Effect of Surgical Adhesion Reduction Devices on the Propagation of Experimental Intra-abdominal Infection

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Hypothesis: The use of certain surgical adhesion reduction devices where there is a risk of concomitant bacterial contamination potentiates intra-abdominal infection.

Design: Evaluation of adhesion reduction devices in an experimental model of intra-abdominal infection.

Setting: Experimental animal model.

Interventions: Adhesion reduction devices were administered at the time of bacterial challenge.

Main Outcome Measures: Animal mortality rate, abscess formation, and bacterial counts in peritoneal fluid and blood cultures.

Results: The use of bioresorbable membrane adhesion reduction devices in the presence or absence of antibi- otic therapy did not alter the disease process as compared with appropriate control groups. However, adhesion reduction gels prepared from sodium hyaluronate and carboxymethylcellulose chemically modified with carbodiimide or ferric ion complexed sodium hyaluronate increased the incidence of peritonitis in treated animals. Gel formulations containing diimide-modified carboxymethylcellulose did not have this effect.

Conclusions: The use of certain adhesion reduction devices resulted in the propagation of intra-abdominal infection in an experimental rat model. This outcome was dependent on the composition of the device employed. The use of adhesion reduction devices should be tested in appropriate models of infection where there is the risk of concomitant bacterial contamination.

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BACTERIAL contamination of the abdominal cavity usually occurs following perforation of the bowel due to penetrating trauma to the abdomen, complications subsequent to abdominal surgery, or underlying bowel disease. Numerous clinical and experimental studies have shown that the release of colonic contents into the peritoneal cavity can lead to widespread septicemia and/or the formation of intra-abdominal abscesses.1-3

Previous studies in a rat model of intra-abdominal infection (IAI) have documented the role of particular intestinal bacterial species that predominate in experimental disease.4-6 Animals challenged via the intraperitoneal route with rat cecal contents closely approximate the disease as it occurs in humans, inducing both bacteremia and abscess formation. Facultative species such as Escherichia coli predominate in the acute septic phase of disease and are associated primarily with mortality, while in surviving animals anaerobes such as Bacteroides fragilis are associated with abscess formation. This model has been used to investigate the efficacy of numerous antibiotics and other novel agents to prevent both peritonitis and abscess formation and has proven to be highly predictive of clinical outcomes.6-9

The development of adhesions is a major cause of morbidity in patients undergoing abdominal or gynecological operations.10 Hyaluronic acid–based adhesion reduction devices have been shown to reduce adhesions after surgery.10-12 Seprafilm and Seprafilm II (Genzyme Corp, Cambridge, Mass) are bioresorbable membranes for use in reducing surgical adhesions in humans. A new generation of gel-based adhesion reduction products are currently under development for this purpose. Because the use of these devices within the peritoneal or pelvic cavities is associated with surgical manipulation, there is the risk of disruption to the bowel and the release of colonic contents into these normally sterile spaces. To assess whether these adhesion reduction devices may have an effect on the progression of bacterial contamination within the peritoneal cavity, we evaluated their use in an animal model of experimentally induced infection.
MATERIALS AND METHODS

EXPERIMENTAL DESIGN

For experiments examining the effect of adhesion reduction devices on mortality and bacterial levels in blood and peritoneal fluid, animals were randomized into groups of 10 or 20, anesthetized with pentobarbital sodium, and prepared for surgery as described here. For experiments with Seprafilm or Seprafilm II, a 2-cm midline incision was made into the abdominal cavity. For experiments with gels, a 0.5-cm midline incision was made. The appropriate bacterial inoculum was inserted with a pipette through the incision into the abdominal cavity. Immediately following challenge, a 3 x 3-cm² section of Seprafilm or Seprafilm II or 2 mL of gel were inserted into the peritoneal cavity. The incision was closed as described below. For experiments using antibiotic therapy, 2 mg of gentamicin and 15 mg of clindamycin was given 2 hours postchallenge and every 8 hours thereafter intramuscularly. Animals were closely monitored every 4 hours thereafter and mortality rates were calculated. Animals that did not survive at least 4 hours after surgery were excluded from the experiment. Results are expressed as a compilation of at least 2 separate experiments.

ANIMAL MODEL FOR IAI AND CHALLENGE INOCULA

A previously published animal model for IAI was used for these studies. Animals were anesthetized with a single intraperitoneal injection of 0.15 mL of pentobarbital sodium (50 mg/mL, Abbott Laboratories, North Chicago, Ill.). An anterior midline incision was made through the abdominal wall and peritoneum, and 0.5 mL of inoculum was inserted into the pelvis. The incision was closed with 3-0 silk sutures.

Three types of bacterial challenge inocula were used for these experiments: (1) cecal contents from meat-fed rats mixed with barium sulfate (10% final concentration wt/vol), (2) B fragilis mixed with sterile cecal contents, and (3) E coli mixed with sterile cecal contents. The cecal contents inoculum was procured from the ceca of meat-fed rats, mixed with peptone-yeast glucose broth to obtain a slurry, and frozen at −80°C until needed. Quantitative and qualitative bacteriology of this inoculum was performed as previously described. This inoculum closely resembles the bacterial flora found in the normal human colon and is used in animals to simulate release of fecal matter from the human intestine. The cecal contents inoculum was mixed with barium sulfate (10% final concentration wt/vol) and titrated in the rat model to yield approximately 50% mortality with abscess formation in 100% of survivors. Following this challenge, animals were examined daily and mortality rates were assessed in each treatment group. Animals typically succumbed to the acute phase of peritonitis within a 48-hour period after challenge, while abscesses required 6 days to form. This inoculum was used to simulate IAI as it occurs in human disease following release of colonic contents into the peritoneal cavity.

A second protocol used an inoculum of sterile cecal contents containing B fragilis NCTC 9343 (5 x 10⁷ colony-forming units). These counts remained relatively constant at both 6 and 24 hours postchallenge.
forming units per milliliter) to assess the specific effect of adhesion reduction devices on abscess formation. This dose was determined to cause abscesses in approximately 30% of untreated control animals. Rats were surgically implanted with the inoculum as described above and then assigned to a saline control group, a group receiving Seprafilm, or a group receiving Seprafilm II.

To facilitate investigation of the effect of gel formulations on peritonitis-induced mortality, an *E coli* challenge inoculum was employed. This inoculum specifically simulates the mortality phase of IAI. This inoculum yields a reproducible mortality rate in animals and was used to discern the effect of compositional changes in adhesion reduction devices that affected mortality. For these studies, \(7.9 \times 10^7\) colony-forming units per animal of *E coli* strain 502501 (Channing Anaerobe Laboratory, Boston, Mass), mixed with sterilized rat cecal contents was used to produce approximately a 50% mortality rate in a given group of animals.

**QUANTITATIVE BLOOD AND PERITONEAL FLUID CULTURES**

Blood and peritoneal samples were obtained from some of the animals at 6 and 24 hours following challenge with the cecal contents inoculum. Blood samples (0.1 mL) were taken from animals by transthoracic intracardiac puncture, placed in molten tryptic soy agar, inverted 4 times, poured into 100-mm Petri dishes, and incubated at 37°C for 24 hours. Peritoneal fluid samples (0.1 mL) were obtained from rats following challenge, diluted appropriately, and plated onto tryptic soy agar. Bacterial counts were performed on all samples and expressed as \(\log_{10}\) colony-forming units per milliliter.

**ADHESION REDUCTION DEVICES**

Seprafilm and Seprafilm II were obtained from the manufacturer as commercially available products. Seprafilm and Seprafilm II are bioresorbable membranes composed of sodium hyaluronate (HA) and carboxymethylcellulose (CMC) that have been chemically modified with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. This chemical modification reduces the water solubility of both the HA and CMC polymers. Reduced aqueous solubility causes membranes or gels prepared from these materials to stay at the placement site longer and in turn function as a barrier to separate traumatized tissue. The adhesion reduction gels, prepared from diimide-modified HA, CMC, or HA and CMC were prepared by previously published methods. All of the carboximidate-modified gels were formulated with pH 4 succinate buffer into an opaque slurry and steam autoclaved. Ferric ion cross-linked HA was prepared in sterile saline under aseptic conditions by published methods. Gel pH was adjusted using sterile filtered (0.22 µm) hydrochloric acid (0.1 normal) or sodium hydroxide (0.1 normal).

**STATISTICAL ANALYSES**

Percent mortality was calculated for each group and compared with the respective control groups. The Fisher exact test was used to calculate differences between experimental and control groups. A comparison of the means for the levels of bacteria within blood and peritoneal fluid between the controls and test samples was calculated by the Student t test (unpaired). \(P<.05\) was considered statistically significant in all experiments. Statistical analyses were performed using Instat Graphpad Software (Graphpad Software Inc, San Diego, Calif).

### Table 1. Effect of Seprafilm and Gentamicin/Clindamycin Therapy on Mortality in a Rat Model of IAI*

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>No. of Animals</th>
<th>Mortality Rate, %</th>
<th>(P)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>38</td>
<td>61</td>
<td>. .</td>
</tr>
<tr>
<td>Saline and antibiotics</td>
<td>36</td>
<td>3</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Seprafilm</td>
<td>38</td>
<td>37</td>
<td>. .</td>
</tr>
<tr>
<td>Seprafilm and antibiotics</td>
<td>39</td>
<td>0</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Seprafilm is manufactured by Genzyme Corp, Cambridge, Mass. A regimen of gentamicin (2 mg every 8 hours) and clindamycin (15 mg every 8 hours) was commenced 2 hours following bacterial challenge. IAI indicates intra-abdominal infection; ellipses, not applicable.†A 3 x 3-cm piece of Seprafilm was placed over the intestines of animals immediately following bacterial challenge.‡Compared with respective control group.

### Table 2. Effect of Seprafilm and Seprafilm II on Experimental Intra-abdominal Abscess Formation Following Challenge With *Bacteroides fragilis*†

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>No. of Animals</th>
<th>Abscess Rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>39</td>
<td>46</td>
</tr>
<tr>
<td>Seprafilm</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Seprafilm II</td>
<td>38</td>
<td>53</td>
</tr>
</tbody>
</table>

*Seprafilm is manufactured by Genzyme Corp, Cambridge, Mass. Animals were challenged with *B fragilis* NCTC 9343 \((5 \times 10^8\) colony-forming units per milliliter) as described in the “Materials and Methods” section.†A 3 x 3-cm piece of Seprafilm was placed over the intestines of animals immediately following bacterial challenge.

**EFFECT OF CHALLENGE INOCULUM IN GEL-TREATED ANIMALS**

*Escherichia coli* is the component of the normal fecal flora that is the major contributor to bacterial peritonitis subsequent to colonic leakage in humans. To investigate whether this organism was responsible for the increased lethality observed in gel-treated animals, we used a cecal contents inoculum that contained a significantly lower *E coli* count than the previously used inoculum. Animals receiving HA/CMC/NAU gel and challenged with this inoculum had a similar mortality rate to animals receiving saline (20% vs 40%, respectively). To confirm the effect of *E coli* on mortality in this model, a monomicrobial challenge inoculum of *E coli* mixed with sterile cecal contents was tested. The mortality rate of animals chal-
of the lethal effect. Animals were given 2 mL of HA/CMC/NAU gel or a 1:4, 1:8, or 1:20 dilution of gel in a total of 2 mL of saline. Administration of 2 mL of saline alone yielded a mortality rate of 45% following challenge with E coli, while injection of 2 mL of undiluted gel resulted in a mortality rate of 100%. All of the gel dilutions similarly resulted in 100% mortality in gel-treated animals.

**EFFECT OF HA/CMC/NAU GEL pH AND BUFFER SPECIES ON MORTALITY**

The HA/CMC/NAU gel is constituted as a 5% opaque gel buffered with pH 4 succinate-buffered saline. We next evaluated whether gel pH or buffer species was responsible for the increase in mortality seen with HA/CMC/NAU gel. In this series of experiments, animals received saline, HA/CMC/NAU gel with pH 4 succinate buffer, pH 4 succinate buffer alone, HA/CMC/NAU gel buffered with pH 7 succinate, pH 4 HA/CMC/NAU gel without succinate buffer, or HA/CMC/NAU gel buffered with pH 7 phosphate. The results of these experiments are presented in Table 4. As seen previously, HA/CMC/NAU gel with pH 4 succinate buffer resulted in a significantly increased mortality rate compared with saline-treated animals (98% vs 54% mortality, respectively, *P* < .001). However, administration of the pH 4 succinate buffer alone did not have this dramatic effect. The HA/CMC/NAU gel buffered with pH 7 succinate or pH 7 phosphate yielded a 100% mortality rate in treated animals. Last, animals given unbuffered HA/CMC/NAU gel alone also had a 100% mortality rate. These data clearly suggested that gel composition and not pH or buffer species was responsible for the increased mortality in gel-treated animals.

**GEL FORMULATION AND ITS EFFECT ON MORTALITY**

Based on the previous experiments, the effect of gel composition on mortality was examined. A variety of gels that were efficacious in reducing adhesions but contained the different components of the HA/CMC/NAU gel were tested in animals. Initially, the HA/CMC/NAU pH 4 succinate-buffered gel was heated at 121°C for increasing periods

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**Table 3. Effect of HA/CMC/NAU Gel and Gentamicin/Clindamycin Therapy on Mortality in a Rat Model of IAI**

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>No. of Animals</th>
<th>Mortality Rate, %</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>70</td>
<td>24</td>
<td>. .</td>
</tr>
<tr>
<td>Saline and antibiotics</td>
<td>34</td>
<td>0</td>
<td>. .</td>
</tr>
<tr>
<td>HA/CMC/NAU gel</td>
<td>54</td>
<td>85</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HA/CMC/NAU gel and antibiotics</td>
<td>35</td>
<td>0</td>
<td>. .</td>
</tr>
</tbody>
</table>

* A regimen of gentamicin (2 mg) and clindamycin (15 mg) every 8 hours was commenced 2 hours following bacterial challenge. Animals were challenged with a rat cecal contents inoculum as described in the "Materials and Methods" section. HA indicates sodium hyaluronate; CMC, carboxymethylcellulose; NAU, N-acyl urea; and ellipses, not applicable.

† Two milliliters of sterile gel was injected into the peritoneal cavity of animals immediately following bacterial challenge.

‡ Compared with saline-treated control group.

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**Table 4. Effects of Gel pH and Buffer Species on Mortality Induced by Escherichia coli in a Rat Model of IAI**

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>No. of Animals</th>
<th>Mortality Rate, %</th>
<th>P‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>70</td>
<td>24</td>
<td>. .</td>
</tr>
<tr>
<td>HA/CMC/NAU gel succinate buffer, pH 4</td>
<td>50</td>
<td>98</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Succinate buffer, pH 4</td>
<td>29</td>
<td>76</td>
<td>.06</td>
</tr>
<tr>
<td>HA/CMC/NAU gel succinate buffer, pH 7</td>
<td>30</td>
<td>100</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HA/CMC/NAU gel, pH 4, without succinate</td>
<td>10</td>
<td>100</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HA/CMC/NAU gel phosphate buffer, pH 7</td>
<td>20</td>
<td>100</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

* Animals were challenged with 7.9 × 10^7 colony-forming units per animal of E coli as described in the "Materials and Methods" section. IAI indicates intra-abdominal infection; HA, sodium hyaluronate; CMC, carboxymethylcellulose; and NAU, N-acyl urea.

† Two milliliters of gel was placed over the intestines of animals immediately following bacterial challenge.

‡ Compared with saline-treated control group.
The development of adhesions is a major cause of morbidity in patients undergoing abdominal or gynecological surgery. Several adhesion reduction devices have been approved for clinical use and new generations of these devices are currently under development. Because the use of these devices within the peritoneal or pelvic cavities is associated with surgical manipulation, there is the risk of disruption to the bowel and the release of colonic contents into these normally sterile spaces. The effect of these devices on the propagation of bacterial peritonitis in these cases is unknown. We examined the effect of hyaluronic acid–based adhesion reduction devices on the propagation of bacterial peritonitis in a rat model of IAI. Results from these studies demonstrated that certain devices resulted in the propagation of IAI and an increase in the severity of disease. This outcome was dependent on the composition of the device employed. The data indicate that adhesion reduction devices should be tested in appropriate models of infection where there is the risk of concomitant bacterial contamination.

to clarify the mixture. Increasing the degree of clarity of the gel reduced the mortality rate in animals (Table 5). The clarity was quantified by placing the gel into a glass tube (length, 5 × 50 mm) and measuring the percent transmittance of light at 610 nm with a UV/visible spectrophotometer. Opaque gels typically gave 4% to 10% transmittance when measured under these conditions. Testing of a 71% or 88% transmittance clarified gel resulted in mortality rates of 100% and 85%, respectively, while clarification of the gel by 97% resulted in a mortality rate that was comparable to the saline control.

Administration of the carbodimide-derivatized HA (HA/NAU) gel buffered with pH 4 succinate resulted in a 100% mortality rate in animals, while administration of a CMC/NAU gel did not exhibit this effect (mortality rate of 55% compared with saline). These data indicated that opaque HA-containing gels exacerbated mortality associated with peritonitis, while gels containing the other major component of the original gel formulation, CMC/NAU, did not enhance mortality in this model.

EFFECT OF HA-IRON GELS ON MORTALITY

Gels containing HA cross-linked to ferric ion were formulated in saline to pHs of 4.6, 6.0, or 7.1. When tested in the infection model, animals receiving these gels all had significantly higher mortality rates following challenge with E coli compared with the saline-treated control group (Table 6).

COMMENT

The development of adhesions is a major cause of morbidity in patients undergoing abdominal or gynecological surgeries. This study examined whether the use of adhesion reduction devices within the peritoneal cavity altered the outcome of experimental IAI. Because these devices are designed for use within the abdominal and pelvic cavities, there exists the possibility of spillage from the bowel while the device is still in the abdominal cavity. Leakage could occur from an anastomosis or an inadvertent and unknown perforation during surgery. Therefore, we believed that the effect of adhesion reduction devices on the propagation of this disease process should be evaluated.

Results of these experiments indicate that use of the adhesion reduction devices Seprafilm and Seprafilm II does not enhance either the mortality associated with the early peritonitis phase of disease or the occurrence of abscesses. Seprafilm and Seprafilm II do not seem to promote a more extensive infectious process when used in this animal model system. Similarly, Seprafilm does not inhibit the efficacy of antibiotic therapy for serious infection.

In contrast, we observed a dramatic increase in the mortality rate of animals receiving HA/CMC/NAU gels following bacterial challenge. It is important to note that the increase in lethality when using the HA/CMC/NAU gels following bacterial challenge was not observed when animals were administered a therapeutic course of gentamicin and clindamycin. These results indicate that some aspect of the gel exacerbated the lethal effect. To address the possible mechanism contributing to the increased lethal effect in HA/CMC/NAU gel-treated animals, we per-
formed blood and peritoneal fluid cultures to determine bacterial numbers present subsequent to challenge. It was clear from these studies that animals receiving gel had significantly more organisms circulating in their blood at both 6 and 24 hours following surgery compared with saline-treated animals. However, bacterial counts in free-flowing peritoneal fluid from all groups were the same. These findings suggest that the increased mortality in gel-treated animals is likely due to a greater number of organisms gaining entry into the bloodstream. The increase in bacterial accessibility to the bloodstream following intra-peritoneal challenge may reflect the host response to bacterial endotoxin followed by induction of proinflammatory cytokines, vasodilatation, and increased vascular permeability. This could facilitate increased translocation of the bacterial load within the peritoneal cavity to peripheral blood, which would eventually lead to bacteremia and death in untreated animals.

The finding that a cecal contents inoculum with a low *E. coli* content did not increase mortality in gel-treated animals while a monomicrobial challenge with this organism did suggested that *E. coli* is a major contributor to the observed effect. While this does not exclude the involvement of other intestinal organisms, *E. coli* is among the most common isolates from clinical cases of bacterial peritonitis and likely leads to enhanced endotoxemia in gel-treated animals.

The HA/CMC/NAU gel is comprised of carbodimide-derivatized HA and CMC that is formulated with pH 4 succinate-buffered saline and autoclaved to yield an opaque viscous slurry. Experiments in the animal model ruled out buffer type and pH as a reason for the enhanced infectivity in gel-treated animals and suggested that the chemically modified HA or CMC component of the gel itself may be responsible for the observed effect. Testing of other adhesion reduction gels containing the different components of the HA/CMC/NAU gel revealed that gels containing HA/NAU typically increased mortality, while gels containing CMC/NAU did not. The exception to this were HA/CMC/NAU gels that had been heated sufficiently to almost completely clarify the formulation. It is not currently known what effect the heating step has on the gel, but it is possible that the molecular weight of the derivatized-HA component of the opaque gel, which is approximately 85 ka, is reduced on heating to a size that approximates the derivatized-HA component in Seprafilm (approximately 50 ka). This may explain why the gel in its opaque form enhances infectivity while Seprafilm does not. This hypothesis is currently under investigation.

Testing of different ferric ion cross-linked HA gels in the animal model showed that these formulations also potentiated bacterial peritonitis. The gels we tested contain between 4 mol/L and 8 mol/L iron, a factor that is known to markedly increase the virulence of any different bacterial species. In addition, certain virulent strains of human pathogens have evolved specialized proteins, known as siderophores, which function to scavenge micromolar concentrations of free iron in the environment for the organism to survive and replicate.

This study was designed to investigate whether the placement of adhesion reduction products within the abdominal cavities at the time of bacterial contamination exacerbates the outcome of disease. These results clearly demonstrate that while some adhesion reduction products such as Seprafilm do not alter the course of disease in a severe infection model, some gels greatly enhance lethality. In the case of the HA/CMC/NAU gels that have this effect, the formulation can be converted to a non-peritonitis-enhancing material by an appropriate heating step. Alternatively, we have shown that other adhesion-reducing gels, such as the CMC/NAU gel formulation, can be used that do not increase mortality in the animal model. The results from these studies underscore the importance of testing such materials in appropriate infection models where there is the risk of concomitant bacterial contamination.

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REFERENCES


