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## METHODS

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# Effects of Sodium Hypochlorite and Ozone on Healing of Intestinal Anastomosis in Simulated Strangulation Colorectal Obstruction

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Strangulation colorectal obstruction was modeled in 60 Wistar rats. Necrotic segment of the intestine was resected under conditions of peritonitis and end-to-end intestinal anastomosis was performed on a PCV catheter conducted through the anus. Sodium hypochlorite and ozone solution were used for sanitation of the abdominal cavity and intestinal lavage, and the intestinal anastomosis was coated with Ozonide (ozonized oil). The use of physico-chemical methods notably reduced the incidence of postoperative pyoinflammatory complications, incompetence of intestinal anastomosis sutures, and animal mortality.

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**Key Words:** *colorectal obstruction; intestinal anastomosis; sodium hypochlorite; ozone; rats*

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Despite recent improvements of suturing technologies and the use of new materials, the incidence of intestinal suture incompetence after colon surgeries reaches 3.5-30.0% and is associated with high mortality [1, 2,6]. Microcirculatory disorders, hypoxia, and bacterial contamination of the suture are the major causes of incompetence of intestinal anastomosis sutures [3,5]. The search for new methods improving the efficiency of intraperitoneal and intrainestinal sanitation, correction of metabolic disorders and morphological changes in the anastomosis zone is now in progress [4,7]. We studied the therapeutic effect of sodium hypochlorite (NaClO) and ozone in rats with experimental acute colorectal obstruction.

## MATERIALS AND METHODS

Experiments were carried out on 60 Wistar rats. Acute strangulation ileus was modeled after median laparotomy under general anesthesia by applying knot liga-

ture on the colorectal loop and mesentery at a distance of 7-8 cm from the anus. After 60 min necrosis of the ligated segment of the colon and peritonitis developed, which was seen visually and was confirmed by laser flowmetry and morphological findings. The segment of the colon was then resected and under laser flowmetry control anastomosis on a PCV catheter (3-4 mm in diameter) was formed from viable colon using a perforating single-row suture with polypropylene 5/0 thread. Microcirculation in the colonic wall was studied using a LA KK-01 laser analyzer of capillary blood flow velocity (Lasma). The transducer was placed on the edge of the studied segment most distant from the mesentery and the microcirculatory blood flow in the zone of the formed colonic anastomosis was evaluated.

In the control group ( $n=30$ ) the abdominal cavity was washed with nitrofurazone before and after the formation of intestinal anastomosis. Nitrofurazone was also used for intraoperative intestinal lavage (4-5 ml through the catheter into the intestine). Before suturing of the operative wound the cavity was repeatedly

washed with nitrofurazone. On the next day after surgery sanitation of the abdominal cavity (through microirrigator) and intestinal lavage (through intestinal catheter) were repeated. The microirrigator and catheter were removed after the procedures.

In the experimental group ( $n=30$ ) the abdominal cavity was irrigated with 0.05-0.06% NaClO and dissolved ozone (4-6 mg  $O_3$ /liter). Enteral lavage was carried out by successive infusion (through the intestinal catheter) of 4-5 ml 0.03-0.05% NaClO; after aspiration of the intestinal contents the same volume of normal saline and 2-3 ml ozone-oxygen gaseous mixture (OOM) with ozone concentration of 20-25 mg/ml was infused. After 3-5 min the intestinal contents was again aspirated and 1-2 ml ozonized oil Ozonide (Medozon) was infused into the anastomosis zone. The abdominal cavity was sutured with a single knot suture with the microirrigator inserted through the operation wound. Sanitation of the abdominal cavity through the microirrigator was repeated on the next day after the operation. Intestinal lavage with ozonized solution was carried out as described above, and after repeated intrainstestinal infusion of ozonide oil the intestinal catheter was removed.

NaClO was prepared by electrolysis of normal saline in EDO-3M and EDO-4 electrolyzers. Ozonized solutions and OOM were prepared using UOTA-60-01 ozone therapeutic device (Medozon).

The treatment efficiency was evaluated by animal survival, visual inspection of changes in the abdominal cavity, anastomosis, and operation wound, dynamics of histomorphological changes, and biomechanical strength of the intestinal anastomosis on days 1, 3, 6, 9, and 12 postoperation. Fragments of tissue from the anastomosis zone were collected for histological analysis in dead and sacrificed animals.

Biomechanical strength of the intestinal anastomosis was evaluated by measuring the intraluminal rupture pressure. The study was carried out using a specially designed device. A fragment of intact or anastomosed intestine was placed into the vessel with fluid. Air or stained fluid was injected into the intestine with a syringe through a catheter at a rate of 1.7 ml/min (the opposite end of the intestine was ligated). The pressure in the examined fragment of the intestine was increased to a level when air or stained fluid started to leak through the anastomosis wound or defect in the intestinal wall. The pressure at which air or fluid leaked, diameter of the intestine, and site of air/fluid leakage were recorded.

## RESULTS

During the first 3 days after surgery air or fluid leaked as a rule at the site of needle pricking at a minimum

pressure (25-45 mm Hg), i.e. intestinal anastomosis was not hermetic (Table 1).

On day 12 the intestinal anastomoses in the main experimental group resisted the pressure of 180 mm Hg vs. 150 mm Hg in the control; the intestine was disrupted outside the anastomosis zone. Hence, in case of an uneventful wound process, anastomoses become stronger than the intestinal tissue soon after their formation. This can be due to the fact that the anastomosis zone is less elastic because of rigid cicatricial tissue and sutures. That is why as the anastomosis acquires certain strength during stretching of the studied segment of the intestine, the intestine is disrupted outside its zone.

We observed the phenomenon of earlier increase of the intestinal wall strength after intrainstestinal infusion of ozone and Ozonide oil. In the main experimental group intestinal wall rupture was observed on days 9-12 at the rupture pressure of 160-190 mm Hg, which corresponded to the strength of the intact intestinal wall (in the control these values were observed only on days 11-14).

Comparative (in the main and control groups) macroscopic evaluation and histological analysis of the intestinal anastomosis, carried out repeatedly during the first 3-5 days after resection of the intestine showed similar inflammatory reaction to injury and foreign body (suturing material). However, differences in the intensity of inflammatory reaction and rate of reparative processes were seen during the early period of observation. Macroscopic study of the intestinal anastomosis area in the main group on day 6 postoperation showed local adhesive process. Serosa was smooth, lustrous, with solitary punctate hemorrhages, while mucosa was slightly hyperemic with few small hematomas. Histological analysis of the suture showed moderate infiltration with neutrophils, plethora, vascular dilatation, and slight edema. The sites of prick-

**TABLE 1.** Mechanical Strength of Intestinal Anastomoses ( $M \pm m$ )

Day of investigation	Group	Anastomosis rupture pressure, mm Hg
3	Main	40.0 $\pm$ 2.1
	Control	30.0 $\pm$ 1.7
6	Main	100.0 $\pm$ 4.2
	Control	85.0 $\pm$ 3.6
9	Main	140.0 $\pm$ 6.1
	Control	118.0 $\pm$ 3.50
12	Main	180.0 $\pm$ 7.4*
	Control	150.0 $\pm$ 6.2

**Note.** \* rupture of the intestine.

ing were enveloped in a thin layer of fibrin. Muscle layers adhered tightly, their structure being intact.

Pronounced loose adhesions were seen in the control group. Fibrin depositions at the site of sutures were clearly seen. Hemorrhages were as a rule seen on the serous membrane around the ligatures. The mucosa was hyperemic and markedly edematous, with necrotic foci. Histological analysis showed pronounced edema in the mucosa and submucosa, foci of pyonecrotic detritus between sutures reached the submucous layer. Erythrocyte imbibition and pronounced infiltration with polymorphonuclear leukocytes were seen around the suturing material, with accumulations of destroyed neutrophils. Diffuse hemorrhagic infiltration involving submucosa and mucosa was observed at some sites. Differences in the severity of inflammatory reaction did not level later, determining the course of anastomosis healing and time of epithelialization.

In the main group wound cleansing from detritus and fibrin depositions started on days 5-6; the intensity of intestinal wall infiltration with leukocytes progressively decreased and the number of fibroblasts increased. Complete epithelialization of anastomosis was as a rule observed by day 12. In the control group stubborn hemo- and lymphostasis in the anastomosis zone, persistent pronounced leukocytic infiltration and foci of necrosis led to the formation of a coarse cicatrice and later epithelialization, which was not over by day 12. Hence, morphological study showed that the use of NaClO and ozone during the formation of intestinal anastomosis under conditions of peritonitis stimulated reparative processes in intestinal anastomoses.

The criteria of efficiency in our studies were the incidence of postoperative intraperitoneal infectious complications, anastomositis, and mortality after resection of the intestine.

The main causes of lethal outcomes in the control group ( $n=30$ ; 13 (43.3%) deaths) were incompetence of anastomosis sutures, peritonitis, adhesive ileus, pyoinflammatory complications (intraperitoneal abscesses, suppuration of postoperative wound with even-tration of the intestine and anastomositis) — 40%.

In the main group five (16.7%) animals died. Four rats died from acute ileus, which developed as a result of anastomositis and adhesive process. In one case the lethal outcome was caused by inadequate anesthesia. Comparative analysis of autopsy data in dead and sacrificed animals showed that adhesive process in the abdominal cavity was far less pronounced in the main group, presenting as flat loose adhesions of the omentum to the interintestinal anastomosis. It is noteworthy that no postoperative suppurative complications developed in this group.

Hence, the use of NaClO and ozone for sanitation of the abdominal cavity and prevention of failure of anastomosis sutures is effective when intestinal anastomoses are formed under conditions of peritonitis caused by acute colorectal obstruction.

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