Prevention of Intra-abdominal Abscesses and Adhesions Using a Hyaluronic Acid Solution in a Rat Peritonitis Model

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Hypothesis: Hyaluronic acid (HA)–based bioresorbable membrane and 0.4% HA solution reduce intra-abdominal adhesion and abscess formation in a rat peritonitis model.

Design: Randomized laboratory experiment.

Setting: A university hospital.

Interventions: In 72 male Wistar rats, a bacterial peritonitis was induced using the cecal ligation and puncture model. Animals were randomized to receive isotonic sodium chloride solution (group 1), HA-carboxymethylcellulose bioresorbable membrane (group 2), or 0.4% HA solution (group 3). Half of each group were killed at day 7 and half at day 21, and adhesions were scored in a blind fashion. The presence and sizes of intra-abdominal abscesses were noted. Cultures were taken for bacterial analysis.

Main Outcome Measures: Intra-abdominal adhesions and abscesses.

Results: The median severity of adhesions was significantly lower in group 3 compared with group 1 rats at day 7 (II [range, I-IV] vs IV [range, I-IV], respectively; \( P = .02 \)) and at day 21 (II [range, I-III] vs IV [range, II-IV], respectively; \( P = .02 \)). There was no significant difference between group 2 and group 1 rats on either day. At day 7, abscesses larger than 2 cm were found in 6 of 12 group 1 rats and in 4 of 12 group 2 rats, but in 0 of 11 group 3 rats (\( P = .01 \)). At day 21, 0 of 11 group 3 rats had an intra-abdominal abscess, in contrast to 4 (33%) of 12 group 1 rats and 5 (45%) of 11 group 2 rats. All cultures of abscesses revealed polymicrobial flora.

Conclusion: Adhesion and abscess formation are reduced using a 0.4% HA solution, and not HA-carboxymethylcellulose bioresorbable membrane, in a rat model of generalized bacterial peritonitis.


INTRA-ABDOMINAL infection is accompanied by fibrin deposition in the abdominal cavity. This fibrin deposition may lead to adhesion and abscess formation. Both entities have significant clinical relevance. Intra-abdominal abscesses are an important cause of morbidity and mortality in patients with generalized peritonitis. Adhesions are the main cause of intestinal obstruction in the developed world. Furthermore, adhesions are responsible for 15% to 20% of cases of infertility and are associated with chronic abdominal and pelvic pain.1-3

Numerous agents have been investigated in the prevention of adhesions, eg, dextran, corticosteroids, phosphatidylcholine, phospholipase inhibitors, non-steroidal anti-inflammatory drugs, heparin, and tissue plasminogen activator.4 Agents particularly interfering with coagulation and fibrinolysis, eg, heparin and tissue plasminogen activator, have also been studied in the prevention of intra-abdominal abscess formation. However, clinical experience with these agents is limited, mainly because of fear of bleeding complications.

Recently, hyaluronic acid (HA) derivatives have been shown to be successful in preventing postsurgical adhesions in experimental and clinical studies.5-11 Hyaluronic acid is a biocompatible, nontoxic, high-molecular-weight polyanionic polysaccharide consisting of repeating units of alternating N-acetylglucosamine and D-glucuronic acid. It is found in all tissues and body fluids of vertebrates.12 Hyaluronic acid has a stabilizing effect on extracellular matrices. Furthermore, it interacts with cell surfaces to modify cell behavior.12,13

Becker et al5 demonstrated a significant reduction of intra-abdominal adhesion formation following colectomy using...
MATERIALS AND METHODS

STUDY DESIGN

Seventy-two male Wistar rats (Harlan Nederland, Zeist, the Netherlands) weighing 250 to 325 g were allowed to become accustomed to laboratory conditions for 1 week before experimental use. Animals were housed at 21°C with a day-night cycle of 12 hours. They had free access to water and standard rodent chow (Hope Farms BV, Woerden, the Netherlands). The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Nijmegen, Nijmegen, the Netherlands.

In all rats, a bacterial peritonitis was induced by performing a cecal ligation and puncture (CLP) procedure, using the methods of Wichterman et al. Food was withheld from the animals for 12 hours before the first operation. On day 0, rats were weighed and anesthetized with a mixture of halothane (Fluothane; Zeneca, Cheshire, England), nitrous oxide, and oxygen. Before the operation, the abdomen was shaved and disinfected with 70% alcohol. Via a 3-cm midline laparotomy, the cecum was dissected without damaging the vascularization and was filled backwards with feces. Thereafter, the cecum was ligated just distal to the ileocecal valve, with a 3.0 polyglactin suture (Vicryl; Ethicon Inc, Somerville, NJ), and at the antisementerial site the cecum was punctured once with a 19-gauge needle. The abdominal wall was closed in 2 layers with a 3.0 polyglactin suture. Immediately after operation, rats received a single dose of gentamicin sulfate (Centrafarm Services BV, Etten-Leur, the Netherlands), 6 mg/kg, intramuscularly and buprenorphine hydrochloride (Temgesic, Reckitt and Colman Products Ltd, Amstelveen, the Netherlands), 0.1 mg/kg, subcutaneously. All animals were resuscitated with 10 mL of isotonic sodium chloride solution administered subcutaneously.

On day 1, animals were weighed, the abdomen was reopened under anesthesia, and peritoneal fluid samples were taken and collected in an envelope (BBL Port-A-Cul; Becton Dickinson, Cockeysville, Md) for microbiological examination. The abdominal cavity was rinsed with 10 mL of isotonic sodium chloride solution, and the cecum was resected. Before closure of the abdomen, animals were randomly assigned to receive 8 mL of isotonic sodium chloride solution (group 1, n = 24), 4 × 4 cm HA-CMC bioreorbable membrane (Seprafilm; Genzyme Corporation, Cambridge, Mass) wrapped around the cecal resection site and 2 × 3 cm under the midline incision (group 2, n = 24), or 8 mL of 0.4% HA solution (Seprocoat Genzyme Corporation) instilled throughout the whole abdominal cavity (group 3, n = 24).

After 1 and 3 weeks, respectively, half of the animals in each group were weighed and killed using carbon dioxide asphyxiation. Adhesions were scored in a blinded manner by one of us (H.v.G.) according to the method of Zuhlke et al, whereby grade 0 means no adhesions and grade IV means firm extensive adhesions that are only dissectable with sharp instruments, with organ damage almost unavoidable. Sites of adhesions scored included the midline, the upper abdomen (liver), the parietal peritoneum, the omentum, and between the bowel loops.

If present, the size of abscesses was noted. An abscess was defined as a walled-off collection containing purulent material. Samples were taken from the abscesses for microbiological examination.

BACTERIAL CULTURES

Samples of peritoneal fluid and abscesses were cultured semi-quantitatively in aerobic and anaerobic conditions. Columbia III agar with 5% sheep blood (Becton & Dickinson, Etten-Leur, the Netherlands), Levine-eosin-methylene blue agar (Oxoid, Haarlem, the Netherlands), and fastidious anaerobic agar (Tapley, Bury, England) with or without kanamycin, were used for cultures. After 24 and 48 hours of incubation at 37°C, bacteria were identified using standard procedures.

STATISTICAL ANALYSIS

The statistical tests used are given within the text. Tests were performed on a personal computer using commercially available software (Stat Xact program; Statcon, Wittenhausen, Germany). Statistical significance was achieved at P < .05. All tests were 2-tailed.

RESULTS

Following CLP, all rats had symptoms of intra-abdominal sepsis. They demonstrated apathetic behavior, ocular exudates, piloerection, and diarrhea. These symptoms resolved within 2 days following the relaparotomy and removal of the necrotic, perforated cecum and peritoneal lavage. Weight loss was observed in the first week. After 1 week, rats regained weight. Differences in weight loss or weight gain were not statistically significant between the groups (P = .62, Mann-Whitney U test). Three (4%) of the 72 animals died. Survival in group 1 was 100% (24 of 24) compared with 96% (23 of 24) in group 2 and 92% (22 of 24) in group 3 (differences were not significant; P = .49, Fisher exact test). One rat died immediately after anesthesia, the other 2 rats, 1 and 4 days after CLP due to unknown causes. None of them died due to bleeding.

ADHESIONS

One week after CLP, significantly fewer rats in group 3 had grades III and IV adhesions (5/11 [45%]) than among group 1 rats (11/12 [92%]) (P = .03; Fisher exact test).
There was no significant difference in the occurrence of grades III and IV adhesions between the rats in group 2 (9/12 [75%]) and group 1 rats. The most frequent sites of adhesions were the omentum, the lateral peritoneum, between the bowel loops, and the midline incision, in that order. Three weeks after CLP, 8 of 12 control rats and 6 of 11 rats in group 2 had grades III and IV adhesions, whereas in group 3 only 2 of 11 rats had grade III adhesions, and none had grade IV adhesions (P = .04; Fisher exact test).

The median severity of adhesions after 1 and 3 weeks is shown in Figure 1. The median severity of adhesions in group 3 rats was significantly lower at 1 week (II [range, I-IV]) and at 3 weeks (II [range, I-III]) compared with group 1 rats (1 week, IV [range, II-IV]; 3 weeks, IV [range, I-IV]) (P = .02 for both comparisons; Mann-Whitney U test). There was no difference in the median severity in group 2 rats after 1 week (IV [range II-IV]) or 3 weeks (III [range, I-III]) compared with group 1 rats.

Involvement of the omentum in adhesion formation was analyzed separately. At week 1, the median severity of omental adhesions was significantly lower in group 3 compared with group 1 animals (P = .02; Mann-Whitney U test). There was no difference in the appearance of omental adhesions between groups 1 and 2. At 3 weeks, no grade III or IV adhesions of the omentum were observed in group 3 rats, whereas in 33% of the group 1 rats and 33% of group 2 rats, grade III and/or IV adhesion of the omentum was found. The difference did not reach statistical significance (P = .06; Mann-Whitney U test).

**ABSCESSES**

Abscesses were predominantly located at the cecal resection site.

At day 7, 6 of 11 rats in group 3 had abscesses, whereas this was 10 of 12 rats in groups 1 and 2; in group 3, no abscesses larger than 2 cm were observed (P = .02; Fisher exact test).

At day 21, no abscesses were found in group 3, whereas 4 of 12 control rats and 5 of 11 rats in group 2 had an intra-abdominal abscess. This difference did not reach statistical significance (P = .09; Fisher exact test). An enterocutaneous fistula in conjunction with an abscess developed in 1 group 1 rat and 2 group 2 rats.

**BACTERIAL CULTURES**

Bacterial cultures taken at the day of cecal resection revealed a mixed aerobic and anaerobic flora of Proteus species, *Escherichia coli*, coliform gram-negative bacilli, anaerobic gram-negative rods, and *Enterococcus* and *Staphylococcus* species in concentrations of 10^7 to 10^9 colony-forming units/mL. A similar flora was found in cultures of abscesses present at day 7.

The predominant bacteria found in abscesses at day 21 were Proteus species and *E. coli*. The concentrations tended to be lower than those at day 7.

**COMMENT**

We demonstrated that 0.4% HA-solution reduces intra-abdominal abscess and adhesion formation in a clinically relevant model of generalized bacterial peritonitis, without adversely affecting morbidity and mortality. Similar results at 1 and 3 weeks after the insult indicate that this effect is long lasting.

Several studies have demonstrated the ability of HA solutions to prevent the formation of postsurgical adhesions in a noninfectious environment.8-10,16,17 Hyaluronic acid solutions reduce serosal trauma by precoating the peritoneal surfaces, which become damaged during surgery. As a consequence, peritoneal surfaces do not adhere to each other with fibrinous deposits and may heal without adhesion formation.

Intra-abdominal infection is a potent stimulus of peritoneal injury, inevitably leading to adhesion formation.18 Hyaluronic acid solution was used 24 hours after initial peritoneal injury to mimic clinical treatment of intra-abdominal infection. At that point, established intrabdominal infection was found with fibrinous exudates and pus in the whole abdomen. Hence, a precoating effect of HA solution does not explain the reduction of adhesions and abscesses, and other mechanisms of action may be involved.

Several lines of evidence suggest that HA decreases inflammation, interferes with fibrin formation, and accelerates the healing of peritoneal tissue (Figure 2).19-21 Inflammation is considered pivotal in adhesion and abscess formation.18,22-24 Hyaluronic acid has been reported to inhibit the release of protease from peritoneal leukocytes and of oxygen radicals from macrophages and to scavenge free oxygen radicals.19,25,26 Macrophages carry a hyaluronate-CD44 receptor on their membrane, which is known to modulate the cytokine response.27,28

Intra-abdominal infection markedly impairs fibrinolysis reflected by high concentrations of plasminogen-activating inhibitors in peritoneal tissue and fluid.23,24,25 Abolishing plasminogen-activating inhibitor activity is important to facilitate fibrin degradation and subsequently to reduce adhesion and abscess formation.30 In another rat experiment, a significantly lower plasminogen-activating inhibitor activity in the abdominal fluid was found 6 hours after instillation of HA solution com-
Hyaluronic acid accelerates the healing of various tissues, including the peritoneum, without excessive growth of connective tissue. Stimulation of mesothelial recovery indeed seems to protect against adhesion formation. Such a mechanism of action seems unlikely, since HA solution has disappeared from the abdominal cavity within 24 hours after instillation, before peritoneal healing takes place.

The surplus of HA solution used in our study may have had a “floating” effect on intra-abdominal organs, which in turn prevented adhesions. Although the same amount of isotonic sodium chloride solution had no influence on adhesion and abscess formation, this does not necessarily refute such an effect, because there is a substantial difference in viscosity and ability to be absorbed between the solutions. Hyaluronic acid installed in the abdominal cavity is probably absorbed similarly to peritoneal fluid, by the diaphragmatic stomata, and degraded in the same manner as endogenous HA, mainly in lymph and blood, but also in liver. Isotonic sodium chloride solution is absorbed by the whole peritoneum. More appropriate solutions to compare with 0.4% HA are methylhydroxypropylcellulose gel and the liquid phase of the HA-CMC bioresorbable membrane. It has recently been shown that methylhydroxypropylcellulose gel did not reduce adhesion and abscess formation in rats with intra-abdominal infection. The HA-CMC bioresorbable membrane that turns into a liquid phase after 24 hours did not seem to reduce adhesion and abscess formation.

The lack of effect of the HA-CMC membrane in comparison with HA solution was surprising. The failure of HA-CMC membrane to reduce adhesions in an infectious environment is in accord with the findings of Medina and associates in a rabbit model of incomplete colon anastomosis and with additional findings in the rat CLP model (J. W. Burns, PhD, and H. V. G., oral communication, June 1998). It may be criticized that the HA-CMC bioresorbable membrane was only placed at operative sites, eg, the cecum resection site and under the midline incision, whereas peritoneal injury was generalized. This initially local therapy compared with HA solution may explain adhesion and abscess formation in other parts of the abdominal cavity. However, most abscesses were found at the sites where the HA-CMC membrane was located. The HA-CMC membrane may have acted as foreign material, which in the presence of bacteria increases the inflammatory reaction. The enterocutaneous fistula, seen in rats treated with the bioresorbable membrane, is a feature of increased inflammation, adhesion to the parietal peritoneum, and abscess formation. A compound of the membrane, CMC, has been reported to increase adhesion formation to injured parietal peritoneum in a rabbit model of peritoneal damage.

Other studies, however, have shown the opposite.

Early reduction of fibrinous adhesions in intra-abdominal infection results in bacteremia and subsequent mortality as described earlier. Increased mortality associated with HA solution was not found, probably as a result of antibiotic treatment. However, an antibiotic effect of HA solution was not ruled out. Recently, it has been suggested that HA confers resistance to phagocytosis of gram-positive organisms.

Treatment of intra-abdominal infection with 0.4% HA solution prevents the formation of adhesions and abscesses. Further experiments are necessary to elucidate the mechanism(s) of action of HA solution in an infectious environment.

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REFERENCES


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**Surgical Anatomy**

The trauma neck zones are as follows: **Zone 1** is that portion of the neck from the cricoid cartilage down to the clavicles; **Zone 2** is the angle of the mandible to the cricoid cartilage; and **Zone 3** is the skull base to the angle of the mandible. These zones are in the same anatomic order as the Le Fort fractures III, II and I.