Influence of the peritoneal lavage with bupivacaine on the survival and resistance of colonic anastomoses performed under fecal peritonitis in rats[1]

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ABSTRACT

PURPOSE: To evaluate the effects of peritoneal lavage with bupivacaine on survival and initial resistance of anastomosis on distal colon, performed under peritonitis or not.

METHODS: Forty rats, weighing from 300 to 350g (321.29±11.31g), were randomly divided in four groups and underwent laparotomy and anastomosis on the distal colon six hours after induction of peritonitis by intraperitoneal injection of autologous fecal material or not. Group 1: No peritonitis and lavage with 3ml NS; Group 2: No peritonitis and lavage with 8 mg.kg-1 (± 0.5 mL) of 0.5% bupivacaine added to 2.5 mL of NS; Group 3: Peritonitis and lavage with 3ml NS; Group 4: Peritonitis and lavage with 8 mg.kg-1 (± 0.5 mL) of 0.5% bupivacaine added to 2.5 mL of NS. Necropsies were performed on the animals that died and the time of death was recorded. Surviving animals were submitted to euthanasia on the fifth post-operative day and Total Energy of Rupture biomechanical test (TER) was applied.

RESULTS: Group 4 showed survival increase compared to Group 3, without statistical significance. Group 3 presented the smallest average TER, with statistical significance.

CONCLUSION: Peritonitis increased mortality and influenced negatively the resistance of colic anastomosis in rats. Peritoneal lavage with bupivacaine increased anastomotic resistance.

Key words: Anesthetics, Local. Bupivacaine. Anastomosis, Surgical. Colon. Peritonitis. Rats.

Introduction

Besides the increasing knowledge of the mechanisms regarding tissue cicatrization, still persist complications due to inadequate healing and infection. The constant research on these detailed mechanisms allows the surgeon to interfere on tissue healing, prevent and anticipate complications.

Ocurrence of intestinal anastomotic dehiscence is associated with significant increase of length of stay, need of reoperations, morbidity, mortality, healthcare costs and patient´s distress[1,2]. Many factors are associated with anastomotic leaking: low rectal cancer, non-specialized surgeons, diabetes mellitus, chronicle obstructive pulmonary diseases, cardiovascular diseases, emergency surgeries, malnutrition, obesity, male sex, smoking, alcohol consumption and perioperative blood transfusion[1-3].

Although many advances have been achieved in surgical techniques and perioperative care, the incidence of anastomotic leaking has not decreased significantly[4]. The incidence of anastomotic leakage after colorectal surgery is around 2.8%[3], which is associated with a 6-22 per cent mortality rate[2].

Along with the previously mentioned factors, peritonitis is a major factor that can negatively influence the cicatrization of colorectal anastomosis. In an international multicenter study, Sartelli *et al*.[5] showed that besides all the advances in diagnostic methods, surgical techniques and antibiotics, the rates of morbidity and mortality related to serious intra-abdominal infections remain excessively high. Frequently, colorectal surgeons face the challenge of performing an anastomosis under condition of peritonitis.

Rocha *et al*.[6] described the decrease of the anastomotic resistance in mice with peritonitis by ligature and cecal punction, when compared to the non-peritonitis group. Also in mice with peritonitis, Naresse and Souza[7] found decreased anastomotic resistance and concentration of collagen, with increased risk for anastomotic leaking and death.

Local anesthetics have been proven to be antiinflammatory substances, interfering in several processes, like the lesions caused by ischemia and reperfusion on the liver[8] and brain[9]. Nevertheless, local anesthetics have antimicrobial properties[10]. Brocco *et al*.[11,12] studied the effect of peritoneal lavage with bupivacaine and lidocaine in mice with fecal peritonitis, which prevented death in 100% of the animals during 11 days, while the group submitted to peritoneal lavage with physiological solution showed a mortality rate of 50%.

Although peritoneal lavage with bupivacaine may cause a positive effect on survival of mice with peritonitis, its action in colonic anastomosis, whether under the presence of peritonitis or not, is unknown.

This study objective is to evaluate the effects of the peritoneal lavage with bupivacaine on the survival and on the initial resistance of the anastomosis performed in the distal colon of rats, under circumstance of peritonitis or not.

Methods

The following study was approved by the Ethical Commission on Animal Experimentation of the Biology Institute (CEEA-IB-UNICAMP) under the protocol number 492-5, according to the ethical code for animal experimentation adopted by the Council for International Organization of Medical Sciences (CIOMS).

Forty male Wistar-line rats (*Rattus norvegicus albinus, Rodentia mammalia*) were used, weighing between 300 and 350 grams and age around 120 days. The animals were submitted to a dark/light cycle with artificial light for a 12-hour period a day. The humidity and temperature were the same as the surrounding environment. The animals had free-access to water and food during all the experiment, without perioperative fasting.

The forty rats were randomly distributed in four groups, with ten animals each. For anesthesia, sodic pentobarbital was administered intravenously in the caudal vein, at the dose of 30 mg/Kg of weight.

Group One: animals had intraperitoneal injection of physiological solution (5 ml/Kg) through a 16G catheter, and after six hours, underwent laparotomy and anastomosis was performed on the distal colon. Peritoneal lavage was made with 3 ml of physiological solution, which remained in the abdominal cavity for three minutes and was dried out with gauze.

Group Two: animals had intraperitoneal injection of physiological solution (5 ml/Kg) through a 16G catheter, and after six hours, underwent laparotomy and anastomosis was performed on the distal colon. Peritoneal lavage was made with 8 mg/Kg of 0.5% bupivacaine, approximately 0.5ml, added to 2.5 ml of physiological solution (final volume solution: 3ml), which remained in the abdominal cavity for three minutes and was dried out with gauze.

Group Three: animals had intraperitoneal injection of 5 mL/Kg of a suspension of 2g of feces recently defecated diluted in 17 mL of physiological solution, which was filtered through gauze to permit the free flow through the needle lumen. After six hours, the animals underwent laparotomy and anastomosis was performed on the distal colon. Peritoneal lavage was made with 3 ml of physiological solution, which remained in the abdominal

cavity for three minutes and was dried out with gauze.

Group Four: animals had intraperitoneal injection of 5 mL/Kg of a suspension of 2g of feces recently defecated diluted in 17 mL of physiological solution, which was filtered through gauze to permit the free flow through the needle lumen. After six hours, the animals underwent laparotomy and anastomosis was performed on the distal colon. Peritoneal lavage was made with 8 mg/Kg of 0.5% bupivacaine, approximately 0.5ml, added to 2.5 ml of physiological solution (final volume solution: 3ml), which remained in the abdominal cavity for three minutes and was dried out with gauze.

The anastomosis was performed in the same way in all groups: end-to-end anastomosis with separate seromuscular stitches (between 16 and 20) with monofilament line of polypropylene 7.0. After the peritoneal lavage, the animals had the omentum and spermatic funiculus resected, and the abdominal wall and skin were closed.

After surgery, there was no administration of supplementary oxygen, antibiotics or parenteral hydration. For the surviving rats, euthanasia was performed on the fifth post-surgical day with a lethal dose of sodic tionembutal (3%), administered in caudal vein. The abdominal cavity was re-opened and the colon segment of four centimeters containing anastomosis was resected and submitted to intraluminal lavage.

These specimens were put in a Becker recipient with physiologic solution (at 37 degrees Celsius) and papaverine chloridrate (250mg/L), to minimize the spasms caused by the handling of the intestinal segments. After 30 minutes, these specimens were removed from solution and submitted to the Total Energy of Rupture biomechanical test (ETR) as described by Wu *et al*.[13,14].

The results of Total Energy Rupture Test (in gf.cm) are described in Table 2. For statistical analysis, it was used the ANOVA (Analysis of Variance) with statistical significance of 5% ($p<0.05$).

TABLE 2 - Statistical analysis of Total Energy Rupture test.

Group	N	Average	Standard-Deviation	Minimum	Median	Maximum	p-value (A)	p-value (B)	p-value (C)
1	10	12736.4	4178.7	4913	14059	17120			
2	10	11410.4	3733.9	4397	12660	15286	<0.00001	0.2507	0.0156
3	5	2693.2	1263.1	890	2985	4220			
4	8	8458	3051.2	3446	9215	11922			

p-value (**A**): effect of fecal peritonitis; p-value (**B**): effect of peritoneal lavage with bupivacaine; p-value (**C**): effect of the interaction between fecal peritonitis and peritoneal lavage with bupivacaine.

Results

Our study had seven deaths, all of them occurred in peritonitis-induced groups. Group 3 showed a 50% mortality-rate, and Group 4 had a 20% mortality-rate, which was not statistically significant (Fisher´s Exact Test: p=0.3498). There were no deaths in Groups 1 and 2. Table 1 summarizes the number of deaths and the days in which occurred, according to the groups. Before their death, these animals stayed in their cage in curved flexion position with little movement, with important abdominal distension and fur erection.

On the fifth post-operative day, the surviving animals were submitted to euthanasia, and the peritoneal cavities were re-opened and observed. There were no anastomosis leakages in neither groups. In Groups 1 and 2 (without peritonitis), the cavities were normal and the anastomosis had no disruptions. Group 3 (peritonitis and peritoneal lavage with physiological solution) showed evident signs of peritonitis: edema and distention of bowel loops, large volume of free peritoneal fluid, diffuse purulent spots, diffuse adherences and unpleasant smell. Group 4 (peritonitis and peritoneal lavage with bupivacaine) showed mild signs of peritonitis: small volume of free peritoneal fluid, few purulent spots and accentuated number of adherences.

TABLE 1 - Mortality in the groups according to the post-operative day.

	Day 1	Day 2	Day 3	Day 4	Total Deaths
Group 1	0	0	0	0	0
Group 2	0	0	0	0	0
Group 3	2	3	0	0	5
Group 4	0	1	1	0	2

Discussion

Anastomotic leakage is a common surgical complication and is related to high rates of morbidity and mortality. With better knowledge on the mechanisms of anastomotic healing under adverse conditions, it may be possible to improve the prevention and treatment of anastomotic dehiscences.

Until this moment, there are no studies about the effect of the peritoneal lavage with bupivacaine on the healing of intestinal anastomosis. Therefore, we cannot compare our results with other reports. However, there are reports about the effects of spinal administration of bupivacaine on colonic anastomosis. Jansen *et al*.[15] showed that epidural analgesia with bupivacaine in dogs submitted to colectomy does not alter the bursting pressure nor changes the colonic transit time. Schnitzler *et al*.[16] used bupivacaine for epidural anesthesia in pigs submitted to colectomy, and reported faster colonic transit time and no changes in bursting pressure.

In our findings, Group 1 (without peritonitis and without peritoneal lavage with bupivacaine) showed the highest values of Total Energy Rupture, with an average of 12736gf.cm. When compared to Group 1, Group 2 (without peritonitis and with peritoneal lavage with bupivacaine) presented inferior values of energy, with an average of 11410gf.cm. After statistical analysis, this difference was not statistically significant (p=0.3486). It is possible that the anti-inflammatory effect of the bupivacaine may have influenced the healing of the anastomosis, leading to decreased values of Total Energy Rupture.

Fecal peritonitis is an important factor that can influence anastomotic cicatrization. In surgical practice, operating patients with peritonitis is a common event. In these conditions, when intestinal anastomosis are required, surgeons fear for the high rates of leakage. When possible, surgeons may choose performing intestinal stomas, which can be definite or temporary.

Rocha *et al*.[6], Naresse and Souza[7] described, in rats, the negative influence of peritonitis on the healing of colic anastomosis, decreasing its resistance and facilitating leakage and death. On the other hand, Orlando *et al*.[17] evaluated rats with peritonitis submitted to ileocolectomy and primary anastomosis, and did not find alteration on anastomotic healing.

In our results, all deaths occurred in animals with peritonitis. Group 3 showed a 50% mortality rate, while Group 4 had a 20% mortality rate. In a metanalysis of 23 studies on experimental peritonitis, Qadan *et al*.[18] found a 48.9% mortality rate in the animals submitted to a peritoneal lavage with physiological solution, which is concordant with our results. Our findings reinforce the benefits of peritoneal lavage with physiological solution in condition of peritonitis. Although the peritoneal lavage with bupivacaine decreased the mortality rate, from 50 to 20%, there was no statistical significance (p=0.3498). We attribute to the anti-inflammatory and antimicrobial properties of bupivacaine the decrease of the mortality rate and the less severe degree of peritonitis in Group 4. Brocco *et al*.[11] reported survival of 100% in rats with peritonitis submitted to peritoneal lavage with bupivacaine. Our hypothesis for a mortality rate of 20% in Group 4 is an increase of morbidity and mortality due to the realization of colic anastomosis.

In our results, fecal peritonitis is associated with decreased anastomotic resistance, independent of the peritoneal lavage with bupivacaine ($p<0.05$). Rocha *et al*.[6] also showed decreased values of Total Energy Rupture under condition of peritonitis, without improvement on anastomotic healing with hyperbaric oxygenation.

Gutman *et al*.[19] compared the healing of colic anastomosis performed under peritonitis between two groups: the first submitted only to surgery and the second group that underwent surgery plus supportive treatment. Their results showed the second group had decreased mortality rate, although bursting pressure was similar between the two groups. They concluded the prognosis of fecal peritonitis depends mainly on the administration of fluids and antibiotics, and not the primary repair itself.

Group 3 (with peritonitis and without peritoneal lavage with bupivacaine) had the lowest values of Total Energy Rupture, with an average of 2693gf.cm. On the other hand, Group 4 (with peritonitis and with peritoneal lavage with bupivacaine) had an average of 8458gf.cm, with statistical significance when compared to Group 3 (p=0.0024). This demonstrates a beneficial effect of the peritoneal lavage with bupivacaine on the resistance of colic anastomosis performed under fecal peritonitis in rats.

Conclusions

Fecal peritonitis increased mortality and influenced negatively the anastomosis in distal colon of rats, decreasing its intrinsic resistance. The peritoneal lavage with bupivacaine increased the resistance of the anastomosis performed under fecal peritonitis in distal colon of rats.

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