Use of a high-molecular-weight carboxymethylcellulose in a tissue protective solution for prevention of postoperative abdominal adhesions in ponies

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Objective—To evaluate efficacy and safety of IP administration of high-molecular-weight carboxymethylcellulose (HMW CMC) for the prevention of postoperative intra-abdominal adhesions in ponies.

Animals—10 ponies.

Procedure—A 1% solution of HMW CMC was instilled intra-abdominally prior to surgery in 5 ponies, whereas 5 control ponies did not receive HMW CMC. Postoperative adhesions were induced by use of a bowel-abrasion method comprising laparotomy, typhlotomy, and abrasion of jejunal serosa at multiple sites with placement of 3 sutures at each site. Day of surgery was day 0. After surgery, ponies were monitored, and hematologic, serum biochemical, and peritoneal fluid analyses were performed on days 1, 2, 3, 5, 7, and 10. On day 10, ponies were euthanatized. Intra-abdominal adhesions were recorded, and tissue samples were collected for histologic examination.

Results—A significantly greater number of adhesions, number of multiple adhesions, and mean incidence of adhesions were identified in control ponies, compared with CMC-treated ponies. Mean peritoneal fluid WBC count on day 7 and serum fibrinogen concentrations on days 5 and 7 were significantly higher in control ponies, compared with CMC-treated ponies. Results of serum biochemical analyses did not differ significantly between the 2 groups.

Conclusions and Clinical Relevance—Intra-abdominal use of 1% HMW CMC during surgery was effective for preventing postoperative adhesions in ponies. Use of HMW CMC did not have detrimental effects on wound healing, intra-abdominal defenses, or patient health. A 1% solution of HMW CMC may be used routinely during abdominal surgery of horses for prevention of postoperative adhesions. (Am J Vet Res 2002;63:1448–1454)

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adhesions. Although CMC is biocompatible, mild transient changes in certain hematologic and biochemical variables have been reported with intra-abdominal use in equids.13,30

Our intention was to develop a safe, economic, and convenient method for preventing intra-abdominal adhesions in horses undergoing abdominal surgery. We formulated the hypothesis that a 1% solution of high-molecular-weight (HMW) CMC applied to tissue surfaces before induction of controlled tissue trauma would significantly reduce postoperative adhesions. We also hypothesized that the intra-abdominal use of CMC would not have significant adverse effects on, or pose a risk to, patients in which it is used. The objectives of the study reported here were to evaluate the efficacy of a 1% solution of HMW CMC in the prevention of postoperative abdominal adhesions in ponies and to evaluate the safety of the intra-abdominal use of such a solution by monitoring clinically relevant variables.

Materials and Methods

Animals—Ten healthy pony geldings and mares of various ages that weighed between 150 and 240 kg were used in the study. The experimental and animal care protocols for this study were approved by the University of Florida Animal Use and Care Committee.

Approximately 4 weeks prior to the study, all ponies were dewormed by administration of ivermectin and inspected for other health problems that might have resulted in exclusion from the study. They were kept on pasture and fed a diet supplemented with sweet feed until surgery. After surgery, they were housed in stalls and fed timothy and alfalfa hay.

Preparation of CMC solution—Iso-osmolar PBS solution was prepared, adjusted to pH 7.1, and then filter-sterilized by use of a 0.5-µm filter. High-molecular-weight CMC was dissolved in the filter-sterilized PBS solution to achieve a final concentration of 1%; the 1% solution then was pressure filtered in 2 stages (initially passed through a 410-µm filter mesh and then through a 10-µm filter mesh to remove gel bodies). The solution was divided into aliquots and placed into 500-ml bottles, which were autoclaved at 115°C for 25 minutes by use of a liquid slow-release cycle. Viscosity of each solution was determined by use of a Brookfield cone and plate viscometer under low-shear conditions (cone/plate, 52; shear rate, 3.75/s).

Surgical procedure—The day before surgery, ponies were weighed and a complete physical examination was performed. Blood samples were collected for determination of total protein (TP) and fibrinogen concentrations, serum biochemical analyses, and determination of electrolyte concentrations and clotting times. On the morning of surgery (day 0), peritoneal fluid was collected for analysis (total and differential cell count, baseline CBC count, measurement of total protein, and platelet viscometer using a liquid slow-release cycle). Viscosity of each solution was determined by use of a Brookfield cone and plate viscometer under low-shear conditions (cone/plate, 52; shear rate, 3.75/s).

Postoperative care and monitoring—Analgesics were not routinely administered after surgery because of concerns about anti-inflammatory effects or effects on gastrointestinal motility that could adversely impact formation of adhesions. However, a pain scoring system was used to assess the ponies for signs of abdominal pain so that analgesics could be administered when needed. The scoring scale used was as follows: 1, sternal or lateral recumbency; 2, recumbent but looking at its flank and restless; 3, standing and intermittent rolling, sweating, and increase in heart rate; and 5, rolling, sweating, and appearing to be extremely uncomfortable.

Ponies that had any of these signs were administered analgesics appropriate to the degree and duration of pain intensity in accordance with the following pain management protocol: 0.5 to 1.0 mg of xylazine/kg, IV, and observe response during subsequent 15 minutes; a combination of 150 mg of xylazine and 0.8 to 1.0 mL of butorphanol, IV, and observe response during subsequent 15 minutes; 0.5 mg of...
flunixin meglumine/kg, IV, and observe response during sub-sequent 20 minutes; 1.0 mg of flunixin meglumine/kg, IV, and observe response during subsequent 20 minutes; or administration of a sufficient amount of xylazine to achieve cessation of the signs of abdominal pain.

All ponies were given a single dose of atropine sulfate (0.3 mg/kg, IV) after recovery from anesthesia to promote ileus, and food was withheld overnight. Ponies were monitored every 4 hours for the first 3 days and then 4 times daily thereafter for colic, fever, appetite, water consumption, and fecal production, and a complete physical examination was performed. Clinical variables such as heart rate, respiratory rate, rectal temperature, and frequency of homborigni were recorded for each pony. Samples of blood and peritoneal fluid were collected for analysis on days 1, 2, 3, 5, 7, and 10 after surgery. Abdominal ultrasonography was performed on days 1, 2, and 3 after surgery to assess the volume of intra-abdominal fluid in each pony. On day 10 after surgery, each pony was weighed and then euthanatized by injection of pentobarbitone (100 mg/kg, IV). Necropsy was performed on each pony.

Necropsy—The entire abdominal cavity was inspected for adhesions, including the celiotomy and typhlotomy incisions. Each abraded site was carefully inspected, and the number and location of adhesions for each pony were recorded and photographed. Each adhesion was classified on the basis of type (bowel-to-bowel, bowel-to-mesentery, bowel-to-omentum, or other) and severity by use of a standardized grading system based on adhesion strength. Adhesions were assigned grades of 1 to 4, with grade 1 (least severe) representing an adhesion that was easily lysed (such as a fibrous adhesion) and grade 4 (most severe) representing a fibrous adhesion that disrupted the adjacent tissue architecture when broken down. Representative full-thickness tissue samples of each abraded site and remote unabraded jejunum were collected from each pony and fixed in neutral-buffered 10% formalin for use in histologic examination. Representative tissues were obtained from each pony at 3 sites (normal small intestine remote from any abraded area or adhesion, an abraded area not involving an adhesion, and an abraded area involving an adhesion).

Statistical analysis—Simple unpaired comparisons were made for the adhesion data. The Fisher exact test was used to analyze total number of adhesions and number of surgical sites with multiple adhesions. The nonparametric Mann-Whitney U test was used to analyze median clinical data (clinical variables determined during physical examinations, such as heart rate and rectal temperature) and mean incidence of adhesions for the treated and control groups. Two-tailed P values were reported in all cases; a value of \( P \leq 0.05 \) was considered significant. Data for hematologic, serum biochemical, and peritoneal fluid analyses were evaluated by use of a 1-way ANOVA, and the Tukey multiple-range test was used to detect significant differences between means for differing time points (\( P < 0.05 \)). Statistical analyses were performed by use of 2 computer software programs.

Results

Clinical data—All ponies recovered from anesthesia without complications. Overall, the ponies were in good health without preoperative abdominal or metabolic compromise at the time of surgery; thus, there was little evidence of postoperative pain. One of the control ponies had a brief period of pawing on the first day after surgery (pain score, 2 to 3) that resolved without treatment. Another control pony had consistent pawing on day 7 after surgery (pain score, 3) and was treated with flunixin meglumine (1.1 mg/kg, IV).

Control ponies had significantly (\( P = 0.001 \)) higher median heart rates and rectal temperatures after surgery, compared with values for CMC-treated ponies. One CMC-treated pony developed a transient heart murmur the day after surgery and had a reduced appetite after surgery. Necropsy of this pony revealed a large intra-abdominal abscess with multiple adhesions that resulted from an unexplained perforation of the jejunum at 1 of the sutures located in an abrasion site. Consequently, data from this pony were excluded from the analysis. Four of 5 control ponies lost weight during the postoperative period (range, 1 to 8% of original body weight). In contrast, only 1 of the 4 remaining CMC-treated ponies lost weight (0.5% of original body weight) during the same period. We did not detect significant differences between control and CMC-treated ponies for any of the other clinical variables measured.

Peritoneal fluid and hematologic analyses—On day 7 after surgery, mean WBC count in peritoneal fluid was significantly (\( P = 0.005 \)) higher for the control ponies, compared with values for the CMC-treated ponies. The percentage of neutrophils and TP concentration in peritoneal fluid also were increased in the control group on day 7, compared with the treatment group, but values did not differ significantly between the 2 groups. Mean TP concentration in peritoneal fluid was increased above the uppermost reference value (reference range, < 2.5 g/dL) in all ponies after surgery. Mean fibrinogen concentration was significantly increased in the control ponies on days 5 and 7, compared with CMC-treated ponies and for all other days. We did not detect significant differences between groups for all other hematologic and serum biochemical variables measured, which included RBC count in peritoneal fluid, PCV, total and differential WBC counts in blood samples, TP concentration, and clotting times. Microscopic examination of blood smears from CMC-treated ponies on days 1 to 3 after surgery revealed a basophilic amorphous material mixed in with the blood components in some of the smears.

Adhesion data—All control ponies developed multiple adhesions at multiple sites. For the 34 potential adhesion sites (1 typhlotomy incision and 6 abraded areas in 4 ponies plus 1 typhlotomy incision and 5
abraded areas in 1 pony), there were 61 adhesions. After adjusting for multiple-adhesion sites (ie, sites at which 1 abraded site adhered to another abraded site, which was counted as 1 adhesion instead of 2), there were 48 adhesions for the control group. This number also included other adhesions that did not involve an abraded area, such as the peritoneum, typhlotomy incision, abdominal incision, or another area of bowel.

For the CMC-treated ponies, there were 11 adhesions for 27 potential sites (1 typhlotomy incision and 6 abraded areas in 3 ponies plus 1 typhlotomy incision and 5 abraded areas in 1 pony). Allowing for multiple-site adhesions, there were 10 adhesions. Overall incidence of total adhesions, which included multiple adhesions at an abraded site, was significantly (P = 0.01) different between the control (141%) and CMC-treated (37%) ponies. In the control group, 16 of 34 (47%) potential adhesion sites had multiple adhesions. This differed significantly (P < 0.001) from the treatment group, which did not have any sites with multiple adhesions (the 1 multiple-adhesion site in the CMC-treated ponies was located at a suture site). Mean ± SD incidence of adhesions in the control group was 11 ± 3.4, which differed significantly (P = 0.02) when compared with the mean incidence (2.5 ± 2.6) for the treatment group. Types of adhesions that formed in control ponies included 19 bowel-bowel adhesions, 13 bowel-mesentery adhesions, 11 bowel-omentum adhesions, and 5 others (Table 1). For the CMC-treated ponies, there was 1 bowel-bowel adhesion, 3 bowel-mesentery-adhesions, 4 bowel-omentum adhesions, and 3 others. All adhesions in the CMC-treated group involved serosal sutures, many of which involved focal attachment to the suture material with minimal involvement of the abraded serosa.

Table 1—Number of adhesions 10 days after surgery to induce intra-abdominal adhesions by use of a modified bowel abrasion method for control ponies and ponies treated with a 1% solution of high-molecular-weight carboxymethylcellulose (HMW CMC)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of abraded sites</th>
<th>Total No. of adhesions*</th>
<th>Adjusted No. of adhesions*</th>
<th>B-B adhesions</th>
<th>B-M adhesions</th>
<th>B-O adhesions</th>
<th>Other†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>34</td>
<td>61a</td>
<td>48a</td>
<td>19a</td>
<td>13a</td>
<td>11a</td>
<td>5</td>
</tr>
<tr>
<td>HMW CMC</td>
<td>27</td>
<td>11b</td>
<td>10b</td>
<td>1b</td>
<td>3b</td>
<td>4b</td>
<td>3</td>
</tr>
</tbody>
</table>

*Adjusted for multiple-adhesion sites (ie, sites at which 1 abraded site adhered to another abraded site, which was counted as 1 adhesion instead of 2). †Other included adhesions involving other intra-abdominal structures such as the bladder, broad ligaments of the ovaries, and peritoneum.


Within a column, values with different superscript letters differ significantly (P < 0.05).
Histologic examination—In regions that had been abraded, the serosa was expanded by granulation tissue, fibrosis, and moderate inflammation characterized by lymphocytes and an occasional eosinophil. Tissue sections that contained suture material had evidence of a severe granulomatous inflammatory response (grade 4) surrounding the suture (Fig 1). In sections without suture material, there was a noticeable difference in serosal thickness between sections from unabraded jejunal serosa and abrasion sites that involved and did not involve an adhesion for both groups of ponies. The serosa in the tissue sections for abraded areas that involved and did not involve an adhesion was significantly thicker than unabraded serosa, and abraded sections also had a higher inflammatory score than unabraded sections. Subjectively, serosa of abraded sections in the control ponies appeared thicker with a greater inflammatory response, compared with the serosa of abraded sections from the treated ponies (Fig 2); however, mean serosal thickness or inflammatory score did not differ significantly between the control and CMC-treated ponies.

Discussion

Results of the study reported here support our hypothesis that a 1% solution of HMW CMC applied to tissue surfaces before inducing controlled tissue trauma during surgery will significantly reduce postoperative adhesions. These results are similar to findings from other studies\(^\text{19,20}\) that involved the use of medium- or low-molecular-weight CMC solutions in equids. In our experience, highly viscous solutions of HMW CMC (molecular weight > 500 kD) are more efficacious at preventing adhesions in rats after cecal abrasion than are less viscous or lower molecular weight solutions. The importance of molecular weight may be that such solutions form a boundary layer that is more difficult to dilute or wipe off, resulting in a longer residence time on the bowel surface. Another study\(^\text{20}\) conducted by our laboratory group revealed that hydrophobic or electrostatic interactions can be an important reason for biophysical adhesion between mesothelial cells and materials used during surgery, such as gloves, instruments, and drapes. Hydrophilic CMC solutions can form a protective and lubricious coating that minimizes these interactions and prevents adhesive and abrasive contacts between the mesothelium and foreign material. The fact that CMC solutions can form a lubricating boundary layer that reduces friction and shearing forces is evidence that such solutions provide a second means of tissue protection.\(^\text{20}\) This boundary layer provides a specific local protective effect that is achieved with a minimal volume of solution and is not enhanced by use of large volumes of solution.\(^\text{20}\) In other studies\(^\text{15,28,29}\) investigator evaluated CMC in ponies and horses by use of up to 7 mL of CMC/kg (approx 3 L for a 450-kg horse) administered IP. In the study reported here, we used only 1 to 2 mL/kg (approx 300 mL/pony) administered IP, which was extremely effective in preventing adhesions for a more severe model than was used in those other studies.

Preemptive use of tissue protective CMC solutions is effective for reducing direct and indirect trauma that occurs during surgery.\(^\text{13}\) Direct trauma occurs as part of the procedure itself (eg, clamping, cutting, and suturing of tissue during bowel resection) and is practically unavoidable. Indirect trauma, also known as adjunctive trauma, occurs concomitant with the surgical procedure but separate from the primary surgical site.\(^\text{13}\) It may be a general phenomenon (eg, tissue desiccation from exposure to air or inappropriate irrigation of tissues as a result of extremes of temperature or volume of fluid\(^\text{32,33}\)), or it may be more localized (eg, holding or blotting of tissues with a sponge).\(^\text{34}\) Adjunctive trauma increases with increasing surgery time and causes mesothelial damage leading to areas of fibrin exudation and potential sites for adhesion formation.\(^\text{32,34-36}\) Because sites at which multiple tissues are damaged favor the development of adhesions,\(^\text{37}\) reducing the number of such sites may reduce the likelihood of adhesion formation after surgery.

The HMW CMC solution was used as a presurgical treatment in the study reported here. It was instilled into the abdomen and used to coat the surgeons’ gloves prior to manipulation of any bowel as well as to coat the jejunal serosa prior to creating the abrasions. In so doing, adjunctive trauma was minimized. In other studies\(^\text{13,27,29}\) that involved evaluation of CMC in horses, investigators have used it primarily as a barrier adjuvant in that it was applied after bowel manipulation and trauma had occurred. Because of differences in adhesion models used and differences in the reporting of results for those studies, it is difficult to compare the effectiveness of CMC for use in prevention of adhesions when used as a tissue protective adjuvant versus its use as a barrier adjuvant. Furthermore, in another study\(^\text{34}\) conducted by our laboratory group, we documented significantly greater efficacy in preventing adhesions with tissue protective solutions than solutions of similar or greater viscosity used as barrier adjuvants after tissue trauma had occurred.

Modifications to the classic serosal abrasion model described elsewhere\(^\text{13}\) for inducing postoperative adhesions led to dramatic, severe adhesion formation in the untreated control group of ponies in the study reported here. One modification we implemented consisted of postoperative administration of atropine sulfate (an anticholinergic drug) to all ponies to induce ileus and promote adhesion formation. Incidence of adhesions in our control group was 141%, which is considerably greater than the values of 33 and 50% reported in other studies\(^\text{14,30}\) conducted by investigators who used the classic serosal abrasion model.

It is difficult to compare the relative effectiveness of the models used to create adhesions between studies because of the various methods used to document adhesions. However, it was reported in 1 study\(^\text{14}\) that only 2 of 6 control horses developed bowel-to-bowel adhesions, and in another study\(^\text{13}\) investigators reported cecal adhesions to the celiotomy incision that were possibly attributable to adjunctive trauma and unrelated to serosal abrasion of the jejunal. In other studies\(^\text{15,39}\) that involved the use of horses, bowel-to-bowel adhesions have not been reported. In the study report-
ed here, all 5 control ponies developed bowel-bowel adhesions, indicating the effectiveness of the modified model for inducing postoperative adhesions.

One treatment pony was eliminated from the study because an intra-abdominal abscess formed at the site of a suture located in an abraded area of jejunum. The cause of the abscess could not be determined because of the extensive omental and mesenteric adhesions associated with it, which distorted the anatomy. It may have been caused by suture material penetrating the bowel lumen during placement, although suture was not found associated with the abscess during necropsy. It is also possible that the abscess resulted from seeding of the abdomen with bacteria during typhlotomy.

All intra-abdominal adhesions are not clinically relevant, and some adhesions (e.g., omental adhesions) may be considered beneficial because they provide blood supply to ischemic areas of serosa or intestine and act as vascular grafts. However, bowel-to-bowel adhesions are far more likely to cause a clinical problem because of kinking or stenosis of the bowel, particularly as fibrous tissue contracts, which leads to partial or complete obstruction. In contrast to the classic model used in other studies, the modified abrasion model used in the study reported here was particularly effective at creating bowel-bowel adhesions. All ponies in the control group developed severe bowel-bowel adhesions at multiple sites, compared with only 1 bowel-bowel adhesion in the CMC-treated group. In fact, all adhesions in the CMC-treated ponies involved a serosal suture, and in 1 pony, the omentum adhered to the catgut sutures at 4 separate abraded sites. The strategic placement of catgut suture in the serosa in our study probably contributed to effectiveness of adhesion formation by creating a persistent inflammatory response, which was evident during histologic examination (Fig 2). The substantial inflammatory reaction invoked by the chronic catgut suture contributed to the inflammatory scores in the CMC-treated ponies and explains the reason that we did not detect significant differences in histologic characteristics between the CMC-treated and control ponies, despite the subjective impression to the contrary (Fig 1). These results confirm the adhesiogenic nature of exposed suture material within the abdomen after surgery and, along with the reactivity of the suture material used, emphasize the surgical principle of avoiding such exposed material, if possible. Persistent inflammation in a postoperative adhesion model will reduce the perceived effectiveness of tissue protective solutions such as HMW CMC and should probably be avoided, as recommended in another study that involved horses.

Results from the study reported here also supported our second hypothesis that it is safe to use HMW CMC solution intra-abdominally in horses. During necropsy, we did not detect evidence of delayed wound healing in any of the CMC-treated ponies at the typhlotomy incision or ventral midline incision. Analysis of results of histologic examination for the CMC-treated ponies confirmed that CMC did not affect wound healing or the formation of granulation tissue. In a recent study, intra-abdominal use of CMC in horses did not adversely affect healing of intestinal anastomosis despite concerns that CMC promotes enteric leakage in rats. Other reported adverse effects of CMC in horses include high blood glucose concentrations, low serum calcium concentrations, and increased fibrinogen concentrations. However, none of those abnormalities were observed in the CMC-treated ponies in our study. In contrast, the control ponies had significantly increased serum fibrinogen concentrations on day 7 after surgery with a concomitant increase in WBC count of the peritoneal fluid, consistent with increased inflammation within the peritoneal cavity.

Prominent precipitate in blood films from horses treated IP with CMC can persist for up to 9 days; it may be dose-dependent and is obvious in foals. Precipitate was observed in blood films of some of the CMC-treated ponies for 48 hours after surgery in the study reported here, but other clinical, hematologic, or serum biochemical abnormalities were not associated with detection of the precipitate. It appears this precipitate was a direct effect of CMC being absorbed from the peritoneal cavity into the bloodstream and admixing with blood components without causing any detrimental clinical effects.

Overall, CMC had good biocompatibility without compromising healing or host-defense mechanisms. Also, we did not detect adverse effects of its use in a clean-contaminated procedure, which was performed to mimic the clinical situation for a colic surgery. None of the CMC-treated ponies developed peritonitis or peri-incisional abscesses, consistent with an in vitro study in which it was documented that CMC is a poor substrate for bacterial growth. Although none of the ponies developed obvious signs of colic after surgery, the control ponies had significant increases in heart rate, rectal temperature, and weight loss, compared with results for the CMC-treated ponies. This suggests that the CMC-treated ponies did better after surgery and that by reducing adhesion formation, preventative coating with CMC improves general patient well-being. We believe a 1% solution of HMW CMC may be advantageous for routine use in abdominal surgery of horses to prevent postoperative adhesions and to preventatively coat the intestinal serosa prior to performing an enterotomy to facilitate removal of foreign material such as ingesta.

References


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