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Protective effects of thymoquinone on the healing process of experimental left colonic anastomosis

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ABSTRACT

Purpose: Colorectal cancer is globally the third most common cancer. Anastomotic complications remain to be an important issue for colorectal surgery. The aim of this study was to investigate the protective effects of thymoquinone (TQ) on the healing process of left colonic anastomosis in an experimental model.

Methods: Thirty-two male rats were divided into two groups, as the TQ group and the control group. TQ was administered to the TQ group, whereas the control group was given a standard feed and water for 2 wk. Following the creation of a left colonic anastomosis, subjects in both groups were sacrificed on the postoperative (PO) third and seventh days. Anastomotic burst pressures were measured mechanically. Immunohistochemical stainings for proliferating cell nuclear antigen, cluster of differentiation (CD) 31, CD45 were performed, and the matrix metalloproteinase-2 levels were measured. Histologic total scores were calculated according to Ehrlich-Hunt model. A value of $P < 0.05$ was considered as statistically significant.

Results: One rat in the control group that died on the PO fourth day was excluded. Anastomotic burst pressures on the PO seventh day were higher in the TQ group than the control group ($P < 0.01$). Histopathological total scores on the PO third and seventh days were higher in the TQ group ($P < 0.01$). In addition, the TQ group revealed lower matrix metalloproteinase-2 scores on the PO third day and higher hydroxyproline levels on the PO seventh day ($P < 0.05$ and $P < 0.01$, respectively).

Conclusions: The use of TQ in colorectal surgery cases with left-sided colonic anastomosis resulted with increased anastomotic burst pressures and increased tissue hydroxyproline levels.

Introduction

Colorectal surgery operations are commonly applied in conditions including ischemic colitis, ulcerative colitis, Crohn's disease, mechanical intestinal obstructions, trauma, recurrent diverticulitis, and especially in colorectal cancer (CRC). Anastomotic leakage is a lethal complication of colorectal surgery, and the incidence is reported to be 1%-19%.[1] The risks of anastomotic leakage and other anastomotic complications increase as the site of the intervention moves

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distally from the Treitz ligament. The risk of leakage after proximal small bowel anastomoses is 1%, but this risk can be up to 16% after anterior colon resections.[2] Possible causes of anastomotic leaks include ischemia, inappropriate surgical technique, tension in the anastomotic line, composition and function of the gut microbiome, local infection, and obstruction distal to the anastomosis.[3,4] Postoperative (PO) mortality due to anastomotic complications is reported to be between 6%-22%.[1]

Recent clinical and experimental studies have shown that thymoquinone (TQ), the active form of Nigella Sativa, has many therapeutic effects such as immunomodulatory, antibacterial, antidiabetic, hepatoprotective, gastroprotective, anticancer, antioxidant, and neuroprotective effects. Attoub *et al*. reported that in cell culture studies, TQ enhanced apoptosis in tumor cells and strongly inhibited the invasion *in vitro*.[5] In their study of an intestinal obstruction model, Kapan *et al*. reported that TQ effectively inactivated gram-positive and gram-negative bacteria by an unknown mechanism and indicated that the number of intestinal villi was increased due to the decrease of bacterial translocation in the TQ group.[6]

Studies that show whether TQ has an effect in an anastomosis model are limited in the literature. The aim of this study was to investigate the protective effects of TQ on left-sided colonic anastomosis by mechanical, histological, immunohistochemical, and biochemical methods in an experimental model.

Methods

This study was carried out at Istanbul University, Experimental Medicine Research Institute, Laboratory Animal Science Department with the approval of the Animal Experiments Local Ethics Committee of Istanbul University. The Animal Research: Reporting of In Vivo Experiments guidelines were followed, and the study was carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Thirty-two male Sprague-Dawley rats, with weights between 300 and 400 g, were used in the study. All subjects were fed with standard laboratory food and water. The subjects were divided into two main groups, as the "TQ group" and the "control group", each of which consisted of 16 rats. TQ (TQ; Sigma–Aldrich, Darmstadt, Germany) was administered at a dose of 50 mg/kg/d orally to the TQ group, whereas the control group was given a standard feed and water for 2 wk.

The subjects were anesthetized with intraperitoneal ketamine HCl (Ketalar; Pfizer, Istanbul, Turkey) injection of 50 mg/kg induced by intraperitoneal xylazine HCl (Rompun; Bayer, Leverkusen, Germany) at 5 mg/kg. Following the preparation of the incision site via shaving and the application of povidon iodine, a laparotomy was created with midline incision. After exploration and the creation of a full-thickness incision on the colon at a level of 3 cm proximal to the peritoneal fold, an anastomosis of the Gambee type was performed with round-pinned 5/0 Vicryl (Vicryl; Ethicon, Lidingö, Sweden) sutures. Subsequent closure of the abdomen was carried out with the use of sharp-pinned 4/0 silk sutures. Two equal doses of 20 mg/kg of subcutaneous meperidine (Aldolan; Gerot Pharmazautika, Vienna, Austria) were administered postoperatively and after 3 h for analgesia. Antibiotherapy was not used. Following the creation of a left colonic anastomosis, half of the subjects in both groups were sacrificed on the PO third day, and the remaining half was sacrificed on the PO seventh day via injection of 135 mg/kg sodium thiopental (Pental Sodium; IE Ulagay Pharmaceutical Industry, Istanbul, Turkey) intraperitoneally. The anastomosed distal colon segment in the previous operation was resected including the proximal and distal 2.5 cm healthy colon tissue of the anastomosis line.

The distal ends of all the resected intestinal segments were tightly bound with silk suture. The proximal end was connected to an air pump by inserting a polyethylene catheter into the lumen, and anastomotic burst pressures were measured mechanically. The intestinal segment was placed in a container filled with water. Air was introduced into the lumen at a rate of 2 milliliters/minute (mL/min). The pressure value calculated at the time of exit of the first air bubble from the anastomosis line was recorded as the anastomotic burst pressure.

Histopathologic examinations of the tissues were performed by an experienced histologist (blind) who did not know which group the samples belonged to.

Duplication of the genome occurs during the synthesis (S) phase of the eukaryotic cell cycle. The proliferating cell nuclear antigen (PCNA) expression represents the S phase of the cell cycle, which reveals the fraction of proliferating cells in the tissue.[7] Analyses of the tissue samples were performed for PCNA (Anti-PCNA antibody–ab2426; Abcam, Cambridge, UK) to measure fibroblastic activity at the site of anastomosis.

CD 31 is a transmembrane glycoprotein type I, a member of the Ig-superfamily of cell adhesion molecules.[8] CD31 expression is observed on endothelial cells and a majority of hematopoietic nonerythroid cells, including platelets, neutrophils, and T and B cell subsets.[8] Accumulated data indicate the role of CD31 in vascular barrier function integration, mechanical stress sensing, apoptosis prevention, and angiogenesis.[9] It has been also widely used as a marker of angiogenesis in immunohistochemical studies.[10] In the present study, CD31 (CD31 Mouse Monoclonal Antibody Colon: JC70; Cell Marque, Rocklin) was used to measure angiogenesis, which is an important component of anastomotic healing.

CD45 is a cell surface glycoprotein with a cytoplasmic tyrosine phosphatase domain that catalyzes the removal of phosphates from tyrosine residues in several substrates, and CD45 is believed to function in both the activation and suppression of lymphocytes as well as in T cell maturation.[11] Examinations for CD45 (CD45 Mouse Monoclonal Antibody LCA Colon: 2B11 & PD7/26; Cell Marque, Rocklin) was carried out to measure the degree of inflammatory infiltration that also takes place during the process of wound healing.

Matrix metalloproteinases (MMPs) are a family of enzymes that play a central role in extracellular matrix turnover during angiogenesis. MMPs 2 and 9, also called gelatinases, appear to be very important during angiogenesis as they are actively involved in the formation of new vasculature by degrading