Does hyperbaric oxygen therapy reduce the effects of ischemia on colonic anastomosis in laparoscopic colon resection?

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Does hyperbaric oxygen therapy reduce the effects of ischemia on colonic anastomosis in laparoscopic colon resection?

BACKGROUND: *An increase in intra-abdominal pressure causes a decrease in the splanchnic blood flow and the intramucosal pH of the bowel, as well as increasing the risk of ischemia in the colon. The aim of the present study is to evaluate the effect of hyperbaric oxygen therapy (HBOT) on the ischemia caused by laparoscopy in colonic anastomosis in an experimental model of laparoscopic colonic surgery.*
MATERIALS AND METHODS: *We divided 30 male Wistar albino rats into three groups: Group A was the control (open colon anastomosis); Group B received LCA (laparoscopic colon anastomosis); while Group C received both LCA and HBOT. Each group contained ten animals. We placed Group C (LCA and HBOT) in an experimental hyperbaric chamber into which we administered pure oxygen at 2.1 atmospheres absolute 100% oxygen for 60 min for ten consecutive days.*
RESULTS: *The anastomotic bursting pressure value was found to be higher in the open surgery group (226 ± 8.8) (Group A). The result for Group C (213 ± 27), which received HBOT, was better than that for Group B (197 ± 27). However, there was no statistically significant difference between Group B and Group C. Group A showed better healing than the other groups, while significant differences in the fibroblast proliferation scores were found between Groups A and B. In terms of tissue hydroxyproline levels, a significant difference was found between Groups A and B and between Groups A and C, but not between Groups B and C.*
CONCLUSIONS: *HBOT increases the oxygen level in the injured tissue. Although HBOT might offer several advantages, it had only a limited effect on the healing of colonic anastomosis in rats with increased intra-abdominal pressure in our study.*

KEY WORDS: Anastomosis, Colon, Hyperbaric Oxygen Treatment, Oxidative Stress

Introduction

Laparoscopy was introduced into colorectal surgery during the 1990s, with Jacobs et al.[1] reporting the first series of laparoscopic colonic resection in 1991. The technique was not widely accepted in the early years due to a combination of insufficient data, technical difficulties, and the detection of tumor dissemination in some cases. Recently, it has been concluded that there is no significant difference between laparoscopy and open surgery with regard to tumor recurrences, distant metastasis, and disease-free survival. This is mainly due to the more common application of laparoscopy and increased experience with large series [2-4]. Anastomotic leakage is a major complication of gastrointestinal surgery, which increases mortality and morbidity, as well as length of hospital stay and costs. The risk has been reported to be 1.8-

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5% in different series [5]. The intra-abdominal pressure (IAP) increases during laparoscopy due to intraperitoneal gas insufflation. Although laparoscopy is more commonly used today, its effect on both healing and the continuity of the colocolic anastomosis has only been evaluated in a few studies. The increase in IAP causes a decrease in the splanchnic blood flow and the intramucosal pH of the bowel, as well as increasing the risk of ischemia in the colon. The ischemia interrupts the healing process of the anastomosis and can result in anastomotic leakage because of bacterial translocation and defective colocolic anastomosis [6]. Hyperbaric oxygen therapy (HBOT) can reverse the negative effects of tissue hypoxia by inducing hyperoxia under high pressures that have beneficial effects on wound healing [7]. The aim of this study is to evaluate the effect of HBOT on the ischemia caused by laparoscopy in colonic anastomosis in an experimental model of laparoscopic colonic surgery.

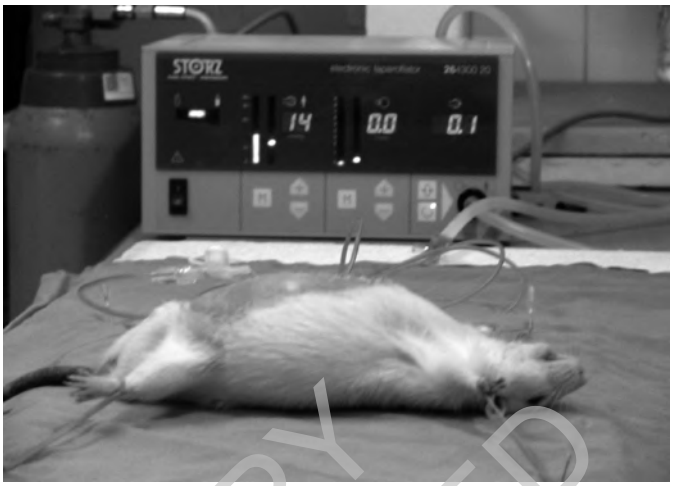

Fig. 1: CO_2 insufflation and creation of 14 mm Hg pressure pneumoperitoneum on rats.

Materials and Methods

The study protocol was designed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. All procedures were reviewed and approved by the Ethical Committee for Institutional Animal Care and Usage of Namik Kemal University. All of the procedures and experiments were performed in the Experimental Research Laboratory at Haydarpasa Education and Research Hospital. Thirty male Wistar albino rats (250 to 325 g) were used in the present study. The rats were housed in a quiet environment for one week prior to the study, with free access to standard laboratory chow and water ad libitum. The rats were maintained on a 12/12 h light/dark cycle.

Experimental design and surgery

The rats were randomized into three groups, with each containing ten animals. Each rat was allocated a code number at the beginning of the study, which was then used to refer to that animal throughout the study. The code number did not identify the particular group to which the animal belonged. The rats were fasted for 24 hours prior to surgery and throughout the entire surgical period to allow adequate time for colonic emptying. The skin over the abdominal wall and neck was shaved and cleansed with povidone iodine solution before the operative procedure. The animals were placed in a supine position on the table. Anesthesia was provided in the form of 75 mg/kg intramuscular ketamine hydrochloride (Ketalar; Eczacibaşi Warner Lambert, Istanbul, Turkey) and 7 mg/kg xylazine (Rompun; Bayer AG, Leverkusen, Germany).

Group A: Control (Open)

The colonic anastomosis was performed using an open surgical method in Group A. A 3 cm midline abdominal incision was made. The rats underwent transection and primary end-to-end anastomosis of the left colon at approximately 3 cm proximal to the peritoneal reflection. The anastomosis was performed using a seromuscular single layer of interrupted sutures with 6-0 polypropylene (Prolene; Johnson and Johnson, Edinburgh, UK). The fascia and skin were closed separately with continuous 4-0 silk sutures.

Group B: LCA (Laparoscopic Colon Anastomosis)

In Group B, a sterile catheter (Mediflon Catheter, 20 × 1.1 × 33 ram; Eastern Medikit, Eastern Medikit Ltd., Gurgaon, India) was inserted into the abdominal cavity. CO2 was insufflated into the peritoneal cavity until the IAP reached 14 mm Hg, and then the IAP was maintained using an electronic gas insufflating system (Electronic Laparoflator 264300 20; Karl Storz, Germany) (Fig. 1). CO2 insufflation was stopped after 60 minutes. The laparotomy and colonic anastomosis processes were then performed as described for the control group.

Group C: LCA and HBOT

The animals in the LCA/HBOT group received ten sessions of HBOT in a steel chamber. The treatment protocol was 100% O2 for 60 minutes at 2.1 atmospheres absolute (ATA). The animals received one session per day, beginning within two hours of the surgery being completed.

Follow-up and Outcome Measurements

The care and feeding of the animals continued postoperatively in the aforementioned manner. All of the rats were killed by an overdose of ketamine anesthesia on the tenth postoperative day. The anastomotic healing and inflammatory response were determined by measuring the anastomotic bursting pressure (ABP), serum and tissue malondialdehyde (MDA), nitric oxide (NO), and catalase (CAT) activity levels, and the tissue hydroxyproline content. A histopathologic examination was performed.

Measurement of ABP

The mechanical strength of the anastomosis was based on the bursting pressure, which was determined in situ without an interruption to the normal blood supply or adhesions of the perianastomotic tissues. A 4 cm segment of the colon, including the anastomosis site, was resected immediately after death in order to allow the measurement of the ABP. A histopathologic evaluation was then performed. A 6-G silastic catheter was installed into the end of the open segment, and both ends of the open segment were tied with a 2/0 silk filament. A pressure manometer (ERKA manometer; Kallmeyer Medizintechnik GmbH & Co., 83646 Bad Tolz, Germany) was connected to the catheter via a three-way stopcock. The intraluminal pressure was measured during normal saline infusion applied via the three-way stopcock. The maximum pressure prior to rupture was recorded as the ABP. The ABP was measured by a team member who was blind to the group characteristics and was reported according to the code number of the animal.

Biochemical analysis

All biochemical measurements were performed in a blind manner. The tissues were placed in petri dishes and stored at –86 °C until they were used. After thawing, each sample was weighed and then homogenized in 0.15 M potassium chloride solution. The samples were centrifuged, and the supernatant was obtained and used to estimate the biochemical parameters, including the NO, MDA, CAT, and hydroxyproline levels.

The NO in the plasma and tissues was measured as the stable metabolites nitrate (NO_3) and nitrite (NO_2). Nitrate was reduced by nitrate reductase into nitrite, and nitrite was determined spectrophotometrically according to the Griess reaction [8]. The plasma MDA was determined spectrophotometrically in accordance with the method of Buege and Aust [9]. The tissue MDA was estimated using the method described by Ohkawa et al.[10] The tissue thiobarbituric acid-reactive material was expressed in terms of nanomoles of MDA per gram of tissue. Goth's spectrophotometric method [11] was used to determine the CAT level in the plasma and tissue. One unit of CAT decomposes 1 mmol of H_2O_2/L min. The results were expressed as kilo units/g of protein. The tissue hydroxyproline content of all of the rats was determined according to the Reddy method [12] and the results were recorded as the micrograms of hydroxyproline per milligram of dry tissue weight. The protein concentration was measured using the Folin phenol reagent method [13].

Histopathologic Analysis

Following the measurement of the ABP, the colonic anastomosis segments were fixed in a 10% formalin solution and embedded in paraffin. The paraffin blocks were cut into 5 mm wide sections. The histopathologic sections were dyed with hematoxylin and eosin, and then evaluated using light microscopy by a single pathologist who was blind to the study groups. A minimum of five histopathologic sections were evaluated for each animal, and the mean value of all of the sections was determined. The formation of the mucosal layer and the severity of inflammation at the colonic anastomosis site were scored according to a semi-quantitative scoring system modified from that described by Hauet et al.[14] The range of scores for mucosal layer formation was: 0 (perfect healing), 1 (healing down to the muscularis mucosae, but not complete), 2 (healing down to the submucosal layer, but not the muscularis mucosae), 3 (healing of the surface epithelium only), and 4 (no healing).

The range of scores for the severity of the inflammatory reaction was: 0 (polymorphonuclear leukocyte infiltration absent), 1 (polymorphonuclear leukocyte infiltration <25%), 2 (infiltration between 25% and 50%), 3 (infiltration between 50% and 75%), and 4 (infiltration extending >75% in a small microscopic area). The longitudinal anastomotic sections were graded on a modified numerical scale, in accordance with Ehrlich et al.[15] The inflammatory cell infiltration, vascular in-growth, fibroblast proliferation, and collagen deposition were graded as: 0 (absent), 1 (occasionally present), 2 (slightly distributed), 3 (abundant), or 4 (confluence of cells or fibers). All of the histopathologic slides were also assessed for evidence of ulceration and perforation. The histopathologic results were reported according to the code number allocated to each animal.

Statistical analysis

The computerization of the data of our study and the statistical analyses were carried out by using PASW Statistics 18 for Windows package software. The conformity of the variables to normal range was controlled by Kolmogorov-Smirnov Test. One-way analysis of vari-

ance was used for the normal ranged group; Tukey's test was used for subgroup comparisons. The Kruskal–Wallis variance analysis and the Mann–Whitney *U*-test were used for the nonparametric data. A p-value of <0.05 was considered statistically significant. The results were calculated as mean ± standard deviation.

Results

No rats died during the experiments, and all of the rats were successfully evaluated on the tenth postoperative day. The distribution of the rats' weights was homogenous between the groups at the beginning of the experiment and no significant weight changes were observed during the experiment (P>0.05). A wound infection developed in one rat in Group B. No anastomotic leakage or massive adhesions were observed in any of the groups. Two rats in Groups A and B showed minimal adhesion around the anastomoses. No adhesion was observed in the rats in Group C.

ABP Results

Table I presents the comparison of the ABP between the groups. The best ABP results were detected in Group A. The result for Group C, which received HBOT, was better than that for Group B. There was a statistically significant difference between Group A and Group B.

TABLE I - *Comparison of Anastomosis Bursting Pressure Among Groups*

	Group A	Group B	Group C	Comparison	P
ABP	226±8,8	197±9,1	213±27	A vs.B	0,0001
				A vs. C	NS
				B vs. C	NS

Data represent the mean±SD of n=10 animals per group.
Group A indicates colon anastomosis only (open group); B, laparoscopic colon operation; C, HBOT and laparoscopic colon operation. ABP indicates anastomosis bursting pressure; HBOT, Hyperbaric oxygen therapy; NS, not significant.

TABLE II - *Serum NO, MDA, and CAT Activity Levels and Tissue NO, MDA, CAT Activity, and Hydroxyproline Levels Among All Groups*

	Group A	Group B	Group C	Comparison	P
Serum NO	38±12.6	64±13.5	60±16.4	A vs.B	0.001
				A vs.C	0.003
				B vs.C	NS
Serum MDA	4±1.3	7±1.2	6±1.4	A vs.B	0.001
				A vs.C	0.005
				B vs.C	0.049
Serum CAT	52±11.2	36±7.3	42±8.9	A vs.B	0.001
				A vs.C	0.039
				B vs.C	NS
Tissue NO	13±3.7	18±3.1	16±3.9	A vs.B	0.004
				A vs.C	NS
				B vs.C	NS
Tissue MDA	1.1±0.3	1.8±0.4	1.7±0.3	A vs.B	0.001
				A vs.C	0.001
				B vs.C	NS
Tissue CAT	2.2±0.5	1.5±0.6	1.8±0.3	A vs.B	0.02
				A vs.C	NS
				B vs.C	NS
Hydroxyproline	206±40.0	268±43.6	265±41.0	A vs.B	0.004
				A vs.C	0.004
				B vs.C	NS

Data representmean ± SD of n=10 animals per group.
Group A indicates colon anastomosis only (opengroup); B, laparoscopic colon operation; C, HBOT and laparoscopic colon operation; NS, not significant. HBOT, Hyperbaric oxygen therapy
CAT indicates catalase; MDA, malondialdehyde; NO, nitricoxide; NS, not significant.

Serum NO, MDA, and CAT activity levels

Table II details the serum and tissue NO, MDA, and CAT levels, as well as the tissue hydroxyproline levels, of the three groups. There was a significant difference in terms of serum NO between Group A and the other two groups, although there was no statistically significant difference between Groups B and C. In terms of the serum MDA level, significant differences were found between Groups A and B, Groups A and C, and Groups B and C. The best serum MDA result was obtained in Group A, while the MDA result of Group C was superior to that of Group B. With regard to the serum CAT level, significant differences were found between Groups A and B and Groups A and C, but not between Groups B and C.

Tissue NO, MDA, CAT activity, and hydroxyproline levels

In terms of tissue NO, significant differences were found between Groups A and B, but not between Groups A and C or Groups B and C. With regard to the tissue MDA levels, significant differences were found between Groups A and B and Groups A and C, but not between Groups B and C. In the case of tissue CAT activity, significant differences were found between Groups A and B, with Group A showing superior levels. No difference was found between Groups A and C or Groups B and C. In terms of the tissue hydroxyproline levels, a significant difference was found between Groups A and B and Groups A and C, but not between Groups B and C.

Histopathologic Evaluation

The data concerning the histopathologic evaluation is summarized in Table III. Wound healing was histologically evaluated according to the modified Ehrlich-Hunt numerical scale. The evaluated characteristics included wound formation, inflammatory cell infiltration, edema, vascular in-growth, fibroblast proliferation, and collagen deposition. Although Group A demonstrated better healing than the other two groups, significant differences in the fibroblast proliferation scores were found between Groups A and B.

Discussion

Laparoscopic colorectal resection has been reported to be more advantageous than open surgery for a number of reasons, including less postoperative pain, less need for analgesia, shorter hospital stay, quicker healing period, and lower mortality and morbidity [16]. The mean operation duration in laparoscopic and open surgery is 150-180 minutes and 95-130 minutes, respectively [16,17]. Patients experience 14 mmHg of IAP for approximately three hours during laparoscopic colorectal surgery. The increase in IAP has side effects in terms of the systemic and splanchnic blood flow, as well as cardiac and renal functions [18-22]. The portal venous flow was shown to decrease by 37% when the IAP increased from 0 to 7 mmHg, and it decreased by 53% when the pressure reached 14 mmHg [23]. We postulated that the decrease in splanchnic blood flow due to the increase in IAP

Table III - *Histopathologic Grading Among the Groups*

	Group A	Group B	Group C	Comparison	P
Formation	2.10±1.20	1.60±0.70	2.10±0.74	A vs.B	NS
				A vs.C	NS
				B vs.C	NS
Infitration with inflammatory cell	2.40±0.97	1.80±0.79	2.20±0.63	A vs.B	NS
				A vs.C	NS
				B vs.C	NS
Edema	2.60±0.97	2.00±0.67	2.40±0.70	A vs.B	NS
				A vs.C	NS
				B vs.C	NS
Vascular in-growth	2.10±0.57	1.90±0.57	2.30±0.48	A vs.B	NS
				A vs.C	NS
				B vs.C	NS
Fibroblast proliferation	2.60±0.70	2.00±0.4	2.30±0.68	A vs.B	0.039
				A vs.C	NS
				B vs.C	NS
Collagen deposition	2.30±0.48	2.00±0.67	2.30±0.68	A vs.B	NS
				A vs.C	NS
				B vs.C	NS

Group A indicates colon anastomosis only (open group); B, laparoscopic colon operation; C, HBOT and laparoscopic colon operation. HBOT, Hyperbaric oxygen therapy; NS, not significant.

would cause bowel ischemia and that HBOT might have a supportive effect on the healing of colonic anastomosis performed laparoscopically. HBOT increases the level of oxygen in the injured tissue. The restoration of oxygenation in sites of inflammation accelerates all the steps of wound healing, including fibroblast proliferation, granulation tissue formation and neovascularization, collagen formation, and epithelialisation [24]. Collagen cannot be synthesized in the absence of molecular oxygen, since fibroblasts need oxygen for their activity [25-28].

Several studies have shown that increased oxygen tension brought about through HBOT not only prevents the adverse effects of ischemia, but also accelerates healing in different types of wounds [29-30]. Oxygen is an essential material for cell metabolism, and it is in especially high demand during reparative processes such as cell proliferation and collagen synthesis. Furthermore, evidence from both animal and cell line studies has shown that hyperbaric oxygen therapy increases growth factor production and accelerates wound healing [31].

Anastomotic leakage following colorectal surgery occurs in 5-15% of patients who undergo this type of surgery and it thus leads to substantial morbidity and mortality [32]. Numerous local and systemic factors affect anastomotic healing, with the most important local factor being the perfusion and oxygenation of the site of anastomosis. Further, intestinal blood flow should exceed 30% [31,33] in order to achieve safe anastomosis.

HBOT, which is applied by means of the intermittent inhalation of 100% oxygen under one atmospheric pressure, has been reported to have favorable effects on intestinal anastomotic healing [5,34,35]. Typically, treatments involve pressurisation to between 2.0 and 2.5 atmospheres absolute (ATA) for periods between 60 and 120 minutes once or twice daily [36]. Several studies have recommended that HBOT be given for at least ten sessions, with each session lasting 90 minutes. A shorter duration of HBOT administration would not result in an adequate tissue oxygen level, while longer applications might lead to unfavorable effects such as central nervous system and pulmonary toxicity [25].

Adas et al. [7] found an increase in both HBOT and ABP in their studies evaluating the healing of ischemic and non-ischemic colonic anastomosis. Yagci et al. [5] investigated the effect of HBOT on ischemic and normal colon anastomosis, and reported that ABP was significantly decreased in the rats that underwent ischemic anastomosis compared to the control rats that underwent normal anastomosis. In the same study, the investigators also reported that adequate tissue oxygenation was the main factor in wound healing and that HBOT therefore had a positive effect on the biochemical and mechanical parameters [5]. Hamzaoglu et al. [35] suggested that postoperative HBOT increased colon anastomosis healing and repaired ischemic damage. In the presented series, the ABP value was found to be higher in the open surgery group (226 ± 8.8) (Group A). The result of Group C (213 ± 27), which received HBOT, was found to be better than that of Group B (197 ± 27). There was no statistically significant difference between Group B and Group C.

Oxidative stress is the imbalance between the production of reactive oxygen species (ROS) and a biological system's ability to readily detoxify the reactive intermediates or else easily repair the resulting damage [37]. Low-level ROS has been shown in both in vitro and in vivo studies to be an essential mediator of intracellular signaling and efficient wound angiogenesis, although excessive production of ROS or impaired detoxification of this aggressive molecule causes oxidative stress, which has been identified as an important feature in the pathogenesis of chronic, non-healing wounds [38-40].Ma et al. [41] did not find an improvement by the 7th day of HBOT in terms of the MDA and CAT analysis in ulcerated tissue, but they did encounter a statistically significant improvement by the 14th day. In the presented series, ten sessions of HBOT were applied and it is thought that more significant results would be obtained with an increased number of sessions.

Shandall et al. [34] demonstrated perianastomotic oxygen tension to be associated with dissociation energy, dissociation tension, and hydroxyproline content. In many studies, the investigators measured the hydroxyproline content of intestinal anastomosis, since it was assumed that the hydroxyproline level is an indicator of the collagen content. However, some studies found that there was no correlation between hydroxyproline concentration and the mechanical strength of an anastomosis [42,43]. HBOT did not alter the hydroxyproline level in the presented series, which we assume to be due to the number and duration of the sessions being insufficient.

It is known that HBOT increases the nitric oxide levels in perivascular tissues via the stimulation of nitric oxide synthase [44,45]. No difference was detected between the groups with regard to the histopathologic values. This might be due to the treatment protocol.

Although HBOT might have several advantages, it had only a limited effect on the healing of colonic anastomosis in rats with increased intra-abdominal pressure in our study. We suggest that further studies are needed, since we believe that HBOT might be a useful treatment modality in the healing of anastomosis, which is negatively affected by intestinal ischemia-reperfusion.

Riassunto

PREMESSA: Un aumento della pressione intraddominale determina una riduzione del flusso splancnico ed del pH intramucoso dell'intestino, come pure un aumentato rischio di eschemia dle colon. Lo scopo di questo studio è quello di valutare l'effetto della terapia con ossigeno iperbarico (HBOT) sull'ischemia provocata dalla laparoscopia sulle anastomosi Coliche in un modello sperimentale di chirurgia laparoscopica del colon.

MATERIALE E METODO: Abbiamo diviso 30 ratti maschi Wistar albini in tre gruppi di 10 animali ciascuno: il Gruppo A di controllo (anastomosi colica ad addome aperto); il Gruppo B di anastomosi colica laparoscopica (LCA); il Gruppo C di anastomosi colica laparoscopica (LCA) trattato con HBOT. Il Gruppo C (LCA + HBOT) è stato introdotto in camera iperbarica con somministrazione di ossigeno puro al 200% a 2,1 atmosfere per 60' per dieci giorni consecutivi.
RISULTATI: Si è riscontrato che il valore della pressione di rottura dell'anastomosi è più elevata nel gruppo A (226 ± 8.8). Lo stesso valore nel Gruppo C, trattato con HBOT) (213 ± 27), è risultato migliore di quello nel Gruppo B (197 ± 27). Comunque non sono state rilevate differenze statistiche significative tra il Gruppo B ed il Gruppo C. Il Gruppo A ha mostrato guarigione migliore rispetto agli altri gruppi, mentre differenze significative relative alla proliferazione fibroblastica sono state rilevate tra Gruppo A e Gruppo B. In termini di livelli di idrossiprolina tissutale è stata rilevata una differenza significativa tra Gruppo A e Gruppo B e tra Gruppo A e Gruppo C, ma non tra Gruppo B e Gruppo C.
CONCLUSIONI: La JBOT accresce il livello di ossigeno sui tessuto traumatizzati. Sebbene la HBOT potrebbe offrire numerosi vantaggi, essa nel nostro studio mostra solo effetti limitati sui processi di guarigione delle anastomosi del colon sui ratti sottoposti ad aumento della pressione intraaddominale.

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Commento e Commentary

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The experimental design of the study presents three questionable elements.
Firstly *why the endo abdominal CO_2 pressure during the LC was maintained at so high level (14 mmHg equal to 19 cm H_2O) while in clinical operations the usual pressure is 12 mmHg (equal to 16,31 cm H_2O): perhaps to stress the experiment?*
Secondly *the duration of the intraabdominal hypertension in the experimental design was present and limited to the operatory times without particular respiratory assistance, while in the clinic situation the respiratory assistance to the patient during a LC assures high values of O_2 during the entire procedure, making the confront with the experimental situation not adequate.*
Thirdly *the application of 100% hyperbaric oxygen during 60' – and not during 90' as the literature suggests – in each of the ten days following the experimental operation seems both too short and perhaps useless, moreover potentially dangerous for so high %, at least mainly for the pulmonary tissue of the rats - without any control - as their survival has been interrupted in the tenth day for the needed measures of bursting pressure.*

* * *

REPLAY

Dear editor,

12 mmHg is the most commonly used pressure in laparoscopic surgery[1] Accordingly, larger and more time consuming abdominal operations are being performed laparoscopically, in which the IAP is maintained at 14mm Hg for 3 hours or more. High IAP and pneumoperitoneum are associated with many struc tural and functional alterations[2], such as reduced portal blood flow (ie, a 27% decrease at an IAP of 10mm Hg)[3] and blood flow in the bowels[4]. We chose pressures of 14 mmHg for the pneumoperitoneum because high IAP and pneumoperitoneum are associated with many structural and functional alterations. This pressure is so high for rat model.(In fact, 5 mmHg in the rat simulates the pressure of 12 mmHg used in humans)[5]. That's way, intraabdominal hypertension in the experimental design was limited to the operatory times.

Typically, treatments involve pressurisation to between 2.0 and 2.5 atmospheres absolute (ATA) for periods between 60 and 120 minutes once or twice daily[6]. Marx[7] assessed the angiogenic properties of normobaric oxygen (100% oxygen at 1ATA for 90 minutes daily) and hyperbaric oxygen (100% oxygen at 2.4ATA for 90 minutes daily for 20 days), as compared with air-breathing controls. Results indicated that normobaric oxygen had no angiogenic properties above the normal revascularization of irradiated tissue than air-breathing controls ($p = 0.89$). Hyperbaric oxygen demonstrated an eight- to ninefold increased vascular density over both normobaric oxygen and air-breathing controls ($p = 0.001$).

Oxygen in high doses is toxic to normally perfused tissue, in particular the brain and lungs. Some adverse effects such as reversible myopia and cataract formation may occur. In addition, rupture of the middle ear or damage to cranial sinuses, teeth, and lungs due to barotraumas have been reported[8]. Hyperbaric oxygen therapy can cause pulmonary oxygen toxicity, resulting in increased sympathetic tone leading to a decrease in LV compliance while increasing afterload, leading to pulmonary edema[9,10]. Therefore it is not possible to expose patients to typical wound treatment pressures for longer than 1 to 2 hours on a regular basis and the question arises as to how such short exposures could be expected to result in a clinical benefit. There are two principal reasons why this might be so. First, elevation of wound oxygen tension may persist for some hours following HBOT and so exert therapeutic effects for rather longer than might be expected[11]. Second, there is experimental evidence that repeated 'on-off' exposures do produce an environment favourable to healing when compared to oxygen or air at normobaric pressure[6].

In experimental design, CO_2 insufflation was stopped after 60 minutes. IAP of 14mm Hg was so high for rat model at that time. That's why the laparotomy and colonic anastomosis processes were performed after 60 minutes.

Sincerely yours
Dr Selim Sözen

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