The effect of Omega-3 fatty acid and ascorbic acid on healing of ischemic colon anastomoses

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PURPOSE: *Many systemic and local factors contribute to gastrointestinal tract anastomoses dehiscence, which is a serious and potentially fatal postoperative complication. The aim of this study was to evaluate the effects of omega-3 fatty acid and ascorbic acid on the healing of ischemic colon anastomosis.*
PATIENTS AND METHODS: *40 Wistar Albino rats weighing between 180 and 220 g were divided into four groups. Groups were assigned as follows; Group 1 (control): anastomosis and no treatment, Group 2: anastomosis plus ascorbic acid, Group 3: anastomosis plus omega-3 fatty acid, and Group 4: anastomosis plus ascorbic acid and omega-3 fatty acid. Colon anastomoses was were performed in all rats. All animals were sacrificed on the 5th postoperative day. Healing of the anastomoses was assessed by measuring the burst pressures (BP) and hydroxyproline levels.*
RESULTS: *No mortality was observed and perianastomotic abscesses were not noted in any rats. The BP was significantly higher in the ascorbic acid plus omega-3 fatty acid combination group than the other groups ($p<0.05$). The hydroxyproline levels were significantly high in ascorbic acid plus omega-3 fatty acid combination group than the other groups ($p<0.05$).*
CONCLUSION: *Dietary supplementation with omega-3 fatty acid and ascorbic acid improved colonic anastomoses healing. Ascorbic acid and omega-3 fatty acid enhance the colonic wound healing process by additive action.*

KEY WORDS: Ascorbic acid, Colon anastomoses healing, Omega-3 fatty acid.

Introduction

Many systemic and local factors contribute to gastrointestinal tract anastomoses dehiscence, which is a serious and potentially fatal postoperative complication.[1,2] Ischemia and local infection are the two most important factors that interrupt healing [3,4]. Connective tissue healing (collagen synthesis) and inflammatory processes are important in colonic anastomosis at ischemic segment. Moreover, the center of each wound is extremely low in oxygen tension compared with the edges and the surrounding normal tissue [5,6,7]. Preventing or reducing the ischemic damage, inflammatory processes and increase of connective tissue are the goals of the surgical intervention.

The effect of nutritional route and enriched diets on colonic anastomoses healing has been studied on rats [8,9,10]. Ascorbic acid and omega 3 are important dietary supplements taking part at wound healing process.

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Ascorbic acid is an important modulator of collagen production, acting as a cofactor for the hydroxylation of proline and lysine residues in procollagen [8]. Dietary omega-3 fatty acid is derived primarily from fish oil. The beneficial effects of omega-3 fatty acids on connective tissue healing and inflammatory disease have been widely reported in many studies [11,12]. The purpose of this study was to examine the effects of ascorbic acid and omega-3 fatty acid on ischemic colon anastomoses in the rat model.

Material and Methods

Animals

Forty adult male Wistar rats weighing between 180 and 220 g were divided into four equal groups (n = 10). The animals were fed with standard laboratory diet and received water ad libitum. A standard left colon resection and end-to-end anastomosis were performed on rats with ischemic colon. Groups were assigned as follows;
Group 1 (control): anastomosis and no further treatment.
Group 2: anastomosis plus dietary supplementation with ascorbic acid starting 5 days preoperatively. (200mg/kg, intraperitoneal, Vitabiol-C, IE, Ulugay, Turkey)
Group 3: anastomosis plus dietary supplementation with omega-3 fatty acid starting 5 days preoperatively. (13 mg/kg, per-oral by gavage, Marinkap cap, Kocak, Turkey)
Group 4: anastomosis plus dietary supplementation with both ascorbic acid (200mg/kg i.p.) and omega-3 fatty acid (13mg/kg p.o.) starting 5 days preoperatively.

Surgical Procedure

The animals were anesthetized by intramuscular ketamine hydrochloride injection (50 mg/kg of body weight, Pfizer). Abdominal wall was shaved and 10% Povidone–Iodine solution was used for disinfection. After the midline abdominal incision, distal colon was found. In all groups, ischemia was formed in a 4 cm-colonic segment, 3 cm proximal to peritoneal reflection, by ligating marginal arteries and vasa recti with 4/0 silk. The ischemic segment was resected at midpoint and reanastomosed end to end with 6/0 polypropylene suture. Then, the abdomen was closed with continuous sutures of 3/0 silk. After surgery, the animals were allowed to feed. All animals were sacrificed on the fifth day.

Measurement of Colonic Burst Pressure

Five days after the operation, the abdominal incision was reopened. The anastomotic line was recognized by polyprolene sutures. Burst pressure (BP) was measured in situ without detaching adhesions unless necessary. At least 2 cm colonic segments apart from anastomose was prepared. After careful removal of feces, the proximal bowel segment was connected to an infusion pump and the distal bowel segment was ligated. The abdomen was filled with saline, and this segment was insufflated with air at a constant rate of 6 mL/min. Burst pressure was measured by an insufflation pump that was sealed intraluminally to the proximal tip of the segment. Insufflation was at a constant value of 6 mL/min under the water, and the pressure level, where air bubbles showed up on the anastomosis line, was accepted as the burst pressure value.
BP was recorded at which leakage of air bubbles was noted.

Hydroxyproline Determination

Perianastomotic bowel segment at least 2 cm in length was kept in a 270°C deep freezer for hydroxyproline determination. Hydroxyproline measurement was performed by spectrophotometer at 560 nm with a technique explained by Prockop and Kivirikko [13].
The colonic segment that was 0.5 cm proximal and 0.5 cm distal to the anastomosis line, was resected, weighed and homogenized with saline into 20% (20% g/mL) homogenates by using a Potter type glass stirrer (Heidolph - RZR 2021, Germany). Homogenates were centrifuged at 1500 rpm for 15 min and obtained supernatants were hydrolyzed by adding hydrochloric acid of equal amounts for 16-18 h. By using the hydroxyproline kit (Hipronisticon, Organon, Holland) and working on the principles of Stegeman and Stadler, hydroxyproline amount was calculated in microgram per milligram of wet tissue by reading the absorbance of solution on spectrometry at 560 nm.

Statistical Analysis

Analysis of variance (ANOVA) was used for statistical analysis and a P value of less than 0.05 was considered to be significant.

Results

No mortality was observed and perianastomotic abscesses were not noted in any rats. All bursts occurred along the anastomotic line in all groups. A statistically significant difference was found between the BP values of the control group and all dietary supplementation groups ($p<0.05$) (Group 1 *vs.* Groups 2, 3 and 4). The BP was significantly higher in the ascorbic acid plus omega-3 fatty acid combination group than the other supplementation groups (Group 4 *vs.* Group 2 and Group 4

TABLE I - *Burst pressure and hydroxyproline levels of all groups*

	Burst Pressure (mmHg) (mean+SD)	Hydroxyproline (mg/100 mg tissue) (mean+SD)
Group 1	0.92+0.08	141.66+24.8
Group 2	1,71+0.19	198.33+18.0
Group 3	1,53+0.16	216.66+28.06
Group 4	1.86+0.26	227.5+28.95

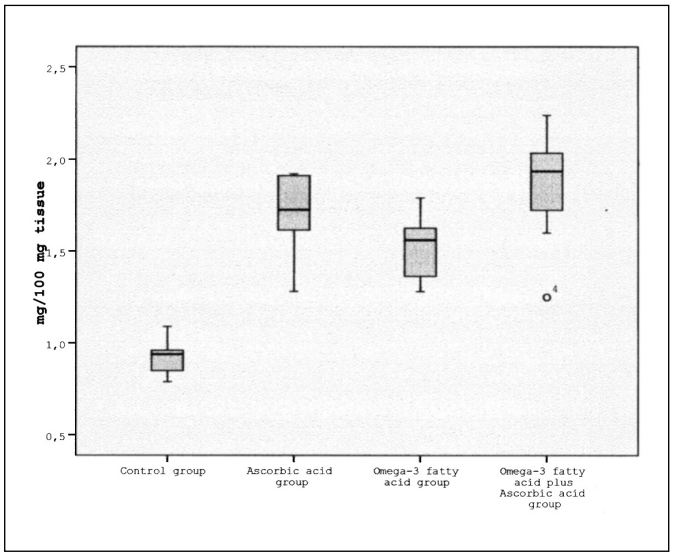

Fig. 1: Hydroxyproline level median values of all groups.

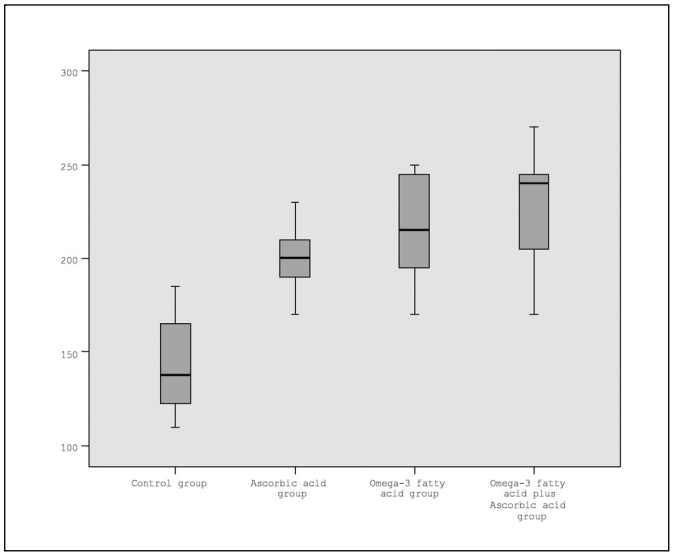

Fig. 2: Burst pressure median values of all groups.

vs. Group 3) (p<0.05). The hydroxyproline levels were significantly higher in all supplementation groups than control (Group 2, 3 and 4 *vs.* Group1) (p<0.05). The hydroxyproline levels were significantly high in ascorbic acid plus omega-3 fatty acid combination group (Group 4) than the other supplementation groups (Group 4 *vs.* Group 2 and Group 4 *vs.* Group 3) (p<0.05). The results are summarized in Table I. Burst pressure and hydroxyproline level median values of all groups are shown in Fig 1, 2.

Discussion

Dehiscence of colonic anastomoses still remains a serious and potentially fatal postoperative complication [14]. The healing process of intestinal anastomosis involves complex biochemical and cellular steps, with collagen synthesis being of fundamental importance. The basic mechanisms involved in the healing process are: aggression, inflammation, collagen metabolism, epithelialization, and wound contraction [15]. Many systemic and local factors contribute to the success or failure of this process [1,2,16]. Acute hemorrhage, hypoproteinemia, deficiency in factor XIII or vitamin C, and disturbances in coagulation may negatively affect wound healing [16,17]. The use of pharmacological agents can affect healing, as previously demonstrated by several authors using corticosteroids, pentoxifylline, antineoplastic drugs, and non-hormonal anti-inflammatories [15]. Furthermore, different dietary supplements have been used to contribute wound and anastomoses healing in many studies [10,18]. Among these, ascorbic acid and omega-3 fatty acid are essential ingredients of the daily human diet and their supplementation have been proven to promote wound healing [19,20]. Ascorbic acid is essential to the process of gastrointestinal tract healing through its role in procollagen secretion from the intestinal smooth muscle. The healed gastrointestinal anastomoses layer shows predominance of type I collagen (68%), with type III (20%) and type V (12%) making up the remainder.[21] Ascorbate contributes to several metabolic processes including efficient hydroxylation of hydroxprolin in elastin, collagen, and proteins with collagenous domains. Its deficiency leads to inability to secrete procollagen into the extracellular space and also decreases the tensile strength in experimental models of wound repair [21-23].

The n-3 and n-6 polyunsaturated fatty acids are essential dietary constituents. Dietary omega-3 fatty acid makes up an appreciable part of the fat intake in diets rich in cold-water fish and seal meat [24]. It represents a class of systemic modulator agents that affect eicosanoid and cytokine production, and influence expression of some membrane receptors or activity of cell activation enzymes such as protein kinase [25,26]. Endres et al. [27] and Billiar et al [28]. have showed that omega-3 fatty acid supplementation have inhibitory effect on the production of cytokines and arachidonic acid metabolites. This effect on eicosanoid synthesis has been associated with an

improved immunocompetence and it reduces inflammatory response to injury [29]. Omega-3 fatty acids have beneficial effects of on connective tissue healing and inflammatory disease[11,12]. The overwhelming clinical effect of diets high in omega-3 fatty acid is a reduction in inflammation [24]. Alexander et al. [30] has shown improved protein synthesis after burn injury in rats receiving omega-3 fatty acid supplementation. Weiss et al. [20] showed a trend toward lower post-surgical infection rates and significantly shorter hospital stay for patients fed with omega-3 fatty acid on perioperative days. Daly et al. [31] demonstrated that postoperative enteral nutrition with supplemental omega-3 fatty acid instead of a standard enteral diet significantly improved immunologic, metabolic and clinical outcomes in patients with upper gastrointestinal malignancies who were undergoing major elective surgery. Therefore, a thorough understanding of the influence of dietary fat intake that may influence the outcome of in the surgical patient is of great importance for gastrointestinal anastomoses. Similarly, in our study, we showed that ascorbic acid and omega-3 dietary supplementations have positive effect on ischemic colon anastomosis both individually and in combination compared with the control group.

In conclusion, dehiscence of colon anastomosis is a serious complication associated with high mortality and morbidity. Therefore, many studies have been carried out for enhancing anastomosis healing. The results of the present study indicated that ascorbic acid and/or omega 3 fatty acid feeding improve anastomosis healing. However further studies are needed to support these findings and randomized clinical trials should be done to emphasize its clinical significance.

Riassunto

Molti sono i fattori sitemici e locali che contribuiscono al verificarsi di deuscenze delle anastomosi del tratto gastrointestinale, che rappresentano una complicazione grave e potenzialmente letale. Lo scopo del presente studio è stato quello di valutare gli effetti degli acidi grassi omega-3 e dell'acido ascorbico sulla guarigione di anastomosi ischemiche del colon.

Lo studio è stato condotto su 40 ratti Wistar Albini del peso compreso tra 180 e 220 g, divisi in quattro gruppi, suddivisi secondo il seguente schema: Gruppo 1 di controllo, con anastomosi senza alcun trattamento; Gruppo 2, con anastomosi e trattamento con acido ascorbico; Gruppo 3, con anastomosi e trattamento con acidi grassi omega-3; Gruppo 4, anastomosi e trattamento con acido ascorbico ed acidi grassi omega-3.

Su tutti i ratti sono state effettuate anastomosi sul colon, e tutti gli animali sono stati sacrificati alla 5a giornata postoperatoria. Lo stato di guarigione delle anastomosi è stato valutato con la pressione di scoppio (BP) e dai livelli di ossiprolina.

Non si sono avuti decessi spontanei nè formazione di ascessi perianastomotici. La pressione di scoppio è risultata significativamente più elevata nel gruppo 4 trattato con acido ascorbico ed acidi grassi omeha-3 rispetto a tutti gli altri gruppi ($p<0,05$). Anche i livelli di ossiprolina sono risultati più elevati nel Gruppo 4 rispetto agli altri gruppi ($p<0,05$).

‹l risultato è che una dieta arricchita con acido ascorbico ed acidi grassi omega-3 appare migliorare la guarigione delle anastomosi coliche, e quella delle ferite del colon con una azione che risulta sommatoria.

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