The Effect of Thermal Preconditioning And Preoperative Warming on Healing of Colonic Anastomosis

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ÖZET

Kolon anastomozlarının iyileşmesinde ısı ile önhazırlık ve preoperatif ısıtma uygulamalarının etkisi

Amaç: Kolorektal cerrahiyi takiben görülen en önemli komplikasyon anastomoz kaçaklarıdır. Bu çalışmada preoperatif ısıtma ve ısıyla ön hazırlık tekniklerinin kısa ve uzun dönemde kolon anastomozu üzerine olan koruyucu etkilerinin araştırılması amaçlanmış ve bu iki yöntem kendi arasında karşılaştırılarak hangisinin üstün olduğu aranmıştır.

Gereç ve Yöntem: 48 adet Wistar-Albino cinsi sıçan her birinde 8 sıçan olacak şekilde 6 gruba ayrıldı. Gruplar kısa (3 gün) ve uzun (7 gün) dönem anastomoz grubu (kontrol), kısa ve uzun dönem ısı ile önhazırlık ve kısa ve uzun dönem preoperatif ısıtma olacak şekilde oluşturulmuştur. Anastomoz değerlendirilmesi, postoperatif 4. ve 7. günlerde anastomoz patlama basıncı, doku hidroksiprolin seviyesi ve anastomoz hattı fibrozis yoğunluğu ölçülerek yapıldı.

Bulgular: Doku hidroksiprolin seviyeleri uzun dönemde kısa döneme göre anlamlı olarak daha yüksek bulundu (p=0.01). Preoperatif ısıtmanın kısa dönemde doku hidroksiprolin seviyeleri üzerine bir etkisi olmadığı bulunmuş olmasına rağmen bu seviyeler ısı ile önhazırlık grubunda preoperatif ısıtma grubundakilere göre daha yüksekti (p=0.01). Ayrıca uzun dönemde patlama basınçlarının tüm gruplarda artmış olduğu bulundu (p=0.01) ancak gruplar arasında kısa ve uzun dönem sonuçları arasında fark bulunamadı. Her iki yöntemde kısa ve uzun dönemde fibrotik indeks bulgularını etkilemedi.

Sonuç: Isı ile ön hazırlık tekniği kullanılarak uygulanan kolon anastomozlarında, iyileşmenin erken döneminde anastomoz hattında kollajen sentezi artmaktadır, ve bu teknik anastomoz sağlamlığının arttırılmasına katkıda bulunabilir. Her ne kadar diğer parametreler bu görüşümüzü desteklemese de, ideal sıcaklık ve uygun zaman ile yapılacak ileri deneysel araştırmaların bu konuda yol gösterici olacağını düşünmekteyiz.

Anahtar kelimeler: Anastomoz iyileşmesi, ısı ile ön hazırlık, preoperatif ısıtma

ABSTRACT

The effect of thermal preconditioning and preoperative warming on healing of colonic anastomosis

Objective: The most important complication following colorectal surgery is the anastomotic leakage. The aim of this study was to investigate the protective effect of thermal preconditioning and preoperative warming in the short and long term manner on anastomotic healing as well as to compare these two procedures to find out which is superior.

Material and Methods: Forty-eight Wistar rats were randomly assigned into six groups, each consisting of 8 rats. The groups were formed as short (3 days) and long (7 days) term anastomoses (control), short and long term thermal preconditioning, and short and long term preoperative warming groups. Anastomotic healing was assessed on postoperative day 4 and 7 by determining anastomotic bursting pressure, tissue hydroxyproline content and histopathological examination.

Results: The hydroxyproline levels were significantly higher in long term than those of short term (p=0.01). While preoperative warming had no effect on the hydroxyproline levels in short term, they were significantly higher in thermal preconditioning (TP) group than those of preoperative warming (PW) (p=0.01). In addition, the long term bursting pressures were found to be increased in all groups (p=0.01, for three groups) without any significant differences between groups both in short and long terms. Neither TP nor PW did affect the fibrotic index in STA and LTA groups.

Conclusion: Colonic anastomoses performed with thermal preconditioning technique, increase the collagen synthesis on the anastomosis line in the early period of healing, and it is obvious that this technique could contribute to the anastomosis stability. Although the other parameters do not support our opinion, we believe further experiments about optimum heat and time would be a guide.

Key words: Anastomotic healing thermal preconditioning preoperative warming

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INTRODUCTION

The most important complication of the colonic anastomoses is leakage, which may cause significant morbidity and mortality (1,2,3). The clinically determined rate of anastomotic leakage changes between 2 and 30% (4). The anastomotic leakage depends on various factors such as type of surgery, patients' overall health and the technique used by the surgeon (5). To decrease the incidence of anastomotic leakage, various methods such as the restoration of the surgical technique, preoperative bowel preparation and nutritional and pharmacological interventions have been used (2,6-9).

The rationale of preoperative warming and thermal preconditioning is based on activation of endogenous

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defense mechanisms in response to thermal stress that has emerged when exposed to temporary sublethal hyperthermia. These mechanisms include cytoskeletal structures, cellular metabolism and macromolecular synthesis (10). On the transcriptional level, hyperthermia induces the heat shock genes, which cause to produce the heat shock proteins (HSPs) (11). In many normal tissues such as heart, muscle, intestine and bone marrow HSPs synthesis have been demonstrated after the thermal procedures (12-15). Synthesis of heat shock proteins (HSPs) in response to a low level of cellular stress results in the adaptation of cells to hierarchical changes in their environment and surviving under unfavorable conditions. Thermal preconditioning has been used successfully to decrease or prevent ischemic tissue damage especially in the heart, small intestine, skeletal muscle, kidneys and the diaphragm in experimental conditions (16,17).

The effect of thermal procedures on colonic anastomoses has not been previously reported. Since the ischemia has been reported as the most important reason of the anastomotic failure either due to vascular compromise or tension (both affect the blood supply adversely and may cause anastomotic failure), the measures that could prevent ischemia or could encourage to prevent ischemic damage (18). Since the thermal preconditioning has been found to decrease or prevent ischemic tissue damage in some organs, the hypothesis underlying the present study is to strengthen the colonic anastomoses by preventing the ischemic damage in an experimental rat model (16,17). We aimed to investigate the effect of thermal preconditioning and preoperative warming on colonic anastomotic healing, as well as to compare two procedures to find out which is superior.

MATERIAL AND METHODS

Forty-eight male Wistar-Albino rats (280-300 grams) were included. Rats were allocated randomly into short-term control (n=8), long-term control (n=8), short-term thermal preconditioning (n=8), long-term thermal preconditioning (n=8), short-term preoperative warming (n=8), long-term preoperative warming (n=8) groups. Rats were kept in cages at 20±2°C constant temperature in 12-hour light/dark cycles and fed with regular rat chow and tap water from drinking bottles. Experimental protocol was designed according to the "Guiding Principles in the Care and Use of Animals" and has been approved by the local ethical committee of animal use.

Thermal preconditioning was induced by raising the core body temperature to 41±0,5°C by partial immersion in a water bath (Grant Instruments, Shepreth, UK), under 40 mg/kg subcutaneous ketamine hydrochloride (Ketalar® flakon, Eczacıbaşı, Istanbul) anesthesia. The animals' body temperature was gradually increased and was continuously monitored with a rectal thermometer. This increased temperature was kept for 15 minutes. Laparotomy was applied 18 hours after the thermal preconditioning (19).

Preoperative warming was applied to the anterior wall of abdomen with a thermophore containing 41±0,5°C hot water for 15 minutes, and after a lag period of 15 minutes, laparotomy procedure was commenced (20).

After overnight fasting, anaesthesia for all animals was induced using 40 mg/kg ketamine hydrochloride (Ketalar® flakon, Eczacıbaşı, Istanbul) subcutaneously. This procedure was followed by laparotomy through a 4-cm midline incision under aseptic conditions. Distal colon was prepared for anastomosis. After resection of a 1-cm segment of the distal colon, anastomosis was performed by construction of inverted, one-layer, seromuscular interrupted sutures with 6-0 polypropylene (Prolene, Johnson and Johnson, Edinburgh, United Kingdom). Fascia and skin were closed separately with continuous 4-0 silk sutures. A resuscitation load with 8 ml of normal saline solution was given subcutaneously. All rats received the same resuscitation load. The operated rats were fed with regular rat chow and tap water from drinking bottle starting from first day after operation. An additional normal saline solution of 2ml/day was given subcutaneously to prevent fluid deficit. No antibiotics were used.

Short-term anastomosis group (STA): Colon anastomoses were performed as explained above. On the fourth day after the anastomoses, the rats were reoperated in the same manner and the anastomoses were evaluated physically, histopathologically and biochemically.

Long-term anastomosis group (LTA): The evaluation was made at the end of day seven after the same procedures in STA group.

Short-term anastomosis (STA)+thermal

preconditioning (TP) group: In addition to the procedures followed in STA group, rats in this group prepared with thermal preconditioning and then colonic anastomoses were done as described above.

Long-term anastomosis (LTA)+thermal preconditioning (TP) group: The evaluation was made at the end of day seven after the same procedures in STA+TP group.

Short-term anastomosis (STA)+preoperative warming (PW) group: Preoperative warming and colonic anastomoses were done as explained above.

Long-term anastomosis (LTA)+preoperative warming (PW) group: The evaluation was made at the end of day seven after the same procedures in LTA + PW group.

Anastomotic healing was evaluated with anastomotic bursting pressure, histopathological examination and tissue hydroxyproline content. Anastomotic bursting pressure (ABP) was determined in situ without interruption of normal blood supply or adhesions of the anastomoses. A colonic segment bordering 2 cm on either side of the anastomosis was resected and the segment was flushed with normal saline to clean luminal contents. An 8-gauge silastic catheter was inserted into the proximal side of colon and tied in position with 2-0 silk with care not to disturb the anastomoses. The distal end was fixed to a pressure transducer and saline with methylene blue was infused through the catheter using a syringe pump (Life Care Pump ®, Abbott/Shaw, Istanbul) at a rate of 10 mL/sec. The pressure was monitored with a disposable pressure transducer and recorded with a Hewlett-Packard Biopac MP-100 Acquisition System, version 3.5.7 (Santa Barbara, CA). Peak pressures detected prior to leakage were recorded as the anastomotic bursting pressures (21).

Colonic segments were dissected along their mesenteries, with 10-mm and 2-mm segments carefully excised on either side of the anastomosis. All samples were than stored at -70°C until further processing. When brought to room temperature, dry weights of the samples were recorded and the amount of hydroxyproline was successively determined. Absorbance was read by a Shimadzu spectrophotometer (UV-120-02; Kyoto, Japan), and the collagen concentration was expressed as micrograms of hydroxyproline per milligrams tissue of dry weight (22).

Paraffin blocks of tissue samples were prepared and six micrometer-thick sections were dyed with Masson-Tricrom and hematoxylin-eosin. Slides were examined by a single pathologist in a double blind fashion by light microscopy. Fibrosis at the anastomoses was evaluated according to the scale mentioned below (23).
(+) Presence of too thin, sparse, irregular collagen fibers
(++) Presence of irregular but denser, thick collagen fibers
(+++) Presence of regular and thick, dense collagen fibers

Statistical analysis

The Mann-Whitney U and chi-square tests were used for continuous and categorical variables respectively. Data were expressed as means±standard deviation (SD). A p value less than 0.05 were considered significant.

RESULTS

The results are shown in Table 1. No rat died and no complications were observed during the experiments.

Bursting pressure: The mean bursting pressures were significantly higher in long term than those of short term in all groups (p=0.01, for all) (Table 2), although there is no significant difference between groups (p>0.05, for all) (Table 3). There was also no difference between groups

Table 1: Results of the study groups (Mean ± SD)

	Bursting Pressure (mmHg)	Hydroxyproline (mg/100 gr wet tissue)	Fibrosis (+, ++, +++)
STA	45±16	41.9±26.5	1.7±0.7
LTA	138.1±22	46.9±19.8	2±0.7
STA + TP	48.7±12.4	66.2±18.3	2.1±0.6
LTA + TP	147.5±29.6	59±19.3	2.1±0.8
STA + PW	46.2±10.6	37.7±7.4	2±0.7
LTA + PW	170±52.7	53.4±20.9	2±0.7

SD= standart deviation, STA= short-term anastomosis, LTA= long-term anastomosis, TP= thermal preconditioning, PW = preoperative warming

Table 2: The comparison of variation between the groups (Mean ± SD)

Groups	Bursting pressures			Hydroxyproline levels			Fibrotic index		
	ST	LT	p	ST	LT	p	ST	LT	p
Control	45±16	138.1±22	0.01	41.9±26.5	46.9±19.8	0.01	1.7±0.7	2±0.7	0.58
TP	48.7±12.4	147.5±29.6		66.2±18.3	59±19.3	0.48	2.1±0.6	2.1±0.8	1
PW	46.2±10.6	170±52.7		37.7±7.4	53.4±20.9	1	2±0.7	2±0.7	1

SD= standart deviation, ST= short term, LT= long term, TP= thermal preconditioning, PW= preoperative warming

Table 3: The p values between the groups in short and long term manner

	Bursting pressures		Hydroxyproline levels		Fibrotic index	
Groups	ST	LT	ST	LT	ST	LT
C vs PW	0.55	0.13	0.75	0.53	0.49	1
C vs TP	0.38	0.49	0.09	0.14	0.27	0.74
TP vs PW	0.70	0.37	0.01	0.4	0.73	0.74

ST= short term, LT= long term, C= control, TP= thermal preconditioning, PW= preoperative warming, vs= versus

in short term (p>0.05, for all) (Table 3).

Hydroxyproline levels: The tissue hydroxyproline levels were higher in STA+TP group than those of STA and STA+PW groups, although it was significant for only STA+PW (p=0.01) (Table 3). There were no significant differences between the groups in long term, although they were higher in TP and PW groups than those of control (Table 2).

Fibrotic index: The fibrotic index values in the groups were not different from each other either in short or long terms (p>0.05, for all) (Table 3). There was also no difference between short and long terms (p>0.05, for all) (Table 2).

DISCUSSION

Anastomotic leakage is one of the most important complications in colorectal surgery (1,2). The factors, which are involved in the development of a leakage include surgical technique, tension of anastomoses, patient's general status, quality of tissue, presence of infection, age of patient, prolonged operation time and surgeon's experience (1,24). But the main reason of anastomotic failure except technical reasons is the tissue ischemia and in order to prevent a leakage, it is very important to apply techniques and methods that increase the stability of anastomosis and prevent tissue ischemia by increasing tissue perfusion (18). Lots of local and systemic factors play important roles in the healing of colon anastomosis. The common characteristics of these factors are related to their effects on the collagen metabolism on the bowel wall (3,25,26). According to qualitative and quantitative characteristics of the bowel wall, it may be possible to assess the stability and health of the bowel anastomosis during the healing period (27,28).

According to experimental studies, heat shock proteins (HSPs) play an important role on the cellular resistance mechanism against stress (17). Pretreatment of cells by supraoptimally elevated temperatures, the expression of HSPs are increased and dramatically upregulated in order to protect cells against higher temperatures that could be lethal (29,30). This phenomenon is named as thermotolerance. Several studies have investigated the mechanism of thermotolerance, which can be induced in some cell lines such as mammalian, tumors and in normal tissues, but it is not well understood (31). It has been suggested that exposure of cells to elevated temperatures causes a rapid increase in the synthesis of HSPs (17). These thermotolerant cells are also protected from lethal exposures such as tumour necrosis factor, ultraviolet light, monocytes and oxidative stress (32,33). Heat shock proteins are divided into six subgroups according to their molecular weights and the 70 kilo dalton heat shock proteins (Hsp70s) are the ones that are most frequently investigated (34). Hsp 70 synthesis, increase the exposed cells ability to overcome the increased concentrations of denaturated proteins under various stress conditions (35). Additionally, by preventing cellular death, apoptosis and necrosis effectively, Hsp 70 results in cell survival when exposed to high levels of

lethal impulse (36,37).

The possible functions of HSPs in ischemic tissue include protection against oxidative stress, repair of damaged proteins and ion channel and suppression of pro-inflammatory cytokines (38,39). It was also found effective in preventing myocardial ischemia in rats (40,41). Since the preventing of tissue ischemia and hypoperfusion is very important in stability anastomosis, prevention of tissue ischemia via thermal preconditioning or preoperative warming could be effective on anastomotic stability and preventing of failure.

In this study, we showed that tissue hydroxyproline contents of the STA+TP group were statistically higher compared to other groups. Our data indicated that heat exposure of thermal procedures resulted in a marked increase only in collagen metabolism and it may be speculated that heat shock caused a large amount of Hsp 47 induction. Heat shock protein 47 is one of the HSPs and has been reported to be associated with the metabolism or processing of procollagen as a molecular chaperone with alpha-1 (IV) collagen (42). Besides, some studies have been found that Hsp 47 is associated with type I-V collagen in vitro and type I-III collagen in vivo (43,44). From our point of view experiments should be conducted in order to clarify the effect of Hsp 47 on healing of colonic anastomoses.

In the study, although the desired physical and histopathological results could not be obtained, especially high tissue hydroxyproline values in the thermal preconditioning group may be the indicator of increased collagen synthesis on the anastomotic line. Although expected significant values could not be acquired, thermal preconditioning and preoperative warming techniques may have a positive effect on the anastomotic stability. As this effect could not yield statistically significant results, it might be related to temperature and duration. Song et al. have investigated the effects of multiple heating (1-5 times) on blood flow of skin and muscle tissues in an experimental study and they found that the blood flow in the muscle and in the skin increased when 2-5 doses (43°C/1 h) were given every 3 days (45). Based on the result of this observation, the possibility of using the thermal procedures for increasing the stability of colonic anastomoses should be analyzed in a different designed research.

In conclusion, colonic anastomoses performed with thermal preconditioning technique, increase the collagen synthesis on the anastomosis line in the early period of healing, and it is obvious that this technique could contribute to the anastomosis stability. Although the other parameters do not support our opinion, we believe further experiments about optimum heat and time would be a guide.

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