THE ROLE OF GLYCEROL AND SODIUM PENTABORATE FORMULATION ON COLONIC ANASTOMOSIS HEALING AND POSTOPERATIVE ADHESIONS IN RATS

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ABSTRACT

Introduction: *We evaluated a new formulation of the mixture of glycerol and sodium pentaborate solution on colonic anastomosis healing.*

Materials and Methods: *Fourteen Sprague Dawley rats were randomly assigned into two equal groups. Segmenter right colon resection and single-layer end-to-end anastomosis was performed for all animals. The mixture of 2 ml 3% glycerol plus 3% sodium pentaborate formulation was injected in the study group, and 2 ml of 0.9% NaCl was injected into the peritoneal cavity in the control group. Rats were sacrificed after 21 days, and anastomosis bursting pressure, histopathological evaluations were assessed.*

Results: *The mean postoperative peritoneal adhesions score was statistically lower (p<0.001) in the study group compared to the control, anastomosis bursting pressure and histopathologic fibrosis scores were high (p <0.001, p<0.0001; respectively).*

Conclusion: *The mixture of 3% glycerol and 3% sodium pentaborate solution is effective for postoperative peritoneal adhesions prevention and inducing anastomosis healing processes.*

Keywords: *Glycerol, sodium pentaborate, peritoneal adhesion, colonic anastomosis.*

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Introduction

Gastrointestinal anastomosis complications and postoperative peritoneal adhesions (PPAs) are still major problems in modern abdominal surgery. Many techniques, methods, materials, and devices were used to prevent these complications. The most common complications of intestinal anastomosis include leakage, bleeding, and stenosis. Anastomotic leakage is a common and grave complication that can lead to high mortality rates(1). The leakage rates of colonic anastomosis were reported as 0.5-30%. Such complications are also causes of emergent colorectal surgeries(2). Septic complications due to anastomotic leakage may cause postoperative mortality(3, 4). Postoperative peritoneal adhesions may cause undesirable complications such as mechanical bowel obstruction, pelvic pain, and female infertility(5). Although different methods were used to prevent PPAs, unsatisfied results have been achieved(6-8). Boron-based stable metallic molecules are identical to carbon-based molecules. Sodium pentaborate has been used for its wound healing and anti-inflammatory effects(9-12).

Glycerol, a central component of lipids, was also scrutinized for its healing effects on colonic anastomosis[8, 13]. We introduced a new formulation of glycerol and sodium pentaborate compounds, and previous investigation revealed its PPA prevention effects in post-adhesion model[10]. In this study ,we aimed to evaluate the effectiveness of the same formulation on PPAs secondary to post-colonic anastomosis and anastomotic healing.

Materials and methods

The protocol for this study was reviewed and approved by the Animal Committee of Yeditepe University School of Medicine (782/2019). Fourteen Sprague Dawley rats (mean weight 270±35 g, mean age 3 months) were used and randomly divided into two groups; a study group and a control group, consisting of 7 rats in each. All animals were managed in accordance with the recommendations of the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Animals and experimental design

The animals were housed in stainless steel cages under controlled temperature (23°C) and humidity conditions, with 12-h dark/light cycles.

Rats were maintained on a standard laboratory diet with tap water ad libitum throughout the experiment, except for an overnight fast before surgery. The rats were divided into the following two groups in a randomized manner: control (n=7), and experimental (n=7).

Surgical procedures

All rats were subjected to fasting for 6 hours before surgery. Prophylaxis before surgery was achieved with 30 mg/kg ceftriaxone intramuscularly. In order to avoid dehydration, 5-mL saline was delivered by subcutaneous injection throughout the surgery. All surgical procedures were performed under sterile conditions using 2.5% povidone-iodine for skin disinfection. Our rodents were anesthetized with ketamine hydrochloride (75-100 mg/kg) and xylazine (10 mg/kg) intramuscularly and were kept on a warm pad throughout the experiment to maintain a constant body temperature of 37°C. A 3-cm long midline incision was made to sufficiently expose the colon.

A 2 cm long right colon segment was resected, and a standard single-layer anastomosis was carried out by end-to-end, separate hand-sewn Lembert pattern suturing method with a polyglactin 910 (Vicryl®5-0; Ethicon, São Paulo, Brazil) suture (Figure 1). In the control group, 2 ml sterile 0.9% NaCl was poured into the peritoneal cavity after the anastomosis. In the study group, the mixture of 2 ml of 3% glycerol and 3% sodium pentaborate formulation was used after anastomosis. In both groups, the laparotomy was closed with a continuous single-plane handsewn suture of polyglactin 910 (Vicryl® 4-0; Ethicon, São Paulo, Brazil) and included the peritoneum and aponeurotic-muscle plane. Skin sutures were made with Perma-Hand®Silk suture 000 (Ethicon, São Paulo, Brazil)

Figure 1: In the research group, the healed colonic anastomosis line (just tip of the forceps) without any PPAs. PPAs: Postoperative peritoneal adhesions.

All the rodents were separated after sugery to prevent cannibalism and kept hydrated with injections of 5-mL saline solution to the dorsal subcutaneous tissue. The rats were allowed to feed normally after twelve hours. The rats were sacrificed by intraperitoneal 100mg/kg sodium pentothal injection on the 21st postoperative day. A midline incision was carried out for abdominal exploration. The PPAs were evaluated by an adhesion grading scoring system (Table 1).

Grades	Definition
0	No adhesion
1	Self-separating adhesions
2	Adhesions separated by traction force
3	Adhesions separated by dissection

Table 1: Adhesion grade scoring of postoperative peritoneal adhesions.

The anastomosis site of 4 cm right colon segment was resected. Stool in the resected colonic segment was cleaned with water. The pressure in the anastomotic area was measured with a transducer

(Alp-K2 Sphygmomanometer®, Norticon Co, Japan). After the measurement of burst pressures, samples with 5 mm borders were resected from each anastomosis line and placed in 10% formalin for histopathological evaluation.

Histopathologic evaluation

Each colonic tissue containing the anastomotic line was sequentially immersed in 10% neutral formaldehyde in 0.1 M phosphate buffer (pH 7.4) for fixation at 40ºC, followed by dehydration in an alcohol batch and finally embedded in paraffin blocks. Paraffin embedded sections of 5 μm thickness were prepared by cutting paraffin tissue blocks using a rotary microtome (Leica RM 2245 model; Leica Instruments, Germany). Tissue sections were mounted on poly-L-lysine coated slides for all rat colonic anastomosis specimens, respectively. Tissues then were stained with hematoxylin-eosin (H&E) and Masson's Trichrom (TCM). The colon sections were investigated under the light microscope (Leica DM 6000 B microscopy system and Leica Application Suite (Leica Microsystems, Wetzlar, Germany). An experienced histologist blinded to the groups graded the H&E and TCM staining sections semi-quantitatively, and evaluated them according to the histopathological fibrosis scoring system (Table 2).

Grades	Definition
0	No Fibrosis: No fibroblasts and/or collagen fibers
1	Mild Fibrosis: Few fibroblasts and/or collagen fibers
2	Moderate Fibrosis: More fibroblasts and/or collagen fibers
3	Severe Fibrosis: Lots fibroblasts and/or collagen fibers

Table 2: Histopathologic fibrosis grading.

Statistical analysis

SPSS 23 was used for the statistical analysis of the data. Descriptive statistical methods (mean, standard error mean) independent t test for control and experimental groups. In this study, the statistical significance level was accepted as p <0.05.

Results

The mean PPAs score, anastomotic burst pressure, and histopathological fibrosis scores of the control and study groups are detailed in Table 3.

Comparison of postoperative peritoneal adhesions

There was a statistically significant difference between PPAs scores of the control and study group (3.0±0.000, 1.4286±0.202, respectively; p: 0.0001).

	Control Group (n=7)	Study Group (n=7)	p
PPA Score (mean ± SD)	3.0±0.000	1.4286±0.202	0.0001
Bursting Pressure Score (mean ± SD)	117.14±2.857	191.42±17.918	0.001
Fibrosis Score (mean ± SD)	4.0±2.00	18.0±5.74	0.0001

Table 3: The mean PPAs, bursting pressure and histopathologic fibrosis scores of the groups. PPAs: Postoperative peritoneal adhesions.

Comparison of anastomotic burst pressure

There was a statistically significant difference between anastomotic burst pressure scores of control and study group (117.14±2.857, 191.42±17.918, respectively; p: 0.001).

Comparison of histopathological fibrosis

There was a statistically significant difference between histopathological fibrosis scores of control and study group (4.0±2.00, 18.0±5.74, respectively; p:0,0001). Figure 2 shows the control and study group anastomotic line fibrotic tissue collections comparatively. In the study group, the fibrotic tissue collection at the anastomotic line is more dense than

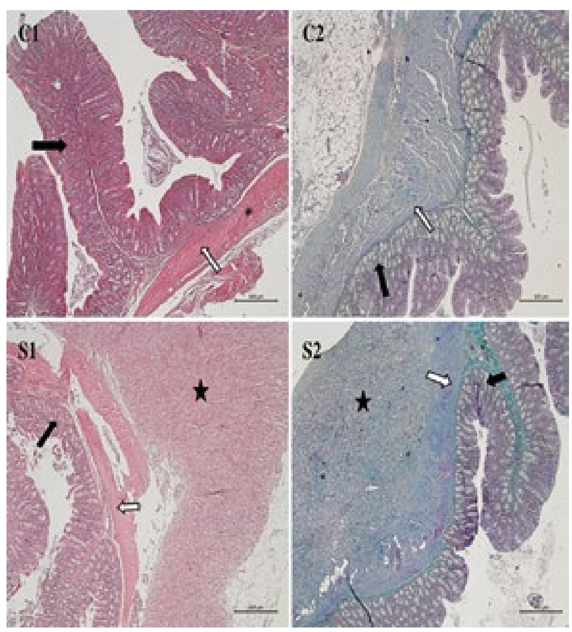

Figure 2: Histological images stained with hematoxylin-eosin (left) and Masson's Trichrom (right) are shown. Intestinal glands are indicated by black arrows, muscle layers by white arrows and fibrosis areas by asterisks. Sections had a scale bar of 400 µm.
(C1: control group, S1: study group).

the control group.

Discussion

Anastomosis leakage is one of the most serious complications of colorectal surgery[14]. Many techniques and methods were used to prevent this complication. The structures that were used to prevent anastomotic leakage include omental graft, the peritoneal graft of dura mater, and various meshes. Unfortunately, despite all attempts, anastomosis leakage remains a common problem after colorectal surgery[15-19].

Postoperative peritoneal adhesions are another serious problem of general surgery and also other surgical branches such as urology, gynecology. Lots of products, materials, and absorbable or non-absorbable solutions were used to prevent PPAs. However, no effective solution has been found effective for definitive treatment[1, 2, 20].

Our team has been focused on preventing PPAs for many years. We evaluated various materials and viscous liquids for this purpose. Postoperative peritoneal adhesions occur in two fundamental ways. The first mechanism is mechanical (surgical manipulation, surgical instruments, benign or malign masses, etc.), and the second mechanism is chemical irritation (intraperitoneal injections, antiseptic materials, ascites, etc.) which occurs by lysis forms of PPAs by different drugs or products. The prevention of adhesion formation is the goal for mechanical causes. The second one is difficult to prevent as it includes lots of mediators, growth factors, and cytokines, and also the roles of these molecules are still unclear. For these reasons, our studies have investigated how to prevent PPAs[5, 10].

We previously evaluated the efficacy of glycerol on PPAs in 2010 by coating peritoneal surfaces with glycerol before and after using the peritoneal trauma model in rats. We found that glycerol is effective in reducing the formation of peritoneal adhesions in two ways. Glycerol was more effective in covering the peritoneal surface in the group that received glycerol prior to trauma, thus reducing the direct effects of trauma on the peritoneal surface[5].

In 2012, different from our previous study, we evaluated the effects of glycerol on the healing process of colon anastomosis[8]. Glycerol caused an increase in mean tissue hydroxyproline levels, which is directly related to anastomotic healing. In this study, we revealed that glycerol could be safely applied for, colonic anastomosis in the peritoneal cavities. In 2016 we revealed that sodium pentaborate increased the wound healing process via inducing cell proliferation, migration, and gene expressions of wound healing growth factors[11].

From our many years of experience, our author team research produced a new formulation for preventing PPAs with, 3% glycerol and 3% sodium pentaborate. In 2020 we evaluated it on a peritoneal adhesion model produced for rats[10]. The PPAs scores of the formulation were statistically lower from the 0.9% NaCl, 3% glycerol, and 3% sodium pentaborate groups for macroscopic and also microscopic evaluations.

As in 2016 when we discovered glycerol as an effective PPAs preventive compound and evaluated its effects on the colonic anastomosis, now in this research, we aim to evaluate its effects on colonic anastomosis of our new formulation. We evaluated anastomosis healing by measuring anastomosis bursting pressure and histopathologic fibrosis scoring. Anastomotic burst pressure indicates the resistance of the anastomotic contour to the intraluminal pressure. At the same time, this pressure is directly related to the amount of mature connective tissue formed by collagen tissue deposition. Histopathologic fibrosis scoring measures the fibrotic tissue deposition on the anastomotic line directly.

Anastomosis causes an acceptable trauma effect during gastrointestinal surgical procedures. This trauma causes prolongation of the time spent in the abdomen and adhesions after interaction with the surrounding tissues. This microenvironmental interaction, which is essential for the healing of the anastomosis, brings with it the adhesion phenomenon, which is an important problem that has survived until the modern surgical era. The dream of every gastrointestinal surgeon is that the anastomosis heals smoothly and that no permanent sequelae occur in the surrounding tissues. However, all efforts made on this subject until today have been insufficient, and post-anastomosis adhesions could not be removed.

The external and internal factors affect anastomosis healing. The safety of the anastomosis depends on many factors such as proper blood supply, tension-free procedure, etc[1]. It is important that these factors be performed in a suitable environment, and this procedure is normally done in peritoneal fluid. It is a known surgical rule that anastomosis healing is interrupted and intraabdominal adhesions increase in abnormal situations where peritoneal fluid is irritated by fluids such as blood, stool, and abscess[5, 10]. Therefore, both increasing the healing of the anastomosis and removing the adhesions after anastomosis are the most optimal goals in gastrointestinal surgery. Our work carries such a goal. It was observed that the formula we used in this study both contributed to anastomosis healing and reduced adhesions after anastomosis.

The new formulation increased anastomosis recovery by reducing PPAs, both by increasing burst pressure and by increasing fibrotic deposition in the anastomosis line. The effect of sodium pentaborate on wound healing by increasing cell proliferation and cell migration may be associated with its effectiveness in anatomical healing. This condition increases anastomosis bursting pressure and increases anastomosis safety.

The decline of PPAs here may seem interesting because the PPAs formation is also a wound healing process, and the formulation we developed can be expected to increase PPAs by increasing fibrotic deposition with similar mechanisms. It should not be forgotten that glycerol is a viscous liquid in the preventive activity of PPAs and has the property of preventing the contact of two surfaces with each other by creating a layer between the peritoneal surfaces. We hypothesized that sodium pentaborate provides rapid wound healing on the peritoneal surfaces, and during this time, glycerol provides separation between the two peritoneal surfaces, which can reduce PPAs. However, in the anastomosis, there is no separation between the surfaces since the two intestines are fixed together by stitches, and glycerol cannot participate in this process and provide separation.

Our study also has its missing parts. This is a rat model, and more studies are needed before it can be applied in humans.

In conclusion, 3% glycerol and 3% sodium pentaborate solution is effective for PPAs prevention and inducing the anastomosis healing process. As a result, our new formulation can be safely applied after colonic anastomosis in peritoneal cavities.

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Authors' contribution:
M.J. contributed to literature searches, study design, data analysis, writing, and critical revision. E.K. contributed to literature searches, study design, data analysis, writing, and critical revision. A.C. contributed to methodology and data analysis. S.O. contributed to data acquisition. E.A. contributed to study design, writing, critical revision, and supervision.

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