Effect of hyperbaric oxygen on anastomoses created under the influence of 5-FU.

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Erenoğlu C, Uluutku H, Emeksiz S, Akin ML, Foley E, Çelenk T, Effect of hyperbaric oxygen therapy on anastomoses created under the influence of 5-FU. Undersea Hyperb Med 2003, 30(4): 321-326. Aim: This study investigates the effects of hyperbaric oxygen (HBO_2) therapy on the healing capacity of colonic anastomoses under the influence of preoperative chemotherapy. Material and Method: Forty male Wistar-Albino rats were divided into four groups of 10. Colonic resection and anastomosis were performed in each group. Group I (control) received no further treatment. In group II, 5-fluorouracil was administered intraperitoneally for five consecutive days preoperatively. Group III received HBO_2 therapy for seven days after the anastomosis. Group IV received HBO_2 therapy following the administration of preoperative 5-fluorouracil. On the seventh postoperative day, all the rats were examined to determine the bursting pressures of the anastomosis and to take tissue sample from the anastomotic line for hydroxyproline measurement. Results: Bursting pressures of the anastomosis in group IV were increased significantly compared to group II. Hydroxyproline levels were significantly increased with the use of HBO_2 in rats, independent of chemotherapy administration. Conclusion: HBO_2 therapy strengthens anastomoses created under the influence of neoadjuvant chemotherapy. This technique might have a future role in the care of colon cancer patients undergoing new multimodality cancer treatments.

Anastomosis; neoadjuvant chemotherapy; hyperbaric oxygen; experimental.

INTRODUCTION

Anastomotic disruption continues to be one of the most feared complications of colorectal surgery. A number of factors have been described as causes of anastomotic leakage, including ischemia, immunosuppressants, anastomotic tension, malnutrition, and surgical technique (1,2,3). The growing use of adjuvant and neoadjuvant treatments in patients undergoing colectomy for cancer has also raised concerns about the negative impact of perioperative chemotherapy on anastomotic healing. Although the results are variable, investigations suggest that perioperatively administered 5-fluorouracil (5-FU)-based chemotherapy may reduce anastomotic strength and thereby increase the risk of anastomotic failure (4,5,6,7,8,9).

On the other hand, there has been growing interest in the use of hyperbaric oxygen (HBO_2) therapy to improve the healing of difficult wounds in a wide variety of clinical situations (10,11). There is evidence that HBO_2 may improve anastomotic wound healing in laboratory animals undergoing colonic resection (12). The impact of HBO_2 therapy on colonic anastomoses altered by the preoperative administration of 5-FU chemotherapy has not been reported. Due to the increasing number of colorectal anastomoses being performed with concomitant 5-FU

chemotherapy, we designed this study to assess the ability of HBO_2 to improve anastomotic integrity in this common and potentially high risk setting.

MATERIALS AND METHODS

This study was performed under the approval of the GATA Haydarpasa Training Hospital Committee of Animal Ethics. Forty male Wistar-Albino rats weighing 200-250g were used and divided into four groups of ten rats each. All animals were fed standard laboratory diet and water (drinking bottle). Following intraperitoneal injection of 1 cc saline solution for five consecutive days preoperatively, group I (control) underwent a 1 cm of distal colonic resection and anastomosis approximately 5 cm proximal to the peritoneal reflection. Group II received 5-FU (20mg/kg) intraperitoneally on five consecutive preoperative days, followed by identical colonic resection. Group III had preoperative saline injection for five consecutive days, underwent colonic resection, received no chemotherapy, and postoperatively completed HBO_2 therapy twice a day for seven postoperative days. Finally, Group IV received the preoperative chemotherapy followed by colonic resection and postoperative HBO_2 therapy.

On the seventh postoperative day, all the rats within the groups were operated again in order to evaluate the bursting pressure of the anastomotic line and to take tissue samples through the anastomoses to measure the hydroxyproline levels of the perianastomotic tissue.

Operative Procedure

A 3-cm midline incision was performed following the administration of anesthesia with an intramuscular injection of ketamine hydrochloride (Ketalar®, Eczacibasi, Turkey) at a dose of 40 mg/kg. A 1 cm segment of distal colon was resected 5 cm above the peritoneal reflection. Colonic continuity was restored by a 1-layer interrupted and inverting end-to-end anastomosis using 6-0 Prolene® (Ethicon) sutures. Abdominal fascia and skin were closed with 4-0 Vicryl® (Ethicon) sutures.

Hyperbaric Oxygen Therapy

Following surgery rats in groups III and IV were immediately taken to an oxygen chamber that was designed to provide 2 ATA (atmosphere absolute) twice daily for seven days. Each supervised treatment of hyperbaric oxygen session lasted 90 minutes.

Bursting Pressure

All the rats were operated on the seventh postoperative day in order to measure *in vivo* bursting pressure of the anastomosis. A catheter that had both an infusion port and a manometer was placed intraluminally 2 cm proximal to the anastomosis without disrupting pericolonic adhesions, and the colon was ligated proximally and distally to isolate a 4-cm perianastomotic segment. The infusion port was started at a continuous rate of 6 ml/min. until leakage of liquid at the anastomosis, or the sudden loss of pressure in manometer indicating rupture was observed by visual inspection. The pressure recorded at these end points was considered the bursting pressure of the anastomosis.

Measurement of Hydroxyproline Levels

Following the bursting pressure measurement, 1 cm of perianastomotic colonic tissue was harvested and immersed in $-30^{o}C$ liquid nitrogen for the hydroxyproline content. Tissue

hydroxyproline levels were determined as described by Switzer which was based on the oxidation of the imino acid to pyrrole 2-carboxylic acid which was converted subsequently to pyrrole with heat (13). Hydroxyproline levels were recorded as microgram hydroxyproline per gram of tissue harvested.

Tissue hydrolysis; Following placement of 25-350 mg wet weight of tissue in dry culture tubes, the samples were dried in an oven at 65°C for 18-24 hours and then the tubes were weighed. By adding 2.0 ml of 6 N HCl, the samples were hydrolyzed at 110°C for 24 hr and then the samples were evaporated to dryness with a stream of nitrogen. 10.0ml of deionized water was added into each tube and mixed well.

Hydroxyproline determination; (a) 0.2 ml of hydrozylate was transferred to a clean, labeled culture tube followed by 1.6 ml deionized water. (b) A set of tubes were prepared containing known amounts of hydroxyproline (1.0-8.0 μg) and water as reagent blank. (c) One ml of 1:5 diluted borate buffer was added to all tubes. (d) Chloramine T of 0.3 ml was added to each tube in a timed sequence to oxidize the hydroxyproline and each tube was mixed well. (e) In order to saturate all tubes, 1.5 g potassium chloride was added. (f) The tubes were then capped and heated in boiling water for 20 minutes. (g) The tubes were cooled to room temperature and added 2.5 ml toluene, and were capped tightly in order to invert the tubes 100 times or shake them about 5 minutes. (h) The tubes were centrifuged at low speed briefly and 1.0 ml toluene extract was transfered to labeled test tubes. (i) 0.4 ml of Ehrlich's reagent was added and the color was allowed to develop by 30 minutes. (j) Finally the absorbances were read at 565 nm against a reagent blank.

Statistical Analysis

Analysis of variance (ANOVA) followed by Tukey post hoc test was used under the SPSS software program for statistical evaluation. *p* values smaller than 0.05 were considered significant.

RESULTS

All rats survived the study. The mean bursting pressures were (mean ± standard deviation) 190.2 ± 18.14 mmHg in Group I, 201 ± 62.10 mmHg in Group II, 221 ± 6.05 mmHg in Group III and 253.4 ± 44.92 mmHg in Group IV (figure 1). HBO_2 therapy increased the bursting pressures of the anastomosis significantly in rats undergoing preoperative chemotherapy (Groups IV vs. II, $p<0.05$). Although there was a similar trend in rats not receiving chemotherapy, this was not statistically significant (Groups III vs. I, $p>0.05$). The mean hydroxyproline levels were 120.20 ± 13.14 μg/g tissue in Group I, 90.09 ± 20.43 μg/g tissue in Group II, 228.76 ± 23.75 μg/g tissue in Group III and 159.64 ± 44.07 μg/g tissue in Group IV. The hydroxyproline levels were significantly increased with the use of HBO_2 in rats both receiving and not receiving chemotherapy (Groups III vs. I, Groups III vs. II and Groups III vs. IV, $p<0.05$, $p<0.05$, $p<0.05$) (figure 2).

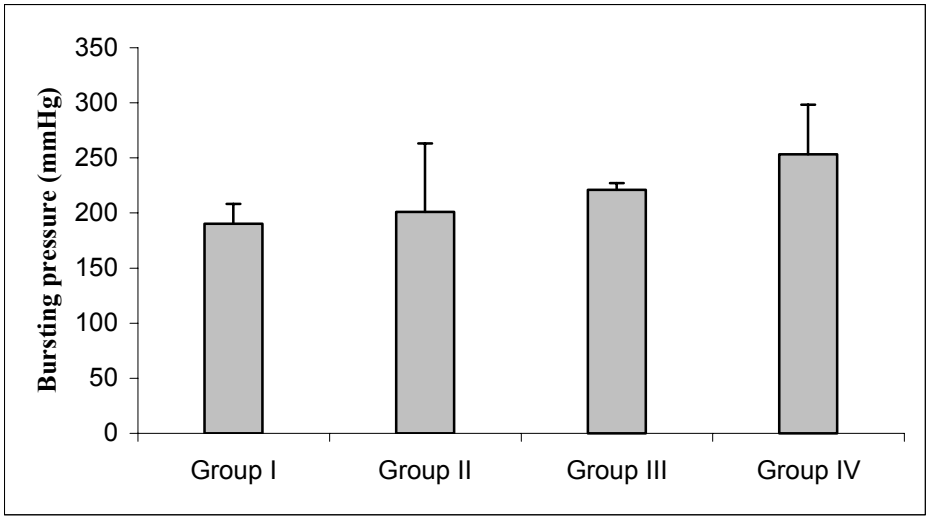

Figure 1. Anastomotic Bursting Pressures (mmHg± S.D): Bursting pressure of Group IV (5-FU + HBO_2) was significantly higher on postoperative day seven compared to Group II (5-FU) (p<0.05).

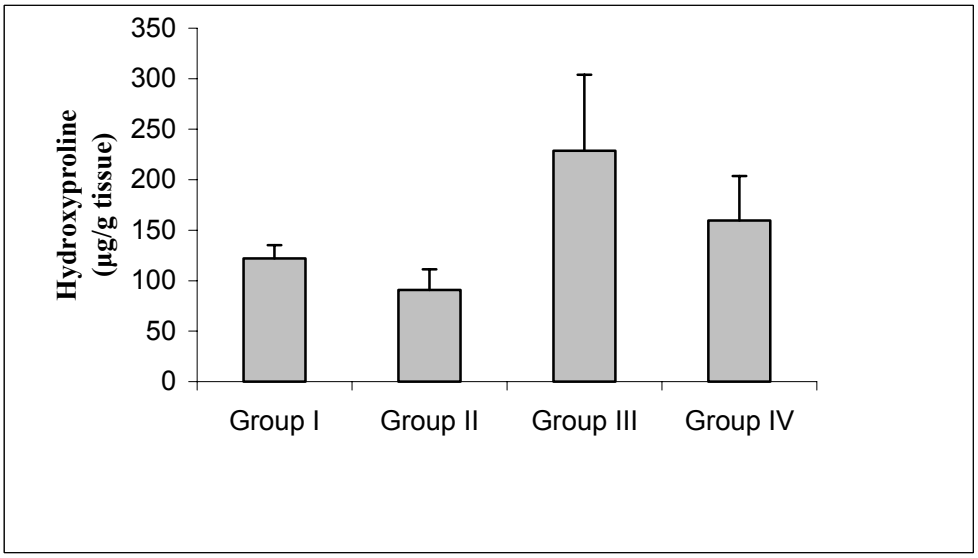

Figure 2. Perianastomotic Hydroxyproline Values (µg/g tissue ± S.D): Hydroxyproline values were significantly higher in the groups treated with HBO_2 (Group I versus III and Group II versus IV) (p<0.05).

DISCUSSION

Anastomotic failure continues to be a major source of morbidity and mortality following colon resection for cancer even though substantial clinical and laboratory investigations have been undertaken to reduce the risk of anastomotic disruption (3). Growing enthusiasm for the use of preoperative systemic chemotherapy in patients with colon cancer has raised some concern about the possibility of increasing morbidity and even mortality associated with the neoadjuvant

approach. This project was undertaken to investigate the efficiency of postoperative HBO_2 therapy in reducing the potentially increased risk of anastomotic complications in patients receiving preoperative systemic chemotherapy. Our data suggests that, based on anastomotic bursting pressures and hydroxyproline levels, HBO_2 may strengthen colonic anastomoses performed after the administration of systemic 5-FU.

Various methods have been described to evaluate anastomotic healing, including bursting pressures, hydroxyproline levels, and histopathologic evaluation (6,14). We adopted the most commonly used and accepted experimental techniques of bursting pressure and hydroxyproline level in this study and performed them using established methods.

It has been well documented that HBO_2 therapy improves wound healing in a variety of difficult clinical situations including necrotizing fasciitis, refractory osteomyelitis, diabetic leg ulcers and compromised skin grafts and flaps (10). In contrast, very few studies are avaliable concerning the effects of HBO_2 therapy on intestinal anastomosis. In a recent study, Hamzaoglu *et al.* described an improvement in the healing capacity of ischemic colon anastomosis in a rat model with HBO_2 therapy in terms of bursting pressures (12). The other study investigating the effects of HBO_2 therapy on anastomosis of crushed microvasculature also claimed beneficial results of HBO_2 in rats (15). Although the exact mechanism of HBO_2 remains unclear, it has been presumed that HBO_2 might be effective in colonic anastomotic healing by both stimulation of angiogenesis and inhibition of bacterial overgrowth (12). Oxygenation of the tissues is essential in the healing process of wounds for angiogenesis and formation of collagen matrix (10,16,17). Moreover there is evidence that HBO_2 may also have antibacterial effect that contributes healing of anastomosis. It has been shown that HBO_2 alone has bactericidial effect on certain pathogenic bacteria, including *Clostridium perfringens*, and bacteriostatic effects on certain species of *Escherichia* and *Pseudomonas* (18,19,20). In addition, HBO_2 facilitates the free radical formation that is normally inhibited during local hypoxia, thereby increasing neutrophil-mediated bacterial killing (21).

HBO_2 therapy is safe when conducted under standard protocols. However, it has a disadvantage of postoperative critical care of a patient who had a major abdominal procedure due to colon cancer. Additionally, HBO_2 therapy itself has some adverse effects secondary to barotrauma such as reversible myopia, perforation of the tympanic membrane, and pneumothorax even though these complications are very rare (10). Although application of HBO_2 therapy in a clinical situation has some difficulties including cost, frequency of application, and difficulty of maintaining medical support, a well-designed and profesionally-operated HBO_2 center operating 24 hours and providing postoperative needs of the patients along with intensive care facilities could be a solution.

The last decade has seen the widespread acceptance of the efficacy of postoperative systemic chemotherapy for colon cancer. Since a significant number of patients with colon cancer will die of recurrent disease despite this multidisciplinary approach, novel methods of combined cancer treatment will be necessary to achieve better and more acceptable oncologic results in this patient population. There has not been widespread use of perioperative or neoadjuvant chemotherapy in colon cancer because of the concerns of increased operative morbidity due in part to anastomotic leakage even though some important benefits of neoadjuvant or perioperative chemotherapy in the treatment of other cancers have been observed (22,23).

Our data suggest that HBO_2 therapy might reduce the negative impact of preoperative 5-FU administration on anastomotic healing, eliminating this concern as a barrier to the

development of combined treatments for advanced colon cancer in the near future. Consequently, HBO_2 might be an adjunct to the neoadjuvant chemotherapy and curative resection and anastomosis in colorectal cancer following further experimental and clinical studies.

REFERENCES

1. Schrock TR, Deveney CW, Dunphy JE.Factor contributing to leakage of colonic anastomoses. *Ann Surg* 1973;177:513-518.
2. Fielding LP, Stewart-Brown S, Blesovsky L, Kearney G. Anastomotic integrity after operations for large-bowel cancer: a multicentre study. *Br Med J* 1980; 281: 411-414.
3. Koruda MJ, Rolandelli RH. Experimental studies on the healing of colonic anastomoses. *J Surg Res* 1990; 48: 504-515.
4. Hananel N, Gordon PH. Effect of 5-fluorouracil and leucovorin on the integrity of colonic anastomoses in the rat. *Dis Colon Rectum* 1995; 38: 886-890.
5. Kuzu MA, Koksoy C, Kale T, Demirpence 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.
6. Kuzu MA, Kuzu I, Koksoy C, 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.
7. Fukuchi SG, Seeburger JL, Parquet G, Rolandelli RH. Influence of 5-fluorouracil on colonic healing and expression of transforming growth factor-beta 1. *J Surg Res* 1999 15; 84: 121-126.
8. Yazdi GP, Miedema BW, Humphrey L. Immediate postoperative 5-FU does not decrease colonic anastomotic strength. *J Surg Oncol* 1998; 69: 125-127.
9. Van der Kolk BM, de Man BM, Wobbes T, Hendriks T. Is early post-operative treatment with 5-fluorouracil possible without affecting anastomotic strength in the intestine? *Br J Cancer*. 1999 ; 79: 545-550.
10. Tibbles Pm, Edelsberg JS. Hyperbaric-oxygen therapy. *N Engl J Med* 1996; 334: 1642-1648.
11. Riseman JA, Zamboni WA, Curtis A, Graham DR, Konrad HR, Ross DS. Hyperbaric oxygen therapy for necrotizing fasciitis reduces mortality and the need for debridements. *Surgery* 1990 ;108:847-850.
12. Hamzaoglu I, Karahasanoglu T, Aydin S, et al. The effects of hyperbaric oxygen on normal and ischemic colon anastomoses. *Am J Surg* 1998 ; 176: 458-461.
13. Switzer BR. Determination of hydroxyproline in tissue. *J Nutr Biochem* 1991; 2: 229-231.
14. Hendriks T, Mastboom WJ. Healing of experimental intestinal anastomoses. Parameters for repair. *Dis Colon Rectum* 1990; 33: 891-901.
15. Shi DY, Zhang F, Kryger Z, Komorowska-Timek E, Lineaweaver WC, Buncke HJ. Effect of hyperbaric oxygen on microvascular anastomosis healing and patency in the rat. *J Reconstr Microsurg* 1999;15: 539-545.
16. Prockop DJ, Kivirikko KI, Tuderman L, Guzman NA. The biosynthesis of collagen and its disorders *N Engl J Med* 1979; 301: 13-23,77-85.
17. Marx RE, Ehler WJ, Tayapongsak P, Pierce LW.Relationship of oxygen dose to angiogenesis induction in irradiated tissue.*Am J Surg* 1990;160: 519-524.
18. Hill GB, Osterhout S. Experimental effects of hyperbaric oxgen on selected clostridial species. I. In-vitro studies.*J Infect Dis* 1972 ;125: 17-25.
19. Brown OR. Reversible inhibition of respiration of Escherichia coli by hyperoxia. *Microbios* 1972; 5: 7-16.
20. Park MK, Muhvich KH, Myers RAM, Marzella L. Hyperoxia prolongs the aminoglycoside-induced postantibiotic effect in Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 1991; 35: 691-695.
21. Knighton DR, Halliday B, Hunt TK. Oxygen as an antibiotic. A comparison of the effects of inspired oxygen concentration and antibiotic administration on in vivo bacterial clearance.*Arch Surg* 1986;121:191-195.
22. Sapunar F, Smith IE. Neoadjuvant chemotherapy for breast cancer. *Ann Med* 2000 ;32: 43-50.
23. Lowy AM, Leach SD. Adjuvant/neoadjuvant chemoradiation for gastric and pancreatic cancer.*Oncology* 1999;13(10 Suppl 5): 121-130.