Abstract: Objective. The purpose of this study was to evaluate the effects of beta-D-glucan on the experimental diabetic rat colon anastomosis model. Background. Beta-D-glucan is a commonly used macrophage activator and promotes wound healing by increasing macrophage infiltration into the wound. The decrease in the function of macrophages and impaired wound healing can be observed in diabetes mellitus (DM). Methods. Eighty Spraque-Dawley rats were divided into 4 groups: colon anastomosis (group 1); colon anastomosis + DM (group 2); colon anastomosis + beta-D-glucan (group 3); and colon anastomosis + beta-D-glucan + DM (group 4). Diabetes was induced with streptozotocin (85 mg/kg), and glycemia was assessed before induction at days 14 and 17. Colon anastomosis was performed at day 14. Beta-D-glucan (100 mg/kg/day) was administered 2 days before colon anastomosis and given orally for 5 days. Relaparotomies were done 3 days after colon anastomosis and given orally for 5 days. Results. There were no differences among groups for hydroxyproline levels. The mean values of anastomotic bursting pressures in group 4 were significantly higher than those of group 2. The mean values of MDA levels in group 2 were significantly lower than those of group 4. Group 2 showed a significant difference in the amount of necrosis, accumulation of polymorphonuclear cells, and edema when compared with groups 1, 3, and 4 (P < 0.001, P < 0.002, and P < 0.001, respectively). Conclusion. This study indicates that oral administration of beta-D-glucan significantly improves the impaired anastomotic healing in rats with diabetes mellitus.

Key words: beta-D-glucan, diabetes mellitus, anastomosis, rat

A nastomotic dehiscence is a recognized complication of abdominal surgery with concomitant high morbidity and mortality rates. Several factors such as ischemia, jaundice, infection, diabetes mellitus (DM), and malignancies can increase the risk of anastomotic dehiscence.1,2

Diabetes mellitus is known to be a contributing factor to impaired wound healing in humans.3,4 Diabetes, through the nonenzymatic glycosylation
mechanism, can have a significant impact on the development of the healing process in colonic anastomosis by impairing its strength and the migration of inflammatory cells, thus delaying reepithelization and reducing the quality of collagen deposition and new vessel formation.5-7 A large body of evidence indicates that the diabetic state is associated with a delayed or reduced repair capacity. Experimental studies with streptozotocin-induced, or genetically diabetic rodents, demonstrate that cutaneous wound strength and gastrointestinal tract strength are decreased similarly.8-10

Beta-D-glucan is a commonly used macrophage activator shown to improve normal wound healing. It is a glucose polymer derived from yeast and employed as an immune stimulant in clinical studies. Poly-branched beta-1,3-D-glucans are naturally occurring polysaccharides, with or without beta-1,6-D-glucose side chains, that are integral in cell wall constituents in a variety of bacteria, plants, and fungi. Glucan receptors that deliver non-self-derived glucan to the immune response have been identified on macrophages, dendritic cells, and other cells. The beta-1,3-D-glucan with beta-1,6-glucan linkage extracted from yeast cell walls (Saccharomyces cerevisiae) has been shown to act as a potent nonspecific immune-activator. Oral, topical, or systemic administration of beta-D-glucan promotes wound healing by increasing macrophage infiltration into the wound milieu, stimulating collagen synthesis and reepithelization. When taken orally, enterocytes facilitate the transportation of beta glucans and similar compounds across the intestinal cell wall into the lymph where they begin to interact with macrophages to activate the immune function. Moreover, some studies have demonstrated that the oral form of beta-D-glucan has similar protective effects as the injected version, including defense against infectious diseases and cancer.11-18

Macrophages are vital to the regulation of immune responses and the development of inflammation. They produce monokines, enzymes, complement proteins, and regulatory factors such as interleukin-1. Macrophages also play an important role in wound healing by producing humoral factors and by increasing fibroplasia, fibrogenesis, and angiogenesis in wounded tissue. The decrease in the function of macrophages has been shown to impair wound healing and can be observed in DM. Similarly, enhanced macrophage function has been demonstrated to accelerate wound healing.

In this study, the authors have evaluated the role of beta-D-glucan on the experimental diabetic rat colon anastomosis model.

**Methods**

The procedures and animal protocols followed in this study complied with the *Guide for the Care and Use of Laboratory Animals*,19 and were approved by the Ethics Committee of the Ankara Oncology Training and Research Hospital, Ankara, Turkey. Eighty Sprague-Dawley rats weighting 200 g - 250 g were randomized into 4 groups: colon anastomosis (group 1, n = 20); colon anastomosis + DM (group 2, n = 20); colon anastomosis + beta-D-glucan (group 3, n = 20); colon anastomosis + beta-D-glucan + DM (group 4, n = 20).

Rats in groups 2 and 4 were rendered diabetic 14 days before surgery by a single intravenous injection of streptozotocin (STZ) (Sigma Chemical Co, St. Louis, MO) at a dose of 65 mg/kg body weight dissolved in citrate buffer solution into the tail vein. Blood glucose levels were collected from rats using a blood glucose meter (Roche

**Table 1. Blood glucose of rats (mg/dL).**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 0</th>
<th>Day 14</th>
<th>Day 17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (anastomosis)</td>
<td>76 ± 5</td>
<td>79 ± 8</td>
<td>77 ± 9</td>
</tr>
<tr>
<td>Group 2 (anastomosis + DM)</td>
<td>78 ± 6</td>
<td>412 ± 92*</td>
<td>423 ± 88*</td>
</tr>
<tr>
<td>Group 3 (anastomosis + beta-D-glucan)</td>
<td>73 ± 5</td>
<td>77 ± 11</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>Group 4 (anastomosis + beta-D-glucan + DM)</td>
<td>78 ± 7</td>
<td>408 ± 103*</td>
<td>421 ± 91*</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Intravenously injected streptozotocin induces rapid hyperglycemia of rats. *P < 0.005, when compared to control groups on day 14 and 17; n = 20 per group.

### Key Points

- Beta-D-glucan is a commonly used macrophage activator shown to improve normal wound healing.
- Poly-branched beta-1, 3-D-glucans are naturally occurring polysaccharides, with or without beta-1, 6-D-glucose side chains, that are integral in cell wall constituents in a variety of bacteria, plants, and fungi.
- The beta-1, 3-D-glucan with beta-1, 6-glucan linkage extracted from yeast cell walls (Saccharomyces cerevisiae) has been shown to act as a potent non-specific immune-activator.
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ACCU-CHEK glucometer, Indianapolis, IN) on day 14 and day 17, and the animals were weighed (Table 1 and 2). For groups 3 and 4, beta-D-glucan (Imuneks, Mustafa Nevzat Pharmaceuticals, Istanbul, Turkey) was given 100 mg/kg/day orally, starting at day 12 for 2 days preoperatively, and continued until the end of the experiments. The beta-D-glucan that was used in this study was 1.3 mg/kg - 1.6 mg/kg beta-D-glucan in microparticulate form, which was prepared from the *Saccharomyces cerevisiae* yeast. The dosage of 100 mg/kg beta-D-glucan was chosen based on the findings of previous studies.18,20

Fourteen days after the STZ injection and confirming that the rats were in diabetic condition for groups 2 and 4 (Table 1), all animals were anesthetized with intramuscular ketamine (40 mg/kg, Ketalar, Pfizer, New York, NY) and xylazine (5 mg/kg, Rompun, Bayer Ag, Leverkusen, Germany). All rats underwent a 4-cm median laparotomy and the left colon was identified and divided (without resection of a segment) 3 cm proximal to peritoneal reflection of the rectum, taking care to preserve the marginal vessels. The bowel was restored by an end-to-end anastomosis with 6 interrupted, inverting sutures of 6:0 polypropylene (Prolene, Ethicon Ltd, Istanbul, Turkey), and the abdominal incision was closed with 2 layers of continuous 4:0 silk sutures. Operations were done in aseptic conditions and neither analgesic nor antibiotics were used during the experiments. Postoperatively, the rats were fed rat chow and water *ad libitum*; also rats from groups 3 and 4 had oral beta-D-glucan administration for 3 more days.

On postoperative day 3 (day 17 after randomization), under similar anesthesia, exploratory laparotomies were made and an en bloc resection done of a 6-cm colonic segment (including the anastomosis in the middle) with adhered tissues (omentum, small bowel, or colon) to preserve the integrity of the anastomosis. The bursting pressure measurements were obtained within 5 minutes of the rat being sacrificed. After measuring the bursting pressure, a ring of tissue 2 cm wide that included the anastomosis, was removed. This tissue was divided into 2 parts. One segment was stored at -20°C for biochemical analyses. The other segment was stored in 10% formaldehyde for a later assessment with immunohistochemistry. Blood samples were taken for monitoring glucose levels and the animals were weighed before anesthesia (Tables 1 and 2).

For anastomotic bursting pressure, distal parts of the segments were closed with 3:0 silk sutures, proximal parts of the colon segments were adapted to an intraluminal pressure manometer (Monitoring kit L978-A07, Abbott Ireland, Sligo), and data were recorded on a digital monitor (Petas K-450, Petas, Ankara, Turkey). The segments were filled with isotonic NaCl solution with continuous infusion (4 ml/min) until the appearance of leakage from the anastomotic site. The appearance of fluid leakage that coincided with pressure decrease monitored by the manometer was accepted as bursting pressure.

A 0.5 cm section of tissue was resected from the anastomosis, kept at -20°C, and immediately sent to the laboratory for evaluation of the hydroxyproline and MDA levels. For the evaluation of hydroxyproline levels, the tissue samples (30 mg - 50 mg) were placed into

**Table 2. Mean weight loss of rats.**

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (anastomosis)</th>
<th>Group 2 (anastomosis + DM)</th>
<th>Group 3 (anastomosis + beta-D-glucan)</th>
<th>Group 4 (anastomosis + beta-D-glucan + DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Day 0</strong> (Pre-streptozotocin injection)</td>
<td>223 ± 12</td>
<td>226 ± 13</td>
<td>227 ± 9</td>
<td>230 ± 11</td>
</tr>
<tr>
<td><strong>Day 14</strong> (Pre-anastomosis)</td>
<td>226 ± 15</td>
<td>196 ± 11*</td>
<td>225 ± 10</td>
<td>200 ± 13*</td>
</tr>
<tr>
<td><strong>Day 17</strong> (Relaparotomy)</td>
<td>214 ± 17</td>
<td>188 ± 9*</td>
<td>217 ± 12</td>
<td>193 ± 14*</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Intravenously injected streptozotocin reduces weights of rats. *P < 0.005 when compared to control groups on day 14 and 17; n = 20 per group.

**Keypoints**

- Eighty Spraque-Dawley rats weighting 200 g - 250 g were randomized into 4 groups: colon anastomosis (group 1, n = 20); colon anastomosis + DM (group 2, n = 20); colon anastomosis + beta-D-glucan (group 3, n = 20); colon anastomosis + beta-D-glucan + DM (group 4, n = 20).
- Rats in groups 2 and 4 were rendered diabetic 14 days before surgery by a single intravenous injection of streptozotocin.
- All rats underwent a 4-cm median laparotomy and the left colon was identified and divided (without resection of a segment) 3 cm proximal to peritoneal reflection of the rectum, taking care to preserve the marginal vessels.
hydrolysis tubes. Fifty mM potassium phosphate buffer pH 7.0 and an equal volume of concentrated HCl were added to each tube and the samples were hydrolyzed at 110°C for 16 hours. The pH of the samples was adjusted to 8.5 by dilution with NaOH and oxidized at room temperature with chloramine-T solution. After 4 minutes, Ehrlich’s reagent was added to the tubes. The color was allowed to develop at 60°C for 25 minutes and the absorbency at 560 nm was determined by the method of Bergman and Loxley.20 The hydroxyproline concentration was calculated as µg/mg wet tissue weight. The level of MDA in tissue homogenate was determined using the method of Mihara and Uchiyama.21 Half a milliliter of MDA in tissue homogenate was determined using the method of Bergman and Loxley.20 The hydroxyproline concentration was calculated as µg/mg wet tissue weight. The level of MDA in tissue homogenate was determined using the method of Mihara and Uchiyama.21

Specimens taken for histopathological evaluation were fixed in 10% formaldehyde, stained with hematoxylin-eosin, and examined under a light microscope by the same pathologist in a random and blinded fashion. Four microscopic fields under 40x magnification were randomly picked in the tissue samples of the anastomosis to evaluate the amount of necrosis, accumulation of polymorphonuclear cells (PMN), edema, healing of the mucosa, and submucosal repair. The counts were made in anastomotic tissue samples, and mean values were calculated for each group and samples. A scoring system was used to evaluate the histopathological results as previously described.25

Results
During the course of experimental protocols, all animals survived the operations. There were no wound infections as assessed by clinical inspection. Mean weight loss in the DM groups was approximately 13%. The mean values of the hydroxyproline levels for the groups were 33.2 ± 1.6, 31.3 ± 1.1, 32.3 ± 1.0, and 30.4 ± 1.7, respectively (Table 3). There were no significant differences among the groups. The mean values of the MDA levels for the groups were 24.2 ± 5.6, 10.9 ± 1.5, 15.8 ± 1.4, and 13.5 ± 1.1, respectively (Table 3). Malondialdehyde levels in the DM group (group 2) were significantly lower than the levels of groups 3 and 4 (P < 0.004).

The mean values of anastomotic bursting pressures were 51.2 ± 2.3, 32.6 ± 2.7, 58.9 ± 2.3, and 44.4 ± 2.5, respectively (Table 3). The mean values of the anastomotic bursting pressures of the DM group (group 2) were significantly lower than those of the control group (group 1) (P < 0.003) and also were significantly lower than those of groups 3 and 4 (P < 0.001 and P < 0.001, respectively). As for histopathological evaluation, the DM group (group 2) showed significant differences in the amount of necrosis, accumulation of polymorphonuclear cells, and edema when compared with groups 1, 3, and 4 (P < 0.001, P < 0.001, and P < 0.002, respectively). There were no differences among groups for healing of the mucosa and submucosal repair (Table 4).

Discussion
Anastomotic dehiscence is a recognized complication of abdominal surgery and has con-

Table 3. Hydroxyproline levels, malondialdehyde (MDA) levels and anastomotic bursting pressures of each group on postoperative day 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Hydroxyproline levels (µg/mg wet tissue)</th>
<th>MDA levels (nmol/g protein)</th>
<th>Anastomotic bursting pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>33.2 ± 1.6</td>
<td>24.2 ± 5.6</td>
<td>51.2 ± 2.3</td>
</tr>
<tr>
<td>Group 2</td>
<td>31.3 ± 1.1</td>
<td>10.9 ± 1.5*</td>
<td>32.6 ± 2.7</td>
</tr>
<tr>
<td>Group 3</td>
<td>32.3 ± 1.0</td>
<td>15.8 ± 1.4</td>
<td>58.9 ± 2.3</td>
</tr>
<tr>
<td>Group 4</td>
<td>30.4 ± 1.7</td>
<td>13.5 ± 1.1</td>
<td>44.4 ± .25</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation. Hydroxyproline content was not changed between groups. MDA levels were decreased in group 2 when compared with group 3 and 4 (P < 0.004). The mean values of the anastomotic bursting pressures were lower in group 2 when compared with groups 1, 3 and 4 (P < 0.003, P < 0.001, and P < 0.001, respectively; n = 20 per group).
comitant high morbidity and mortality rates. Therefore, factors contributing to poor anastomotic healing are of clinical importance and have been the subject of several earlier studies. According to other studies, the effects of a multitude of experimental conditions and substances include infection, hypovolemia, lavage, pectin, prostaglandin, vitamin A, aprotinin, cytostatics, and nutrition. Diabetes mellitus has been reported as an important risk factor causing the impairment of anastomotic wound healing. The effects of DM on anastomotic wound healing have been suggested to be a result of decreased mononuclear cell inflammation, neovascularization, and collagen synthesis. Also the absence of diabetic control endangers anastomotic integrity and enhances the possibility of leakage and its subsequent severe complication. Patients with poorly controlled DM show an increased susceptibility to infection, possibly caused by the suppression of certain immunological functions. Since it has been reported that collagenase activity is enhanced in wounds from diabetic animals, it seems conceivable that a limited and localized degradation of collagen fibrils may loosen the structure of the matrix, thereby diminishing its capacity to retain the sutures and leading to loss of strength and eventually to anastomotic leakage.

The authors observed the parameters of the anastomotic wound healing only on the third postoperative day, because recent studies of the effects of diabetes on anastomotic wound healing reported that the deleterious effects are limited to the early postoperative period. It has also been reported that diabetes impairs anastomotic wound healing during the early (ie, inflammatory) healing phase by delaying the migration of inflammatory cells.

According to recent studies, oral administration of beta-D-glucan improves impaired anastomotic wound healing in rats treated with long-term corticosteroids. Beta-D-glucan enhances macrophage function and stimulates collagen synthesis and angiogenesis. Beta-D-glucans are being referred to as biological response modifiers because of their ability to activate the immune system. However, it should be noted that the activity of beta-D-glucan is different from agents that stimulate the immune system. Agents that stimulate the immune system can push the system to over-stimulation, and hence are contraindicated in individuals with autoimmune diseases, allergies, or yeast infections. Beta-D-glucans seem to make the immune system work more effectively without becoming overactive. They accomplish this by activating phagocytes, which are immune system cells that function to trap and destroy foreign substances in our bodies such as bacteria, viruses, fungi, and parasites. In addition to enhancing the activity of phagocytes, beta-D-glucans also reportedly lower elevated levels of LDL cholesterol, aid in wound healing, help prevent infections, enhance natural killer cell function, and help in the prevention and treatment of cancer. The possible mechanisms are the promotion of neoangiogenesis, increase in cell infiltration, stimulation of collagen synthesis, and decrease in bacterial infection. Neoangiogenesis may prevent the anastomosis from the ischemia and maintain blood supply. Thus, these factors alone or in combination can mediate the beneficiary effects of beta-D-glucan on anastomotic wound healing.

Macrophage activity is known to play a key role in wound healing from surgery or trauma. In both animal and human studies, therapy with beta-D-glucan has provided improvements such as fewer infections, reduced mortality, and stronger tensile strength of scar tissue.

The commonly used parameters to assess the intrinsic resistance of an anastomosis to rupture are bursting pressure, bursting wall tension, and tensile strength. Earlier studies indicate that in the early phase of wound healing,
bursting pressure is more helpful in assessing the functional status of anastomosis, and closely approximates to the clinical situation. Additionally, the authors measured the circumferences of the anastomotic line after failure of the anastomosis. Variation in the circumferences of the anastomoses among the groups was lower than 3%. The differences in the mean length of circumferences of the anastomoses among the groups were not significant. Therefore, if the bursting wall tensions had been calculated, the results of the study would not have changed. Thus, the authors chose the bursting pressure for anastomotic wound healing as a mechanical parameter. In this study, the mean values of bursting pressures were significantly higher in group 4 (anastomosis + beta-D-glucan + DM) as compared to group 2 (anastomosis + DM) (P < 0.001).

The authors also chose the hydroxyproline and MDA levels for anastomotic wound healing as a parameter for histobiological examination. Lipid peroxidation is a well-established mechanism of cellular injury in humans, and MDA is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides, derived from polyunsaturated fatty acids, are unstable, and decompose to form a complex series of compounds. These include reactive carbonyl compounds, which is the most abundant MDA. Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation. Increased levels of lipid peroxidation products have been associated with a variety of diseases in both humans and model systems. In this study, hydroxyproline content was not changed between groups. The mean values of MDA were significantly higher in group 4 (anastomosis + beta-D-glucan + DM) when compared with group 2 (anastomosis + DM) (P < 0.004). The elevated level of MDA was suppressed by increased macrophage infiltration that was stimulated by beta-D-glucan, indicating that beta-D-glucan reduces lipid peroxidation and thereby supports the maintenance of cellular integrity in diabetic rat colon anastomosis.

Conclusion

This study found that oral administration of beta-D-glucan improves impaired anastomotic wound healing in rats with DM. The deleterious effects of DM on anastomotic wound healing can be treated with oral administration of beta-D-glucan.

References

13. Hong F, Hansen RD, Yan J, et al. Beta-glucan functions as...


