EVALUATION OF THE CICATRIZATION OF LEFT COLON ANASTOMOSES IN THE PRESENCE OF PERITONITIS. AN EXPERIMENTAL STUDY ON RATS[1]

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SUMMARY: To monitor the evolution of anastomoses performed on the distal colon in a situation of experimental peritonitis, 37 Wistar-Tecpar male rats aged 114 to 130 days and weighing on average 298 g were divided into two lots: group S (control, N = 18) and group P (experimental, N = 19). P rats were submitted to laparotomy and peritonitis was induced by perforating the cecum with a needle, and S rats were only submitted to manipulation of the cecum. Twenty-four hours later the animals were resubmitted to laparotomy and distal colectomy was performed 1.5 cm distal to the peritoneal reflection. End-to-end anastomosis was performed on a single extramucosal plane using 8 separate stitches with 5-0 polypropylene sutures. The anastomoses were checked on the 3rd and 7th postoperative day. Upon opening the peritoneal cavity, the presence of alterations such as peritonitis or abscesses, adhesions, organs involved, fistulas or dehiscences was recorded. A 4.0 cm segment of the colon containing the anastomosis was resected and rupture pressure was measured. Epithelialization of the mucosal wound was evaluated and the material was studied histopathologically for inflammatory reaction and scar condition. Upon relaparotomy, peritonitis was detected in all P animals and fibrin was observed in the cavity of all animals. Adhesions were present in 2 groups, without significant differences between them. Mean rupture pressure was 108.7 mm Hg in group S and 112.0 mm Hg in group P on the 3rd day and 205.0 mm Hg in group S and 206.6 mm Hg in group P on the 7th day, with no significant difference between groups. Microscopic evolution was similar in the two groups. These results permit us to conclude that peritonitis induced by this method does not modify the healing process of distal colon anastomoses in rats.

SUBJECT HEADINGS: Surgical anastomosis.Infection. Colon.

INTRODUCTION

In view of the high incidence of infection of the peritoneal cavity in surgical practice, many investigators have studied the relationship between peritonitis and healing of intestinal anastomoses. Contradictory results have been obtained in studies of the influence of infection on the development of colon anastomosis healing in experimental animals, with some investigators reporting high rates of dehiscence and mortality[6,8,21] and others reporting little or no effect of infection on the anastomotic lesion[12,18,20]

Thus, the present study was conducted to determine the effect of peritonitis on the healing of experimentally induced colon anastomoses in rats.

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METHOD

The study was conducted on 40 male rats (*Rattus norvegicus albinus, Rodentia mammalia*) of the Wistar-Tecpar strain aged 114 to 130 days and weighing on average 298 g. The animals were divided into groups S (control) and P (experimental).

The animals were anesthetized with ether, weighed and identified. The ventral abdominal wall was shaved and a median 4 cm laparotomy was performed. In P animals, the induction of peritonitis consisted of partial ligation of the cecum immediately below the ileocecal triangular fold with 5-0 polypropylene sutures in order to increase pressure inside this portion of the intestine without provoking ischemia, and also to permit at the same time the free transit of the fecal bolus from the small intestine to the large bowel. The cecum was then perforated at 10 random points with a 40 x 13 venipuncture needle. The abdominal wall was closed with continuous 4-0 cotton sutures on two planes, i.e., peritoneum-muscle-aponeurosis and skin. After recovery from anesthesia the rats were returned to their cages where they had free access to water and ration.

In group S animals the first procedure consisted of manipulation of the cecum by passing 5-0 polypropylene suture through its base without performing ligation. Laparorrhaphy was the same as performed in P rats.

Twenty-four hours after the first surgery, the animals were anesthetized and resubmitted to laparotomy. Total left transverse colotomy was performed 1.5 cm from the peritoneal reflection, followed by end-to-end anastomosis on a single extramucosal plane with 8 separate stitches using 5-0 propylene sutures. Laparorrhaphy was performed on two planes as done in the first surgery, and the animals were allowed to recover from anesthesia and returned to their cages with free access to water and ration until the day for verification.

The animals were sacrified with a lethal dose of sulfuric ether on the 3rd and 7th postoperative day. Changes in the peritoneal cavity, such as presence of purulent or fibrinoid secretion indicating the presence of peritonitis, adhesions and organs involved, fistulas or dehiscences, were recorded. A 4-cm colon fragment containing the anastomosis was removed and submitted to measurement of rupture pressure by the method of CRONIN, JACKSON and DUNPHY (1968). The surgical piece was opened along the antimesentery border and the epithelialization of the mucosal wound was evaluated. We also determined the possible presence of a mucosal ulcer (term used to define the anastomosis line still without reepithelialization and covered with fibrin), an internal spur and surgical sutures in the mucosa.

The colon segments were stretched out on filter paper, fixed in 10% formalin and submitted to histopathological study by the hematoxylin and eosin and Mallory trichrome methods.

The inflammatory reaction was scored as acute, acute-chronic and chronic according to the predominance of cell types and according to scar organization in terms of the arrangement of the tunicae as disorganized, organizing and organized.

The presence of mucosal reepithelialization, mural abscess, necrosis and granulation tissue was determined microscopically.

Data were analyzed statistically by the Mann-Whitney test for analysis of the means and by the exact Fisher test for 2 x 2 tables, with the level of significance set at 0.05 or 5.00%.

RESULTS

Two animals from the 3-day control group and 1 animal from the 7-day experimental group were excluded due to technical problems, so that 37 rats were left in the experiment.

MACROSCOPIC FINDINGS

PERITONEAL CAVITY

Peritonitis was absent in all S animals and present in 100.0% of P animals on the day of the second intervention and at both evaluation times.

Adhesions were present in 100.0% of the animals of both groups on the 3rd and 7th days, involving fat of the spermatic funiculus, seminal vesicle, posterior parietal peritoneum, intestinal loops and mesentery of the small intestine. In both groups, adhesions involved 3/4 to 4/4 of the circumference on the 3rd day, and 1/4, 3/4 or 4/4 on the 7th day.

No fistulas or dehiscences were observed in either group at any time studied.

Figure 1 shows details of the abdominal cavity and adhesions in the animals of the two groups at the two evaluation times.

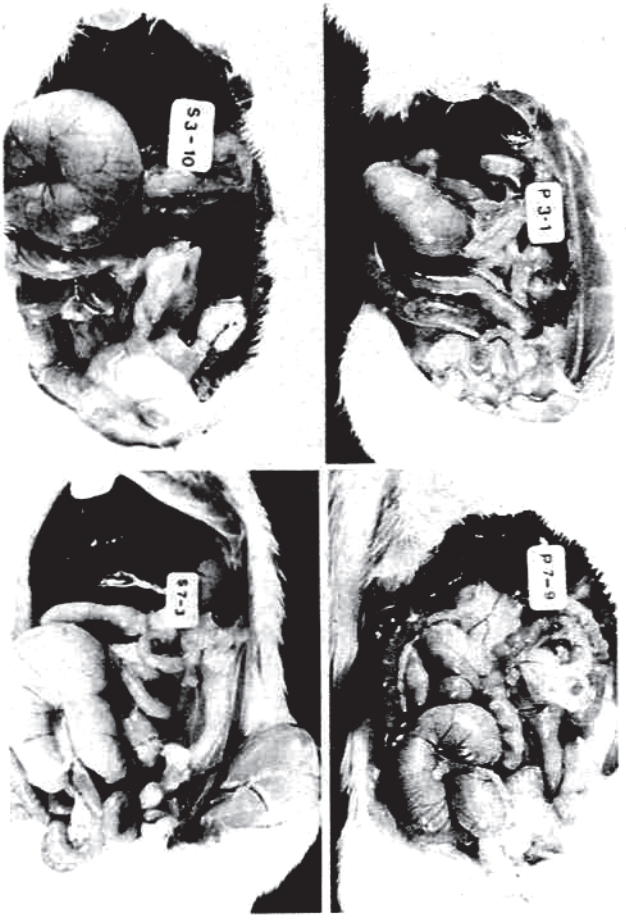


Fig. 1 - Aspect of the abdominal cavities of the animals of both groups on the days of evaluation. S 3-10 = control animal, 3rd day. P 3-1 = experimental animal, 3rd day. S 7-3 = control animal, 7th day. P 7-9 = experimental animal, 7th day

SURGICAL PIECE

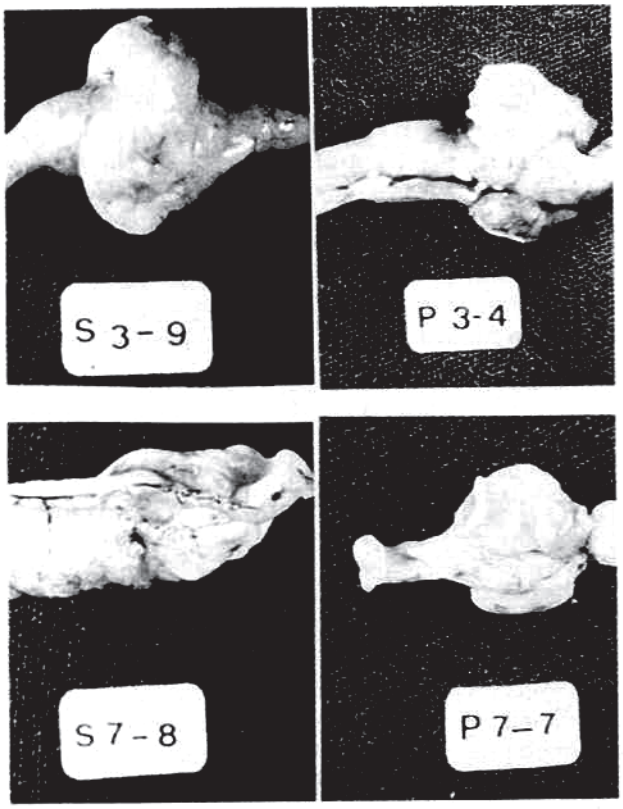
On the 3rd and 7th day the outer surface presented adhesions, as described earlier.

Analysis of the mucosal surface revealed the presence of an internal spur in all surgical pieces from groups S and P.

Mucosal ulcer was identified in 100.0% of the pieces from group S and 90.0% of the pieces from group P on the 3rd day (p = 0.3573), and in 60.0% of the pieces from group S and in 100.0% of the pieces from group P on the 7th day (p = 0.0376).

Sutures turned towards the mucosa were detected in 25% of the pieces from group S and in 30.0% of those from group P on the 3rd day (p = 0.6176) and in 70.0% of the pieces from group S and 77.7% of those from group P on the 7th day (p = 0.5557).

Figure 3 illustrates aspects of the mucosal surface of the anastomoses of the animals in both groups at the two evaluation times.



Flig. 2 - Details of the anastomoses performed in the two groups, seen from the serosal surface. S 3-9 = piece from a control animal, 3rd day. P 3-4 = piece from an experimental animal, 3rd day. S 7-8 = piece from a control animal, 7th day. P 7-7 = piece from an experimental animal, 7th day.

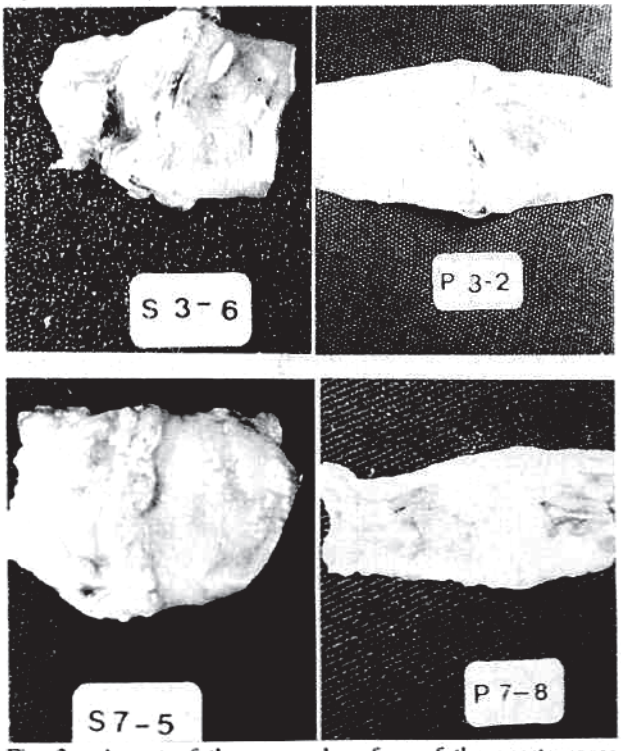


Fig. 3 - Aspect of the mucosal surface of the anastomoses performed in both groups. S 3-6 = colon from a control animal, 3rd day. P 3-2 = colon from an experimental animal, 3rd day. S 7-5 = colon from an experimental animal, 7th day. P 7-8 = Colon from an experimental animal, 7th day.

RUPTURE PRESSURE

According to SMITH, CONNOLY and GILMORE (1982), anastomoses that rupture at pressures of less than 60.0 mm Hg have flaws, even though these are undetectable by being blocked by nearby structures. If we consider this statement to be correct, no detectable flaws were present in the pieces from the two groups submitted to measurement of rupture pressure.

On the 3rd postoperative day, pressures of 70.0 to 150.0 mm Hg (mean = 108.75 mm Hg) were obtained for group S and pressures of 60 to 150 mm Hg (mean = 112.0 mm Hg) were obtained for group P (Table I).

TABLE I - Rupture pressure on the 3rd postoperative day in the colons of the animals of both groups (mm Hg)

	GROUPS	
ANIMAL	S3	P3
1	80	120
2	90	100
3	*	120
4	*	80
5	70	140
6	120	60
7	150	90
8	70	150
9	150	130
10	140	130
MEAN	108,75	112,0

*Animals excluded

Mann-Whitney test
Calculated U = 38.5
Critical U = 17.0

On the 7th day, rupture pressure ranged from 90.0 to 270.0 mm Hg (mean = 205.0 mm Hg) for group S and from 140.0 to 240.0 mm Hg (mean = 206.6 mm Hg) for group P (Table II).

TABLE II - Rupture pressure on the 7th postoperative day in the colons of the animals of both groups (mm hg)

	GROUPS	
ANIMAL	S7	P7
1	240	240
2	200	230
3	150	*
4	190	230
5	220	200
6	90	220
7	210	220
8	230	190
9	250	190
10	270	140
MEAN	205,0	206,6

*Animal excluded

Mann-Whitney test
Calculated U = 41
Critical U = 20

MICROSCOPIC FINDINGS

On the 3rd and 7th day, microscopic analysis revealed the presence of polymorphonuclear cells, vascular congestion, macrophages, lymphocytes and monocytes, characterizing an inflammatory reaction of the acute-chronic type in all histological sections examined.

On the 3rd day the tunicae of the wall were disorganized in 75.0% of the histological sections in group S and in 80.0% of those of group P (p = 0.6176). On the 7th day, they were organizing in 70.0% of group S animals and in 56.0% of group P animals (p = 0.4299). Figures 4 and 5 presents photomicrographs of the histological sections.

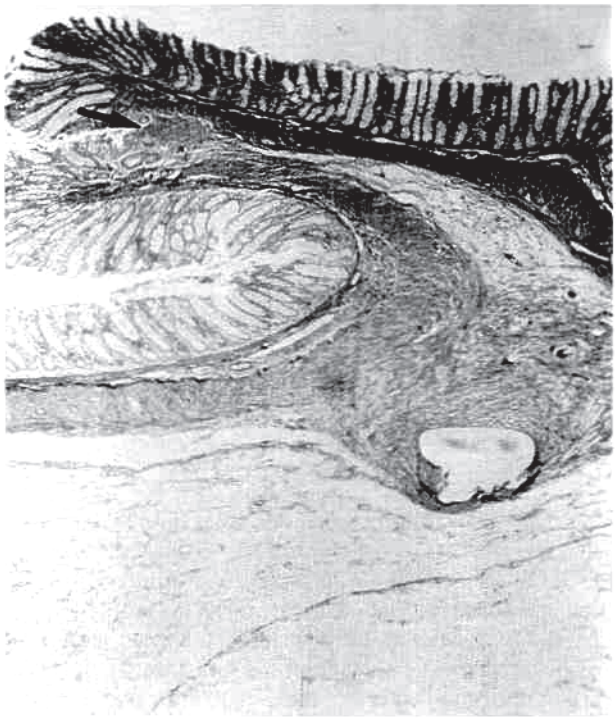


Fig. 4 - Photomicrographs of histological sections of colon obtained of control. animal, 7th day (100x). Incomplete reepithelialization, acute-chronic. inflammatory infiltrate and organizing tunicae.

A mural abscess was identified in 30.0% of the histological sections from group S and in 62.5% of the sections from group P on the 3rd day (p = 0.1842). On the 7th day, a mural abscess was observed in 40.0% of the histological sections from group P and no abscesses were observed in group S (p = 0.0542).

Necrosis was observed in 10.0% of the histological sections from group S and in 11.0% of the sections from group P on the 3rd day (p<0.7059). On the 7th day there was no necrosis in either group.

Granulation tissue was detected in 100% of the histological sections both on the 3rd and on the 7th day.

Fig. 5 - Photomicrographs of histological sections of colon obtained of experimental animal, 7th day (100x). Absence of epithelialization, intense acute-chronic inflammatory infiltrate and disorganized tunicae.

DISCUSSION

Several authors consider infection to be one of the major causes of failure of colon anastomoses. COHN Jr. (1970) emphasized the role of infection in anastomosis dehiscence. DUNPHY (1970) and MASTBOOM et al. (1989) suggested that the presence of bacteria on the surface of the anastomosis promotes collagenolytic activity and accelerates dehiscence. HAWLEY et al. (1970), in a study on rats and rabbits, demonstrated a higher collagenase concentration near the infected intestinal sutures and in the mucosa of the colon and rectum. In a study on the effect of infection on colon anastomoses in the rat, IRVIN (1976) demonstrated increased intracellular collagen lysis during the early phases of healing. According to YAMAKAWA et al. (1971), there was a decrease in collagen in dogs with simulated diverticulitis. MORGENSTERN et al. (1972) reported a decrease in collagen synthesis in contaminated dog anastomoses. IRVIN and HUNT (1974), in an investigation of the cause of dehiscence in traumatized rats, concluded that infection is the immediate cause of anastomosis failure. NAHAI et al. (1977), in a study on dogs, observed that infection of the peritoneal surface along the suture line precedes dehiscence.

TORNQVIST et al. (1990) demonstrated in rats that infection is less important in terms of stimulation of collagen metabolism and that the mechanical factor of obstruction has a greater effect in this situation. In the present study, although peritoneal infection was present it was not sufficient to promote fistulae or dehiscences, in agreement with data reported by RYAN (1970) and IRVIN and GOLIGHER (1973) who observed that gross infection did not prevent healing.

However, CLEAVER et al. (1974) reported delayed healing in the presence of peritonitis. HESP et al. (1984), in a study of infected intestinal anastomosis healing, concluded that infection reduced the hydroxyproline content at the site of anastomosis leading to a delay in recovery of local resistance.

The infection induced in the present experiment did not reduce the resistance of anastomosis, as demonstrated by the measurement of rupture pressure, which was higher than 60.0 mm Hg in both the control and experimental groups. This permitted us to propose that there was no damage at the level of the anastomoses since, according to SMITH, WEHE, BADGER and PIRKLE (1974), above this pressure limit the anastomoses do not present healing failure. However, mucosal reepithelialization was delayed in the peritonitis group compared to the control on the 7th day ($p = 0.0376$).

After inducing peritonitis by cecal perforation, BERMAN et al. (1974) reported 79.4% mortality within 24 hours and ALMDAL et al. (1985) reported 100% mortality after 3.0 mm perforations. LAZAR Jr. et al. (1987), CASTILLO et al. (1991) and ZALOGA et al. (1992), using combined perforation and ligation of the cecum below the ileocecal triangular fold, reported mortality rates ranging from 34 to 100%.

MARSHALL et al. (1988), using ligation and needle perforations, obtained peritonitis with edema and a 14% death rate. In the present study carried out using the same methodology there were no animal deaths, although the method proved to be efficient for obtaining peritonitis.

CONCLUSION

Analysis of the results obtained permits us to conclude that the course of healing of distal colon anastomoses in the presence of peritonitis is not significantly altered in rats.

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