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EXPERIMENTAL STUDY

The effect of neostigmine on postoperative ileus and the healing of colon anastomoses

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Abstract: *Objectives:* The aim of this study was to assess the effects of neostigmine on the postoperative ileus and the healing of colon anastomoses.
Background: Anastomotic leaks and postoperative ileus are among the most important complications in colorectal surgery. Nutritional status of the patient is one of the most important factors of the anastomoses healing.
Methods: Forty Wistar-albino rats were randomly assigned to (1) colon anastomoses + protein-enriched diet + 1 µg neostigmine, (2) colon anastomoses + protein-enriched diet, (3) colon anastomoses + 30 % dextrose + 1 µg neostigmine, (4) colon anastomoses + 30% dextrose. Body weights, quantities of the consumed products and feces weight change were measured at the first and second 48 hours postoperatively. All rats were sacrificed on the 5th postoperative day. Then, the healing of anastomosis was evaluated by measuring of body weights, burst pressure and hydroxyproline level.
Results: There were significant differences between the groups 1 and 2 ($p<0.001$), and the groups 3 and 4 regarding the preoperative and postoperative mean weight ($p<0.001$). The group 2 did not show any weight loss, whereas the group 1 demonstrated a significant weight gain ($p<0.001$). The group 4 showed a significant weight reduction compared to the preoperative period, while the group 3 exhibited a significantly lower weight loss compared to the group 4 ($p<0.001$). There were significant differences regarding the burst pressure and hydroxyproline level values of groups with and without neostigmine administration ($p<0.001$).
Conclusion: Starting the early intake of nutrition with neostigmine in the postoperative period reduced the postoperative ileus and increased the healing of colon anastomoses *(Tab. 4, Fig. 5, Ref. 27).* Full Text in free PDF *www.bmj.sk.*
Key words: neostigmine, anastomoses healing, postoperative ileus.

Colon surgery comprises a high number of patients treated in the gastrointestinal surgery department. Surgical and medical postoperative complications may be diverse. Anastomotic leaks and postoperative ileus are among the most important complications in the colorectal surgery. Local and systemic factors such as infections, bleeding, suture materials, surgical techniques, systemic diseases, sepsis, and nutritional status, all affect the process of anastomoses healing (1, 2). Among them, nutritional status of the patient is one of the most important factors. Even short-lasting malnutrition adversely affects the anastomoses healing (3, 4). Therefore, early enteral feeding and treatment of postoperative ileus have drawn the attention of research in the recent years. However, tolerance of oral intake can sometimes pose problems due to late recovery of gastrointestinal motility. Due to this reason, a duration of postoperative ileus is important to early feeding and discharge. If duration of postoperative ileus is longer, hospital stay periods lengthen and hospital costs increase. Currently available therapeutic options for the management of delayed gastric emptying and feeding intolerance in patients are prokinetic therapy, postpyloric feeding, or total parenteral nutrition. Motility improving acetylcholinesterase inhibitors, such as neostigmine, cause an increase of the cholinergic activity in the intestinal walls leading to more contraction (5, 6). Hence, we thought that following the enteral feeding in the early postoperative period, intestinal motility can be stimulated with neostigmine to ensure distal passage. Therefore, in the present study, we aimed to assess the effects of neostigmine on postoperative ileus and colon anastomoses healing.

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Methods

This experimental study was held in the Cerrahpasa Medical Faculty and was approved by the ethical committee of the Cerrahpasa Medical Faculty. Forty female Wistar-albino rats of 130–220 g were randomly assigned to one of the four groups of 10. All rats were kept in constant environmental conditions.

All animals were kept in metabolic cages and body weights were measured prior to the beginning of the study. 1 µg of neostigmine (Neostigmine, Adeka Drug Company, Samsun, Turkey, 0.5 mg/1 ml amp) was delivered subcutaneously to the groups 1 and 3 three times a day for four days. Groups 1 and 2 were fed with protein-enriched diet (Alitraq, Abbott Lab, Columbus, Ohio) (1 kcal/ml), whereas groups 3 and 4 were fed with 30 % dex-

trose (Dextrose, Eczacibasi-Baxter) (1.02 kcal/ml) orally. The feeding of all the animals was carried out relative to their groups, and the daily liquid nutrient consumption of each rat was recorded.

Group 1: colon anastomoses + feeding with Alitraq + Neostigmine (n=10)

Group 2: colon anastomoses + feeding with Alitraq (n=10)

Group 3: colon anastomoses + feeding with 30% Dextrose + Neostigmine (n=10)

Group 4: colon anastomoses + feeding with 30% Dextrose (n=10)

Body weights, quantities of the consumed products and feces weight change were measured at the first and second 48 hours postoperatively. With the end of the test period (postoperative 5th day), the healing of anastomosis was evaluated by measuring of body weights, burst pressure and hydroxyproline level.

Surgical procedure

Without making a colonic preparation, all subjects were anesthetized with intramuscular ketamine (Ketalar 50 mg/kg) and ether inhalation. The weight of the rats were measured and recorded preoperatively. Operations were performed in a randomized fashion. Abdominal wall was shaved and 10 % of Povidine–Iodine solution was used for disinfection and then a median laparotomy was performed. A transsection was formed 3 cm proximal to peritoneal reflection and reanastomosed end-to-end with 6/0 polypropylene suture. Following that, abdominal fascia and skin were closed.

All animals were sacrificed on the 5th postoperative day under the ether anesthesia and their weights were measured. Relaparotomy was carried out. The colon was clamped at 2 cm distal to anastomosis and resected at 2 cm proximally. A burst pressure was measured by an insufflation pump that was sealed intraluminally to the proximal tip of the segment. Insufflation was at a constant value of 6 mL/min under the water, and the pressure level, where air bubbles showed up on the anastomosis line, was accepted as the burst pressure value.

Biochemical procedure

The colonic segment 0.5 cm proximal and 0.5 cm distal to the anastomosis line was resected, weighed and homogenized with saline into 20 % (20 % g/mL) homogenates by using a Potter type glass stirrer (Heidolph – RZR 2021, Germany). Homogenates were centrifuged at 1500 rpm for 15 min and obtained supernatants were hydrolyzed by adding hydrochloric acid of equal amounts for 16–18 h. By using the hydroxyproline kit (Hipronisticon, Organon, Holland), hydroxyproline amount was calculated in microgram per milligram of wet tissue by reading the absorbance of solution on spectrometry at 560 nm.

Statistical analysis

The values were expressed as the mean ± SD. The differences between the groups were evaluated with ANOVA, whereas intra-group comparisons were performed with the instant t test. The level of significance was set at $p<0.05$.

Tab. 1. Preoperative and postoperative mean weight.

	Pre-operative weight (grams) (mean+SD)	Post-operative weight (grams) (mean+SD)	p value
Group 1	154.40+7.79	156.90+7.90	<0.001 *
Group 2	154+9.25	153.60+8.82	>0.05
Group 3	154+8.62	150.10+8.54	<0.001 *
Group 4	154+9.94	148.30+9.58	<0.001 *

* The level of significance is p< 0.05.

Results

No mortality occurred during anesthesia or the surgical procedure. Preoperative and postoperative mean weights of the groups are shown in Table 1 and Figure 1. There were significant differences between the groups 1 and 2 ($p<0.001$), and the groups 3 and 4 ($p<0.001$). The group 2, which was fed with Alitraq, did not show any weight loss, whereas the group 1, which was fed with Neostigmine and Alitraq, demonstrated a significant weight gain ($p<0.001$). The group 4, in which only 30 % dextrose was administered to the rats, showed a significant weight reduction compared to the preoperative period, while the group 3, which included a delivery of Neostigmine as well, exhibited a significantly lower weight loss compared to the group 4 ($p<0.001$). Feeding with Alitraq made a significant difference compared to the feeding with 30 % dextrose, and the groups including neostigmine administration exhibited a significantly higher amount of weight gain compared to the other groups.

When the groups were compared with respect to the change in feces weight and the consumption of dietary intake within the first and second 48 hours, there were significant differences in

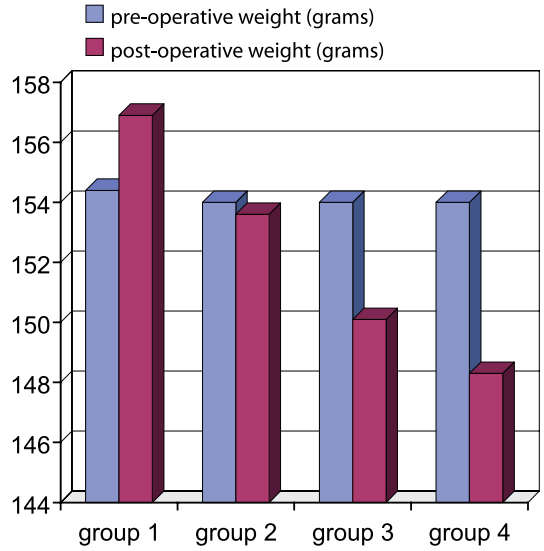


Fig. 1. Preoperative and postoperative mean weight.

Tab. 2. Changes in gaita weight at first and second 48 hours in all groups.

	First 48 hours gaita weight (grams)	Second 48 hours gaita weight (grams)	t test
Group 1	6.21+0.8	0.58+0.27	p<0.001*
Group 2	1.90+0.59	4.42+0.34	p<0.001*
Group 3	4.92+0.43	0.54+0.44	p<0.001*
Group 4	1.57+0.44	4.24+0.28	P<0.001*

* The level of significance is p< 0.05.

Tab. 3. Quantities of the consumed products.

	Quantities of the consumed products (the first 48 hours) (mL) (mean+SD)	Quantities of the consumed products (the second 48 hours) (mL) (mean+SD)	t test
Group 1	85.70+5.8	97.40+4.01	p<0.001*
Group 2	61.1+4.93	70.20+5.45	p<0.001*
Group 3	83.0+5.89	91.8+4.44	p<0.001*
Group 4	55.60+4.81	68.5+3.81	p<0.001*

* The level of significance is p< 0.05.

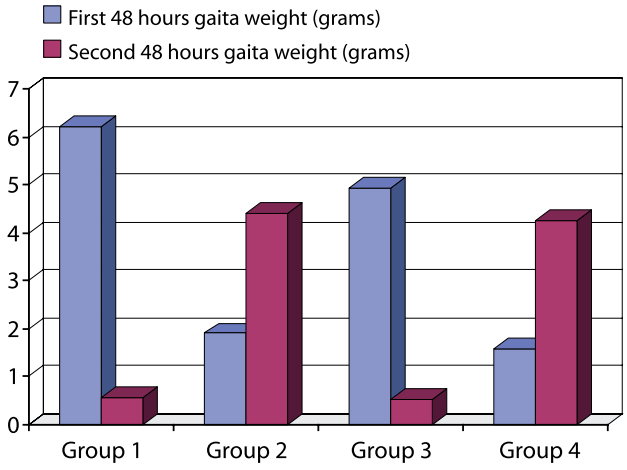


Fig. 2. Feces weight change at the first and second 48 hours in all groups.

the results of all treatment groups (groups 1 and 3) compared to those of the non-neostigmine groups (groups 2 and 4) (p<0.001) (Tab 2 and 3, Figs 2 and 3). Moreover, the amount of feces in the groups 1 and 3 within the first 48 hours was statistically higher than the amount of feces in the second 48 hours (p<0.001).

In all subjects, the burst of the colon was on the anastomotic line. The burst pressure and hydroxyproline level values and the standard deviations of groups are shown in Table 4 and Figs 4 and 5. A significant difference (p<0.001) between the results of the groups 1 and 2 was found, whereas the results of the groups 3 and 4 showed a significant difference in this regard, as well (p<0.001).

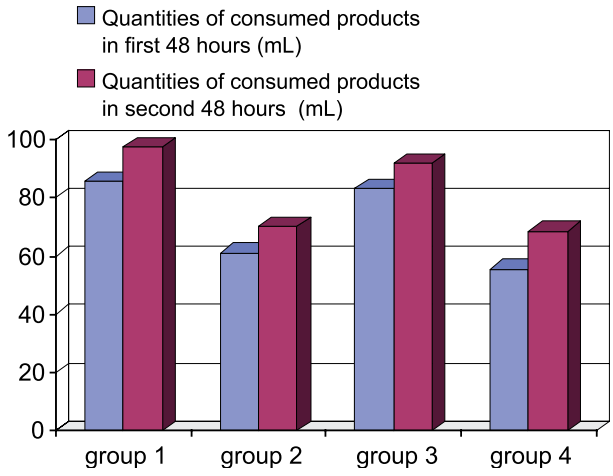


Fig. 3. Quantities of the consumed products.

Discussion

After the abdominal surgery, postoperative ileus and anastomoses healing are frequent, frustrating situation for patients and surgeons. A reduction of this condition is very important in clinic. Although a growing research on colonic anastomoses healing has been carried out, there is no single strategy to reduce the postoperative ileus. Most of the morbidity and mortality after colon surgeries are associated with anastomotic leakages (1). Even today; there are ongoing significant efforts to reduce anastomotic leaks. A number of positive and negative factors are known to affect the healing of anastomosis including infections, perfusion, suture materials, surgical technique, sepsis, and malnutrition. A nutritional support is important to patients. The enteral route has become the preferred method of nutrient delivery, since it is cheaper, has less septic complications, and is associated with better intestinal mucosal barrier function than the parenteral route (7). Enteral feeding was found to contribute positively to the healing process of the disease, and besides immunoglobulin A, transferrin, and serum protein levels were reported to be high, whereas sepsis and mortality rates were noted to be low (8, 9, 10).

At the beginning of this century, postoperative recovery protocols, mainly after colorectal surgery, known as the fast-track

Tab. 4. Burst pressure and Hydroxyproline levels in all groups.

	Burst pressure(mmHg) (mean+SD)	Hydroxyproline level (mg/100 g of wet tissue) (mean+SD)
Group 1	67.10+6.17	13.93+1.74
Group 2	45.90+4.20	9.11+1.02
Group 3	43.00+4.29	8.25+0.89
Group 4	32.10+5.78	6.70+1.50
ANOVA test	F=79.68 p<0.001	F=54.54 p<0.001

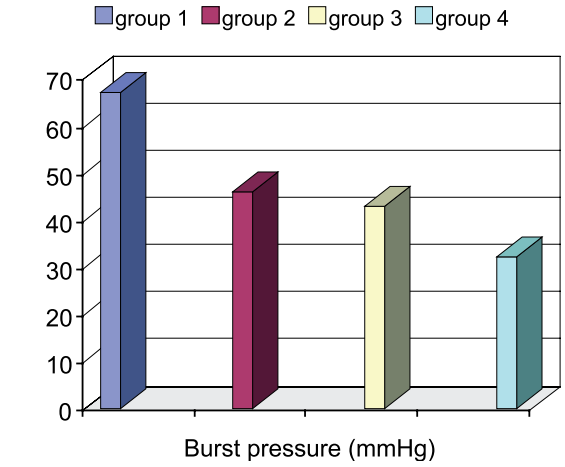


Fig. 4. Burst pressure levels in all groups.

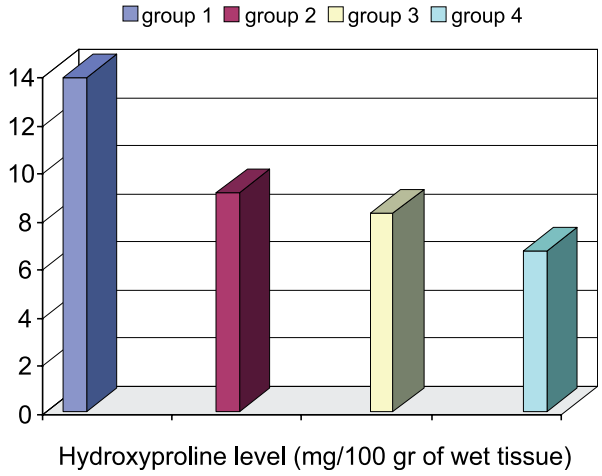


Fig. 5. Hydroxyproline levels in all groups.

were described (11, 12, 13). Later, a similar, but a more complex protocol was introduced by the Enhanced Recovery after Surgery Group (ERAS) (14, 15, 16). The main objectives of both protocols were to reduce postoperative complications, mortality, ileus period and hospital stays. These protocols included early oral feeding, epidural analgesia, prophylaxis of nausea and vomiting, opiate-free analgesia, early postoperative ambulation, etc. These actions, as outlined in the goals of the protocols, reduced postoperative ileus period and introduced a good tolerance to early oral nutrition. Furthermore, even before, a positive tolerance of early enteral nutrition was demonstrated by Sagar et al (17). Other authors demonstrated the positive effects of early enteral feeding, such as direct oral or through nasoenteral tubes, after the gastrointestinal surgery. So the concept that there is no obvious advantage in keeping patients at "nil by mouth" following the gastrointestinal surgery has been in existence with the adoption of fast-track conception, the diet tolerance has virtually ceased to be a problem (18, 19, 20).

Postoperative ileus has a neural and inflammatory physiopathology. Physiological ileus seen in the postoperative period lasts for a few hours in the small intestine, 24 to 48 hours in the stomach, and 48 to 72 hours in the colon following the abdominal surgery. As there are no objective criteria regarding the recovery of gastrointestinal functions, a restarting of oral intake shows a delay, which in turn prolongs the hospital stay of the patient and increases the hospital costs (21, 22). An initiating prokinetic therapy was used to accelerate the gastric emptying (23). Methylnaltrexone, mitemcinal, ghrelin agonists and dexloxiglumide have potential advantages, but require further investigation before a widespread clinical practice (24). Neostigmine was used in this study to ensure that the gastrointestinal functions were recovered early and to shorten the postoperative physiological atony passage, thereby aiming to avoid these complications, and early enteral feeding was performed through the oral route, which is the most physiological way. Acetylcholinesterase inhibitors, such as neostigmine, cause an increase in the cholinergic activity in the intestinal walls, leading to more frequent intestinal contractions (5, 6, 24, 25, 26). The use of neostigmine in the reduction of postoperative ileus is controversial. Kreis et al (27) used a manometry/barostat system and found an elevated colonic motility in patients who received neostigmine following the colorectal surgery. The gastrointestinal postoperative physiological atony may relate with absorption problem. Some studies have shown moderate effects in acute colonic pseudo obstruction (6, 25, 26), but adverse effects, including abdominal cramps, excess salivation, vomiting and bradycardia, may limit the clinical usefulness of neostigmine postoperatively. With this respect, neostigmine was used in the present study (groups 1 and 3) in order to evaluate and assess such negative effects on nutrition.

The significant increase in the consumption of dietary fluid and the amount of feces in groups 1 and 3 was in accordance with the absence or lower occurence of postoperative ileus. Furthermore, hydroxyproline and burst pressure values in groups 1 and 3 were found to be statistically significant as a result of the elevated dietary intake due to the neostigmine usage. We can attribute this to the status of nutrition, which was less negatively affected.

In conclusion, starting the early intake of nutrition with neostigmine in the postoperative period reduced the postoperative atony passage and increased the colon anastomoses healing. The eventual increase in the amount of weight and feces improved the anastomosis healing. Further in vivo studies should be conducted on the patient's satisfaction and other physiological changes in order to validate these results.

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