ORIGINAL ARTICLE

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

Arthur O. Tzianabos, PhD; Ronald L. Cisneros, MS; Julia Gershkovich, MS; Jeff Johnson, MS; Robert J. Miller, PhD; James W. Burns, PhD; Andrew B. Onderdonk, PhD

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 antibiotic 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.

Arch Surg. 1999;134:1254-1259

From Channing Laboratory, Brigham and Women's Hospital, Harvard Medical School, Boston, Mass (Drs Tzianabos and Onderdonk and Mr Cisneros); and Genzyme Corp, Cambridge, Mass (Drs Miller and Burns, Ms Gershkovich, and Mr Johnson).

Dr Tzianabos and Dr Onderdonk do not hold an equity position in Genzyme Corp, but do serve as consultants. Ms Gershkovich, Mr Johnson, and Drs Miller and Burns are all employees of Genzyme Corp.

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.[3-6] 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 3×3-cm^2 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.[3-6] All animal experiments were performed in accordance with the Animal Care and Use Committee guidelines set forth by the Brigham and Women's Hospital and Harvard Medical School, Boston, Mass. Briefly, male Wistar rats (180-200 g, Charles River Laboratories, Wilmington, Mass) 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.[4] 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 (5×10^7 colony-

Continued on next page

RESULTS

EFFECT OF SEPRAFILM AND SEPRAFILM II ADMINISTRATION ON IAI

Animals challenged with cecal contents and saline (control) demonstrated a 61% mortality rate 48 hours following surgery, while animals receiving gentamicin showed a reduction in mortality to 3% (**Table 1**). Animals receiving Seprafilm alone had a mortality of 37%, while those receiving Seprafilm and gentamicin had no mortality.

To address whether animals given Seprafilm or Seprafilm II potentiated the formation of intra-abdominal abscesses, animals were challenged with *B fragilis* NCTC 9343. Forty-six percent of animals challenged with this inoculum developed abscesses, while animals implanted with Seprafilm or Seprafilm II had abscess rates of 50% and 53%, respectively (**Table 2**).

EFFECT OF HA/CMC/NAU GEL AND ANTIBIOTIC THERAPY ON MORTALITY ASSOCIATED WITH IAI

The effect of HA/CMC/*N*-acyl urea (NAU) gel on mortality in this model was evaluated. Animals were challenged with the cecal contents inoculum and immediately given 2 mL of gel via the intraperitoneal route. Animals receiving the gel had a significantly higher mortality rate compared with the saline-treated control group (85% vs 24%, $P<.001$, **Table 3**). However, animals given a therapeutic course of gentamicin and clindamycin in addition to saline or the gel did not succumb to the infection (0% mortality in each group) (Table 3).

BACTERIAL COUNTS IN BLOOD AND PERITONEAL FLUID OF HA/CMC/NAU GEL-TREATED ANIMALS

To investigate the basis for the increased mortality rate associated with HA/CMC/NAU gel usage, bacterial counts were performed on blood and peritoneal fluid of animals challenged with the cecal contents inoculum and treated with the gel. In animals challenged with the cecal contents inoculum, there was no difference in bacterial counts in peritoneal fluid cultures between animals treated with HA/CMC/NAU gel vs controls (**Figure**, top). These counts remained relatively constant at both 6 and 24 hours postchallenge.

In contrast, blood cultures obtained from animals treated with the gel yielded significant increases in total bacterial counts at 6 and 24 hours postchallenge compared with animals receiving saline ($P<.03$, Figure, bottom). Gel-treated animals had approximately an order

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 50% 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, 7.9×10^7 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.[13] 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.[13] All of the carbodiimide-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.[14] Gel pH was adjusted using sterile filtered (0.22 µm) hydrocholric 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*

Treatment†	No. of Animals	Mortality Rate, %	P‡
Saline	38	61	. . .
Saline and antibiotics	36	3	<.001
Seprafilm	38	37	. . .
Seprafilm and antibiotics	39	0	<.001

**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 × 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**

Treatment†	No. of Animals	Abscess Rate, %
Saline	39	46
Seprafilm	40	50
Seprafilm II	38	53

**Seprafilm is manufactured by Genzyme Corp, Cambridge, Mass. Animals were challenged with* B fragilis *NCTC 9343 (5×10^7 colony-forming units per milliliter) as described in the "Materials and Methods" section.*

†A 3 × 3-cm piece of Seprafilm was placed over the intestines of animals immediately following bacterial challenge.

of magnitude more organisms in their blood at 6 hours postchallenge and greater than 2 orders of magnitude more bacteria at 24 hours postchallenge compared with animals treated with saline.

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-

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

Treatment†	No. of Animals	Mortality Rate, %	P‡
Saline	70	24	. . .
Saline and antibiotics	34	0	. . .
HA/CMC/NAU gel	54	85	<.001
HA/CMC/NAU gel and antibiotics	35	0	. . .

*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.

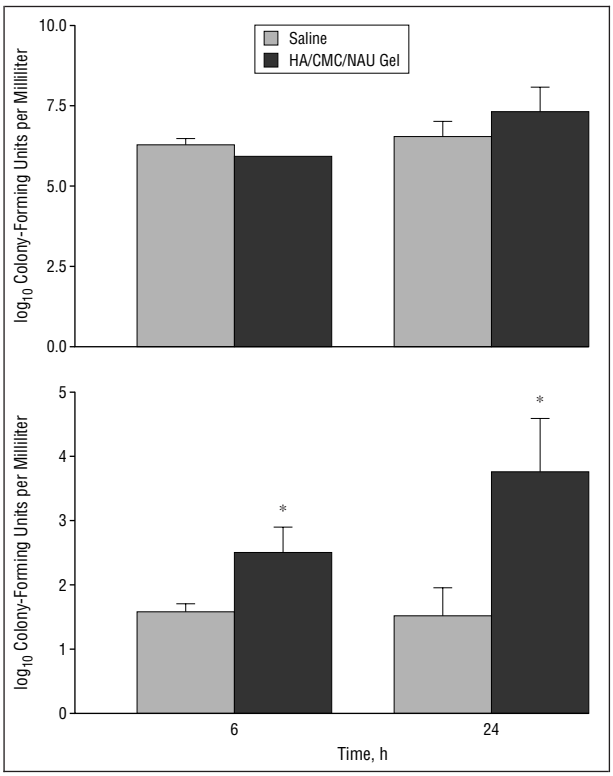
Table 4. Effects of Gel pH and Buffer Species on Mortality Induced by *Escherichia coli* in a Rat Model of IAI*

Treatment†	No. of Animals	Mortality Rate, %	P‡
Saline	50	54	. . .
HA/CMC/NAU gel succinate buffer, pH4	50	98	<.001
Succinate buffer, pH 4	29	76	.06
HA/CMC/NAU gel succinate buffer, pH7	30	100	<.001
HA/CMC/NAU gel, pH4, without succinate	10	100	<.001
HA/CMC/NAU gel phosphate buffer, pH7	20	100	<.001

*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.



Total bacterial levels in peritoneal fluid and blood from animals treated with saline or sodium hyaluronate/carboxymethlycellulose/N-acyl urea (HA/CMC/NAU) gel. Top, Peritoneal fluid cultures showed that bacterial levels in the abdominal cavity following challenge and concomitant placement of HA/CMC/NAU gel were the same as saline-treated animals at 6 and 24 hours postchallenge. Bottom, Blood cultures of animals treated with HA/CMC/NAU gel showed a significant increase in the number of bacteria at both 6 and 24 hours postchallenge (P<.03 vs saline-treated animals, Student t test).

lenged with *E coli* and receiving HA/CMC/NAU gel was significantly higher than similarly challenged animals receiving saline (97% vs 47% mortality, $P<.001$).

TITRATION OF LETHAL EFFECT

Different concentrations of HA/CMC/NAU gel were tested in the *E coli* inoculum model to determine the potency 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

Statement of Clinical Relevance

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 carbodiimide-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

Table 5. Effect of Gel Formulation on Mortality Induced by *Escherichia coli* in a Rat Model of IAI*

Treatment†	No. of Aminals	Mortality Rate, %	*P*‡
Saline	79	52	. . .
HA/CMC/NAU gel, nonclarified	39	100	<.001
HA/CMC/NAU gel, 71% clarified	20	100	<.05
HA/CMC/NAU gel, 88% clarified	20	85	.01
HA/CMC/NAU gel, 97% clarified	59	53	>.99
HA/NAU gel	20	100	<.05
CMC/NAU gel	40	55	.85

*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; ellipses, not applicable; 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.

Table 6. Effect of HA-Iron Gel Formulations on Mortality Induced by *Escherichia coli* in a Rat Model of IAI*

Treatment†	No. of Animals	Mortality Rate, %	*P*‡
Saline	39	49	. . .
HA/iron gel, pH 4.6	40	90	<.001
HA/iron gel, pH 6.0	40	90	<.001
HA/iron gel, pH 7.1	19	100	<.001

*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.

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

‡Compared with saline-treated control group.

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 intraperitoneal 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 carbodiimide-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 kd, is reduced on heating to a size that approximates the derivatized-HA component in Seprafilm (approximately 50 kd). 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.[15-18] 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.

This work was supported by a grant from Genzyme Corp, Cambridge, Mass.

We would like to thank Matt Lawlor, BS, for expert technical assistance.

Corresponding author: Arthur O. Tzianabos, PhD, Channing Laboratory, 181 Longwood Ave, Boston, MA 02115 (e-mail: atzianabos@channing.harvard.edu).

REFERENCES

1. Gorbach SL, Thadepalli H, Norsen J. *Anaerobic Microorganisms in Intra-abdominal Infections*. Springfield, Ill: Charles C Thomas Publisher; 1974.
2. Nichols RL. Intra-abdominal infections: an overview. *Rev Infect Dis*. 1985;7 (suppl):S709-S715.
3. Onderdonk AB, Weinstein WM, Sullivan NM, et al. Experimental intra-abdominal abscesses in rats: quantitative bacteriology of infected animals. *Infect Immun*. 1974;10:1256-1259.
4. Onderdonk AB, Bartlett JG, Louie T, et al. Microbial synergy in experimental intra-abdominal abscess. *Infect Immun*. 1976;13:22-26.
5. Weinstein WM, Onderdonk AB, Bartlett JG, et al. Antimicrobial therapy of experimental intra-abdominal sepsis. *J Infect Dis*. 1975;132:282-286.
6. Louie TJ, Onderdonk AB, Gorbach SL, Bartlett JG. Therapy of experimental intra-abdominal sepsis: a comparison of four cephalosporins with clindamycin and gentamicin. *J Infect Dis*. 1977;135:518-522.
7. Cisneros RL, Bawdon RE, Onderdonk AB. Efficacy of ampicillin/sulbactum for the treatment of experimental intra-abdominal sepsis. *Curr Ther Res*. 1990;458: 1021-1029.
8. Cisneros RL, Gibson FC, Tzianabos AO. Passive transfer of poly-(1-6)-b-glucotriosyl-(1-3)-b- glucopyranose glucan protection against lethal infection in an animal model of intra-abdominal sepsis. *Infect Immun*. 1996;64:2201-2205.
9. Tzianabos AO, Kasper DL, Onderdonk AB. Structure and function of *Bacteroides fragilis* capsular polysaccharides: relationship to induction and prevention of abscesses. *Clin Infect Dis*. 1995;20(suppl):S132-S140.
10. Becker JM, Dayton MT, Fazio VW, et al. Prevention of postoperative abdominal adhesions by a sodium hyaluronate–based bioresorbable membrane: a prospective, randomized, double-blind multicenter study. *J Am Coll Surg*. 1996;183: 297-306.
11. Burns JW, Skinner K, Colt J, et al. Prevention of tissue injury and postsurgical adhesions by precoating tissues with hyaluronic acid solutions. *J Surg Res*. 1995; 59:644-652.
12. Seeger JM, Kaelin LD, Staples EM, et al. Prevention of postoperative pericardial adhesions using tissue-protective solutions. *J Surg Res*. 1997;68:63-66.
13. Burns JW, Cox S, Walts AE. *Water Insoluble Derivatives of Hyaluronic Acid*. Cambridge, Mass: Genzyme Corp; 1991.
14. Huang WJ, Johns DB, Kronenthal RL. *Ionically Crosslinked Carboxyl-Containing Polysaccharides for Adhesion Prevention*. Chaska, Minn: Lifecore Biomedical Inc; 1996.
15. Zhang JP, Normark S. Induction of gene expression in *Escherichia coli* after pilus-mediated adherence. *Science*. 1996;273:1234-1236.
16. Neilands JB. Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem*. 1995;270:26723-26726.
17. Torres AG, Payne SM. Haem iron-transport system in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol*. 1997;23:825-833.
18. Biosca EG, Fouz B, Alcaide E, Amaro C. Siderophore-mediated iron acquisition mechanisms in *Vibrio vulnificus* biotype 2. *Appl Environ Microbiol*. 1996;62: 928-935.