

The healing effect of shark cartilage in rabbits after colonic anastomosis



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Barlas Sulu*, Mete Cihan**, Yusuf Gunerhan*, Mahmut Sozmen***

*Kafkas University Faculty of Medicine, Department of General Surgery, Kars, Turkey

**Kafkas University Faculty of Veterinary Medicine, Department of Surgery, Kars, Turkey

***Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Pathology, Samsun, Turkey

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AIM: Shark cartilage has anti-inflammatory, analgesic, anti-angiogenic, anti-tumoral, and immunomodulatory properties. We studied the effect of shark cartilage on the healing of colonic anastomoses, which are among the gastrointestinal system anastomoses that most commonly cause leakage.

MATERIAL OF STUDY: Rabbits were divided into two groups of seven as the study and control groups. A normal diet was given to both groups before and after right colonic anastomosis. Shark cartilage tablets were given orally to the study group for five days before and after the anastomosis. Bursting pressures, hydroxyproline levels and translocation of the intestinal flora in anastomosis region were evaluated on the 6th day by operating on both groups.

RESULTS: Bursting pressure and hydroxyproline levels were higher in the experimental group compared to the control group ($p < 0.05$). An increase in connective tissue and vascularization without growth of microorganisms was observed in the experimental group on microbiological examination.

CONCLUSIONS: Shark cartilage given orally to rabbits increased anastomotic healing and did not cause serious consequences such as bacterial translocation.

KEY WORDS: Anastomosis, Colon, Rabbits shark cartilage, Surgery

Introduction

The postoperative separation of a gastrointestinal system anastomosis is an important and serious complication. Prolongation of hospital stay, increase in treatment cost, one or more re-operations to control sepsis and an intestinal stoma are expected results¹. The mortality rate after leakage has been reported to be 10-15%².

Large bowel anastomoses differ from anastomoses of gastrointestinal system in other localizations in terms of the intestinal flora and the balance between collagen production and destruction. The risk of anastomosis leakage is higher in the large intestines than the other regions due to both the higher number of pathogenic microorganisms and the high collagenase enzyme activity. Anastomosis leakage is observed at a rate of 1% in the small intestines but can be as high as 30% in the large intestines³⁻⁵.

Changes in colonic flora are also known to affect anastomosis healing. The preparations before elective colon resections negatively affect mucosal healing by changing colonic flora and also lead to translocation^{6,7}.

Shark cartilage is used in China as a health product for a thousand years. Cartilage is an acidic mucopolysaccharide/protein complex with avascular properties that contains components such as collagen, glycosaminoglycans and chondroitin sulfate. Shark cartilage was found

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Correspondence to: Corresponding Author: Barlas Sulu, Kafkas University Faculty of Medicine, Pasacayiri 36100, Kars/Turkey (e-mail: barlas.sulu36@gmail.com)

to have anti-analgesic and anti-inflammatory characteristics, and to protect against bacterial, viral and fungal infections by stimulating the immune response and T cells⁸⁻¹⁰. It is therefore now used as an alternative treatment in diseases such as psoriasis, osteoarthritis, and diabetic retinopathy. Each year, 30 million dollars are spent on shark cartilage products used by nearly 50.000 Americans^{11,12}.

We studied the effect of shark cartilage on healing criteria such as bursting pressure, hydroxyproline level and bacterial translocation in rabbits that had undergone right colonic anastomosis after colon preparation and fed shark cartilage before and after the surgery.

Materials and Methods

ANIMAL MATERIAL

14 adult New Zealand Rabbits with an average weight of 3 kg (2.7-3.2) were used in the study. The necessary permission to use animals for experimental purposes within the scope of the study was obtained from the Kafkas University Ethics Committee (Ethics Committee Code: 14.05.2009/12).

PREOPERATIVE PROCEDURES

Rabbits were fed a normal diet for 5 days. During this period, they were checked in terms of food intake and defecation and a physical examination was performed. At the end of this period, the rabbits were divided into two groups of 7 as the study and control groups. One tablet of a preparation containing 750 mg shark cartilage powder per tablet (Shark Cartilage 750 mg., GNC) was given orally each day for 5 days to all rabbits in the experimental group while the normal diet was continued. The control group was fed with their normal diet during the same period. Colon preparation was performed by causing diarrhea with 1ml/kg anthraquinone (Indian Oil) the day before the surgery in both groups. No food was provided except water to all rabbits for 12 hours before the surgery.

SURGICAL PROCEDURES

All rabbits were anesthetized with the application of Xylazine HCl (Alfazyne) 5 mg/kg IM and then Ketamine HCl (Ketalar, Pfizer, Turkey) 30 mg/kg IM. Following the shaving and disinfection of the abdominal region, laparotomy was performed from the median-line. The colon was externalized from the incision. A 2-3 cm-long piece was resected from the colon. The ends exposed after the resection were reconnected with an end-to-end anastomosis technique. Ten to twelve interrupted 6/0 polypropylene (Prolene, Ethicon) inverting sutures were used in the anastomosis. The abdominal fascia was closed with 3/0 continuous polyglactine (Polysorb, Tyco) sutures, and the skin was closed by continuous suture with 3/0 silk.

POSTOPERATIVE PROCEDURES

Antibiotic administration (40mg/kg, ceftriaxone disodium IM, FAKO, Turkey), wound care and routine clinical examinations were performed for 5 days after the operation. During this period, rabbits in both the study and control groups were fed a normal diet. At the same time, one tablet of shark cartilage was given orally to the rabbits in the study group each day. The rabbits were anesthetized at the 6th day following the operation. The abdominal cavity was opened and the anastomosis region was controlled by bringing outside of the abdominal cavity. Materials were collected from blood, intestinal content and regional lymph nodes for microbiological examination. The anastomosis pressure was measured and subsequently a piece of tissue was taken from the anastomosis region for histopathological examination.

BURSTING PRESSURE MEASUREMENT

A ligature was performed 3 cm distal to the anastomosis that was exteriorized. A hole was created 4 cm proximal to the region. The air pump and pressure gauge tubes were inserted from here and bound tightly. The anastomosis region was put into a container full of water and then air was pumped slowly. Pumping was continued until the air came out of the anastomosis region. The last pressure at the time of bursting was recorded. The unit of pressure used was mmHg.

HYDROXYPROLINE MEASUREMENT

After the bursting pressure was determined, the perianastomotic region was cleared. A 1-cm colon segment (0.5 cm from each side of the anastomosis) was resected for hydroxyproline determination and stored at -40°C. Hydroxyproline content determinations of the samples were performed as described by Jamall et al¹³.

HISTOPATHOLOGICAL EXAMINATION

Anastomosed intestine samples were fixed in 10% buffered formalin and embedded into paraffin. 5 micron thick sections were obtained from each paraffin block included in routine tissue follow-up and stained with hematoxylin-eosine (HE) for histopathological and light microscope examination. Histomorphological evaluation was performed by looking at 10 different sites, as mild (+), moderate (++) and severe (++)¹⁴.

MICROBIOLOGICAL EXAMINATION

Samples taken from the intestinal content, blood serum and mesenteric lymph nodes of rabbits were cultured onto 7% defibrillated human blood agar (Blood Agar Base, Oxoid) under aerobic conditions. They were incubated at 37°C for 24 hours and analyzed according to routine criteria.

STATISTICAL ANALYSIS

SPSS for Windows® 10.0 was used for the statistical analysis. The differences between the groups in terms of bursting pressure and hydroxyproline content were

assessed using the Mann-Whitney U test. The statistical significance was set to $p < 0.05$.

Results

No adverse clinical finding was observed in the preoperative and postoperative checks of the rabbits. A mild adhesion to the surrounding tissues in the anastomosis region was detected on postmortem examinations in one rabbit in the control group. No other pathological feature was found.

MICROBIOLOGICAL FINDINGS

No pathogenic species were found in either group in intestinal content, lymph node and blood serum cultured on 7% defibrillated human blood agar under aerobic conditions. No growth was observed in cultures from serum while gram-positive and gram-negative bacilli were occasionally observed in mesenteric lymph nodes. Non-hemolytic gram-negative bacilli were detected throughout the intestinal content.

BURSTING PRESSURE MEASUREMENTS

139.29 mmHg (125-150 mmHg) of pressure was obtained in the experimental group, 85.00 mmHg (68-102 mmHg) pressure was obtained in the control group ($p < 0.05$) in bursting pressure measurements performed in anastomosis regions.

HYDROXYPROLINE LEVELS

Hydroxyproline levels were 4.59 ± 0.26 (Mean \pm SD g/mg tissue) in the shark cartilage group and 2.55 ± 0.21

(Mean \pm SD g/mg tissue) in the control group ($p < 0.05$).

HISTOPATHOLOGICAL RESULTS

Histopathological findings are summarized in Table 1 according to their severity. Histopathological lesions of various degrees were found in both groups (Table 1). The intestinal lamina propria and submucosa showed hemorrhage and edema as well as leukocyte infiltration with mononuclear cells consisting (MNC) mainly of plasma cells and lymphocytes, ranging from mild to moderate, and accompanying eosinophils and neutrophils in the both control (Fig. 1) and experimental groups. A more significant connective tissue reaction (Fig. 2), together with elevated vascularization (Figs. 2, 3) were noticed in the study group compared to the control group. A distinct neutrophil leukocyte infiltration, necrotic regions (Fig. 4), bleeding and bacterial colonies were observed in the muscle layers of the study group rabbits.

Discussion

Healing of colon anastomosis is a model of wound healing including different processes. The key factor determining the strength of colonic anastomosis is the balance between the synthesis and degradation of collagen.

TABLE 1 - Histopathological grading scores among the control and experimental groups.

Histopathological Findings	Control group	Experimental group
<i>Lamina propria</i>		
MNC infiltration	++/+++	+/++
PMNC infiltration	+	+/++
Edema	+	+/++
Hemorrhage	+	++
<i>Submucosa</i>		
MNC infiltration	+++	+/++
PMNC infiltration	+/+++	++/+++
Edema	+	+++
Hemorrhage	+	+/++
Connective tissue proliferation	+/++	+++
Neovascularization	++/+++	+++
Dilatation of lymphatics	-	+++
<i>Tunica muscularis</i>		
PMNC infiltration	+++	+++
Necrose	+++	++/+++
Hyalinization	+	+
Bacterial aggregates	+	+/++
Hemorrhage	+	+/++

Legend: MNC: mononuclear cells; PMNC: polymorphonuclear cells.

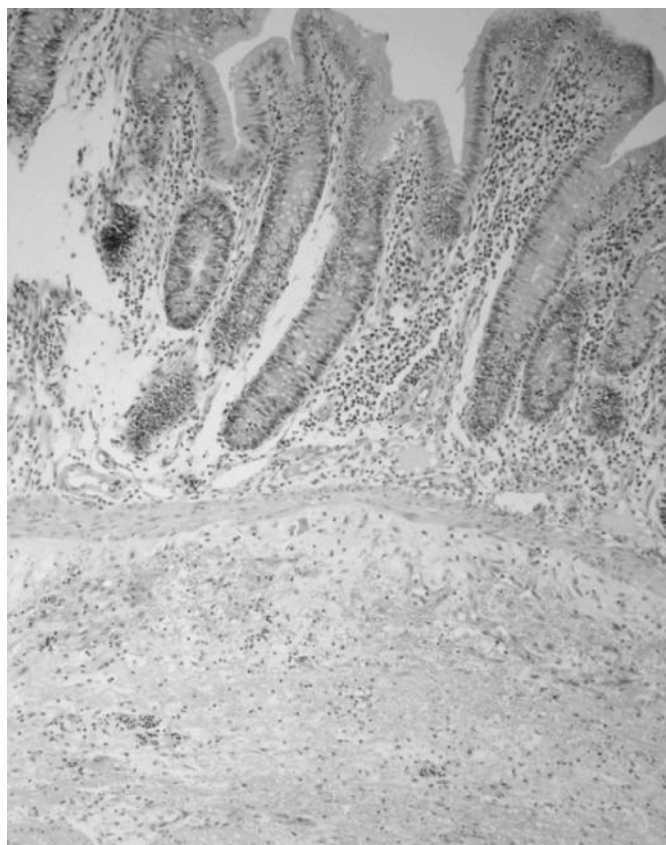


Fig. 1: MNC infiltrations in the lamina propria. Edema and hemorrhage in the submucosa. Control Group, HEx100.

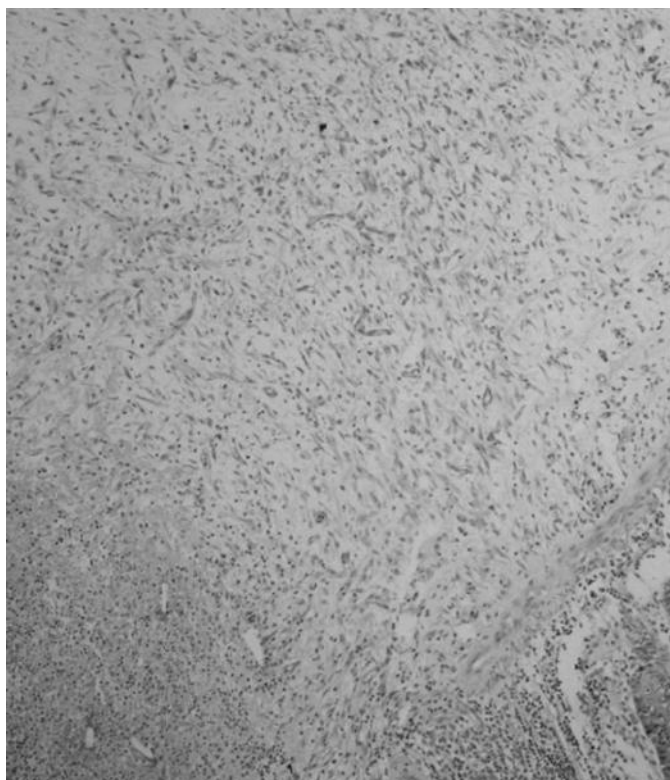


Fig. 2: MNC infiltrations in the lamina propria. Connective tissue proliferation vascularisation and edema in the submucosa. Study Group, HEx100.

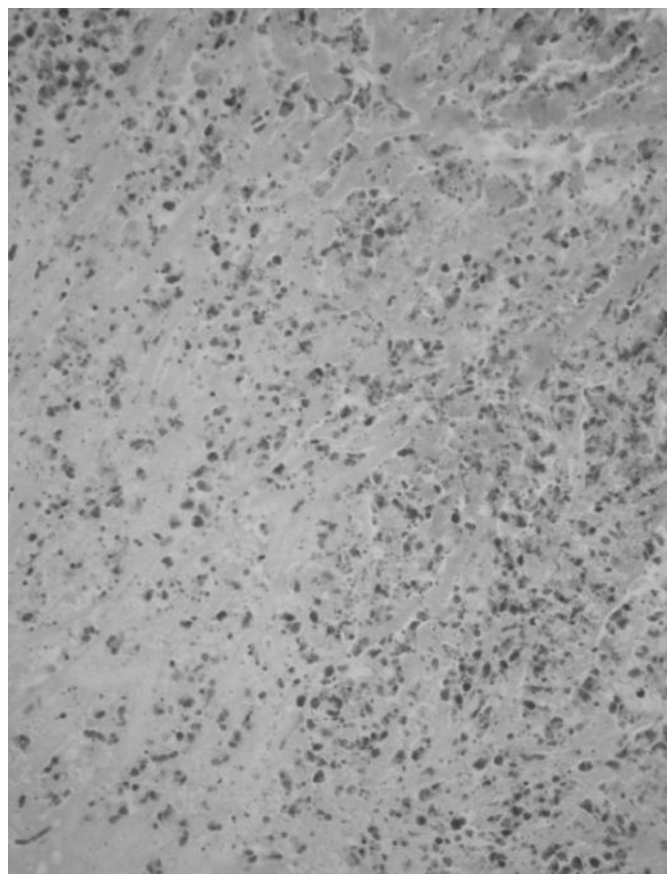


Fig. 4: Degenerated neutrophils and necrosis in muscle fibers. Study Group. HE x400.

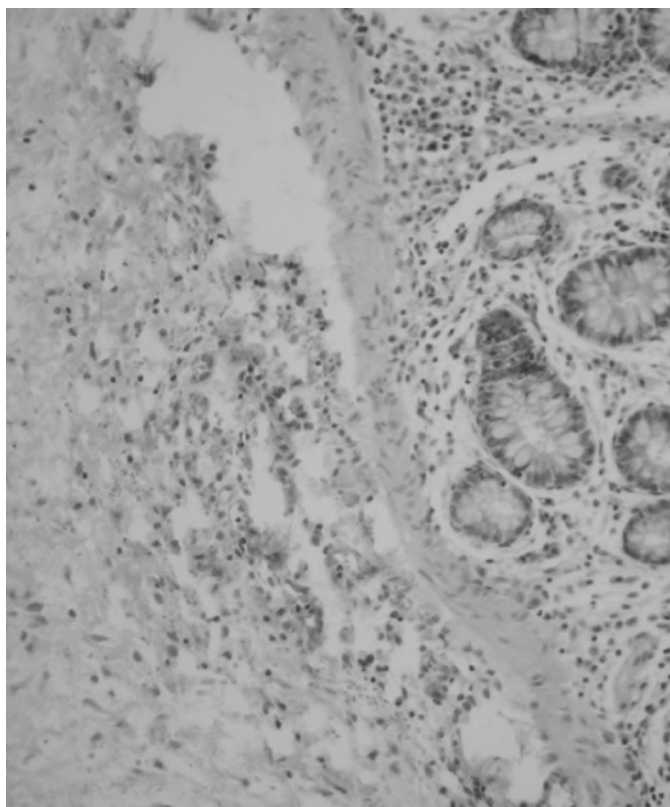


Fig. 3: Increased vascularization and edema in the submucosa. Study Group. HE x200.

Collagenase activity, which has a role in collagen degradation, has been reported to be more active in colon mucosa than other regions of the gastrointestinal system¹⁵. Degradation is more prominent in the first few days of wound healing while the strength of the anastomosis increases in a manner parallel to increasing collagen synthesis in the following days. Patient-related factors such as malnutrition, obesity, steroid use, advanced age, history of radiation, the presence of Crohn's disease or diverticulitis; and surgical factors such as poor surgical technique, prolonged operation time, blood loss, bowel preparation, and use of a pelvic drain are known to be able to adversely affect this course and thus cause anastomotic leakage^{1,16}.

Shark cartilage has been used in experimental studies in order to heal the wound and inhibit inflammation since the early 1950s¹⁷. Prudden et al. have reported that cartilage affects the reticulum, fibroblast density and collagen synthesis during tissue repair and accelerates healing in the first three days after the injury. The wound has been reported to heal 20% faster than the control group with tensile strength increased by 40%, and herniation and disintegration of the wound reduced¹⁰. Studies per-

formed in the following years reported shark cartilage's anti-inflammatory mechanism of action^{9,18} Fontenele et al. have reported that the anti-inflammatory impact occurs via inhibiting nitric oxide biosynthesis and Lee et al. have reported that it occurs via inhibiting the vascular endothelial growth factor^{9,18}. In addition, shark cartilage extract has also been identified to have a fibrinolytic effect on fibrinogen and fibrin, which play major role in events such as wound healing, inflammation, and blood clotting^{19,20}.

Studies related to the oral use of shark cartilage have increased in recent years. The oral intake of chondroitin sulfate and glucosamine sulfate obtained from shark cartilage were determined to show absorption and bioavailability^{21,22}. Uebelhart et al. reported that orally given chondroitin sulfate has a preventive effect on cartilage degeneration in rabbits²³. The oral use of Neovastat (AE-941), a shark cartilage extract, was reported to reduce the tumor volume and number of nodules in mouse lungs²⁴.

Despite these reports, the effect of cartilage on the gastrointestinal system is unknown. The role of shark cartilage on colon anastomosis was evaluated for the first time in this study and its effects on the gastrointestinal system evaluated. Experimental studies have shown that there is a correlation between the strength of anastomosis, collagen synthesis and bursting pressure¹⁵. The strength of an anastomosis is therefore assessed by measuring the bursting pressure. Orally given shark cartilage in the pre-operative and post-operative periods was determined to significantly increase the hydroxyproline levels, indicating collagen synthesis, and increase the bursting pressure in a parallel manner. Furthermore, it was also histologically determined to have a positive effect on wound healing in the anastomosis region. In particular, an increase in connective tissue and new vessel generation was observed compared to the control group. Shark cartilage's accelerative effect on wound healing and effect on increasing collagen formation are among the possible reasons for this.

In experimental studies, shark cartilage was shown to inhibit matrix metalloproteinases (MMPs) that are effective in the formation of inflammatory diseases such as ulceration of the stomach, skin and cornea, vascular diseases, bacterial meningitis and rheumatoid arthritis^{25,26}. MMPs show extracellular protease properties and are composed of enzymes such as collagenases, gelatinases, and stromelysin involved in the degradation of intercellular matrix²⁶. Collagenases play important roles in anastomosis integrity and suture holding capacity in the first days of wound healing. The collagenase level increases and suture holding capacity decreases to 80% at the 3rd day after anastomosis. Inhibition of collagenase activity was shown to reduce collagen degradation, indirectly increase collagenase in the anastomosis region and accelerate wound healing in experimental studies²⁷. A significant increase in bursting pressure depending on the hydroxyproline amount showing collagen synthesis in the

anastomosis region compared to the control group suggests that it may be due to the effect of collagenase inhibition of shark cartilage in this study. The presence a more significant histological connective tissue reaction in the experimental group also seems to support this situation. Increased vascularization in the anastomosis region on histological examination shows that shark cartilage may have an angiogenesis inducing property in the gastrointestinal system, contrary to common opinion.

The effect of shark cartilage on intestinal flora and translocation were also examined in our study. Bacterial translocation is defined as intestinal intraluminal live bacteria to pass from epithelial mucosa to lamina propria, and from here to mesenteric lymph nodes and distant organs²⁸. Deterioration of intestinal flora and proliferation of bacteria in the intestinal lumen are important causes of translocation. This situation may lead to sepsis and multi-organ failure in patients who undergo surgery. Seehofer et al. have determined bacterial translocation at high rates in mesenteric lymph nodes after colon anastomosis in their study on rats²⁹. In our study, while bacterial growth was not observed in blood samples, partial bacterial growth in one of the mesenteric lymph node in each of both groups and determination of no bacteria other than the bacteria in normal flora show that shark cartilage did not cause bacterial translocation in the colon.

In conclusion, the effect of shark cartilage on gastrointestinal system was analyzed for the first time in this study. Shark cartilage was determined to increase the strength of the anastomosis by leading collagen synthesis and better vascularization in the anastomosis region in the study. In addition, it was determined to not disturb the intestinal flora and not cause translocation.

Riassunto

Le proprietà antinfiammatorie, analgesiche, antiangiogeniche, antineoplastiche ed immunomodulatrici della cartilagine di squalo sono note. Noi abbiamo studiato l'effetto di questa cartilagine sul processo di guarigione delle anastomosi coliche, che rappresentano la più comune incidenza di deiscenze nell'ambito dell'apparato gastroenterico.

Per lo studio sono stati impiegati 14 coniglio divisi in due gruppi di 7 ciascuno, uno destinato allo studio sperimentale e l'altro a gruppo di controllo. Prima e dopo l'esecuzione dell'anastomosi è stata somministrata a tutti gli animali una dieta normale. Compresse di cartilagine di squalo sono state somministrate al gruppo sperimentale per i cinque giorni precedenti e successivi alla confezione dell'anastomosi. Al sesto giorno postoperatorio sono stati studiati in entrambi i gruppi la pressione di rottura dell'anastomosi, i livelli di idrossiprolina e la traslocazione di flora intestinale a livello della zona dell'anastomosi.

La pressione di rottura ed i livelli di idrossiprolina sono risultati più elevati nel gruppo di studio rispetto a quello di controllo ($p < 0,05$). Nel gruppo di studio si è registrato un aumento del tessuto connettivo e della vascolarizzazione, senza aumento di crescita dei microrganismi.

Si conclude che la cartilagine di squalo somministrata oralmente ai conigli migliora i processi di guarigione postanastomotica senza gravi conseguenze quali la traslocazione batterica.

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