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Effect of sodium carboxymethylcellulose and methylprednisolone on the healing of jejunal anastomoses in rats

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Abstract

Sodium carboxymethylcellulose (SCMC) has been effective in reducing adhesion formation and corticosteroids reduce the inflammatory process. The objective of this study was to define the intraperitoneal (*ip*) effects of SCMC combined with intramuscular (*im*) methylprednisolone on peritoneal adhesion formation and on jejunal anastomosis healing in rats. Twenty Wistar rats (200-350 g) were divided into four groups (N = 5): groups I and III (controls) 5 and 21 days of treatment before sacrifice, respectively; groups II and IV (experimental groups) 5 and 21 days of treatment, respectively. SCMC (1%) was infused into the abdominal cavity and methylprednisolone (10 mg kg^{-1} day^{-1}) was injected *im* daily from the day before surgery for animals of groups II and IV. All rats were submitted to a jejunal anastomosis. Sections of the anastomosis were prepared for routine histopathological analysis. The abdominal adhesion of group IV was less intense when compared with group III ($P<0.0008$). Anastomotic resistance was higher in groups II and IV when compared with groups I and III, respectively ($P<0.05$). There was no histological difference between groups I and II (exuberant granulation tissue on the serosal surface). Group III presented little peritoneal fibrinous tissue, with numerous thick collagen fibers. Group IV presented extensive although immature young fibrous tissue with rare thick collagen fibers. Sodium carboxymethylcellulose combined with corticosteroids seemed to diminish peritoneal adhesion but did not reduce anastomotic resistance.

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- Sodium carboxymethylcellulose
- Corticosteroid
- Histology
- Wound healing

Introduction

Fibrinous adhesions link intestinal loops to other abdominal viscera and peritoneum in the first hours after surgery, inflammation or abdominal trauma. The fibrin may be completely reabsorbed or organized with the ingrowth of capillaries and fibroblasts establishing fibrous adhesions (1).

Peritoneal adhesions have been considered to be the main cause of intestinal obstruction. This phenomenon has been extensively studied, but some of its characteristics are still not fully understood. Adhesions may not depend only on peritoneal mechanical injuries, but also on the presence of ischemic tissue which must result from pulling the edges of the tissue together with sutures. Contaminants in the peritoneal cavity at the time of laparotomy are responsible for both

granuloma formation and development of fibrous adhesions (2,3).

Procedures against adhesions involve prevention of fibroblastic proliferation, prevention of fibrin formation, mechanical and enzymatic removal of fibrin, use of fibrinolytic agents, separation of surfaces, and inhibition of fibroblastic proliferation with antihistaminic drugs or cortisone. Numerous therapeutic agents have been used in the attempt to prevent postoperative adhesions (2). Recently, it has been shown that sodium carboxymethylcellulose (SCMC) is effective in reducing adhesion formation (4). Solutions of this material can be prepared to mimic the viscosity of body fluids such as synovial fluid and aqueous humor. The high molecular weight (350,000) and slow peritoneal absorption of SCMC enhance its ability to separate serosal and peritoneal surfaces during epithelial regeneration (1,5-8). The postulated mechanism of action for SCMC is the creation of a "flotation bath" during the period of epithelial regeneration which prevents the apposition of serosal and peritoneal surfaces blocking the formation of adhesions (1,3,5,6,9). Another tenable explanation is that SCMC produces its beneficial effect by curtailing fibroblast activities or proliferation and preventing fibrin deposition on the serosal surfaces of the injury (10). It may also inhibit the movement of inflammatory cells and cellular elements during the period of peritoneal repair (10,11).

Another factor that interferes with peritoneal healing after a trauma is cortisone, which reduces the initial inflammatory process and delays its subsequent stages. Cortisone also reduces the formation of granulation tissue and inhibits fibroblastic proliferation (2,12). However, the literature presents contradictory data about the effects of cortisone on wound healing (13-17). Some studies have suggested that wound healing and integrity of the anastomosis are unaffected by steroids (2,18). In 1994, we showed that methylprednisolone reduces the resistance of intestinal anastomosis in rats (12). Previous studies have demonstrated that betamethasone retards fibroplasia (19) and dexamethasone retards the healing process of anastomosis in rats (20). Consequently, the use of cortisone is accompanied by a high incidence of anastomotic fistulas, abscesses and sepsis.

The purpose of the present study was to identify the effects of SCMC administered intraperitoneally (*ip*) in combination with intramuscular (*im*) methylprednisolone on peritoneal adhesion formation as well as on jejunal anastomosis resistance in rats.

Material and Methods

The present study was carried out on 20 adult Wistar rats of both sexes weighing 200-350 g from the animal facilities of the Medical School, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil. The animals were randomly divided into four groups of five or six animals per cage and received ration and water *ad libitum*.

Group I (control). Five animals studied on the 5th postoperative day without *ip* application of SCMC or methylprednisolone.

Group II. Five animals studied on the 5th postoperative day after *ip* infusion of 12 ml 1% SCMC. Intramuscular methylprednisolone (10 mg kg^{-1} day^{-1}) was administered daily from the first day before surgery to the end of the experiment.

Group III (control). Five animals studied on the 21st postoperative day without *ip* application of SCMC or methylprednisolone.

Group IV. Five animals studied on the 21st postoperative day after *ip* infusion of 12 ml 1% SCMC. Intramuscular methylprednisolone (10 mg kg^{-1} day^{-1}) was administered daily from the first day before surgery to the end of the experiment.

SCMC was obtained from a local pharmacy; 0.12 g was weighed and transferred to empty penicillin vials. The vials were sterilized in an autoclave and the SCMC in each

vial was diluted in 12 ml distilled water immediately before its administration. The substance was cultured for the detection of bacteria and fungi before use. No microorganism was detected.

The surgical procedure was performed under inhalatory anesthesia with ether. All animals were submitted to the same technique. Through a median laparotomy, the jejunum was cut 10 cm from the duodenojejunal flexure and anastomosed end-to-end in one layer with 5-0 catgut. The abdominal wall was closed in two layers with continuous 2-0 Vicryl suturing.

After the scheduled time (5th or 21st postoperative day), the animals were killed with an ether overdose and the abdominal cavity was examined. The extent of the adhesions was graded according to the following classification: grade 0 - complete absence of adhesions, grade I - adhesions restricted to the anastomosis area, grade II - adhesions close to anastomosis, and grade III - adhesions far from the anastomosis not related to the suture.

Bowel resistance was tested on the 5th and 21st postoperative days. This parameter was evaluated by measuring the rupture tension of two 10-cm bowel segments: one containing the anastomosis in its center and the other consisting of an intact segment of jejunum located immediately after the first segment. A three-way set of communicating tubes was used to measure the rupture tension. It included a tube containing mercury graduated in ml, a second tube introduced into the proximal part of the jejunal segment, and a third tube connected to an air pump. The distal part of this segment was closed with 2-0 silk. Air was gradually and uniformly insufflated into the jejunal segment until its suture burst. The pressure that provoked the burst was determined in the graduated tube.

A segment of the anastomosis was removed from all rats and prepared for routine histopathological analysis after staining with hematoxylin and eosin.

The data for the groups were compared statistically by ANOVA, with the level of significance set at $P<0.05$.

Results

Table 1 shows the pressures of jejunal rupture and the grade of adhesion in both control and experimental groups. The resistance of the anastomosis was significantly lower compared to the intact jejunum in both the control and experimental groups after 5 days ($P<0.03$). Groups I and II presented lower anastomotic resistance than groups III and IV. The resistance of the intact jejunum did not differ among the four groups.

Anastomotic resistance was higher for groups II and IV when compared with groups I and III, respectively ($P<0.05$). It should be emphasized that after 21 days there was no difference in resistance between the anastomosis and intact segment in either group. Comparison of groups III and IV did not show a difference in jejunal wall resistance.

The abdominal adhesion of group IV was significantly less intense when compared with group III ($P<0.0008$).

There was no histological differences between groups I and II. Both presented exuberant granulation tissue on the serosal surface with intense vascular formation; fibroblasts and inflammatory mononuclear infil-

Table 1 - Resistance of jejunal anastomosis and intact jejunum and grade of adhesions in the presence of sodium carboxymethylcellulose (SCMC) and methylprednisolone in rats.

Group I (control) - 5 days; group II - 1% SCMC (12 ml) + methylprednisolone (10 mg kg^{-1} day^{-1}) for 5 days; group III (control) - 21 days; group IV - 1% SCMC (12 ml) + methylprednisolone (10 mg kg^{-1} day^{-1}) for 21 days. Data are reported as means ± SEM. *$P<0.03$ compared with the corresponding intact bowel; $^{0}P<0.05$ compared with group II; $^{\#}P<0.002$ compared with group III; $^{+}P<0.0008$ compared with group IV (ANOVA)

Parameters	Group I	Group II	Group III	Group IV
Anastomosis	7.8 ± 2.7$^{*0\#}$	12.2 ± 3.1^{*+}	23.6 ± 1.8^{+}	26.6 ± 2.1
Intact jejunum	22.8 ± 3.7	23.2 ± 4.7	22.8 ± 3.3	23.8 ± 2.7
Adhesion grade	1.2 ± 1.1	2.2 ± 0.5^{+}	2.0 ± 0.0^{+}	0.4 ± 0.6

tration were also observed. The animals of the control group studied on the 21st postoperative day presented some peritoneal fibrinous tissue, relatively well organized, with numerous thick collagen fibers, few inflammatory cells, and little vascular formation. The animals submitted to SCMC and corticoid treatment showed extensive although immature fibrous tissue with rare thick collagen fibers (Figure 1).

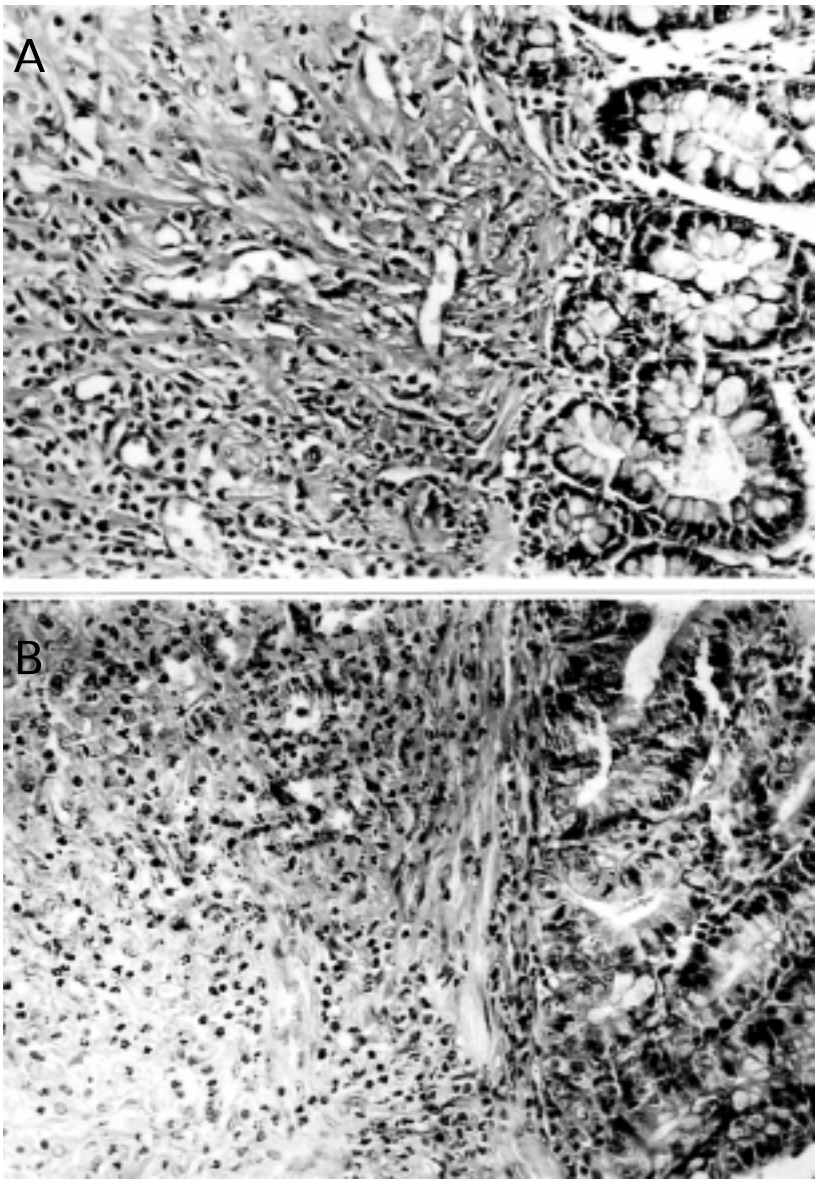

Figure 1 - Jejunal anastomosis of the control group (A) and animals submitted to sodium carboxymethylcellulose and a corticoid (B) on the 21st day after surgery. *A*, Observe the scarce peritoneal fibrinous tissue, relatively well constituted, with numerous thick collagen fibers, few inflammatory cells, and little vascular formation. *B*, Note the extensive although immature fibrous tissue with rare thick collagen fibers. Hematoxylin and eosin. A and B, Magnification = 200X.

Discussion

The peritoneum covers the abdominal organs, and one of its main functions is to prevent adhesions. This role is impaired when inflammatory or trauma processes affect integrity of the serosa (2). In the present study, the dose of methylprednisolone was chosen on the basis of our previous studies and on literature data that demonstrated an inhibitory effect of the drug on wound healing (12,17). According to the literature, the harmful effects of corticoids are more evident when the agents are administered before surgery and maintained during the postoperative period (12,21,22).

As expected, there was less peritoneal adhesion in the animals submitted to SCMC and methylprednisolone. However, in contrast to reports by others, this condition increased the anastomotic resistance after the 5th postoperative day, with no difference from the intact wall by the 21st postoperative day. The histological findings for the groups submitted to SCMC and corticosteroids presented thin healing tissue with disorganized collagen fibers although containing thicker fibrous tissue.

We cannot explain the physiopathology of the increasing resistance in the presence of SCMC and corticosteroids. *In vitro* experiments have demonstrated that SCMC phagocytosis does not affect the production of oxygen free radicals by peritoneal macrophages. It is possible, however, that SCMC quells the peritoneal inflammatory response by preventing macrophage adhesion and cytokine production (3). In any case, the absence of differences between the anastomotic resistance on the 21st postoperative day and resistance of the intact jejunal segment suggests that anastomosis healing had reached full resistance by this time in the

presence or absence of adhesions. It is well known that in clinical practice most anastomotic complications occur during the first postoperative week. The late complications observed in intestinal surgeries are obstructions due to adhesions or, very rarely, due to anastomotic hyperplastic fibrosis.

In conclusion, the presence of SCMC combined with corticosteroids reduces peritoneal adhesion formation and increases anastomotic resistance. Further studies are necessary in order to explain the role of adhesions on intestinal healing.

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