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The Effect of Postoperative Ileus on the Gastrointestinal Motility of Rats

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ABSTRACT

Postoperative ileus (POI) is defined as a prolonged absence of bowel function which can occur after an abdominal surgical procedure. Due to the complications caused by POI, hospital stays and related costs have been shown to increase. The purpose of this study was to determine the effects of POI on the gastrointestinal (GI) motility of rats. Sprague-Dawley rats were given a meal with phenol red and POI was induced via abdominal surgery. The experimental and the naive control group had stomachs and small intestines (SI) isolated and each SI was divided into 7 equal-length pieces. Each created a homogenous mixture and the amount of phenol red was determined. Positive differences for SI 2 to 8 indicated delayed intestinal motility in the experimental group. The stomachs in the experimental group had a significantly larger ($p<0.05$) amount of meal inside (0.362 ± 0.031 absorbance) compared to the stomachs of the control rats (0.176 ± 0.032 absorbance), suggesting a slower rate of food passage in the experimental group. These findings suggest that surgically induced POI causes a decrease in GI motility in rats. Future studies could focus on the effects of neostigmine on the decrease of POI in rats, for the purpose of eliminating discomfort during survival surgeries in rat models.

Introduction

Postoperative ileus (POI), which develops after an abdominal surgical procedure, is a physiological arrest of gastrointestinal (GI) transit in response to surgical stress[1]. Due to its role in postoperative morbidity and increased hospital stay and cost, POI has become a public health problem[1]. Around $1 billion annually is incurred because of these complications[2]. The symptoms of POI include vomiting, nausea, abdominal pain, reduced borborygmi, and an increased transit time for the passage of stool[2]. The pathophysiology of POI is complex involving pharmacological neural, and immune-mediated mechanisms[3]. Repeated handling of the small intestine during abdominal surgery is believed to be one of the causes of POI[3, 4]. Even though efforts to prevent its occurrence have been made using the knowledge of its pathophysiology, 20% to 30% of patients still develop POI after abdominal surgery[5]. A rat model is commonly used to develop novel surgical techniques. Thus, it is vital that artifacts are not created by POI or the discomfort that POI can cause.

The hypothesis for this experiment is that the induced POI will negatively impact normal GI motility, such that the rate of food passage through the GI tract is decreased compared to the negative control.

Methods

Materials

Materials included resources for rats' environment (cages, water, lighting, etc.), 5% and 2% isoflurane, all instruments required for surgery (including rectal probe, recovery chamber, hemostats, etc.), two 3-0 nylon

threads with needles, 1.5mL of 1.5% methylcellulose and 50mg/100mL phenol red solution, 600 mL of 0.1M NaOH solution, 1000p 200p and 100p pipettes, measuring instruments (100mL graduated cylinder, beaker, tubes, weight, ruler, tape measurer), 2.2mL trichloroacetic acid of 20% m/v, centrifuge and vortex machines, and 1.375mL of 0.5M NaOH solution.

Animals

A total of 13 female and 2 male Sprague-Dawley rats (190-360g) between 6-9 months old supplied by the Surgical Sciences Research Laboratory at Englewood Hospital were used for the study. The control group consisted of 2 male and 5 female Sprague-Dawley rats and the experimental group consisted of 8 female Sprague-Dawley rats. The animals used in the study were housed in controlled temperatures and humidity and maintained under a light/dark cycle in plastic/stainless steel cages, with food and water provided *ad libitum*. The rats used in the experiments fasted for 24 hours prior to the experiments with access to water.

Surgical Preparation

The naive control group did not undergo induced POI. Rats from the experimental group were anesthetized with 5% isoflurane and the surgical site was shaved. Antiseptic was used to prevent infection. The surgical site for the induced POI was the central surface of the abdomen. The body temperatures were maintained throughout the surgical procedures at 37° C. Body temperature was monitored using a rectal probe and adequate anesthesia was maintained with 2% isoflurane.

Induced POI in the Experimental Group

After the necessary preparations for surgery were made, an incision along the abdomen was performed on the experimental group. The small intestine and cecum were exteriorized and fanned out on saline-covered gauze. With saline-moistened cotton-tip applicators, the intestine was manipulated from the stomach end to the cecum end twice for 5 minutes. The small intestine and cecum were then returned back to the abdominal cavity, and the muscle and skin tissues were closed up with running stitches using a 3-0 nylon both times.

Isolation of Samples

The phenol red method was performed as described in Taché *et al*[6]. Specifically, a solution of 1.5% methylcellulose and 50mg/100mL phenol red was prepared and 1.5mL of the liquid meal was administered to the rats by oral gavage. The rats were given 40 minutes in the recovery chamber to digest the meal and recover from anesthesia. Afterward, the rats were euthanized in a CO_2 chamber. An incision was made, cutting from the lower abdomen to the chest cavity. The diaphragm was cut through and the liver was pushed toward the lungs. The esophagus and pyloric sphincter were located and hemostats were placed on each end to isolate the stomach, which was subsequently removed. The small intestine was isolated from the mesentery and also removed. The stomach was weighed and placed in a 90mL solution of 0.1M NaOH. The displacement was recorded. The same solution was brought up to 100mL with 0.1M NaOH. The small intestine was stretched from the pyloric to the cecal end and cut into 10 equal pieces. Each section was placed into a tube with 50mL of 0.1M NaOH. Leakage throughout the procedure was prevented using hemostats.

Assay for The Measurement of Gastric Motility

The intestines and stomach were homogenized and 8mL of each homogenate were centrifuged at 4000 rpm for 10 minutes. Then 2mL of the supernatant was taken from each tube and 0.2mL trichloroacetic acid of 20% m/v was added to each sample. The samples were vortexed and the proteins were precipitated. The samples were centrifuged at 4000 rpm for 10 minutes, and 125µL of 0.5M NaOH was added to 125µL of supernatant in a 96-well plate with three replicates done per sample. A microplate reader was used to determine the amount of phenol red at 560 nm.

Data and Statistical Analysis

In this experiment, the data are expressed as the mean ± standard error of the mean (SEM). Data was analyzed in EXCEL using t-test. P-value of < 0.05 were considered statistically significant.

Results

Data from Figure 1 shows a positive difference for SI 2, 3, 4, 5, 6, 7, and 8 meaning that there was delayed intestinal motility in the experimental group. According to Figure 1, SI 2, 3, 4, 5, 6, 7, and 8 have positive differences, the largest difference being at SI 7 at 0.135, meaning that the experimental group had a higher phenol red absorbance than the control group at these segments. The smaller phenol red absorbance numbers indicate that the experimental group experienced slower gastric motility throughout the SI. Additionally, the SI 7 and SI 8 absorbance means in Figure 2 show that the control group had significantly more meal content compared to the experimental group in these two segments. Since the meal traveled further down the GI tract in the control group, the experimental group had slower gastric motility. Figure 2 shows that the stomachs of the rats in the experimental group had a significantly larger ($p<0.05$) amount of meal inside (0.362 ± 0.031 absorbance) compared to the stomachs of the rats in the control group (0.176 ± 0.032 absorbance). The large difference in amounts indicates that the experimental group also experienced a slower rate of food passage through the GI tract.

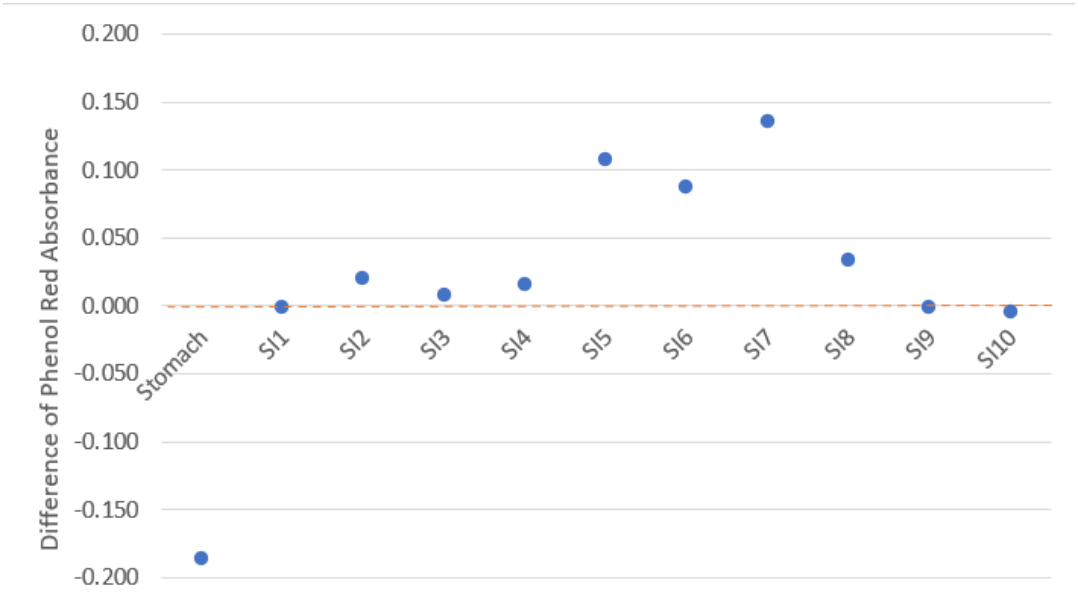

Figure 1. Difference between phenol red absorbances of rats with induced post-operative ileus and rats from corresponding control groups without induced post-operative ileus

The average phenol red absorbances for each GI tract segment and stomach in the experimental group were subtracted from its counterpart in the control group (ex: ave. absorbance SI2 control - ave. absorbance SI2 experimental). Negative values below 0 indicate that the phenol red absorbance in the control group was less than in the experimental group. Positive values above 0 indicate that phenol red absorbance in the control group was higher than in the experimental group.

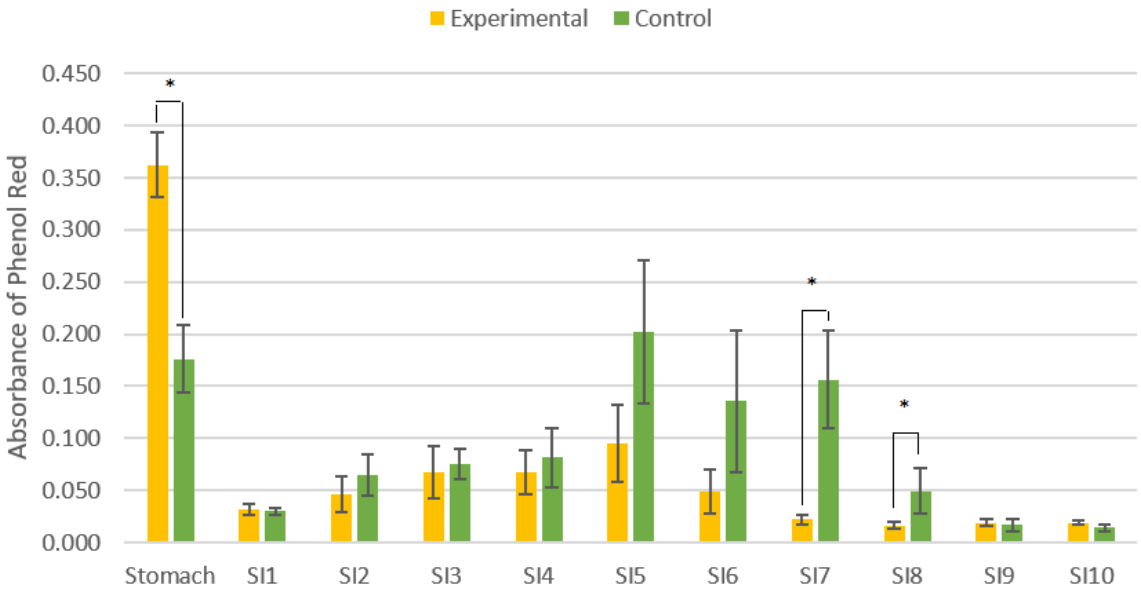

Figure 2. Absorbances of phenol red in different segments of the small intestine (SI) and stomach compared between the experimental and control groups.

The absorbance of the phenol red moved from the stomach down the small intestine pieces labeled 1-10 in both the control group and the experimental group. The amount of phenol red in the stomach of the experimental group was significantly more (meaning $p<0.05$) than the amount of phenol red absorbed in the control group. The amount of phenol red in SI 7 and SI 8 of the control group was significantly more ($p<0.05$) than the amount of phenol red absorbed in the experimental group.

Discussion

The smaller absorbances of phenol red in the majority of the experimental groups' small intestines, as well as the large absorbances of the meal in the stomachs of the experimental group versus the control group, indicate that the rats with induced POI had slower gastric motility than the non-induced POI rats. The results of the experiment support the hypothesis that POI negatively impacts normal GI motility, specifically slowing it down. With the issue behind the occurrence of post-surgical decreased GI motility known, future studies could focus on strategies to prevent POI from occurring and decrease the amount of postoperative morbidity, increased hospital stay, and costs caused by POI.

For example, one such solution could be the drug neostigmine, commonly known as Bloxiverz or Prostigmin[7]. Neostigmine is a water-soluble compound that acts as an acetylcholinesterase reverse inhibitor[8].

Its FDA indication is for reversing the post-surgical effects of non-depolarizing neuromuscular blocking agents[7]. Despite its benefits for patients after anesthesia, Neostigmine is more commonly used as a treatment for a muscle disease called myasthenia gravis[9].

Mechanism of Action

Acetylcholine is a type of neurotransmitter, which is produced, stored, and released at the tips of motor nerve endings. Acetylcholinesterase is the enzyme responsible for metabolizing acetylcholine by hydrolyzing it in the neuromuscular junction. Neostigmine acts as an oxy-diaphoretic inhibitor of the acetylcholinesterase enzyme, meaning that it works by inhibiting via acid-transferring. By inhibiting acetylcholinesterase, neostigmine prevents the enzyme from metabolizing acetylcholine into choline and acetic acid. Due to the lack of acetylcholine metabolism, acetylcholine builds up at the neuromuscular junction and overcomes the inhibitory effect of non-depolarizing blocking drugs[10, 11, 12].

Post-Operative Uses

Due to the muscular rousing characteristics of neostigmine, it could be used to assist with GI motility. Some studies suggest that prokinetic drugs, one of which is neostigmine, improve GI motility[13, 14, 15, 16].

Although the effects of it on the human model have been studied, neostigmine could be used in a rat model in a similar sense. Many animals used in survival surgeries, specifically rats, can undergo discomfort due to POI[17, 18]. These discomforts and prolonged absence of bowel function could become artifacts in data gathering. Future studies could test if neostigmine could assist with post-operative GI motility in rat survival surgeries.

Conclusions

A rat model was tested for the effects of POI on GI motility. The results support that GI motility is negatively impacted by surgically induced POI. Future research can be done to determine the benefits of neostigmine on GI motility during POI in a survival surgery rat model.

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