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The Effect of Single-Dose Intraperitoneal Bevacizumab on Peritoneal Adhesion Formation

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ABSTRACT

Background: Intra-abdominal adhesions and their complications following abdominal surgery are serious problems, with an incidence of 67-93%. Prevention of peritoneal adhesion formation may eliminate the need for surgical intervention, decreasing complications, morbidity, and cost. Bevacizumab is a recombinant monoclonal antibody which specifically binds vascular endothelial growth factor, an important cytokine in adhesion formation, and neutralizes its biological activity. We developed an experimental model in rats to determine the effect of bevacizumab in preventing adhesion formation and analyzed its effect both micro- and macroscopically. **Methods:** We used 32 Wistar rats randomly divided into two groups: Group A (control) and Group B (bevacizumab), with 16 rats each. A modified cecum abrasion model was developed; 0.9% NaCl solution was administered intraperitoneally to Group A and bevacizumab to Group B. On day 15, adhesion formation was evaluated both macro- and microscopically. **Results:** Both micro- and macroscopic adhesion grades in Group B were significantly lower than those of control Group A; macroscopic grades were 2.69 ± 0.95 and 0.69 ± 0.8, and microscopic grades were 2.25 ± 1.06 and 0.5 ± 0.52 for Groups A and B, respectively. **Conclusions:** Bevacizumab was effective in preventing intraperitoneal adhesion formation in our study; however, its inhibitory effects on embryogenesis and the hematopoietic, endocrine, and immune systems may limit its clinical use. (REV INVEST CLIN. 2018;70:279-84)

Key words: Peritoneal adhesion. Bevacizumab. Vascular endothelial growth factor.

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INTRODUCTION

Intra-abdominal adhesions and their complications following abdominal or pelvic surgery are serious problems. The most frequent causes of intra-abdominal adhesions are laparotomies and intra-abdominal infections. These adhesions increase the risk of injury in subsequent intra-abdominal and pelvic surgeries[1]. The incidence of adhesion formation following intra-abdominal surgeries is 67-93%[2,3]. A direct relationship between surgical procedures and adhesion formation has been shown in various studies, suggesting that adhesions due to previous surgeries are the most important cause of postoperative intestinal obstructions[4]. There are numerous macrophages, eosinophils, basophils, and mast cells in the intraperitoneal cavity which originate from monocytes in the blood. These cells have a significant amount of plasminogen activators, allowing blood to be collected in the intraperitoneal cavity uncoagulated. Although the fibrinolytic activity of the mesothelium decreases, fibrin and fibrinous adhesion formation increases in trauma, ischemia, and infections. Adhesion formation is a normal process of peritoneal healing. Mechanical trauma, venous stasis, bacterial contamination, and ischemia are some of the main causes of postoperative peritoneal adhesion (PPA) formation[5-7]. A large number of systemic or intraperitoneal medical treatments have been proposed to decrease PPA[8,9]. Vascular endothelial growth factor (VEGF) is not only a potent angiogenic factor but also an important cytokine affecting adhesion formation. Remodeling and fibroblast function in newly formed tissues are the effects of VEGF on the inflammatory process[10]. Bevacizumab is a recombinant monoclonal antibody which specifically binds VEGF and neutralizes its biological activity[11]. It has antiangiogenic and antitumoral effects. An important delay in tumor progression has been observed with bevacizumab, which also decreases VEGF levels to undetectable limits[12,13]. Angiogenesis and adhesion can be blocked by inhibiting VEGF[14,15]. In an experimental model in rats, we used bevacizumab to block intra-abdominal adhesion formation, an important cause of morbidity and mortality, and tried to demonstrate its VEGF neutralization property both micro- and macroscopically.

METHODS

This experimental study was approved by the Local Ethics Committee on Animal Experiments. A total of 32 adult female Wistar rats with a mean weight of 200 g, fed with standardized rat food, were randomly divided into a control group (Group A) and a bevacizumab group (Group B), of 16 rats each. Surgery was performed after 12 h of fasting. A combination of intramuscular ketamine hydrochloride 30 mg/kg (Ketalar Pfizer, Turkey) and 5 mg/kg xylazine (Rompun Bayer, Turkey) was used as anesthesia. A cecum abrasion model was developed, based on the one described by Ignjatovic et al[16]. Following anesthesia, the abdominal skin was shaved and scrubbed with povidone-iodine 10% solution. Then, 3 cm-long median laparotomy incisions were made. The cecum was located, and 2 cm^2 of its wall and 2 cm2 of the neighboring peritoneum were abraded by scrubbing with gauze until they bled. Bevacizumab (0.1 mL of 2.5 mg/kg bevacizumab diluted to 5 mg/mL with 0 9% NaCl solution) was administered intraperitoneally in Group B, and 0.9% NaCl solution of 0.1 mL volume was administered intraperitoneally in control Group A. The same degree of abrasion was made in all rats, so the drug dose administered did not depend on the degree of abrasion but on the rat's weight. Oral feeding and drinking began freely following the operation. On day 15, all rats were sacrificed under anesthesia, and relaparotomy was performed. The investigators who sacrificed the rats were blind to bevacizumab or placebo administration. The adhesions formed were evaluated by two surgeons who were also blind to the sample groups. The macroscopic evaluation was based on the Zuhlke Method, ranging from 0 to 4 (Table 1 and Figs. 1 and 2)[17]. Following macroscopic evaluation, the tissues were excised *en bloc,* including all intestinal adhesions, and then fixed in 10% formalin solution for histopathological analysis. Hematoxylin and eosin stained samples were evaluated under light microscope by an expert pathologist on gastro intestinal system. Microscopic scoring was done using the method of Ignjatovic et al. (Table 2)[16].

Statistical analysis

The SPSS statistics 22 program (IBM SPSS, Turkey) was used in statistical analysis. The Shapiro–Wilk test was used to check the normal distribution of parameters. The Mann–Whitney *U*-test was used to compare the quantitative parameters between the groups which did not show normal distribution.

Table 1. Zuhlke method for macroscopic evaluation of adhesions.

Grade	Findings
0	No adhesion
1	Thin adhesion, membrane-like adhesions which can be separated by blunt dissection easily. There is no vascularization
2	Strong adhesion which may be separated slightly by blunt dissection but may need sharp dissection (vascularization begins)
3	Strong adhesion which can only be separated by sharp dissection; marked vascularization is present
4	Very strong adhesion fixing organs firmly which can only be separated by sharp dissection and high risk of organ injury during dissection

Table 2. Ignjatovic et al. score system for microscopic adhesions.

Grade	Findings
Grade 0	Normal, fibrosis absent
Grade 1	Focal slight fibroblast activation, minimal vascular proliferation
Grade 2	Widespread slight fibroblastic and vascular proliferation
Grade 3	More marked fibrosis, vascular proliferation
Grade 4	Widespread, severe fibroblastic proliferation, vascular proliferation, and inflammatory granulation tissue development

Figure 1. Grade 0 macroscopic adhesion in Group B (bevacizumab).

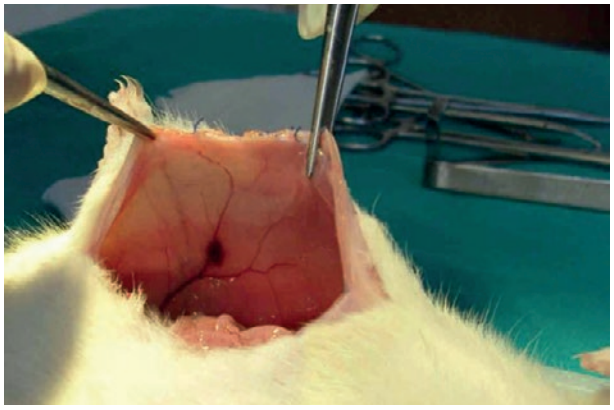
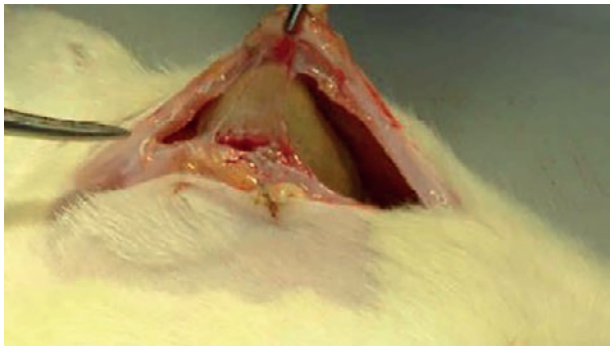


Figure 2. Grade 3 macroscopic adhesion in Group A (control).



Spearman's rho correlation analysis was used to evaluate the relationship between parameters that did not show normal distribution. Significance level was accepted as $p < 0.05$.

RESULTS

There was no operative or postoperative mortality, wound infection, or wound dehiscence. The groups were analyzed for microscopic and macroscopic adhesions. Both micro- and macroscopic adhesion grades in Group B were lower than those of control Group A (Fig. 3 and Table 3). Macroscopic grades were 2.69 ± 0.95 and 0.69 ± 0.8; microscopic grades were 2.25 ± 1.06 and 0.5 ± 0.52 for Group A and B, respectively. Micro- and macroscopic grades within each group were evaluated for correlation (Table 4). There was a 73.4% positive correlation between micro- and macroscopic grades for Group A, which was statistically significant ($p < 0.001$) (Fig. 4). There was an 84.7% positive correlation between micro- and macroscopic grades for Group B, which was statistically significant ($p < 0.001$) (Fig. 5).

Figure 3. Macroscopic and microscopic comparison graph of adhesion formation between Group A (control) and B (bevacizumab).

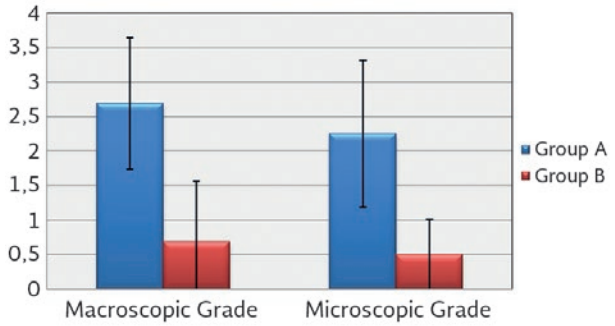


Figure 4. Positive correlation between macroscopic and microscopic grades in Group A (control).

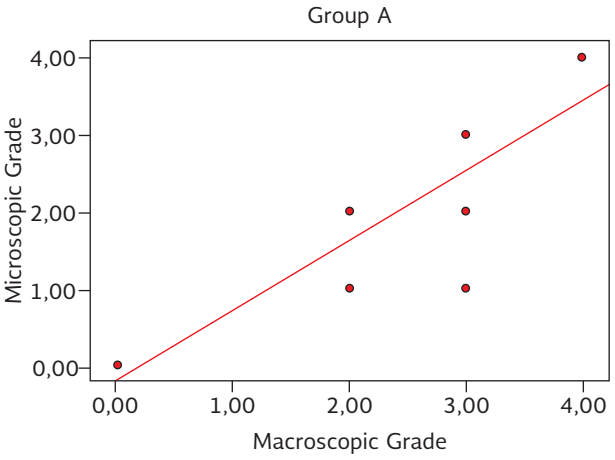


Figure 5. Positive correlation between macroscopic and microscopic grades in Group B (bevacizumab).

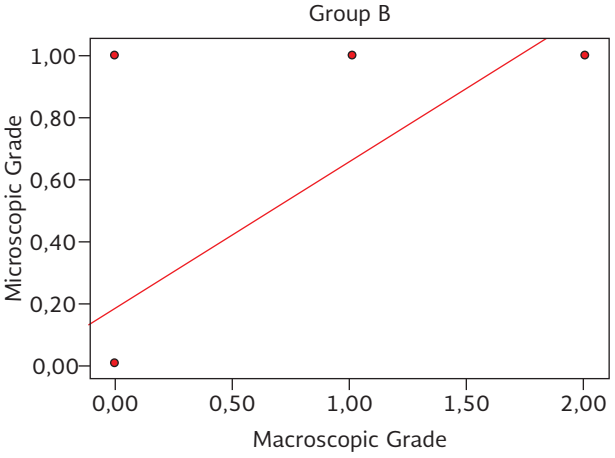


Table 3. Comparison of macroscopic and microscopic grades for each experimental group (Group A, control, and Group B, bevacizumab).

Group	Macroscopic grade	
	R	*p*
Group A		
Microscopic grade	0.734	0.001*
Group B		
Microscopic grade	0.847	0.000*

Spearman's rho correlation analysis, *$p<0.05$

Table 4. Comparison of macroscopic and microscopic grades between the experimental groups (Group A, control, and Group B, bevacizumab).

Grade	Group A (*n*=16)	Group B (*n*=16)	*p*
	Median (Q1-Q3)	Median (Q1-Q3)	
Macroscopic grade	3 (2-3)	0 (0-1.8)	0.000*
Microscopic grade	2 (2-3)	0.5 (0-1)	0.000*

Mann–Whitney *U*-test. *$p<0.05$

DISCUSSION

Peritoneal adhesions following abdominal operations are results of normal wound healing and constitute a major problem in surgery. Research on methods to prevent post-operative adhesion formation has been an important field for surgeons[18,19]. Factors such as trauma, infections, foreign bodies, and ischemia may cause PPA[20]. Intra-abdominal adhesions may also result in chronic pain, intestinal obstruction, fistula formation, and infertility. A method to prevent peritoneal adhesion formation may eliminate surgical intervention with the associated complications, morbidity, and cost[21]. VEGF is an important factor in peritoneal adhesion formation. It plays other roles in various physiological events and etiologies of pathological events within the body, including tumor growth and spread, and the promotion of proliferation, migration, and differentiation of endothelial cells.[22] Besides its crucial importance in angiogenesis, VEGF also plays a critical role in many endothelial cell functions. VEGF is

the most specific mitogenic factor known for endothelial cells, plays an important role in vasculogenesis and angiogenesis, and helps tumor growth and hematogenous spread[23]. VEGF is a basic factor in wound healing and is responsible for adhesion formation[24]. Thus, the inhibition of VEGF and neutralization of its effects are important in preventing peritoneal adhesions[14,15]. Bevacizumab is a recombinant IgG1 type monoclonal antibody which binds VEGF and inhibits its biologic activity[16]. It has recently been used intravitreally by ophthalmologists in treating proliferative diseases of the eye without any remarkable toxicity[25,26]. Studies report that inhibiting the biologic activity of VEGF with bevacizumab may limit adhesion formation following intra-abdominal surgery in rats[16]. Our observation that the formation of serious adhesions in all rats in the control group following cecal and serosal abrasions supported the role of serosal injury in adhesion formation. In our study, in spite of making serosal abrasions in the bevacizumab group, the finding of a statistically significant decrease in abrasion formation in both macroscopic and microscopic evaluations may be related to VEGF inhibition. This result suggested that bevacizumab may have been an efficient agent in preventing intra-abdominal adhesions. Taking its disadvantages into consideration, the most important adverse effects of bevacizumab are gastrointestinal perforation risk and negative effect on wound healing[27]. Allergy, intestinal bleeding, hypertension, leukopenia, abdominal pain, diarrhea, constipation, nausea, vomiting, and proteinuria are frequently seen side effects of intravenous bevacizumab[27]. We did not observe any prominent side effects in our study, leading us to suspect that these adverse effects might be dose dependent. Angiogenesis during wound healing promotes peritoneal adhesion formation. Inhibition of VEGF, which is responsible for angiogenesis, is promising in the prevention of adhesion formation. Different studies have also shown that bevacizumab was effective in preventing intraperitoneal adhesion formation, both macroscopically and microscopically[16,28]. Recently, intraperitoneal bevacizumab was reported to be well tolerated but not effective in symptom control of gastrointestinal malignancy-related ascites[29]. They reported that intraperitoneal bevacizumab is safe. The more serious side effects of intravenous bevacizumab were reported in a review[30]. As a result, we showed that intraperitoneal application of bevacizumab was effective in decreasing adhesion formation, which could prevent post-operative adhesion-related intestinal obstructions. However, more randomized prospective clinical studies are needed before bevacizumab is used for this purpose because of its serious side effects.

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