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ORIGINAL RESEARCH

The Effects of Duration of CO_2 Pneumoperitoneum on Colonic Anastomosis

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ABSTRACT The aim of this study is to evaluate the effects of duration of carbon dioxide (CO_2) pneumoperitoneum on experimental colonic anastomosis. Forty-eight male Sprague–Dawley rats were used. The rats were divided into three groups. The rats in group 1 ($n = 16$) underwent laparotomy and colonic anastomosis without pneumoperitoneum. The rats in group 2 ($n = 16$) and group 3 ($n = 16$) were subjected to 2 and 4 hours of 12 mm Hg pneumoperitoneum, respectively, before laparotomy and colonic anastomosis. Half of the rats were sacrified on the third postoperative day; and the other half, on the seventh postoperative day. A colonic segment including anastomosis site was resected for histopathologic and biochemical evaluation. On day 3, hydroxyproline levels of the three groups were similar. The edema score of group 2 was significantly higher than that of group 1, and the necrosis score was higher in group 2 than in group 3. The scores of the other histopathologic parameters were similar. On day 7, group 3 showed significantly higher hydroxyproline levels than group 1, and group 1 showed a higher necrosis score than group 3. In conclusion, CO_2 pneumoperitoneum of 12 mm Hg for 2 and 4 hours did not result in impaired healing of experimental colonic anastomosis.

KEYWORDS laparoscopy, pneumoperitoneum, duration, experimental, colon, anastomosis

Widely application of laparoscopic cholecystectomy demonstrated the advantages of minimally invasive surgery such as less postoperative pain, early ambulation, shorter hospitalization, and better cosmesis. Demonstration of these advantages has resulted in application of laparoscopy for various types of more extensive and time-consuming operations including antireflux operations, splenectomy, and bowel resections. However, carbon dioxide (CO_2) pneumoperitoneum has been shown to have some adverse effects including reduced cardiac output, increased mean arterial pressure, vena caval pressure, and pulmonary arterial wedge pressure in experimental studies [1]. In addition, decreased portal blood flow and splanchnic ischemia have been demonstrated during CO_2 pneumoperitoneum [2, 3].

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Decreased blood flow to the small and large intestines during laparoscopic surgery may lead to impaired anastomotic healing. Despite the increased number of laparoscopic colorectal procedures, studies evaluating the effects of CO_2 pneumoperitoneum on colonic anastomoses are lacking. We were able to find only a few numbers of experimental studies evaluating the effects of pneumoperitoneum on anastomotic wound healing [4–7].

The aim of this study is to evaluate the effects of duration of CO_2 pneumoperitoneum on colonic anastomoses, and to our knowledge this is the first controlled experimental study evaluating this subject.

MATERIALS AND METHODS

Forty-eight male Spraque–Dawley rats weighing 275–300 g were used. The Animal Studies Ethics Committee of Faculty of Veterinary of Ankara University approved the protocol. All rats were kept under constant temperature (20°C–24°C), 12 hours of light and dark cycles, and fed a standard rat diet with free access to water.

The rats were divided into three groups. After an overnight fast, they were anesthetized with intramuscular ketamine (50 mg/kg) and intramuscular Rompun (7 mg/kg). The abdominal region was shaved and cleansed with povidone iodine. The abdominal cavity was punctured with a 20-gauge intravenous canula and the canula was connected to an electronic insufflator (Storz & Company, Tutthufen, Germany). Pneumoperitonium was induced at a pressure of 12 mm Hg and a flow rate of 0.2 liters/min, maintained for 2 and 4 hours, and then released completely. Laparotomy was performed as a midline incision. The colon was transected 5 cm distal to the caecum and anastomosed by the same surgeon with interrupted one-layer sutures using 4/0 polyglactin throughout the experiment. The abdominal muscle layer and skin incision were closed with running sutures separately. The three groups were designed as follows: group 1 ($n = 16$), laparotomy and colon anastomosis without CO_2 pneumoperitonium; group 2 ($n = 16$), laparotomy and colon anastomosis after 2 hours of CO_2 pneumoperitonium at 12 mm Hg pressure; group 3 ($n = 16$), laparotomy and colon anastomosis after 4 hours of CO_2 pneumoperitonium at 12 mm Hg pressure.

After the operations, water was given to the rats on the same day and they were fed by standard diet and water as during the preoperative period. In each group, half of the rats were killed on the third postoperative day; and the other half, on the seventh postoperative day. A 4-cm colonic segment including the anastomosis site was resected and cut longitudinally. One half was reserved for histopathologic evaluation; and the other half, for biochemical evaluation.

Histopathologic Evaluation

Histopahologic evaluation was performed by a single pathologist who did not know to which group the specimen belonged. Colonic segments were fixed in 10% formaldehyde solution and, after routine procedures, embedded in paraffin blocks. The paraffin blocks were cut into 4-micron-wide sections, and these tissue sections were stained with hematoxylin–eosin. Histopathologic scoring was performed according to the scale previously used by Verhofstad et al. [8]. This scale was modified by uniting lymphocyte and macrophage infiltration as mononuclear cell infiltration and addition of granulation tissue (Table 1).

TABLE 1 **Histopathologic parameters used to analyze wound healing**

Score	Necrosis	PMN's	Mononuclear cells	Edema	Mucosal epithelium	Submucosal, muscular layer	Granulation tissue
0	None	Normal number	Normal number	None	Normal glandular	Good bridging	None
1	Small patches	Slight increase	Slight increase	Some	Normal cubic	Average bridging	Some
2	Some patches	Marked infiltration	Marked infiltration	Marked	Incomplete cubic	Poor bridging	Marked
3	Massive	Massive infiltration	Massive infiltration	Severe	Absent	No bridging	Severe

(PMN's: Polymorphonuclear leucocytes)

Biochemical Evaluation

Tissue hydroxyproline was determined on the hydroxilate by a modification of the method of Jamall et al. [9].The concentrations of hydroxyproline were defined as microgram of hydroxyproline per milligram of tissue.

Statistical Analysis

Differences between hydroxyproline levels and histopathologic scores were analyzed using Kruskall–Wallis variance analysis, and *p* values below .05 were considered statistically significant. When significance was detected for any parameter, the Mann–Whitney U test was performed to analyze the group causing the difference. Bonferroni correction was done to increase the confidence of the test, and *p* values below .017 were considered to be significant.

RESULTS

Postoperative Period

All rats survived during the experiment, and during scarification no anastomotic leakage was observed.

Hydroxyproline Levels

Mean hydroxyproline levels of the groups on days 3 and 7 are shown in Table 2. On day 3, the difference between the groups was not statistically significant. On day 7 there was a statistically significant difference between group 1 and group 3. Group 3 was found to have higher hydroxyproline levels when compared to group 1.

Histopathologic Evaluation

At day 3, edema and necrosis scores of group 2 show a statistically significant increase when compared to those of group 1. The scores of the other parameters except mononuclear cells displayed a tendency to increase slightly; however, these increases were not statistically significant. In group 3, scores of all of the parameters were lower than those of group 2, but except for the necrosis score, these differences were not significant, either.

At day 7, differences among the three groups were not significant, but necrosis was not observed in group 3. This was a significant difference when compared to the necrosis score of group 1. However, it was not a significant difference when compared to that of group 2. Interestingly, the scores tended to decrease in the CO_2 pneumoperitoneum groups, but the differences were not significant. Histopathologic scores are shown in Table 3.

TABLE 2 **Mean hydroxyproline levels of the groups on day 3 and 7 (μg/mg tissue)**

Group	Day 3 mean ± SD	Day 7 mean ± SD[a]
1	0,416 ± 0,09	0,320 ± 0,06*
2	0,429 ± 0,09	0,342 ± 0,04
3	0,383 ± 0,06	0,414 ± 0,06*

SD: Standard deviation.

[a]P < 0,05 (difference between groups, Kruskal Wallis Variance Analysis).

*P < 0,017 (difference between groups 1 and 3, Mann Whitney U test).

TABLE 3 **Histopathologic scores of the groups on day 3 and 7**

Parameter Group	Day 3 Mean ± SD (median)	Day 7 Mean ± SD (median)
Submucosa, muscular layer		
1	1,75 ± 1,28 (2)	2,13 ± 0,64 (2)
2	2,57 ± 0,53 (3)	1,50 ± 0,92 (3)
3	2,43 ± 0,79 (3)	1,71 ± 1,11 (3)
Epithelium		
1	1,63 ± 0,92 (2)	2,00 ± 0,54 (2)
2	2,14 ± 0,38 (3)	2,00 ± 0,00 (2)
3	2,00 ± 0,00 (2)	1,71 ± 0,49 (2)
Edema[a]		
1	1,75 ± 0,46* (2)	1,88 ± 0,35 (2)
2	2,71 ± 0,49* (3)	1,88 ± 0,64 (2)
3	2,57 ± 0,53 (3)	2,00 ± 0,81 (2)
Mononuclear cells		
1	1,25 ± 0,46 (1)	1,13 ± 0,35 (1)
2	1,14 ± 0,38 (1)	1,00 ± 0,00 (1)
3	1,00 ± 0,00 (1)	1,00 ± 0,00 (1)
Polimorphonuclear cells		
1	2,13 ± 0,35 (2)	2,13 ± 0,35 (2)
2	2,43 ± 0,54 (2)	2,00 ± 0,00 (2)
3	2,00 ± 0,00 (2)	2,00 ± 0,00 (2)
Necrosis[a]		
1	1,63 ± 0,74 (1,5)	1,25 ± 1,04* (1)
2	1,86 ± 0,38* (2)	0,75 ± 0,89 (0,50)
3	1,00 ± 0,58* (1)	0,00 ± 0,00* (0)
Granulation		
1	0,88 ± 0,99 (0,5)	1,25 ± 0,89 (1)
2	1,57 ± 0,53 (2)	1,00 ± 0,76 (1)
3	1,14 ± 0,69 (1)	0,86 ± 0,38 (1)

SD: Standard deviation.

[a]P < 0,05 (difference between the groups, Kruskal Wallis Variance Analysis).

*P < 0,017 (difference between groups 1 and 3, Mann Whitney U test).

DISCUSSION

The adverse effects of CO_2 pneumoperitoneum on cardiac, respiratory, intestinal, renal, and hepatic functions have been shown in various studies [1, 10–12]. All abdominal cavity organs are subjected to a high-pressure environment with pneumoperitoneum. In an experimental dog model by Ishizaki et al. [13], at 16 mm Hg intra-abdominal pressure a significant decrease was observed in portal venous and superior mesenteric arterial blood flows, but no significant change was observed at 8 and 12 mm Hg. At 16 mm Hg intra-abdominal pressure, portal venous flow, and superior mesenteric artery (SMA) flow decreased progressively as the duration of pneumoperitoneum increased. Decreased visceral blood flow during elevated intra-abdominal pressure (IAP) has been shown in some other studies, and all abdominal organs were shown to be affected [14, 15]. Eleftheriadis et al. [3] reported that hepatic microcirculation and gastric mucosal pH were found to decrease during laparoscopic cholecystectomy at 12 mm Hg CO_2 pneumoperitoneum. Tsuboi et al. [16] observed decreased blood flow values for the stomach small and large intestines, pancreas, spleen, and mesentery during 1 hour of CO_2 pneumoperitoneum at 8 mm Hg IAP in cirrhotic rats. Elevated intra-abdominal pressure was shown to lead to oxygen-free radical production and bacterial translocation [17]. Abdominal insufflation and deflation was thought to be a model of ischemia and reperfusion [3], which was shown to impair colonic anastomotic healing [18]. However, Posma et al. reported that prolonged reperfusion after transient ischemia did not impair anastomotic wound healing in the rat [19]. According to the data obtained from these studies, microcirculation of abdominal viscera may be disturbed during abdominal insufflation.

Effects of increased IAP on colonic anastomoses were first studied by Polat et al. [4]. An IAP increased to 20 mm Hg and higher was found to result in impaired strength and wound healing in colonic anastomoses. They suggested that an IAP of 14 mm Hg was the upper limit of IAP to be reached during laparoscopic colorectal procedures [4]. However, duration of CO_2 pneumoperitoneum in this study was shorter than that in laparoscopic colorectal procedures. Duration of laparoscopic colorectal procedures may exceed 4 hours. Increased duration of CO_2 pneumoperitoneum may lead to prolonged splanchnic ischemia, and this may result in impaired anastomotic healing. To our knowledge, the effects of duration of CO_2 pneumoperitoneum have not been evaluated previously. We have evaluated the effects of duration of CO_2 pneumoperitoneum within a constant intraabdominal pressure, which is widely used in clinical practice. Histopathologic wound healing parameters and tissue hydroxyproline levels as a measure of collagen in the tissue are used to evaluate anastomotic healing in this study. Histopathologic evaluation of the anastomoses and collagen accumulation in the anastomotic area has been widely used as parameters of wound healing in experimental studies [8, 19–23].

The process of wound healing is similar in all wounds. Gastrointestinal wound healing slightly differs because it is a controlled, multilayered full thickness injury in which intestinal continuity is reconstructed with sutures. Intestinal wound healing is affected by the peritoneum, omentum, multilayered structure of intestinal wall and inversion or eversion of mucosa [17, 20]. In addition, the healing of colonic anastomoses is slower and is accompanied by more complications than elsewhere in the gastrointestinal tract [17]. Dehiscence of intestinal anastomoses is mostly seen during the first week after operation. Immediate postoperative wound strength mainly depends on extracellular matrix components, particularly collagen fibers and sutures [8]. After this period, wound strength is repaired by deposition of new collagen fibers, produced by fibroblasts. The submucosal layer is mainly responsible for the mechanical strength of the anastomoses because of its collagen content [20, 24].

During wound healing coordinated action of several cell-type and biochemical events takes place [20]. Anastomotic healing begins with a strong inflammatory reaction [17]. The first group of cells is granulocytes, which appear 3 hours after wounding [20]. Then, monocytes, macrophages, and lymphocytes appear in the wound. These cells reflect the immune reaction to tissue injury. Granulocytes and lymphocytes almost disappear in the later stages of wound healing [8]. Polymorphonuclear and mononuclear cell infiltration persisted at seventh day in this study and was not significantly affected by pneumoperitoneum.

The local vascular response to trauma results in edema and necrosis in the anastomotic area during the first postoperative week [17]. On day 3, the edema score of group 2 was higher than that of group 1, but on day 7, this score declined to the level of that of the control group. Interestingly, on day 3, the necrosis score of

group 3 was lower than that of group 2, and necrosis disappeared on day 7 as the duration of pneumoperitoneum increased.

Fibroblasts emerge during the later stages of the inflammatory phase [20] and are responsible from collagen production. The proliferative stage of healing is characterized by granulation tissue. Scores of granulation in the groups were similar on days 3 and 7. In a study from Zuidewijn et al. [21], granulation tissue emerged in the first days of wound healing and reached to maximum on the seventh day. Granulocytes and macrophages were found to be positively correlated to the presence of granulation tissue [21]. These three parameters were similar among the groups at days 3 and 7 in our study.

Good apposition of wound edges is supposed to be the result of good technique and expected to be better in later stages of wound healing [21]. Two and 4 hours of pneumoperitoneum did not seem to affect submucosal and muscular repair and healing of the mucosa in this experiment. In addition, slight but statistically insignificant improvement is observed on day 7 in the pneumoperitoneum groups.

Hydroxyproline levels were similar among the three groups on day 3. On day 7 the mean hydroxyproline level of the three groups was significantly higher than that of the first group. This difference may be correlated with the histopathologic scores, but as the differences between the histopathology scores were not significant, we are not able to make definite conclusions. The tendency of increased histopathology scores with 2 hours of CO_2 pneumoperitoneum that showed a statistically insignificant decrease, as the duration of pneumoperitoneum reached 4 hours, is not a significant finding but may be regarded as interesting. This may be a subject of a new experimental study.

CONCLUSION

In conclusion, experimental CO_2 pneumoperitoneum at 12 mm Hg intraabdominal pressure for 2 or 4 hours did not have deleterious effects on colonic anostomotic healing in the means of histopathologic parameters and hydroxyproline levels. Further studies are needed to explain the probable causes and mechanisms of these findings.

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