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Mechanical Bowel Preparation Does Not Affect Anastomosis Healing in an Experimental Rat Model

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Background: Mechanical bowel preparation before colorectal surgery is commonly performed, but its benefits are controversial. The aim of this study was to compare the effects of mechanical bowel preparation on healing of colonic anastomosis and tissue strength.





Material/Methods: After institutional review board approval, 20 adult Wistar albino rats were randomly divided into 2 groups of 10 animals each. Mechanical bowel preparation including sodium phosphate was performed on the experimental group via a feeding tube, whereas no bowel preparation procedures were performed on the control group. Transverse colon resection and anastomosis were performed on all rats under general anaesthesia. On postoperative day 5, re-laparotomy was performed and the anastomotic areas were resected. Animals were killed, after which bursting pressure and tissue hydroxyproline concentrations were measured, histopathological examination was performed, and we evaluated and compared the results.

Results: There were no differences between control and experimental groups in bursting pressure, tissue hydroxyproline concentrations, or histopathological examination results ($P > 0.05$).

Conclusions: Our study demonstrated no significant difference between bursting pressures, tissue hydroxyproline levels, or modified wound healing score at postoperative day 5 between rats undergoing and not undergoing mechanical bowel preparation. Mechanical bowel preparation is not essential for healing or strength of colonic anastomosis in rats.

MeSH Keywords: **Anastomosis, Surgical • Cathartics • Colorectal Surgery**

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Background

Mechanical bowel preparation (MBP) is often used before elective colon and rectal surgery. Its primary aim is to decrease infectious complications by minimizing the bacterial load of the surgical field [1,2]. The need for MBP before surgery is debated, but many surgeons use it routinely [3,4]. While the literature reports that MBP leads to a decrease in anastomosis leakage, surgical site infections, hospital stay, and similar complications, some studies have reported no difference between patients who underwent MBP and those who did not [5–9]. There are also many animal studies supporting the use of MBP [10,11]. In the present study we evaluated the effect of MBP on colon anastomosis and surgical wound healing.

Material and Methods

After institutional review board approval (Date: 02.03.2011 No: 164), 20 Wistar albino rats weighing 350–400 g were randomized into 2 groups of 10 rats each. Group 1 (control) did not receive MBP before colon surgery and group 2 (experimental) received MBP. The experimental group received 2.4 g sodium phosphate (monobasic) and 0.9 g/5 ml sodium phosphate (dibasic) via a 6 Fr feeding catheter at 2 ml/kg/min, 8 h before surgery. MBP was continued until clear fluid was seen coming from the anus. No procedure was performed in the control group.

Surgical procedure

All rats received 4 mg/kg of 23.32 mg/mL xylazine hydrochloride and 20 mg/kg ketamine intramuscularly in the leg muscle. Following induction of anesthesia, the abdominal area was shaved in the supine position. Asepsis was applied using povidone-iodine solution and a 2-cm midline incision was made. The transverse colon was located and a 3-cm resection was performed followed by anastomosis of ends using separate single-layer 4/0 silk sutures. Abdominal closure was performed using continuous 3/0 silk sutures (Figure 1). The procedure was performed by the same surgeon for all rats and sterile instruments were used each time. No complications were observed postoperatively. The same anesthesia procedure was used on all rats on postoperative day 5. After re-laparotomy, the anastomosis was resected with a 2-cm margin at either side. Rats were killed by decapitation. Mechanical, biochemical, and histopathological parameters were used to evaluate anastomosis healing. Bursting pressure was measured as described below. Thereafter, the anastomosis was opened longitudinally and two 0.5-cm samples from the anastomosis line were taken. One sample was placed in 10% formaldehyde for histopathological examination and the other was wrapped in aluminum foil and stored for determination of hydroxyproline level.

Bursting pressure

The resected colon segment was cleaned of fecal material and closed at one end using 3/0 silk sutures. The opposite end was

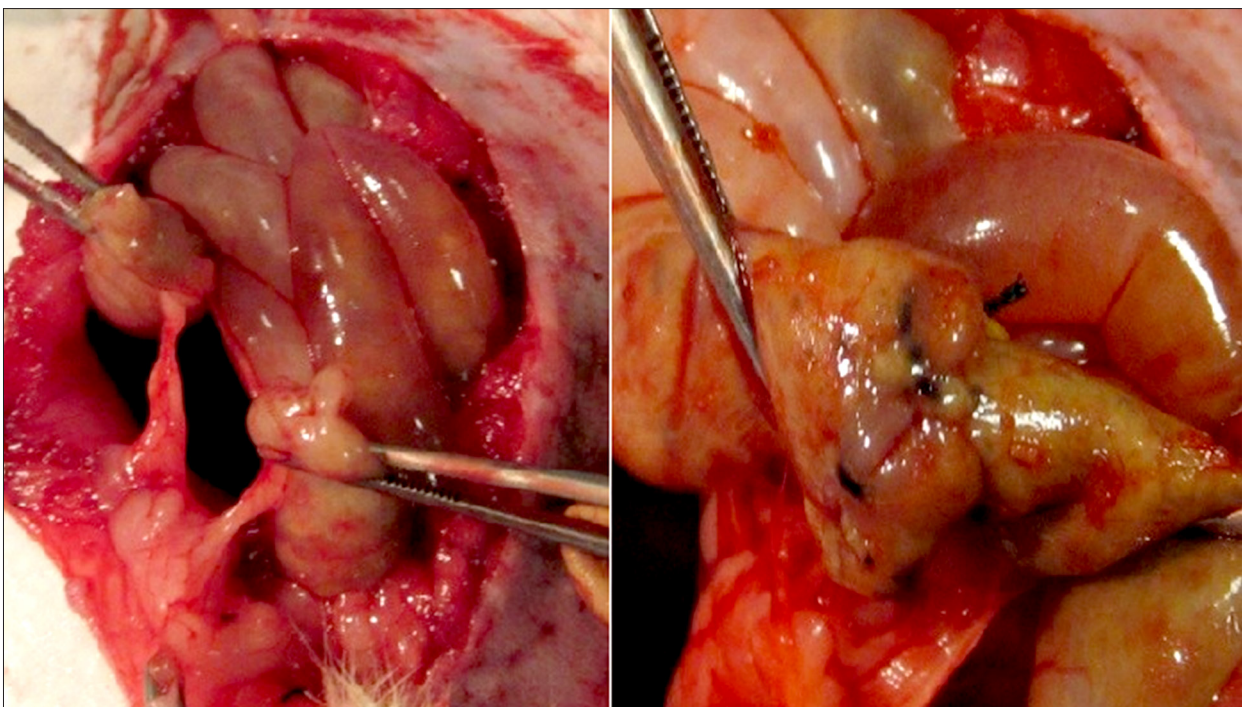


Photo 1. Segmenter resection and anastomosis.

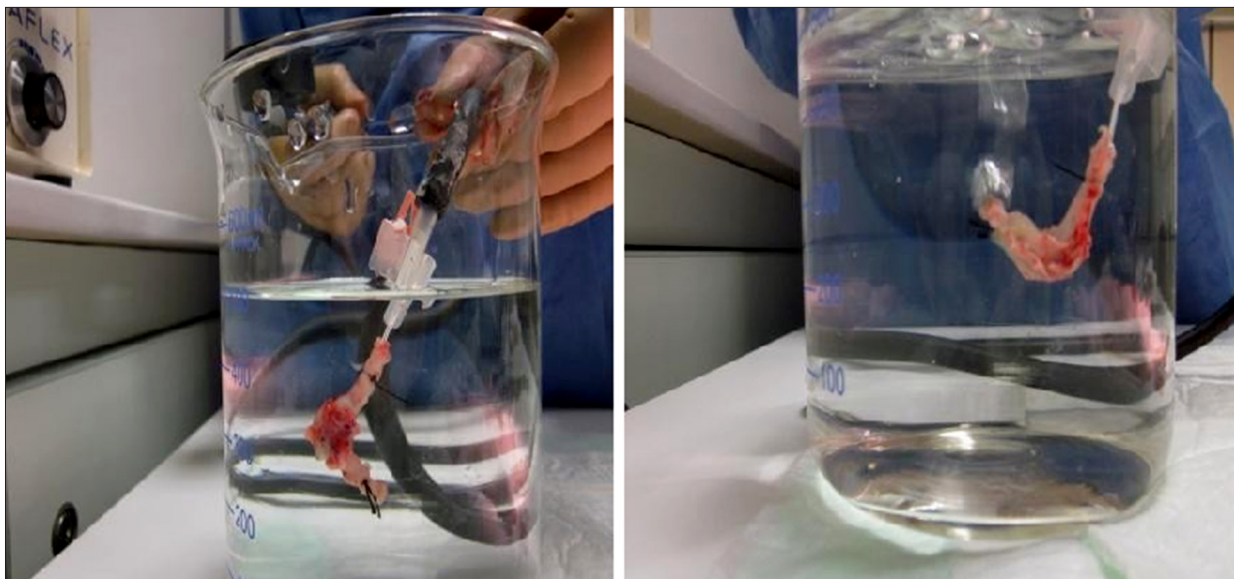


Photo 2. Mechanical measurement of anastomosis bursting pressure.

Table 1. Modified scoring system of wound healing.

Score (S)	Concentration of Inflammatory Cells	PMNL Infiltration	Neo vascularization	Fibroblastic activity	Collagen fibers	
1	Bad	+++	+++	-/+	-	
2		+++	+++	+ /+++	+ /+++	-
3	Good	++	+ /+++	+++	+++	+
4		+ /+++	- /+	+ /+++	+ /+++	++
5		- /+	- /+	+	+ /+++	+++

sealed over an infusion pump. A stationary pump and manometer were used. After submerging the sample under water, air was pumped into the segment at 2 ml/m. The pressure at which air bubbles were observed at the anastomosis line was accepted as bursting pressure (Figure 2).

Hydroxyproline measurement

After defrosting of samples at room temperature, moisture on samples was removed using blotting paper. Samples were weighed with a precision scale and then submerged in saline. A Potter-type glass homogenizer (Heidolph-RZR 2021, Germany) was used to prepare 20% homogenates (20 g/ml). The homogenates were centrifuged for 15 min at 1500 rpm. An equal amount of HCl was added to the obtained supernatants and they were hydrolyzed for 16–18 h. We used a hydroxyproline kit (Hypronsticon, Organon, Holland) based on the principle defined in 1967 [12,13]. The absorbance of released painted compound was evaluated by spectrophotometric technique at 560 nanometers and hydroxyproline levels were measured as microgram/mg in wet tissue.

Histopathological examination

Tissues including the anastomotic site were fixed in 10% formaldehyde. After routine techniques, sections were embedded in paraffin. Preparations were stained with hematoxylin and eosin (HE) dye and examined under a light microscope. Anastomosis site healing was semi-quantitatively recorded. Microscopic findings were then scored using a modified scoring system for wound healing [14,15] (Table 1). This scoring system includes the concentration of inflammatory cells, PMNL infiltration, fibroblastic activity, collagen fibers, and neovascularization. Each component is scored as bad (1–2) or good (3–4–5), with a maximum score of 25. The total score was evaluated as good if it was larger than or equal to 10.

Statistical analysis was conducted using NCSS (Number Cruncher Statistical System) 2007 & PASS 2008 Statistical Software (Utah, USA). Apart from descriptive statistical methods (mean and standard deviation), comparison of quantitative data with normal distribution was performed with the *t* test

Table 2. Modified wound healing score, bursting pressure, and hydroxyproline measurement comparison.

	Control group (Ave ±SD)	Experimental group (Ave ±SD)	P
Wound healing score	13.50±2.40	14.50±1.09	0.631*
Bursting pressure (mmHg)	88.0±7.49	88.0±11.07	1.000**
Hydroxyproline (mcg/mg)	3.85±0.69	3.87±0.66	0.933**

Mann-Whitney U test*, t test**.

and comparison of quantitative data without normal distribution was performed with the Mann-Whitney U test. Qualitative data was compared using Fisher's exact and chi-square tests. Statistical significance was accepted as $P < 0.05$.

Results

Anesthesia was well tolerated by all rats and no deaths occurred due to anesthesia. No purulent wound infection was observed. No rats were lost postoperatively. In all rats, omentum was observed to have adhered to the anastomosis site, although these adhesions were easily removed with blunt dissection. No macroscopic findings suggestive of peritonitis or anastomosis leakage were observed.

Histopathological scoring

The average histopathological score was 13.5 ± 2.40 for group 1 and 14.5 ± 1.09 for group 2. There was no statistical significant difference, as shown in Table 2.

Bursting pressure

The average bursting pressure in group 1 was 88.0 ± 7.49 mmHg and for group 2 was 88.0 ± 11.07 mmHg. There was no statistically significant difference, as shown in Table 2.

Hydroxyproline evaluation

The average hydroxyproline level was 3.85 ± 0.69 mcg/mg for group 1 and 3.87 ± 0.66 mcg/mg for group 2. There was no statistical significant difference, as shown in Table 2.

Discussion

The major complications seen after colorectal surgery are wound infection, intra-abdominal or pelvic abscesses, and anastomosis leakage. MBP, oral nonabsorbable antibiotics, perioperative intravenous antibiotics, and modern surgical techniques

have decreased these complication rates [12]. However, the usefulness of MBP is still debated [5–9].

A study performed on 63 patients by Saha et al. [6] concluded that there was no difference in anastomosis leakage in patients receiving MBP and those who did not. Similarly, Scabini et al. [9] followed 244 patients undergoing colorectal surgery for 30 days and compared anastomotic and intra-abdominal complications in those who received MBP and those who did not, concluding that colorectal surgery without MBP was safe. Kim et al. [7] also suggested that surgery for left- or right-sided colon tumors was safe without MBP. A Cochrane review published in 2011 found that MBP offered no advantage regarding anastomosis leakage or wound infection rates [17]. The present study also showed that colorectal surgery is safe without MBP.

O'Dwyer et al. [18] examined the effect of MBP on anastomosis and anastomosis leakage pressure in dogs. While anastomosis defects at postoperative day 9 were seen in 13% of dogs undergoing MBP, this rate was 47% in those not receiving MBP; the authors concluded that MBP could actually increase the rate of complications. In 2012, Regadas et al. [11] reported a study of 42 dogs that were separated into 2 groups. The authors found no difference in anastomosis bursting pressure at postoperative day 21 between dogs undergoing MCT and those who did not. The authors reported that MBP did not decrease mortality or postoperative complication rates in dogs.

In 2006, Mersin et al. [10] published a study in which 3 groups of 9 rats each were compared for anastomosis bursting pressure and hydroxyproline levels at postoperative day 4 after anastomosis. The anastomosis bursting pressure was reported as 87 mmHg in the control group, 88 mmHg in the sham group, and 76 mmHg in the group receiving MBP. Hydroxyproline was reported as 3.25 mcg/ml, 4.15 mcg/ml, and 3.52 mcg/ml in these groups, respectively. The differences were not statistically significant and the authors concluded that MBP was not necessary to increase anastomotic integrity. While our study shows similarities to the study by Mersin et al. [10], we also performed histopathological examination of anastomosis site tissues. The

3 studies previously mentioned in this paragraph [10,11,16] all examined anastomotic bursting pressure as a measure of anastomosis safety. Although O'Dwyer et al. [18] found a significant decrease in anastomotic leakage pressure in animals not undergoing MBP, Regadus et al. [11], Mersin et al. [10], and the present study found no difference in bursting pressure between the groups. Although our findings for hydroxyproline levels correlate well with those of Mersin et al. [10], it is interesting that no statistical significance was found.

Previously, a histological scoring system was validated that was based on the qualitative and quantitative aspects of healing – re-epithelialization, granulation tissue formation, presence of inflammatory cells, and angiogenesis [19]; however, we used a modified score that evaluates the healing of intestinal mucosa. This modified scoring system includes the concentration of inflammatory cells, PMNL infiltration, fibroblastic activity, collagen fibers, and neovascularization [14,15]. To the best of our knowledge, there are no studies that included

histopathological data when analyzing anastomosis safety and healing. While finding similar results to previous publications, our study also adds histopathological data. This data and all our findings support that anastomosis safety and healing are not positively or negatively affected by MBP performed before colorectal surgery.

Conclusions

Our study demonstrated no significant difference between bursting pressures, tissue hydroxyproline levels, or modified wound healing score at postoperative day 5 between rats undergoing and not undergoing MBP. Further studies are required to determine if MBP is required before colorectal surgery.

Disclosure of conflict of interest

None.

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