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The Effects of Hyperbaric Oxygen Therapy on Experimental Colon Anastomosis After Preoperative Chemoradiotherapy

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The aim of the present study was to investigate the effect of hyperbaric oxygen therapy (HBOT) on colon anastomosis after chemoradiotherapy (CRT). Sixty female Wistar-Albino rats were divided into 5 groups and underwent left colon resection and end-to-end anastomosis. CRT simulation was performed on 2 sham groups before the anastomosis, and 1 of these groups was administered additional postoperative HBOT. Two groups were administered CRT before the anastomosis, and 1 of them received additional postoperative HBOT. On postoperative day 5, all groups underwent relaparotomy; burst pressure was measured and samples were obtained for histopathologic and biochemical analysis. There was a significant weight loss in the CRT groups and postoperative HBOT had an improving effect. Significantly decreased burst pressure values increased up to the levels of the controls after HBOT. Hydroxyproline levels were elevated in all groups compared to the control group. Hydroxyproline levels decreased with HBOT after CRT. No significant difference was observed between the groups regarding fibrosis formation at the anastomosis site. However, regression was observed in fibrosis in the group receiving HBOT after CRT. Preoperative CRT affected anastomosis and wound healing unfavorably. These unfavorable effects were alleviated by postoperative HBOT. HBOT improved the mechanical and biochemical parameters of colon anastomosis in rats.

Key words: Anastomosis – Chemoradiotherapy – Hyperbaric oxygen – Rat – Burst pressure

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Colorectal cancer is an important health problem that accounts for approximately 50,000 and 200,000 deaths every year in the United States and Europe, respectively.[1,2] Local recurrence or distant metastasis develops in two thirds of the patients undergoing curative resection for colorectal cancer. Nearly 85% of relapses occur within the first 2.5 years after surgery.[2] Although adjuvant therapy has been a well-accepted procedure to prevent metastasis and local recurrence after surgical resection, more recently neoadjuvant therapy has gained popularity to enable secondary curative resection with clear surgical margin and to prolong overall and recurrence-free survival.[2] It has been reported that local recurrence rate is lower, toxicity is rare, and the anal sphincter is more likely to be preserved in those undergoing preoperative radiation.[1,2]

Despite the current advances in surgical techniques, anastomotic leakage is a severe complication leading to morbidity and mortality after intestinal surgery.[3–6] Chemoradiotherapy (CRT) has the potential to lead to inadequate vascularization in tissues, a well-known risk factor for intestinal anastomosis leak. Impaired oxygenation as a result of hampered vascularization may lead to delay in wound healing, which ultimately impairs anastomotic durability. Studies on factors that enhance wound healing to prevent anastomotic leakage in colon anastomosis have been accelerated in the past decade. In a limited number of studies performed for this purpose, hyperbaric oxygen therapy (HBOT), which is applied by means of intermittent inhalation of 100% oxygen under 1 atmosphere pressure, has been reported to have favorable effects on intestinal anastomotic healing.[7–9] However, whether HBOT can reverse potential side effects of CRT on would healing and anastomotic durability is unclear. The aim of the present study was to investigate the effects of HBOT on the biochemical and mechanical parameters of anastomosis healing after colon anastomosis in rats after preoperative CRT.

Materials and Methods

Experimental Animals

The present study was approved by the Gulhane Military Medical Academy Ethical Committee for Animal Experiments. In the present study, 60 Wistar-Albino female rats, weighing between 150 and 220 g, were used. In the course of the study, the animals were kept in the same laboratory conditions and fed with commercial rat food and ordinary tap water. The rats were weighed at the beginning of the study, during the first operation, and during relaparotomy. The rats were randomized into 5 groups by simple random sampling, therefore 12 rats in each group (one control, two sham and two study groups). All of the groups underwent standard left colon resection and single layer end-to-end anastomosis. In 2 sham groups (groups 2 and 3), chemotherapy (CT) and radiotherapy (RT) simulation was performed before anastomosis and 1 of these groups received additional postoperative HBOT. In 2 CRT groups (groups 4 and 5), CT plus RT was performed before anastomosis and 1 of these groups received additional HBOT. On postoperative day 5, all of the groups underwent relaparotomy and burst pressure (BP) was measured. Tissue samples were taken for histopathologic examination and biochemical analyses. Procedures applied to the experimental groups are summarized in Table 1.

In the course of the study, 1 of the rats in the sham group and 1 of the rats in the CRT group died on the postoperative second and third days, respectively. Postmortem laparotomies of these experimental animals revealed no intra-abdominal pathology. Considering that dehydration and weight loss might have led to mortality, the groups were completed replacing the dead experimental animals with the new ones.

Procedures

CT application

Eight days before the anastomosis, fluorouracil (5-FU) was intraperitoneally injected at a dose of 10 mg/kg for 5 days.

RT application

Radiotherapy, under anesthesia, was applied 8 days before the anastomosis, anesthesia was applied and totally 20 gy RT (Cobalt 60 Theratron 780 E, Theratronics, Canada) was given at a dose of 4 gy/day for 5 days. Anesthesia was also given on the day of surgery.

CT simulation

Eight days before the anastomosis, intraperitoneal saline was injected for 5 days at the same volume as CT. Procedures were applied at the same time to the groups receiving CT.

Table 1 Procedures applied to the experimental groups

Procedure	Group 1 Control	Group 2 Sham	Group 3 Sham+HBOT	Group 4 CRT	Group 5 CRT+HBOT
Preoperative CT and RT				X	X
Preoperative CT and RT Simulation		X	X		
Colon Resection and anastomosis	X	X	X	X	X
Postoperative HBOT			X		X
Postoperative fifth day Relaparotomy and measurements	X	X	X	X	X

CRT, chemoradiotherapy; CT, chemotherapy; HBOT, hyperbaric oxygen treatment; RT, radiotherapy.

RT simulation

Anesthesia was applied daily, but RT was not given. Procedures were applied at the same time to the groups receiving RT.

Anesthesia applied during RT and RT simulation

Xylazine HCl 2% injection vial (Alfazyne, Alfasan International BV, Woerden, Holland) was given at a dose of 10 mg/kg and ketamine HCl 10% injection vial (Alfamine, Alfasan International BV) was given intramuscularly at a dose of 90 mg/kg.

Colon resection and anastomosis

All rats fasted for 12 hours before operation and only water intake was allowed for 24 hours after the operation. Anesthesia induction included 60% O2, 40% nitrogen protoxide, and 5% to 6% sevoflurane. Maintenance of anesthesia included 40% O2, 20% nitrogen protoxide, and 1.8% to 2% sevoflurane. The abdominal skin of the rats was shaved after anesthesia and the operation site was cleaned with povidone-iodine. The abdomen was opened through a median incision of 3 cm, the left colon was explored, and colonic content was manually evacuated, not damaging the mesoderm and surrounding tissues. One centimeter of the left colon was resected at 4 cm proximal of the peritoneal reflection. Thereafter, end-to-end anastomosis was performed by single layer introverted sutures using 6/0 polypropylene (Ethicon Inc, Gargrave, United Kingdom). During the operation, sterile gauzes damped with Ringer lactate solution were used to protect intra-abdominal organs from drying out. Before closing the abdomen, 5 mL of Ringer lactate solution was injected into the abdomen to prevent dehydration. Peritoneum and the fascia were closed by continuous suturing with 3/0 silk suture, whereas the skin was closed with single suture using 3/0 silk.

HBOT application

HBOT application was initiated on postoperative day 1 and continued for 5 days with 2 sessions per day (each session, 90 minutes). A cylindrical hyperbaric chamber (diameter, 24.5 cm; length, 38 cm) composed of nickel, steel, and chromium and tested for durability against 6 atm pressure, was used for hyperbaric application. Tubes including pure oxygen under high pressure were connected to the pressure room. The strength of the current could be observed and was maintained at 1.5 to 2 L/min. After the animals were placed into the pressure room, the medium was rapidly flushed with oxygen. Absorbent granules, known as soda lime, composed of sodium hydroxide and calcium hydroxide in varying ratios, were placed in the pressure room to uptake CO2 that might have accumulated in the medium as a result of ventilation of the animals. The pressure room was then closed and the inner pressure was increased up to the application level at a rate not exceeding 1 atm pressure per minute. As soon as reaching to the application level was reached, the amount of input and output O2 was stabilized and the time was kept.

Relaparotomy

Relaparotomy was performed under inhalation anesthesia on postoperative day 5 of anastomosis. Anastomosis line was found based on the presence of Prolene sutures and adhesions at anastomosis site were evaluated. BP was measured, and samples were obtained for biochemical analysis and histologic examination.

Measurements

BP measurement

Of the anastomotic line, 2 cm distal and proximal were cautiously prepared and stool content of the colon was evacuated manually. The mechanism prepared for BP included infusion pump (Micro-

Table 2 Weight monitoring of the groups

	Weight (g) mean ± SD		
	Baseline	After operation	After relaparatomy
Group 1 control	161.33 ± 7.39	162.75 ± 7.50	157.25 ± 6.24
Group 2 sham	164.58 ± 5.40	149.92 ± 3.94	147.83 ± 5.06
Group 3 sham+HBOT	172.67 ± 7.88	161.67 ± 6.08	162.17 ± 5.42
Group 4 CRT	173.42 ± 4.80	143.17 ± 10.25	144.83 ± 15.79
Group 5 CRT+HBOT	176.17 ± 12.52	153.33 ± 12.43	162.25 ± 10.41

CRT, chemoradiotherapy; HBOT, hyperbaric oxygen treatment; SD, standard deviation.

macro Life Care Infusion Pump, Abbott, Berkshire, United Kingdom) and bedside monitor (Cardiocap/Critical Care Monitor, GE healthcare, New Jersey). A serum containing 0.9% NaCl was attached to the infusion pump with infusion set. Three-way tap was attached to the end of the infusion set. One end was extended to the intestinal segment, of which the pressure would be measured, and the other end was attached to the pressure measuring transducer with display (Monitoring Kit, Transpac IV, Abbott Critical Care Systems Abbott, Dublin, Ireland). The change in pressure was monitored, while the serum was being infused with a constant speed of 4 mL/min. As the serum was observed to leak from anastomosis site into the abdomen, the highest pressure measured on the monitor was recorded as the BP. The burst site was evaluated as "on the anastomotic line" and "out of the anastomosis." Then, the anastomosis segment extending 1 cm distal and proximal of the anastomotic line was excised and divided into 2 by opening along the mesenteric margin. One segment sample was put into a tube, frozen in liquid nitrogen, and stored in deep freeze at –80°C for hydroxyproline analysis. The other segment sample was stored in a tube containing 10% formaldehyde for histopathologic examination.

Hydroxyproline analysis

After 1 mL of 6M hydrochloric acid was added into the tubes used to collect tissue samples, they were hydrolyzed at 150°C for 1 hour (Hycel Thermal Block, Hycel Inc, Houston, Texas) and tissue hydroxyproline hydrolysates were prepared. Of this hydrolysate, 10 μL was taken and put into a separate tube and left for drying for 24 hours. Hydroxyproline standards of 0.4, 0.8, and 1.5 μmol were prepared in 50% isopropanol and 450 μL of chloramine T was added and kept in room temperature for 25 minutes to provide oxidation. Thereafter, 500 μL of Erlich reactive was added and they were kept at 65°C for 20 minutes. At the end of the procedure, all of the samples were read at 559 nm against a reagent blank.

Histopathologic examination

The samples were fixed with 10% formalin for 24 hours, paraffin blocks were prepared, and 6-μm sections were obtained from the tissue samples. They were then stained with Masson's trichrome and hematoxylin-eosin. The preparations were evaluated under the light microscope by a blinded pathologist. Fibrosis on the anastomosis line was evaluated using a scale according to the severity of fibrosis (0: minimal, 1: mild, 2: moderate, 3: severe).

Statistical analysis

Statistical analyses were performed using statistical package for the social sciences for Windows 10.0 (SPSS Inc, Chicago, Illinois). Descriptive analyses were represented as mean ± standard deviation, median, and percentage. Statistical difference between the median values of the groups was analyzed using Kruskal Wallis test. From which groups the differences emerged was analyzed using Bonferroni adjusted Mann-Whitney U test. The comparison between the changes in weight (initial, during anastomosis, and relaparatomy) was performed using Wilcoxon signed ranks test. Histopathologic findings were evaluated using χ^2 test. A P value < 0.05 was considered significant.

Results

In the groups receiving CRT, food intolerance and weight loss were observed after the treatment was started, and diarrhea was observed on the days after treatment. When HBOT was administered to the rats in the CRT groups, it was observed that food intolerance was rapidly improved and weight gain began. Weight monitoring of the groups is summarized in Table 2 and the change in weight after

Table 3 Weight changes among groups

	Baseline—operation			Baseline—relaparotomy			Operation—relaparatomy		
	Weight difference			Weight difference			Weight difference		
	g	%	*P* value	g	%	*P* value	g	%	*P* value
Group 1 control	+1.42	+0.88	0.573	−4.08	−2.52	0.004	−5.5	−3.37	0.013
Group 2 sham	−14.66	−8.90	0.002	−16.75	−10.17	0.238	−2.09	−1.39	0.002
Group 3 sham+HBOT	−11.0	−6.3	0.003	−10.5	−6.08	0.929	+0.5	+0.30	0.002
Group 4 CRT	−30.25	−17.44	0.002	−28.59	−16.48	0.694	+1.66	+1.15	0.002
Group 5 CRT+HBOT	−22.87	−12.9	0.002	−13.92	−7.9	0.060	+8.95	+5.83	0.015

(–), weight loss; (+), weight gain; CRT, chemoradiotherapy; HBOT, hyperbaric oxygen treatment.

operation and relaparotomy is presented in Table 3. It was determined that CRT caused remarkable weight loss in experimental animals and weight loss stopped and weight gain started in the groups who underwent HBOT; weight gain was quite significant in group 5.

When adhesions at anastomosis line were evaluated during relaparotomy, it was observed that urine bladder, oviducts, and omentum showed extensive adhesion to the colon. During BP measurements, burst sites other than the anastomotic line were determined in 2 rats in the control group, in 1 rat in the sham group, and in 1 rat in the CRT+HBOT group. Burst pressure and tissue hydroxyproline values are presented in Table 4. Burst pressure was lower in the sham and CRT groups compared to the control group. The mean BP value of the control group was 132.58 mmHg, whereas it was 110.67 mmHg in the sham group, indicating that the anastomosis operation led to decrease in BP. BP was higher in groups 3 and 5 that received additional HBOT compared to their own control groups (groups 2 and 4; Fig. 1).

There was a significant difference between the groups in terms of BP($P = 0.004$). In paired comparisons, the decrease in BP in the sham group was not significant compared to the control group ($P = 0.085$), whereas it was significant in the CRT group ($P = 0.003$). Although it was not statistically significant, BP was found higher in group 5 that received HBOT compared to group 4 ($P = 0.06$).

There was a significant increase in tissue hydroxyproline levels in all groups compared to the control group. In addition, a significant difference was found between the groups in terms of tissue hydroxyproline levels ($P = 0.001$). Paired comparison of the groups showed that tissue hydroxyproline levels in the CRT group were significantly higher than that in the control group ($P = 0.001$). Although it was not statistically significant, tissue hydroxyproline level was lower in group 5 that received HBOT compared to that in group 4 ($P = 0.128$; Fig. 2). The severity of fibrosis in the groups is presented in Table 5. Severe and moderate fibrosis were most commonly observed in group 4 in 3 and 8 rats, respectively, whereas fibrosis was rarely seen in the control group. When all the groups were taken into consideration, no significant difference was found between the groups in terms of fibrosis ($P = 0.222$). In paired comparisons, it was observed that fibrosis was more common in the CRT group compared to the control group ($P = 0.008$). When group 4 was compared with group 5, it was determined that HBOT reduced fibrosis; however, it was not statistically significant ($P = 0.057$).

Table 4 Burst pressure and tissue hydroxyproline values of the experimental groups

	Burst pressure (mmHg)		Tissue hydroxyproline (μM/mg tissue)	
	Mean ± SD	Median	Mean ± SD	Median
Group 1 control	132.58 ± 26.99	137.00	12.05 ± 4.14	10.91
Group 2 sham	110.67 ± 18.57	106.50	14.96 ± 5.84	13.71
Group 3 sham+HBOT	133.67 ± 29.66	141.50	17.31 ± 6.60	14.41
Group 4 CRT	100.83 ± 21.15	102.50	24.28 ± 7.95	22.92
Group 5 CRT+HBOT	123.42 ± 18.75	127.00	19.43 ± 7.50	17.68

CRT, chemoradiotherapy; HBOT, hyperbaric oxygen treatment; SD, standard deviation.

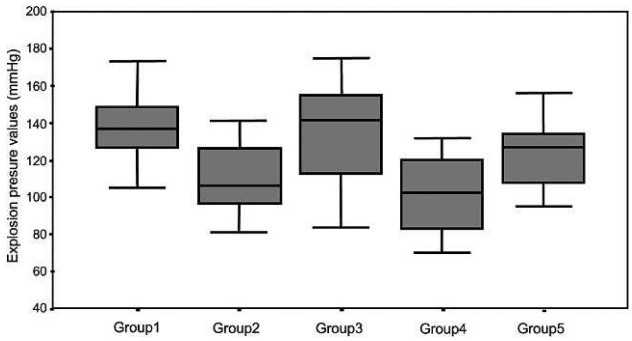

Fig. 1 The mean burst pressure values of the groups.

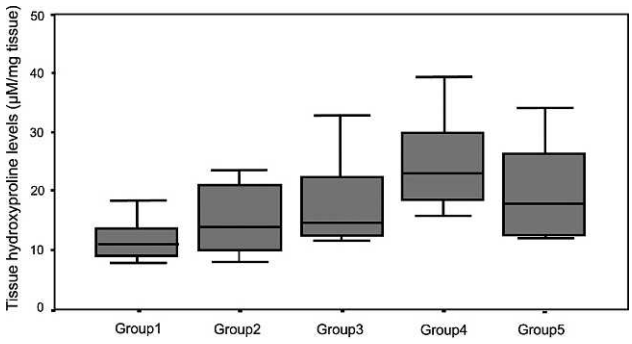

Fig. 2 The mean tissue hydroxyproline levels of the groups.

Discussion

Because colorectal cancer surgery requires anastomosis and anastomotic leakage is an important cause of morbidity and mortality, and as well as influences cancer-related outcomes and survival, the risk factors for anastomotic leakage and the causes affecting wound healing have been an attractive issue to be investigated.[10,11] In addition to the surgical treatment, adjuvant treatment modalities and their influence on wound healing and anastomotic leakage have been topics of research in some experimental and clinical studies.[12–14] However, the effect of HBOT on colon anastomosis after neoadjuvant CRT has not been studied. In the present experimental study, we investigated the effects of HBOT on biochemical and mechanical parameters of anastomosis healing after colon anastomosis in rats that underwent preoperative CRT. Before 1970, preoperative pelvic radiation (4000–6000 cGy) was performed to enable surgical removal of rectal tumors that invaded into adjacent organs and successful results were obtained in 50% to 80% of the cases.[15–17] Along with preoperative RT, radiation-induced toxicity decreases and the potential for sphincter-preserving surgery increases.[18–22] It has also been suggested that preoperative RT causes enteric complication less frequently than postoperative RT.[23] In addition, it has been shown that better survival can be obtained with a decrease in tumor mass and lymph node involvement.[24–26] Recent studies have evaluated the effects of CRT on resectability. In 4 series of patients who underwent preoperative combination therapy, complete histologic response has been obtained by 12% to 29% with surgery performed 4 to 6 weeks after full dose radiation and with combined CRT performed for 5 or 6 weeks. After a follow-up period of 22 to 39 months, local recurrence rate was between 0 and 5% and grade 3 toxicity rate was 21%.[27–30] Despite all of these studies showing that neoadjuvant CRT is an effective treatment modality in the treatment of colorectal cancer, risk for potential negative effects of antineoplastics and RTs on wound healing leads to a reluctance in using them. El-Malt *et al*[31] reported that preoperative CRT had no harmful effect on the mechanical strength of colonic anastomosis. Besides the studies defending that RT has negative effects on anastomosis healing,[5,32,33] there are also studies[34–36] reporting that RT does not impair wound healing. Various outcomes have been reported in the studies investigating the effects of 5-FU on wound healing.[31,37–42] Nonetheless, 5-FU is the cytotoxic agent of choice in colorectal cancer CT and has the characteristics of existing on the peritoneal surface for long term and passing into the portal circulation in high amounts.[42] In the present study we used 5-FU together with preoperative RT.

Ceelen *et al*[43] investigated the effect of preoperative high dose RT on colon anastomosis healing.

Table 5 The severity of fibrosis in the groups

Severity of fibrosis	Group 1 Control	Group 2 Sham	Group 3 Sham+HBOT	Group 4 CRT	Group 5 CRT+HBOT
0 (minimal)	2	—	1	—	1
1 (mild)	7	4	3	1	2
2 (moderate)	3	7	6	8	7
3 (severe)	—	1	2	3	2

Data are presented as number.

CRT, chemoradiotherapy; HBOT, hyperbaric oxygen treatment.

They found no difference in postoperative morbidity between the groups receiving 40, 60, and 80 Gy of RT. They also reported significant weight loss in the RT group compared to the control group; however, the changes in BP were not statistically significant. El-Malt *et al*[44] determined significant weight loss in the rats that underwent colon anastomosis after preoperative CRT compared to the control and sham groups and suggested that weight loss was higher in the groups receiving higher doses of CRT. The investigators also observed that intra-abdominal adhesion scores were higher in the groups receiving higher doses of CRT and CT only, compared to the control group. No significant change was observed either at high or at low dose in the group receiving CT only, and as well as no significant difference was reported between the control group and the other groups in terms of anastomotic BP. Biert *et al*[40] investigated the effect of combined preoperative RT and direct postoperative CT on healing of early period of colonic anastomosis. They reported a significant weight loss in the groups receiving CT and a significant decrease in BP and tissue resistance in the CRT groups. In their experimental study, de Meerleer *et al*[45] administered high doses of preoperative unilateral and bilateral RT and found that weight loss and decrease in anastomotic BP were more frequent in the rats receiving RT compared to the control group. On the other hand, Seifert *et al*[46] gave high doses of intraoperative RT and determined a weight loss of approximately 9% on postoperative day 1. In the present study, when we compared the control groups with the sham groups, we determined that weight loss was present in all groups due to the surgical stress. However, it was more significant in the group received CRT, in which diarrhea and food intolerance was also observed. No weight loss was observed in the control group within the period from baseline to operation. There was a significant weight loss (8.9%) in the sham group within the period from baseline to operation, and it was 1.39% within the period from operation to relaparotomy. A significant weight loss (6.3%) was also observed in the sham+HBOT group within the period from baseline to operation. However, in the same group a significant weight gain, by 0.30%, was observed within the period from operation to relaparotomy. Among all groups, the highest weight loss was observed in the CRT group by 17.44% within the period from baseline to operation. In the CRT+HBOT group, significant weight loss was observed (12.9%) within the period from baseline to operation, as well as the highest weight gain was observed (5.83%) in the same group within the period from operation to relaparatomy compared to the other groups. Thus, CRT led to food intolerance, diarrhea, and remarkable weight loss in the rats. There was weight gain when HBOT was started.

The time for BP measurement is controversial in the studies performed on rats. Hendriks and Mastboom[4] reviewed many studies on this issue and concluded that the burst occurred outside of the anastomosis line during BP measurement performed on day 4 of anastomosis. In addition, other studies have suggested that burst occurring outside of the anastomosis line is frequent during BP measurements performed after postoperative day 5.[47–51] In the present study, BP was measured on the postoperative day 5. It was observed that burst occurred outside of the anastomosis line in 4 rats. BP values were lower in the sham and CRT groups compared to the control group. In addition, the mean BP was significantly lower in the CRT group than in the control group (100.38 mmHg, 132.58 mmHg, respectively; $P = 0.003$). Although it was not statistically significant, BP was higher in group 5 that received HBOT compared to group 4 ($P = 0.06$). Higher BP values in the groups receiving HBOT (groups 3 and 5) compared to their own control groups (groups 2 and 4) suggested that HBOT had a positive effect on tissue healing.

It has been shown that tissue healing is inversely affected by low oxygen levels.[9,25,52–54] HBOT shows its effect by a double mechanism—increasing antibacterial and tissue partial oxygen pressure in the tissues with impaired integrity. Adequate tissue oxygen pressure plays an important role in the collagen matrix production, which forms the basis of angiogenesis. Collagen matrix production and tissue oxygen pressure, which play a role in angiogenesis, are provided more effectively with HBOT compared with normobaric oxygen in the tissues exposed to radiation. Adequate tissue oxygenation enhances the efficacy of RT.[19,20] There have been studies on HBOT in the recent years. In these studies, it has been recommended that HBOT be given at least for 10 sessions, with each session lasting 90 minutes. A shorter duration of HBOT administration could not provide adequate tissue oxygen level and that longer applications might lead to unfavorable effects such as central nervous system and pulmonary toxicity.[52] In the present study, HBOT was given twice a day at 8-hour intervals, each session lasting 90 minutes. Studies investigating the effect of HBOT on colon anastomoses are limited.

Shandall *et al*[7] showed that perianastomotic oxygen tension was associated with dissociation energy, dissociation tension, and hydroxyproline content. Hamzaoğlu *et al*[9] suggested that postoperative HBOT increased colon anastomosis healing and repaired ischemic damage. In another study from our institute, Yagci *et al*[8] investigated the effect of HBOT on ischemic and normal colon anastomosis and reported that BP was significantly decreased in the rats that underwent ischemic anastomosis compared to the control rats that underwent normal anastomosis. In that particular study, the investigators also reported that adequate tissue oxygenation was the main factor in wound healing and that HBOT had positive effect on the biochemical and mechanical parameters. In the present study, we obtained a decrease in BP values in the CRT and sham groups compared to the control group. The mean BP value was 132.58 mmHg in the control group, whereas it was 110.67 mmHg in the sham group and 100.83 mmHg in the CRT group. The mean BP values were similar in the group that received HBOT with those in the control group. The mean BP value was 133.67 mmHg in the sham+HBOT group and 123.42 mmHg in the CRT+HBOT group. The BP value was significantly lower in the CRT group compared to the control group ($P = 0.003$). Although it was not statistically significant, the BP value was higher in group 5 receiving HBOT compared to that in group 4 ($P = 0.06$).

Analyzing collagen level is one of the methods that is extensively used to assess wound and anastomosis healing. Almost all of the hydroxyproline is found in collagen in vertebrates. Thus, hydroxyproline is a favorable indicator to assess collagen metabolism and collagen level.[4,5] In the present study we used tissue hydroxyproline level to indicate the collagen level in the healing wound. Biert *et al*[40] determined a significant increase in tissue hydroxyproline level in the therapy groups compared to the control groups. In another study by Biert *et al*,[55] a decrease in the BP values was reported on postoperative day 3. They detected that the burst usually occurred in the suture line, but outside of the suture line after postoperative day 7, and reported that hydroxyproline level was significantly increased on postoperative day 7, whereas no significant increase was observed on postoperative day 3. In another study,[46] it was reported that hydroxyproline level increased between months 6 and 18 due to collagen deposition in the anastomosis site in the groups receiving RT; however, similar increase was also observed in the control group. In the present study, hydroxyproline level was significantly higher in the CRT group compared to the control group (12.05 versus 24.28 μM/mg tissue; $P = 0.001$). Hydroxyproline level was 19.43 μM/mg tissue in the group receiving CRT+HBOT. Although it was not statistically significant, decrease in the hydroxyproline level values in the CRT+HBOT group compared to those of the CRT group suggested that HBOT positively affected tissue healing. Kuzu *et al*[56] investigated histologic changes in anastomosis healing after CT and RT and found an increase in the necrosis and inflammatory exudate formation in the therapy groups. They showed that delay in granulation tissue formation and fibroblast proliferation significantly influenced the therapy groups. Rocha *et al*[57] showed that hyperbaric oxygen therapy did not alter the mechanical resistance of anastomosis in distal colon of rats in the presence of peritonitis. Murray[33] found that impairment due to an increase in the collagen and protein synthesis in the early postoperative colon surgery after radiation returned to the normal levels on the fourth month. The investigator also reported that stricture occurred in the collagen 1 year later and functional changes occurred, and that a decrease was observed in the compliance of intestinal wall due to the radiation. Seifert *et al*[46] detected an increase in fibrosis with RT dose. In the present study, histopathologic examination revealed higher amounts of fibrosis in the CRT group compared to that in the control group ($P = 0.008$). It was detected that HBOT decreased the fibrosis formation; however, the difference was not statistically significant.

In conclusion, our results suggest that preoperative CRT has unfavorable effects of colon anastomosis healing. Such unfavorable effects are decreased with HBOT, which influences biochemical and mechanical parameters of anastomosis healing positively.

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References

1. Wolpin BM, Meyerhardt JA, Mamon HJ, Mayer RJ. Adjuvant treatment of colorectal cancer. *CA Cancer J Clin* 2007;**57**:168–185

2. Andre N, Schmiegel W. Chemoradiotherapy for colorectal cancer. *Gut* 2005;**54**:1194–1202
3. Kingham TP, Pachter HL. Colonic anastomotic leak: risk factors, diagnosis, and treatment. *J Am Coll Surg* 2009;**208**:269–278
4. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Parameters for repair. *Dis Colon Rectum* 1990;**33**: 891–901
5. Koruda MJ, Rolandelli RH. Experimental studies on the healing of colonic anastomoses. *J Surg Res* 1990;**48**:504–515
6. Petersen S, Freitag M, Hellmich G, Ludwig K. Anastomotic leakage: impact on local recurrence and survival in surgery of colorectal cancer. *Int J Colorectal Dis* 1998;**13**:160–163
7. Shandall A, Lowndes R, Young HL. Colonic anastomotic healing and oxygen tension. *Br J Surg* 1985;**72**:606–609
8. Yagci G, Ozturk E, Ozgurtas T, Gorgulu S, Kutlu OC, Topal T *et al*. Preoperative and postoperative administration of hyperbaric oxygen improves biochemical and mechanical parameters on ischemic and normal colonic anastomoses. *J Invest Surg* 2006;**19**:237–244
9. Hamzaoğlu I, Karahasanoğlu T, Aydin S, Sahin DA, Carkman S, Sariyar M *et al*. The effects of hyperbaric oxygen on normal and ischemic colon anastomoses. *Am J Surg* 1998;**176**:458–461
10. Ptok H, Marusch F, Meyer F, Schubert D, Gastinger I, Lippert H. Impact of anastomotic leakage on oncological outcome after rectal cancer resection. *Br J Surg* 2007;**94**:1548–1554
11. Khan AA, Wheeler JM, Cunningham C, George B, Kettlewell M, Mortensen NJ. The management and outcome of anastomotic leaks in colorectal surgery. *Colorectal Dis* 2008;**10**:587–592
12. Blouhos K, Pramateftakis MG, Tsachalis T, Kanellos D, Zaraboukas T, Koliakos G *et al*. Healing of colonic anastomoses after immediate postoperative intraperitoneal administration of oxaliplatin. *Int J Colorectal Dis* 2008;**23**:1185–1191
13. Fang CB, Klug WA, Capelhuchnik P. Preoperative cobalt60 irradiation delays the healing of rectal anastomoses in rats. *Braz J Med Biol Res* 2005;**38**:895–899
14. Zacharakis E, Demetriades H, Pramateftakis MG, Lambrou I, Zacharakis E, Zaraboukas T *et al*. Effect of IGF-I on healing of colonic anastomoses in rats under 5-FU treatment. *J Surg Res* 2008;**144**:138–144
15. Duraker N, Bender O, Memişoğlu K, Yalçiner A. Intraoperative bowel irrigation improves anastomotic collagen metabolism in the left-sided colonic obstruction but not covering colostomy. *Int J Colorectal Dis* 1998;**13**:232–234
16. Percarpio B, Bitterman J, Sabbath K, Alfano F, Ruszkowski R, Bowen J. Combined modality preoperative therapy for unresectable rectal cancer. *Conn Med* 1992;**56**:7–9
17. Sugarbaker PH, Cunliffe WJ, Belliveau J, de Bruijn EA, Graves T, Mullins RE *et al*. Rationale for integrating early postoperative intraperitoneal chemotherapy into the surgical treatment of gastrointestinal cancer. *Semin Oncol* 1989;**16**:83–97
18. Marks G, Mohiuddin M, Goldstein SD. Sphincter preservation for cancer of the distal rectum using high dose preoperative radiation. *Int J Radiat Oncol Biol Phys* 1988;**15**:1065–1068
19. Marsh PJ, James RD, Schofield PF. Adjuvant preoperative radiotherapy for locally advanced rectal carcinoma. Results of a prospective, randomized trial. *Dis Colon Rectum* 1994;**37**: 1205–1214
20. Minsky BD, Cohen AM, Enker WE, Sigurdson E. Phase I/II trial of pre-operative radiation therapy and coloanal anastomosis in distal invasive resectable rectal cancer. *Int J Radiat Oncol Biol Phys* 1992;**23**:387–392
21. Minsky BD, Cohen AM, Enker WE, Paty P. Sphincter preservation with preoperative radiation therapy and coloanal anastomosis. *Int J Radiat Oncol Biol Phys* 1995;**31**:553–559
22. Rouanet P, Fabre JM, Dubois JB, Dravet F, Saint Aubert B, Pradel J, *et al*. Conservative surgery for low rectal carcinoma after high-dose radiation. Functional and oncologic results. *Ann Surg* 1995;**221**:67–73
23. Påhlman L, Glimelius B. Pre- or postoperative radiotherapy in rectal and rectosigmoid carcinoma. Report from a randomized multicenter trial. *Ann Surg* 1990;**211**:187–195
24. Mohiuddin M, Ahmad N, Marks G. A selective approach to adjunctive therapy for cancer of the rectum. *Int J Radiat Oncol Biol Phys* 1993;**27**:765–772
25. Thornton FJ, Barbul A. Healing in the gastrointestinal tract. *Surg Clin North Am* 1997;**77**:549–573
26. Reis Neto JA, Quilici FA, Reis JA Jr. A comparison of nonoperative vs. preoperative radiotherapy in rectal carcinoma. A 10-year randomized trial. *Dis Colon Rectum* 1989;**32**:702–710
27. Chari RS, Tyler DS, Anscher MS, Russell L, Clary BM, Hathorn J *et al*. Preoperative radiation and chemotherapy in the treatment of adenocarcinoma of the rectum. *Ann Surg* 1995; **221**:778–787
28. Grann A, Minsky BD, Cohen AM, Saltz L, Guillem JG, Paty PB *et al*. Preliminary results of preoperative 5-fluorouracil, low-dose leucovorin, and concurrent radiation therapy for clinically resectable T3 rectal cancer. *Dis Colon Rectum* 1997; **40**:515–522
29. Rich TA, Skibber JM, Ajani JA, Buchholz DJ, Cleary KR, Dubrow RA *et al*. Preoperative infusional chemoradiation therapy for stage T3 rectal cancer. *Int J Radiat Oncol Biol Phys* 1995;**32**:1025–1029
30. Stryker SJ, Kiel KD, Rademaker A, Shaw JM, Ujiki GT, Poticha SM. Preoperative "chemoradiation" for stages II and III rectal carcinoma. *Arch Surg* 1996;**131**:514–519
31. El-Malt M, Ceelen W, van den Broecke C, Cuvelier C, Van Belle S, De Neve W *et al*. Healing of experimental colonic anastomoses: effects of combined preoperative high-dose radiotherapy and intraperitoneal 5-fluorouracil. *Int J Cancer* 2001;**96**:297–304
32. Morgenstern L, Sanders G, Wahlstrom E, Yadegar J, Amodeo P. Effect of preoperative irradiation on healing of low colorectal anastomoses. *Am J Surg* 1984;**147**:246–249
33. Murray JC. Radiation-induced fibrosis: the structure/function relationship. *Scanning Microsc*. 1994;**8**:79–87

34. Weiber S, Jiborn H, Zederfeldt B. Preoperative irradiation and colonic healing. *Eur J Surg* 1994;**160**:47–51
35. Biert J, Hoogenhout J, Wobbes T, Hendriks T. High-dose preoperative irradiation without detrimental effect on early repair of anastomoses in the colon of the rat. *Radiat Res* 1997; **147**:362–368
36. Karliczek A, Zeebregts CJ, Benaron DA, Coppes RP, Wiggers T, van Dam GM. Preoperative irradiation with 5×5 Gy in a murine isolated colon loop model does not cause anastomotic weakening after colon resection. *Int J Colorectal Dis* 2008;**23**: 1115–1124
37. Ersoy E, Akbulut H, Moray G. Effects of oxaliplatin and 5-fluorouracil on the healing of colon anastomoses. *Surg Today* 2009;**39**:38–43
38. Ozel L, Ozel MS, Toros AB, Kara M, Ozkan KS, Tellioglu G. Effect of early preoperative 5-fluorouracil on the integrity of colonic anastomoses in rats. *World J Gastroenterol* 2009;**15**:4156–4162
39. Kuzu MA, Köksoy C, Kale T, Demirpençe E, Renda N. Experimental study of the effect of preoperative 5-fluorouracil on the integrity of colonic anastomoses. *Br J Surg* 1998;**85**:236–239
40. Biert J, Wobbes T, Hoogenhout J, de Man B, Hendriks T. Combined preoperative irradiation and direct postoperative 5-fluorouracil without negative effects on early anastomotic healing in the rat colon. *Radiother Oncol* 1996;**41**:257–262
41. Janjan NA, Crane CN, Feig BW, Cleary K, Dubrow R, Curley SA *et al*. Prospective trial of preoperative concomitant boost radiotherapy with continuous infusion 5-fluorouracil for locally advanced rectal cancer. *Int J Radiat Oncol Biol* Phys 2000;**47**:713–718
42. de Waard JW, Wobbes T, Hendriks T. Early post-operative 5-fluorouracil does not affect the healing of experimental intestinal anastomoses. *Int J Colorectal Dis* 1993;**8**:175–178
43. Ceelen W, El Malt M, Cardon A, Berrevoet F, De Neve W, Pattyn P. Influence of preoperative high-dose radiotherapy on postoperative outcome and colonic anastomotic healing: experimental study in the rat. *Dis Colon Rectum* 2001;**44**:717–721
44. El-Malt M, Ceelen W, de Meerleer G, Verstraete A, Boterberg T, van Belle S *et al*. Influence of preoperative combined radiochemotherapy on surgical outcome and colonic anastomotic healing: experimental study in the rat. *Int J Radiat Oncol Biol Phys* 2001;**50**:1073–1078
45. De Meerleer G, Pattyn P, Fortan L, de Wever N, Cuvelier C, van Renterghem K *et al*. High-dose preoperative radiotherapy does not alter the strength of unilaterally irradiated colon anastomoses in rats. *Int J Radiat Oncol Biol Phys* 1999;**44**:163–170
46. Seifert WF, Biert J, Wobbes T, Verhofstad AA, Hoogenhout J, Hendriks T. Late effects of intraoperative radiation therapy in anastomotic rat colon. *Int J Radiat Oncol Biol Phys* 1998;**42**:623–629
47. Hesp FL, Hendriks T, Lubbers EJ, deBoer HH. Wound healing in the intestinal wall. A comparison between experimental ileal and colonic anastomoses. *Dis Colon Rectum* 1984;**27**:99–104
48. Kiyama T, Onda M, Tokunaga A, Efron DT, Barbul A. Effect of matrix metalloproteinase inhibition on colonic anastomotic healing in rats. *J Gastrointest Surg* 2001;**5**:303–311
49. Kuzu MA, Tanik A, Kale IT, Aşlar AK, Köksoy C, Terzi C. Effect of ischemia/reperfusion as a systemic phenomenon on anastomotic healing in the left colon. *World J Surg* 2000;**24**:990–994.
50. Månsson P, Zhang XW, Jeppsson B, Thorlacius H. Anastomotic healing in the rat colon: comparison between a radiological method, breaking strength and bursting pressure. *Int J Colorectal Dis* 2002;**17**:420–425
51. van Geldere D, Fa-Si-Oen P, Noach LA, Rietra PJ, Peterse JL, Boom RP. Complications after colorectal surgery without mechanical bowel preparation. *J Am Coll Surg* 2002;**194**:40–47
52. Grim PS, Gottlieb LJ, Boddie A, Batson E. Hyperbaric oxygen therapy. *JAMA* 1990;**263**:2216–2220
53. Khoury GA, Waxman BP. Large bowel anastomoses. I. The healing process and sutured anastomoses. A review. *Br J Surg* 1983;**70**:61–63
54. Witte MB, Barbul A. General principles of wound healing. *Surg Clin North Am* 1997;**77**:509–528
55. Biert J, Seifert W, de Man B, Wobbes T, Hoogenhout J, Hendriks T. Combined preoperative irradiation and local hyperthermia delays early healing of experimental colonic anastomoses. *Arch Surg* 1996;**131**:1037–1042
56. Kuzu MA, Kuzu I, Köksoy C, Akyol FH, Uzal D, Kale IT *et al*. Histological evaluation of colonic anastomotic healing in the rat following preoperative 5-fluorouracil, fractionated irradiation, and combined treatment. *Int J Colorectal Dis* 1998;**13**:235–240
57. Rocha AA, Leal RF, de Ayrizono ML, Chung WF, Coy CS, Lee HD *et al*. Hyperbaric oxygen therapy and mechanical resistence of the colonics anastomosis in rats with peritonitis. *Acta Cir Bras* 2010;**25**:368–374