgery: the uterine horn model and the small bowel laceration model.

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MATERIAL AND METHODS

NOCC. NOCC is a derivative of chitosan, which is a long-chain polysaccharide. The addition of carboxymethyl groups to chitosan's nitrogen and oxygen centers produces a water-soluble, negatively charged biocompatible polymer that is hydrophilic, lubricious, and viscoelastic. NOCC is nontoxic, either in vitro in fibroblast culture assays or in vivo in experimentation with intraperitoneal, oral, or subcutaneous treatment at concentrations higher than described in this experiment. NOCC was administered as a sterile 2% solution initially spread evenly over the peritoneal contents in the area of the incision and then over the entire peritoneal area before closure. NOCC was also administered as a sterile cross-linked gel directly to the site of the incision.

Uterine horn model. Adult female Sprague-Dawley rats (Harlan Sprague Dawley) weighing 200 to 250 gm were lightly anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg), and a midline incision was made in the abdomen. With a stainless steel retractor the left uterine horn was revealed. A portion

of the uterine horn serosa measuring approximately 5 mm was excised. In addition, an area of approximately 5 mm^2 of retroperitoneum was abraded with a scalpel until petechia could be detected. The damaged area of the uterine horn was then approximated to within 5 mm of the abrasion and was held in place with a single 6.0 Prolene (Ethicon, Inc., Peterborough, Ontario, Canada) suture. Two groups of 10 animals were used. One group served as an untreated control, whereas the other was treated with 1 ml 1% NOCC gel directly to the site of the incision and 3 ml 2% NOCC solution distributed evenly across the incision site and peritoneal contents before closure. Ten days later the animals were killed, and a blinded observer examined the severity of the adhesions.

The size of adhesion planes was measured with engineer's calipers, and the severity of adhesions was assessed by gentle retraction after the sutures were removed. A scale of 1 to 4 was used to score the strength of adhesive plaques. The scale is defined as follows: (1) adhesion was filmy and easily torn with very light pressure, (2) adhesion was substantial and needed moderate pressure to tear, (3) adhesion was heavy and required significant pressure to rupture, and (4) adhesion was very heavy and needed substantial pressure to rupture.

Small bowel model. Male Sprague-Dawley rats (Harlan Sprague Dawley) weighing 250 to 300 gm were anesthetized with sodium pentobarbital (60 mg/kg). A midline incision was made in the abdominal cavity, and the abdomen was exposed with a stainless steel retractor. Under an operating microscope the cecum was located and drawn posteriorly out of the animal onto a pad of sterile, saline solution–soaked gauze. A site was located approximately 3 cm from the ileocecal junction for sectioning. This site was always distal to the last ileal Peyers patch and away from major omental vessels. The ileum was cut with microsurgical scissors to an extent of 50% of its circumference. The cut was always opposite the omental attachment and was repaired with five 5.0 polyglactin 910 sutures. Each suture was left untied until all were in place to ensure that the back wall of the ileum was not mistakenly included in the suture placement. The animal was closed with running 4.0 nylon sutures. This procedure was adapted from Adams et al.[12] with slight modification. Three groups of 10 animals were in the study: a control group that was untreated, a group receiving 1 ml 1% cross-linked NOCC gel applied directly to the site of the incision and 3 ml 2% NOCC solution intraperitoneally (as described previously) after operation, and a group receiving 3 ml 0.4% HA[6, 8-10] (Sigma Chemical Company, St. Louis, Mo.) intraperitoneally after operation.

Fourteen days after operation the animals were killed, and a blinded observer assessed the extent of adhesion formation. The frequency and intensity of adhesions were recorded. Adhesion intensity was graded between 0 and 5 as follows: 0, no adhesions; 1, thin filmy adhesion; 2, more than one thin adhesion; 3, thick adhesion with focal point; 4, thick adhesion with planar attachment; and 5, very thick vascularized adhesions or more than one planar adhesion.

In addition to recording the frequency and intensity of adhesions, we recorded the frequency of adhesions resulting in the intestine folding back on itself at the suture site. This folding back, or "kinking," occurred because of adhesions on the omental side of the intestine across to the suture site. Mild kinking was recorded when the angle of the fold was less than 90 degrees, moderate kinking was defined as greater than 90 degrees but less than 160 degrees, and severe kinking approximated a 180-degree folding back of the intestine. Obvious blockage of intestinal flow by this severe kinking was recorded.

Statistics. Data were analyzed by either the Mann-Whitney *U* test or Fisher's exact (two-tailed) test depending on the distribution of the data points within the groups.

RESULTS

Uterine horn. Analysis of animals in the uterine horn model revealed a reduction in adhesion formation in the NOCC-treated group compared with the control group. The mean planar width of adhesions in the control group was 3.38 ± 0.48 mm, whereas the mean size in the NOCC-treated group was significantly lower at 1.30 ± 0.68 mm (Fig. 1, *A*) ($p < 0.001$). In addition, adhesion strength (Fig. 1, *B*) in the control group was substantial. Four of the ten animals had scores of 4, and the mean strength was 3.4 (± 0.4) in this group. The mean strength of adhesions in the NOCC-treated group was 1.0 (± 0.6), with only one animal exhibiting an adhesion score of 2 or greater.

Small bowel model. The results of the small bowel model are presented in Fig. 2. Fig. 2, *A* shows the incidence of adhesions involving the omentum, a local part of the small bowel causing kinking (kink), and the cecum. In both the NOCC- and the HA-treated groups a marked and highly significant reduction occurred in the percentage of animals with adhesions of all types ($p < 0.004$).

Of particular interest, kinking of the small bowel was observed in 40% of the control group, in 40% of the HA-treated group, but in only 10% of the NOCC-treated group. The severity of the kinking in the one (of 10) animal in the NOCC-treated group was low, and although at least one clear incidence of bowel obstruction was observed in both the control and HA-treated groups, the kinking in the animal in the NOCC-treated group was not nearly severe enough to cause obstruction.

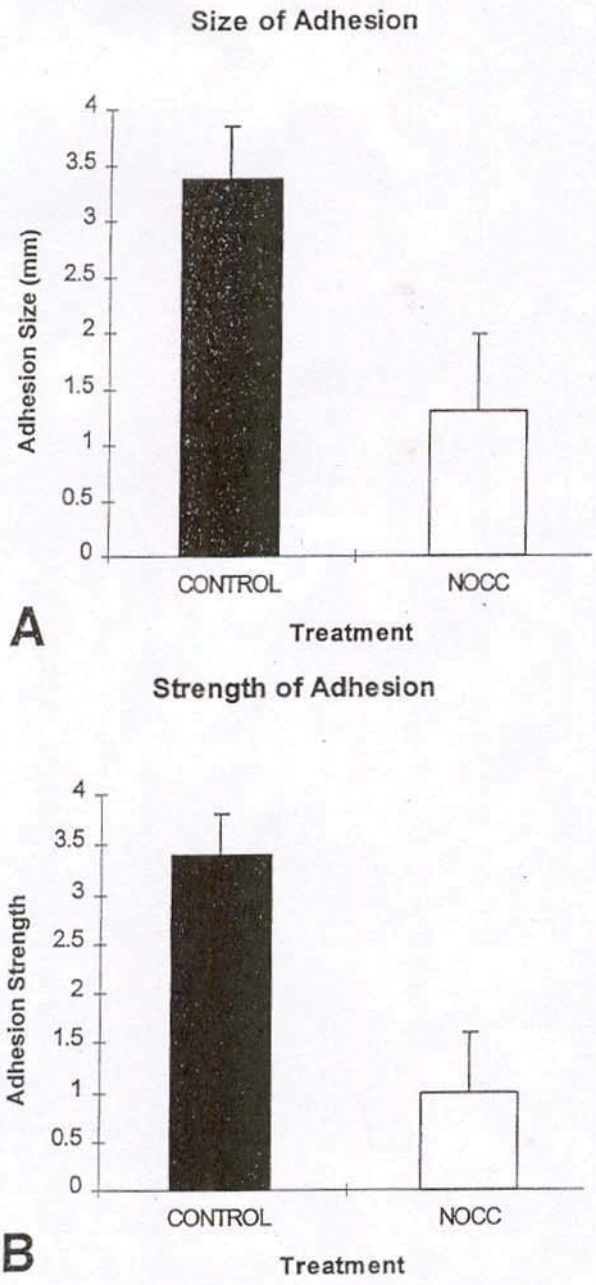

Fig. 1. Effect of NOCC on size (**A**) and strength (**B**) of adhesions in uterine horn model. Rats were subjected to surgical manipulation designed to induce adhesion formation in the uterine horn. One group was treated after operation with 1 ml 1% NOCC gel and 3 ml 2% NOCC solution at site of operation, whereas the other group served as untreated control. Ten days later adhesion size (**A**) was measured with engineer's calipers, and adhesion strength (**B**) was scored with scale of 1 to 4.

The severity of the adhesions to the omentum was highly variable in all groups but was consistently reduced in the NOCC-treated group. The mean score for the omental adhesions was 2.5 (±2.1) for the control group, 0.75 (±1.5) for the NOCC-treated group, and 0.75 (±1.5) for the HA-treated group. Because of the high variability reflected in the standard deviation, the observed reduction in the mean score for the NOCC-treated group did not reach significance when compared with that of the control group ($p = 0.07$).

The results for the cecal adhesions are shown in Fig. 2, *B*. Again, high variability was seen in the severity of adhesions in the control and HA-treated groups. Adhesions in the NOCC-treated group, however, were significantly reduced compared with those in the control group ($p < 0.01$).

DISCUSSION

NOCC significantly reduced the incidence and intensity of postoperative adhesions in both the uterine horn and the small bowel laceration models. Because many of the cells involved in adhesion formation are also involved to some extent in normal wound healing,[13] it was of concern that NOCC would nonspecifically limit healing required for healthy recovery after operation. In other experiments, however, we have demonstrated that wound healing at the site of both intestinal anastomosis and skin wound is not deleteriously affected by treatment with NOCC. No significant differences from the untreated control group were seen, for example, in the bursting strength of a repair of a large intestinal incision (at 4, 7, or 14 days after repair) with the use of NOCC at the same levels and route as described in this experiment (unpublished data). The mechanism of action of NOCC is still unclear. Adhesion formation is essentially an inflammatory reaction, with factors released that increase vascular permeability, leading to fibrinogen influx and fibrin deposition. Permanent fibrous adhesions result from the subsequent ingrowth of fibroblasts, deposition of collagen, and local neoangiogenesis.[14, 15]

In addition to these events, cells of the myeloid and granulocytic lineage have been implicated in the regulation of fibrin deposition in wound repair. Neutrophils can be seen at the wound site by 6 hours after injury, and macrophage numbers peak at 24 hours.[16, 17] Macrophages are known to accumulate locally in response to a variety of inflammatory stimuli. Macrophages affect the acellular response by secreting plasminogen activators,[18-20] which degrade fibrous deposits through their enhancement of the plasmin pathway. However, macrophages could also promote the adhesiogenetic response by activating fibroblasts.[21] Thus macrophages may have the potential to enhance or limit adhesion development.

There are several mechanisms by which NOCC may exert its antiadhesive activity including diluting fibrin in the original inflammatory exudate, affecting the activities of the inflammatory factors, or acting as a barrier by

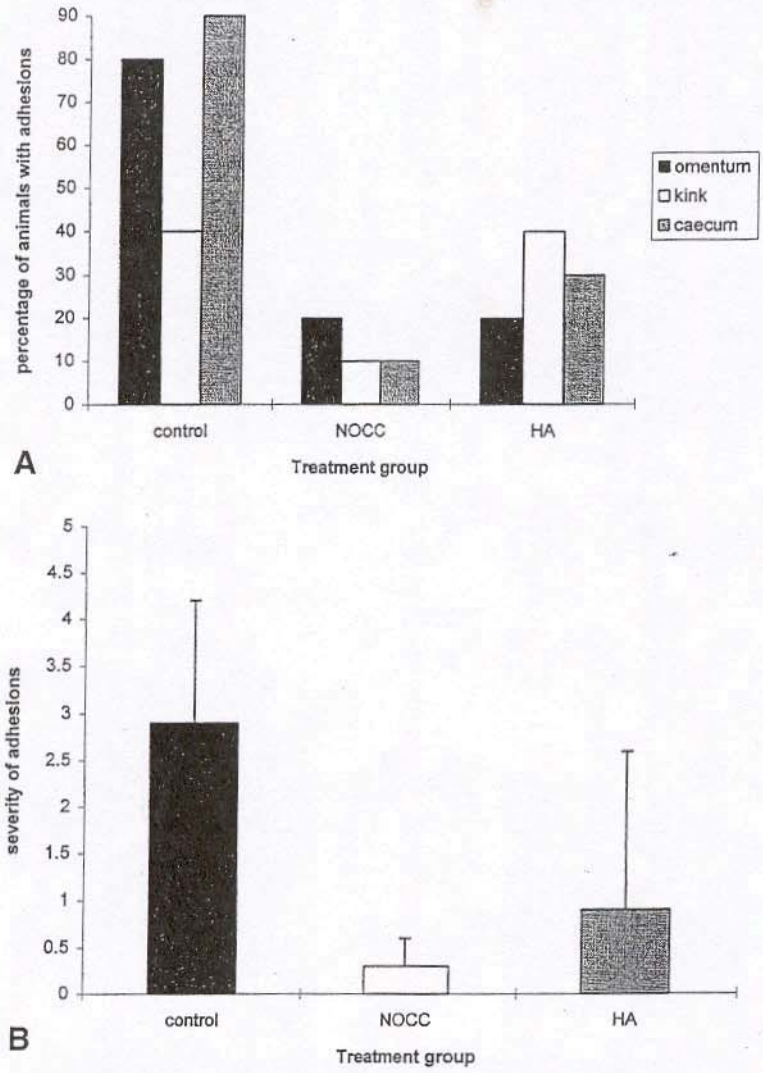

Fig. 2. Effect of NOCC on incidence (A) and severity (B) of adhesions in small bowel laceration model. Rats were subjected to surgical manipulation designed to induce adhesion formation in small bowel. One group was treated with NOCC after operation, one group was treated with HA, whereas control group was untreated. Frequency of adhesions to omentum and cecum were recorded (A). Incidence of intestinal kinking was also recorded (A). Severity of adhesions involving cecum is shown in (B).

coating the visceral surfaces. Because peritoneal trauma results in a loss of plasminogen activator activity[15] and viscous macromolecular solutions can inhibit the removal of plasminogen activator from areas of localized trauma,[22] it is possible that NOCC exerts its impact in this manner. It has been shown in other models that direct inhibition and stimulation of fibronolytic activity increase or decrease postoperative intraperitoneal adhesion formation, respectively.[23] It is interesting that the critical time course for experimental adhesion formation in the rat is approximately 36 hours,[24] a period well within the range of depressed plasminogen activator activity levels.[15]

It is also possible that NOCC may reduce adhesions by forming a barrier to the activities of inflammatory cells or factors released from them. Alternatively, it is possible that fibrin deposition may be retarded with the addition of NOCC. Because coagulation and fibrin matrix development have been shown to coincide[14] and because HA has been shown to inhibit platelet aggregation,[7] it is reasonable to speculate that this is a potential target for NOCC's antiadhesion activity.

In conclusion, the administration of NOCC reduces experimental peritoneal adhesion formation in the rat uterine horn and small bowel laceration models. We hypothesize that NOCC forms a barrier to the activation of the normal pathways of fibrin deposition, and this prevents adhesions but does not affect normal wound healing.

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