Effect of carboxymethylcellulose and a hyaluronate-carboxymethylcellulose membrane on healing of intestinal anastomoses in horses

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Objective—To evaluate the effect of sodium carboxymethylcellulose (SCMC) or a hyaluronate-carboxymethylcellulose membrane (HA membrane) on healing of the small intestine in horses.

Animals—18 healthy adult horses.

Procedure—Midline celiotomy and 2 jejunal resection-and-anastomosis surgeries were performed. In treated horses, SCMC (n = 6) or a HA membrane (6) was applied to the jejunum to cover the anastomosis. There were 6 untreated control horses. Horses were euthanatized 10 days after surgery. For each horse, 1 anastomosis was used for histologic examination, and the second was used to determine intestinal bursting strength. Intestinal bursting tension, serosal granulation tissue, serosal fibrin deposition, and width of the fibrous seal at the anastomosis were compared among groups.

Results—3 control horses had adhesions associated with the anastomosis, but none of the treated horses had adhesions associated with the anastomosis. Mean thickness of fibrin deposited on the serosal surfaces for the SCMC and HA-membrane groups was significantly less than that for control horses. Mean thickness of serosal granulation tissue, width of fibrous seal between inverted musculature, inflammatory cell infiltrate scores, and bursting tension did not differ significantly among groups.

Conclusions and Clinical Relevance—Use of SCMC or application of a HA membrane to small intestinal anastomoses in horses resulted in fewer adhesions and decreased fibrin deposition, and it did not adversely affect anastomatic healing. In horses at increased risk for intra-abdominal adhesions, SCMC or application of HA membranes may decrease the frequency of adhesions without adversely affecting healing of small intestinal anastomoses. (Am J Vet Res 2000;61:369–374)

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ntra-abdominal adhesions that result in secondary intestinal obstruction are a common cause of postoperative morbidity and mortality in horses. Adhesions reportedly cause intestinal obstruction and signs of abdominal pain in 18 to 22% of horses that survive abdominal surgeries performed to correct primary small intestinal lesions. Intra-abdominal adhesions may lead to intestinal constriction, incarceration, and obstruction as well as secondary intestinal volvulus. Clinical studies have documented that horses with intra-abdominal adhesions have a high incidence of recurrence of adhesions and poor prognosis for survival.

Numerous investigations have been performed to determine pharmacologic and surgical methods of minimizing adhesions after surgery. Intraperitoneal administration of sodium carboxymethylcellulose (SCMC), a high molecular weight, substituted polysaccharide, has been used successfully in models to prevent adhesions in laboratory animals, ewes, ponies, and horses. Intraperitoneal administration of SCMC can prevent reformation of adhesions following surgical lysis. Its beneficial effect is believed to be 2-fold: it minimizes serosal trauma during intestinal manipulation and mechanically prevents apposition of serosal or peritoneal surfaces during early postoperative healing.

Recently, a bioresorbable hyaluronate-carboxymethylcellulose (HA) membrane was developed for use in reducing adhesion formation after surgery in people. The translucent, flexible HA membrane is placed on the serosal surface of the intestines or parietal peritoneum, forming a temporary protective barrier against serosal-serosal or serosal-peritoneal adhesion formation during the early postoperative period. In studies on animals, the HA membrane reduced the frequency and severity of adhesions after surgery to parietal and visceral peritoneal surfaces and the pericardium. In a prospective clinical trial of people undergoing abdominal surgery, use of the HA membrane significantly decreased the incidence and severity of adhesions after surgery.

Not all adhesions are considered detrimental. Adhesions may provide an additional blood supply to ischemic serosa or intestine. In that role, adhesions may function as vascular grafts, protecting areas of intestine with marginal viability. Adhesions associated with enteric anastomoses are also believed to be important in preventing leakage of the suture line. Treatment aimed at preventing adhesions may eliminate the protective functions of adhesion formation and predispose to local intestinal necrosis and peritonitis. In one study, intraperitoneal administration of SCMC led to
an increased incidence of perianastomotic abscesses and generalized peritonitis, which corresponded to an increase in mortality. This suggests that SCMC and a HA membrane could promote leakage associated with enteric anastomoses, thereby increasing the risk of anastomotic dehiscence and fatal generalized peritonitis. Therefore, the use of agents that prevent intra-abdominal adhesion formation in horses has been accompanied by concerns of their effect on normal healing of the intestines and peritoneum. The effect of SCMC and a HA membrane on healing of small intestinal anastomosis in horses has not been evaluated. The objectives of the study reported here were to evaluate the effect of intraperitoneal administration of 1% SCMC or a HA membrane on strength and healing of anastomotic sites in the small intestine of horses. We hypothesized that administration of 1% SCMC or application of a HA membrane would not adversely affect strength or healing of anastomotic sites in the small intestine of horses.

Materials and Methods

Preparation of SCMC—A 1% solution of SCMC was prepared by boiling 200 ml of sterile water and, using constant stirring, adding 10 g of SCMC powder. After the SCMC was in solution, additional sterile water was added to achieve a final volume of 1 L. The SCMC solution was then transferred into 500-ml glass bottles and autoclaved at 121 C for 20 minutes.

Experimental protocol—Eighteen healthy adult horses that ranged from 3 to 19 years old (mean, 7.8 years) and weighed between 345 and 575 kg (mean, 454 kg) were used in the study. Experimental procedures and animal care were approved by an institutional Animal Care and Use Committee. Horses (n = 6/group) were randomly assigned to control, SCMC, or HA-membrane groups. Blood and serum samples were obtained from each horse for hematologic and biochemical evaluation.

Food was withheld for 12 hours before surgery. One hour before induction of anesthesia, administration of potassium penicillin G (22, 000 U/kg of body weight, IV, q 6 h), gentamicin sulfate (6.6 mg/kg, IV, q 24 h), and flunixin meglumine (1.1 mg/kg, IV, q 12 h) was initiated. Horses were induced with xylazine hydrochloride (1.1 mg/kg, IV) followed by ketamine hydrochloride (2.2 mg/kg, IV), and anesthesia was maintained with halothane in oxygen in a semi-closed circle system. Lactated Ringer’s solution (10 ml/kg/h, IV) was administered during the surgical procedure. Horses were positioned in dorsal recumbency and prepared for aseptic abdominal surgery.

Ventral midline celiotomy and systematic exploration of the abdominal cavity were used to facilitate examination of viscera. The jejunum was exteriorized and examined from the abdominal incision, the abdominal cavity, and anastomotic sites were evaluated. In each horse, one of the anastomotic sites was randomly selected for histologic evaluation, and the second anastomotic site was used for determination of intestinal bursting strength.

Determination of bursting strength—A 14-cm segment of jejunum containing the anastomotic site was harvested and flushed with saline solution to remove intestinal contents. Intestinal bursting wall tension was used as a biomechanical indicator of anastomotic strength. Measurements of bursting pressure were performed on samples coded to conceal the identity of the horse from which they were obtained and the treatment used. Pressure within the intestinal segment was recorded continuously on a chart recorder from a pressure transducer attached to the tubing used to deliver air to the intestinal segment (Fig 2). The segment of intestine was distended by air delivered at a constant rate of 0.5 L/min until bursting was evident by the appearance of air bubbles at the anastomotic site or the intestinal segment ruptured. The maximum pressure at the time of bursting was recorded as the bursting pressure. Bursting wall tension was then calculated by using the Law of Laplace:

\[ \text{bursting wall tension} = \text{bursting pressure} \times \text{radius} \]

Assuming that the distended intestinal segment approximated a cylinder, the radius (r) was calculated from the
cells/HPF, 3 =
inflammatory cells/HPF, 2 = 10 to 30 inflammatory
time of the serosal bed of granulation tissue was
3 measurements. In addition, intensity of the inflamma-
variables: thickness of the serosal bed of granulation tissue,
the sections were obtained evaluated the tissue sections.
hematoxylin and eosin. A single pathologist (BGH) who did not
bursting pressure. Bursting wall ten-
sion was then calculated, using the Law of La Place and the fol-
tion was then calculated, using the Law of La Place and the fol-
to chart recorder (upper left corner) from a pressure
Figure 2—Diagram of the method used to evaluate intestinal
bursting pressure. A 14-cm segment of intestine that contained
constant rate until bursting of the anastomotic site or the intestine.
Pressure within the intestinal segment was recorded continu-
ously on a chart recorder (upper left corner) from a pressure
tubing used to deliver air to the intestinal segment. Maximum pressure at the time of intestinal
rupture was recorded as bursting pressure. Bursting wall ten-
sion was then calculated, using the Law of La Place and the fol-
volume (v) of water displaced using the formula:
\[ r = \text{square root} \left( \frac{v}{l_{sa}} \cdot \pi \right) \]

Microscopic evaluation—Tissue sections of the anas-
romatotic sites were fixed by immersion in neutral-buffered
10% formalin, processed routinely, embedded in paraffin,
sectioned at a thickness of 3 µm, and stained with hema-
toxylin and eosin. A single pathologist (BGH) who did not
have knowledge of the experimental groups from which the
sections were obtained evaluated the tissue sections.
For each anastomotic site, measurements were obtained by
use of a micrometer at 3 locations for each of the following
variables: thickness of the serosal bed of granulation tissue,
thickness of fibrinous exudate on the serosal surface, and
width of the fibrous seal between the inverted muscula-
ture. Mean value for each variable was calculated from the
3 measurements. In addition, intensity of the inflamma-
atory response in the serosal bed of granulation tissue was
scored subjectively, using the following scale: 1 = < 10
inflammatory cells/HPF, 2 = 10 to 30 inflammatory
cells/HPF, 3 = > 30 inflammatory cells/HPF.

Statistical analyses—Means for intestinal bursting ten-
sion, serosal granulation tissue, and fibrin thickness at anas-
tomotic sites were compared among groups, using an
ANOVA. Significant differences of means among groups were
compared by use of the Fischer Protected Least Significant
Difference test for multiple comparisons. Inflammatory cell
infiltrate scores for anastomotic sites were compared among
groups, using a Kruskal-Wallis test. Significance was estab-
lished at \( P < 0.05 \).

Results
Hematologic and biochemical values in samples
obtained before surgery from all horses were within
reference ranges for our laboratory. All horses recov-
ered from surgery without complications. Two con-
trol horses, 2 SCMC-treated horses, and 1 HA-mem-
brane-treated horse had a single episode of abdominal
pain after surgery, but all horses responded to medical
treatment.
Postmortem examination revealed that all of the
anastomotic sites had evidence of normal healing. We
did not detect evidence of leakage from anastomotic
sites, intra-abdominal abscesses, stricture at the
anastomotic site, or diffuse peritonitis in any of
the horses. Three control horses had fibrous adhe-
sions extending from the anastomotic site to adjacent
mesentery. These adhesions were firm attachments of
the jejunal serosa to the adjacent mesentery. One
control horse had a fibrous adhesion extending from
one of the anastomotic sites to the omentum. None
of the adhesions resulted in stricture of the jejunal
lumen. Horses in the SCMC and HA-membrane
management treatment groups did not have adhesions associated
with any of the anastomotic sites. We did not detect
evidence of HA membrane remaining or persisting at
the anastomotic sites.

Intestinal bursting tension did not differ signifi-
cantly among groups. Values (mean ± SD) for the con-
trol, SCMC, and HA-membrane groups were
890 ± 143, 1,017 ± 90, and 939 ± 104 dynes/cm,
respectively. In all horses, all of the jejunal segments
ruptured at a point distal to the anastomotic site.
Representative tissue sections of the anastomotic
sites from all groups had evidence of granulation tis-
uce with macrophage invasion and fibroblastic prolif-
eration, similar to that typically seen in the early
stages of wound healing. Anastomotic sites were char-
acterized by a profound increase in the thickness of
the serosa, muscularis, and submucosa at the incision
sites. This increase in thickness was attributed to
inversion of the muscularis externa and interna along
with granulation tissue in the submucosa and serosa.
The inverted muscularis from each side of the incision
was bound together by a thin but dense band of
fibrous tissue that extended from mucosa to serosa. At
the serosal surface, the granulation bed was covered
with a thin layer of fibrin.
Mean thickness of fibrin on the serosal surface at
the anastomotic site for the SCMC and HA-mem-
brane groups was significantly (\( P < 0.001 \)) less than
that for horses in the control group (Table 1). Mean
thickness of the serosal bed of granulation tissue
(\( P = 0.175 \)), thickness of the fibrous seal between

\[ \text{bursting wall tension} = \frac{\text{bursting pressure} \times \pi}{2r^2} \]

\[ r = \sqrt{\frac{v}{l_{sa}}} \]
inverted musculature ($P = 0.787$), and inflammatory cell infiltrate scores ($P = 0.422$) for the anastomotic sites were highly variable and did not differ significantly among groups.

### Discussion

With the advent of more sophisticated anesthetic and surgical techniques, abdominal surgery for the treatment of intestinal obstruction or strangulation in horses has become commonplace. Unfortunately, the clinical importance of intra-abdominal adhesions concurrently has become more apparent.1-3,17,19 Recurrent bouts of colic associated with intra-abdominal adhesions often necessitate additional surgery or euthanasia.

Morbidity associated with adhesions after surgery has prompted extensive research efforts into potential preventative treatments. Intra-abdominal infusion of high molecular weight solutions and application of temporary physical barriers are directed at mechanical- high molecular weight solutions and application of preventative treatments. Intra-abdominal infusion of has prompted extensive research efforts into potential complications of adhesion prophylaxis. In the small intestinal anastomosis model used in the horses of this study, we attempted to mimic a clinical situation to evaluate the effect of SCMC or a HA membrane on strength and healing of anastomotic sites. Analysis of results of this study suggested that neither 1% SCMC nor a HA membrane significantly decreased the strength or quality of healing of anastomoses of the small intestine of horses.

Adhesions may be beneficial in their ability to provide an additional blood supply to ischemic serosa or intestine. Perianastomotic adhesions may prevent leakage of suture lines and isolate inflammatory debris, thereby preventing generalized peritonitis.2,3,12 By pharmacologically altering the response of the peritoneal or serosal surface to insult, the normal healing response of the abdominal cavity may be impaired. In the study reported here, all anastomoses healed without evidence of leakage, intra-abdominal abscesses, or diffuse peritonitis. These results are in agreement with studies in which investigators evaluated the effect of a hyaluronic acid solution on intestinal anastomoses in rabbits.12,13 In those studies, wound strength and healing of anastomotic sites in the small intestine were not compromised by topical application of a hyaluronic acid solution. Therefore, intraperitoneal administration of SCMC or application of a HA membrane to an anastomotic site in the small intestine does not appear to compromise normal healing.

Wound strength is the most important functional property of the healing wound and may be measured at any stage of healing. Biomechanical patterns of wound healing for luminal organs have been evaluated in various tissues by use of bursting strength.11,13,18 Although mechanical strength is low within the first 3 to 6 days after wounding, it increases rapidly between 5 and 10 days.12 In our study, intestinal bursting tension was used as a biomechanical indicator of strength of the anastomatic site. Intestinal segments were tested 10 days after surgery so that measurements would represent early tensile wound strength yet allow sufficient time for the development of complications at the anastomoses, such as adhesion formation, intestinal stricture, or perianastomotic abscess formation. We did not detect significant differences in intestinal bursting tension among groups. In all groups, all of the jejunal segments ruptured at a point distal to the anastomotic site. The point of intestinal failure was always at either the proximal or distal end of the intestinal segment, at the point of fixation to the bursting apparatus. The noncompliant nature of the intestine at the fixation points may have predisposed the intestine to rupture at these sites. These results indicated that, in this experimental model, neither SCMC nor the HA membrane adversely affected strength at anastomotic sites in the small intestine of horses.

Early in serosal healing, polymorphonuclear and mononuclear cells rapidly infiltrate the area of injury,12 and fibrin accumulates within the serosa and on the denuded serosal surfaces. As normal wound healing progresses, fibroblasts migrate into the fibrin and form

### Table 1—Mean (± SD) values for variables assessed histologically in tissue sections obtained from the anastomotic site in control horses and horses treated by use of sodium carboxymethylcellulose (SCMC) or a hyaluronate carboxymethylcellulose-membrane (HA membrane)

<table>
<thead>
<tr>
<th>Group</th>
<th>Thickness of fibrin exudate on serosal surface (mm)</th>
<th>Thickness of serosal bed of granulation tissue (mm)</th>
<th>Width of fibrous seal between inverted musculature (mm)</th>
<th>Intensity of inflammatory response*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>0.81 ± 0.21a</td>
<td>1.2 ± 0.48</td>
<td>0.54 ± 0.05</td>
<td>2.3 ± 1.00</td>
</tr>
<tr>
<td>SCMC (n = 6)</td>
<td>0.05 ± 0.13a</td>
<td>1.2 ± 0.31</td>
<td>0.57 ± 0.23</td>
<td>2.2 ± 0.75</td>
</tr>
<tr>
<td>HA membrane (n = 6)</td>
<td>0.06 ± 0.15a</td>
<td>0.85 ± 0.27</td>
<td>0.51 ± 0.10</td>
<td>1.7 ± 0.82</td>
</tr>
</tbody>
</table>

*Scored subjectively, using the following scale: 1 = < 10 inflammatory cells/HPF, 2 = 10 to 30 inflammatory cells/HPF, 3 = > 30 inflammatory cells/HPF.

a,b Values with different superscript letters differ significantly ($P < 0.05$).
a layer of granulation tissue in the original serosal layer. Primordial mesenchymal cells become fibroblasts or differentiate into mesothelial cells and cover the granulation tissue. Under normal conditions, fibrin is dissolved by the fibrinolytic system, and adhesions are resolved before there is fibrous maturation. In the study reported here, microscopic evaluation of the anastomotic sites from all groups revealed evidence of granulation tissue with macrophage invasion and fibroblastic proliferation, as is typically seen in normal wound healing. However, accumulation of fibrin on the serosal surface at the anastomotic sites for the SCMC and HA-membrane groups was significantly less than for the control horses. It cannot be determined from this study whether increased peritoneal fibrinolysis or decreased fibrin deposition was responsible for the decrease in fibrin thickness on the serosal surface in the treatment groups. The proposed mechanism of action of intraperitoneal administration of SCMC and the HA membrane is mechanical separation of peritoneal and serosal surfaces during early healing. Considering this, it appears that decreased fibrin deposition at the anastomotic sites is a more likely mechanism for the decreased fibrin accumulation observed in the treated horses. This decrease in fibrin deposition at the anastomotic site of SCMC- and HA-membrane-treated horses, although significant, did not compromise strength or healing of the small intestine in this experimental model. The thickness of the serosal bed of granulation tissue, thickness of the fibrous seal between inverted musculature, and inflammatory cell infiltrate scores at the anastomotic site did not differ significantly among groups.

In this study, intraperitoneal administration of SCMC or application of a HA membrane to anastomatic sites in the small intestine of horses did not adversely affect strength or healing. In horses at increased risk for formation of intra-abdominal adhesions, use of intraperitoneal administration of SCMC or HA membranes during exploratory celiotomy may reduce morbidity and mortality associated with intra-abdominal adhesions after surgery.

References