Effect of Beta-Glucan on Intestinal Anastomoses in a Rat Model

Abstract

Objectives. The authors aimed to investigate the effect of β-Glucan on healing of an experimental left-sided colon anastomosis model.

Material and Methods. Twenty adult male Wistar albino rats were randomized into two groups which had colonic transection and end-to-end anastomosis. Group I (Control): anastomosis group, received no treatment (n = 10); group II, anastomosis + β-Glucan (50 mg/kg/day within seven days after surgical procedure). Bursting pressure, hydroxyproline levels and histopathological characteristics of the anastomosis were analyzed.

Results. The average burst pressure of Groups I and II were 106.67 ± 5.00 and 148.00 ± 11.35 mm Hg and hydroxyproline levels were 0.85 ± 0.14 and 1.45 ± 0.46 µg/mg, respectively. Both the burst pressure and hydroxyproline levels in group II were statistically significantly higher (p < 0.05). Histopathological examination revealed less epithelial damage in group II (p < 0.05). Though not statistically significant, less edema and damage to the submucosal-muscular layer was seen in Group II (p = 0.079).

Conclusions. Due to significant increases in anastomotic bursting pressures and tissue hydroxyproline levels and considering the inhibitory effect of β-Glucan on epithelial damage, edema, and submucosal-muscular layer damage, β-Glucan was thought to contribute to the healing of the anastomosis (Adv Clin Exp Med 2013, 22, 2, 157–163).

Key words: colon anastomosis, β-Glucan, burst pressure, hydroxyproline.

Streszczenie

Cel pracy. Autorzy zbadali wpływ β-glukanu na gojenie eksperymentalnego modelu lewostronnego zespolenia okrężnicy.

Materiał i metody. Dwadzieścia dorosłych samców szczurów albinosów szczepu Wistar podzielono losowo na dwie grupy, u których wykonano przecięcie okrężnicy i zespolenie koniec do końca. Grupa I (kontrolna): wykonano zespolenie, bez leczenia (n = 10), grupa II, wykonano zespolenie i podano β-glukan (50 mg / kg / dzień w ciągu siedmiu dni po zabiegu). Oceniono ciśnienie rozrywające, stężenie hydroksyproliny i cechy histopatologiczne zespolenia.

Wyniki. Średnie ciśnienie rozrywające grupy I i II wynosiło 106,67 ± 5,00 i 148,00 ± 11,35 mm Hg, a stężenie hydroksyproliny wynosiło 0,85 ± 0,14 i 1,45 ± 0,46 ng / mg. Zarówno ciśnienie rozrywające, jak i stężenie hydroksyproliny w grupie II były statystycznie istotnie większe (p < 0,05). Badanie histopatologiczne wykazało mniejsze uszkodzenie nabłonka w grupie II (p < 0,05). Chociaż nie było to istotne statystycznie, mniejszy obrzęk i uszkodzenie warstwy podśluzówkowo-mięśniowej zaobserwowano w grupie II (p = 0,079).
Anastomotic leakage after colorectal surgery is a major cause of morbidity and mortality. It is also associated with high rates of local recurrences and survival after curative resection of colorectal cancers. Anastomotic leak rates range from 0.5% to 30% in the literature [1–3]. Although there are differences among surgeons, the ratio is expressed as 3.4–6% amongst colorectal cancer surgery experienced surgeons [4]. Many factors, such as poor blood supply of the anastomosis, location of anastomosis, lack of technical capacity, colonic bacteria (bacterial flora of the colon, colonic bacteria distribution or content), inflammation, age, obesity, smoking, hypoalbuminemia, cardiovascular disease (CVD) and chemotherapy, and use of drugs such as dexamethasone, adversely affect the healing of the anastomosis [1, 4].

β-Glucan is a glucose polymer found in the cell walls of yeast, fungi and cereals. It has beneficial effects on the immune system and is accepted as a booster for the immune system, and it has no toxic or side effects [5, 6]. The biological activity of β-Glucan is by binding to β-Glucan receptors on monocytes, leukocytes and macrophages, increasing the release of cytokines and arachidonic acid metabolites, and stimulation of hematopoiesis [7, 8]. The immune reaction and the formation of collagen in the early period is important for wound healing and this happens with fibroblast proliferation and collagen synthesis [9]. A study by Hyun Joo Son et al. [10] showed an in-vitro positive effect of β-Glucan use on fibroblast proliferation. For this reason, β-Glucan use is thought to have a positive effect in preventing intestinal anastomotic leaks with its effects of strengthening the immune system and enhancing fibroblast proliferation, and this study was planned.

The aim of this study is to investigate, in an experimental rat model, the effects of the β-Glucan on the healing of left colon anastomoses.

Material and Methods

Animals

The study was approved by the Ondokuz Mayýs University Local Ethics Committee for Animal Experiments (Date: Dec. 27, 2010, number: 2010/78). Twenty Wistar Albino male rats, weight ranging 250–300 g, were used in the study. All rats were fed ad libitum with standard rat chow and tap water. All subjects were kept in 12 hour darkness and 12 hour light before and after study (at a standard temperature of 22°C).

All animals were given water only on the first postoperative day; standard rat chow and water ad libitum were provided on the second postoperative day. There was no difference in food intake between groups.

Experimental Groups

The rats were randomly divided into two groups, each containing ten rats. Group I (control): anastomosis group, received no treatment. Group II (β-Glucan): 50 mg/kg was suspended in 2 ml of saline and given by intragastric gavage to the glucan groups within seven days after surgical procedure (Mustafa Nevzat Company, Istanbul, Turkey). The dose of β-glucan was decided by taking the studies of Sener et al. [8, 11] into consideration.

Surgical Procedure

50 mg/kg ketamine (Ketalar, Pfizer, Turkey) with 10 mg/kg xylazine HCl (rompun, Bayer, Turkey) were given to all animals for anesthesia. The abdominal skins of all subjects were shaved and painted with iodine povidone under general anesthesia. All surgical procedures were performed under sterile conditions. Laparotomy was performed with a midline incision of approximately 3 cm. After the left colon was transected horizontally, 4 cm proximal to the peritoneal reflection, an end-to-end anastomosis was performed with a single layer manner with 5/0 polyprolene sutures (Prolene, Ethicon). The fascia and skin were closed separately by 4-0 silk. In the control group, one rat died on the sixth day. This animal was excluded from the study. All the rats were sacrified with a high dose of anesthetic on the 7th day after surgery. After being sacrified, a repeat laparotomy was immediately made through the same midline incision and the peritoneal cavity was opened.

Measurement of Bursting Pressure

The anastomosis line was found and the intestine was cut out 2 cm proximally and 2 cm distally to the anastomosis region by the same method used by Li et al. [12]. One end was sutured and the other end was tied after an 18 g intraluminal cath-
eter placement. A three-way cannula was placed at the tip of the catheter. One end was connected to intraluminal side and the other end was connected to the manometer. Methylene blue, diluted with saline, was administered at 6 ml/min speed with an infusion pump. The pressure when the blue-colored fluid was seen or the time when a sudden fall in pressure was seen had been defined as the burst pressure and was recorded in mm Hg.

Measurement of Hydroxyproline Level

Tissue samples were put into an appropriate PBS (Phosphate Buffer Solution, 10 mM, pH 7.2) after being homogenized manually in porcelain mortar and pestle with liquid nitrogen. Tissue samples were stored frozen at −80°C in individual aliquots after being sonicated for 1 min at +4°C in 220V (Fisher Sonic Dismembrator; Mosel 300). The homogenates were centrifuged at +4°C for 5 minutes with 15000 g (Sigma 3K30, S. No: 76262, Germany) and the supernatants were used for analysis. Hydroxyproline levels were determined using a commercially available Hydroxyproline Assay Kit (BioVision Incorporated, Milpitas, USA), based on an analysis of chromogenin as a result of the reaction at 560 nm spectrophotometrically. The range of the kit was 0.1–2 μg. The amount of protein in tissue homogenates was determined by the Lowry method and hydroxyproline level was expressed as μg per mg protein (μg/mg.prot). The principle of the Lowry method is based on the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin-Ciocalteay phosphomolybdic phosphotungstic acid to heteropolybridge by the copper-catalyzed oxidation of aromatic acids [13].

Histopathologic Evaluation

Tissue samples were taken from the anastomosis region, and histopathological examination

Table 1. Scores used to analyze healing of the anastomosis line in ileum semi-quantitatively

<table>
<thead>
<tr>
<th>Score (Punktacja)</th>
<th>Necrosis (Martwica)</th>
<th>PMN (Linfocyty)</th>
<th>Macrophages (Makrofagi)</th>
<th>Edema (Obrzęk)</th>
<th>Mucosal epithelium (Nabłonek błony śluzowej)</th>
<th>Submucosal-muscular layer (Warstwa podśluziówkowo-mięśniowa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>none</td>
<td>normal number</td>
<td>normal number</td>
<td>none</td>
<td>normal glandular</td>
<td>good bridging</td>
</tr>
<tr>
<td>1</td>
<td>small patches</td>
<td>slight increase</td>
<td>slight increase</td>
<td>some</td>
<td>normal cubic</td>
<td>average bridging</td>
</tr>
<tr>
<td>2</td>
<td>some patches</td>
<td>marked infiltration</td>
<td>marked infiltration</td>
<td>marked incomplete cubic</td>
<td>poor bridging</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>massive infiltration</td>
<td>massive infiltration</td>
<td>massive infiltration</td>
<td>severe</td>
<td>absent</td>
<td>no bridging</td>
</tr>
</tbody>
</table>
was performed by a pathologist blinded for all groups. Tissue samples were fixed for 12 hours in a 10% buffered neutral formaline solution. After a routine follow-up, samples were embedded in paraffin blocks and sections of 4–5 µm thickness were stained with hematoxylin and eosin (H&E) and finally examined under a light microscope.

A semi-quantitative scale as described by Biert et al. [14], containing various parameters involved in wound healing, was used (Table 1). The amount of necrosis was expressed as none (0 points), only small patches (1 point), some patches (2 points) or massive (3 points). In the anastomotic area, accumulation of polymorphonuclear cells (pmns), macrophages and lymphocytes was also assessed in terms of none or normal number (0 points), slight increase (1 point), marked infiltration (2 points) and massive infiltration (3 points). Edema, expressed as the ratio of maximum thickness of the wall at the anastomosis and the thickness of the normal intestinal wall as present at the end of the section, was established in terms of none (0 points), slight (1–1.5× normal thickness; 1 point), marked (1.5–2× normal thickness; 2 points) and severe (> 2x normal thickness; 3 points). Healing of the mucosa was expressed as normal, i.e. mucosa with restored glandular epithelium (0 points), intact mucosa with cubic epithelium but without glands (1 point), mucosa only partially covered by cubic epithelium (2 points) and mucosa completely devoid of epithelial coverage (3 points). Submucosal-muscular repair was assessed in terms of good (0 points), average (1 point), poor (2 points) or no (3 points) fibroblast stretching and bridging the anastomotic wound. This way, in each anastomosis, two observations from the mesenterial and two from the anti-mesenterial side were obtained.

Statistical Analysis

The data was encoded, then transmitted to a computer and analyzed using the SPSS15.0 package program. Descriptive characteristics of the data were expressed as mean ± standard deviation. The Mann-Whitney U test was used to compare the groups. Statistical significance level was considered as p < 0.05.

Results

Anastomotic Bursting Pressure and Hydroxyproline Content

Anastomosis bursting pressure was found to be 106.67 ± 5.00 mm Hg in Group I and 148.00 ± 11.35 mm Hg in Group II. The bursting pressure in group II was significantly greater than the bursting pressure in Group I (p < 0.001). When the groups were compared in terms of peri-anastomotic tissue hydroxyproline levels, it was measured at 0.85 ± 0.14 µg/mg.prot in group I and 1.45 ± 0.46 µg/mg.prot in group II. The hydroxyproline level in Group II was significantly greater than in Group I (p = 0.003). When the differences between groups in terms of anastomotic bursting pressures and tissue hydroxyproline levels were taken into account, it is possible the use of 50 mg/kg β-Glucan for 7 days in the postoperative period contributes to anastomotic healing. The bursting pressures and tissue hydroxyproline levels of the groups are shown in Table 2.

Histology

Histopathological examination of tissue samples taken from the peri-anastomotic region in the group using β-Glucan showed less necrosis in the anastomosis (p = 0.024). However, in terms of inflammation, lymphocytes and macrophage infiltration were statistically significantly less in the control group (p = 0.022 and p = 0.023). Less mucosal epithelial damage was found in group II and this difference was found to be statistically significant (p = 0.012). Tissue polymorphonuclear leukocyte levels (PNL) were found to be similar between the two groups and the difference was insignificant (p = 0.274). Edema and submucosal-muscular layer damage were less in group II, but the difference was not significant (p = 0.079). Histopathologic examination results are given in Table 3.
Discussion

Anastomotic leakage is an important complication of colon surgery and is associated with morbidity and mortality [15]. Despite improvements in perioperative and surgical techniques, the levels of gastrointestinal anastomotic leaks have not fallen to negligible levels [16]. For this reason, anastomotic leaks still continue to be an annoying situation and surgeons are expected to prevent leaks. Anastomotic healing is a complex process involving a series of biological events. In this process, coordination of cellular activity and humoral factors are necessary [9]. The degree of inflammatory response, mucosal re-epithelialization rate, amount of newly synthesized collagen, resistance and impact affect anastomotic healing [17]. Anastomosis strength is associated with collagen fibers and their maturation with the submucosal layer [17]. Fibroblast proliferation and collagen synthesis occurs in the submucosal layer [9]. The synthesis of collagen by fibroblasts is at maximum 5–7 days. Therefore, taking into account the experimental models in the literature, this study terminated on the 7th day [17, 18]. Similarly, Kosmidis et al. [18], in their study, showed the key role of myofibroblast in the healing process of colonic cancer anastomosis and expressed that the detachment of anastomosis is reduced after 7 days.

In the literature, β-Glucan was found to have a positive contribution to the wound healing process, indirectly by increasing the release after binding to specific receptors on macrophages and by means of glucan-specific receptors expressed on human fibroblasts [7, 19]. Similarly, β-Glucan is expressed to stimulate monocytes, macrophages and natural killer (NK) cells [20]. In another clinical study, Demir et al. [20] expressed enhancement in proliferation and stimulation of peripheral blood monocytes after short term (two weeks) use of β-Glucan without any evidence of side effects in breast cancer patients. In this present study, the histopathologic examination of tissue samples from the anastomosis region revealed statistically significantly increased macrophage, neutrophil and lymphocyte infiltration in rats fed with β-glucan compared to the control group. Hyun Joo Son et al. [10] stated in their study that there were positive effects on fibroblast proliferation.

Anastomotic bursting pressure and peri-anastomotic tissue hydroxyproline levels are the parameters used in experimental studies related to colonic cancer [9]. Collagen has an important role in all stages of wound healing and tissue strengthening with integrity [21]. Strength of the anastomosis and quality depends on the strength of synthesized collagen [22]. Lack of collagen leads to weak anastomosis [23]. In this study, a statistically significant increase in hydroxyproline levels, which is a parameter of collagen synthesis, was determined in the peri-anastomotic tissue samples of β-glucan-fed rats compared to the control group. Likewise, the anastomotic bursting pressure of the rats fed with β-glucan was statistically significantly higher than in the control group. According to these results, the authors can say that it has a positive effect on anastomotic bursting pressure and hydroxyproline levels. The authors think the significant increase in both the burst pressure and hydroxyproline levels is probably the result of an incremental effect of β-glucan on fibroblast proliferation.

<table>
<thead>
<tr>
<th></th>
<th>Group I (Grupa 1)</th>
<th>Group II (Grupa 2)</th>
<th>P value (Istotność statystyczna)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Necrosis (Martwica)</td>
<td>2.22 ± 0.667</td>
<td>1.50 ± 0.527</td>
<td>0.024</td>
</tr>
<tr>
<td>PNL</td>
<td>1.89 ± 0.601</td>
<td>2.20 ± 0.632</td>
<td>0.274</td>
</tr>
<tr>
<td>Lymphocytes (Limfocyty)</td>
<td>1.56 ± 0.527</td>
<td>2.30 ± 0.675</td>
<td>0.022</td>
</tr>
<tr>
<td>Macrophages (Makrofagi)</td>
<td>1.44 ± 0.527</td>
<td>2.10 ± 0.568</td>
<td>0.023</td>
</tr>
<tr>
<td>Edema (Obręcz)</td>
<td>1.89 ± 0.601</td>
<td>1.40 ± 0.516</td>
<td>0.079</td>
</tr>
<tr>
<td>Mucosal epithelium (Nabłonek błony śluzowej)</td>
<td>1.89 ± 0.333</td>
<td>1.30 ± 0.483</td>
<td>0.012</td>
</tr>
<tr>
<td>Submucosal-muscular layer (Warstwa podśluzówkowo-mięśniowa)</td>
<td>1.78 ± 0.667</td>
<td>1.20 ± 0.632</td>
<td>0.079</td>
</tr>
</tbody>
</table>
lease of wound growth factors with subsequent modulation of fibroblast activity including collagen biosynthesis.

In conclusion, anastomotic leaks remain a major problem for surgeons today. Both experimental and clinical studies will continue for reducing the anastomotic leakage rates. To authors’ knowledge, in this first experimental study of β-glucan on anastomotic healing, when significant increases in anastomotic bursting pressures and tissue hydroxyproline levels and the inhibitory effect of β-Glucan on epithelial damage, edema, and submucosal-muscular layer damage are taken into account, β-Glucan was thought to contribute to healing of the anastomosis. However, it will be possible to have a clearer idea of the effects of β-glucan on anastomotic healing when the results of similar tests are compared.

References


Address for correspondence:
Kasim Caglayan
Bozok University, Faculty of Medicine
Department of Surgery, Yozgat
Turkey
Tel.: +90-354-2127949
E-mail: kasimcaglayan@hotmail.com

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