Intra-abdominal administration of bevacizumab diminishes intra-peritoneal adhesions

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**Abstract**

**AIM:** To determine the effect of a single dose of bevacizumab on adhesion formation in the rat cecum abrasion model.

**METHODS:** The cecum and parietal peritoneum of 38 male Wistar rats were abraded to promote adhesion formation. The rats were randomized into 2 groups: group 1 received bevacizumab (2.5 mg/kg) intraperitoneally, and group 2 received saline. On day 30 animals were killed, adhesions scored, and histopathological samples taken.

**RESULTS:** There was no wound dehiscence; there were 2 incision hernias (5.3%), 1 per group. Thirty-seven animals developed adhesions (97.4%). Adhesion grade and severity scores were significantly different between groups 1 and 2 at 2.7:1.6 ($P = .018$) and 3.8:2.7 ($P = .007$), respectively. There was no difference in adhesion square area (27.7:25.0%; $P = .16$), location ($P = 1.00$), or number (2.1:1.3; $P = .06$). Histopathology confirmed the statistical difference between groups ($P = .049$), and a highly significant correlation between results was shown ($r = .758$; $P = .0001$).

**CONCLUSION:** A single dose of intraperitoneal bevacizumab significantly reduces grade and severity of abdominal adhesions in the cecum abrasion rat model.

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**KEYWORDS:** Vascular endothelial growth factor; Adhesion; Postoperative; Peritoneum; Wistar rats; Comparative animal study

Abdominal adhesions remain a common clinical problem. They are often the reason for lengthy hospital stays both in cases of surgery for acute bowel obstruction, as well as for costly, time-consuming diagnostics and treatment of chronic abdominal pain. In acute small bowel obstruction mortality rates can be as high as 30%.\textsuperscript{1} Data for 1988 reveal 948,000 hospital days and 1.18 billion dollars for this treatment.\textsuperscript{2} This makes the effective prevention and treatment of abdominal adhesions not only a professional issue but also a financial burden for the health system.\textsuperscript{2}

The effect of vascular endothelial growth factor (VEGF) is not confined only to a potent angiogenic cytokine, but rather this substance has multiple effects on several crucial mechanisms in adhesion formation. The role of VEGF has been demonstrated in restorative tissue processes such as early inflammatory responses, wound repair, and remodeling through its effect on fibroblast function.\textsuperscript{3} Leukocytes, macrophages, and T lymphocytes have also been implicated in this process and data demonstrate that CD4$^+$ T cells play a central role in adhesion formation.\textsuperscript{4,5} Apart from angiogenesis, VEGF facilitates increased vascular permeability and the deposition of fibrinogen and subsequent cellular
migration. Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to and inhibits the biological activity of human VEGF.6

The aim of this study is to evaluate the effect of a single 2.5-mg/kg intraperitoneal dose of bevacizumab on adhesion formation in the cecum abrasion rat model.

Materials and Methods

License

The study was performed at the Laboratory Animal Unit at the Norwegian School of Veterinary Science in Oslo, Norway. The animal unit is licensed by the Norwegian Animal Research Authority (NARA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The study was approved by the animal unit local competent person, by NARA, and by the Animal Unit Ethics Committee of the Institutional Animal Care and Use Committee (IACUC).

Animals, housing, and husbandry

The animals were 42 male Wistar rats from Charles River Laboratories, 12 to 13 weeks of age, weighing 250 to 300 g. The rats were housed in type IV Macrolon rat cages, from North Kent Plastic Cages (Rochester, Kent, UK), in groups of 4. They lived on standard aspen bedding from Scanbur BK (Nittedal, Norway) and they were given Cellawatt nesting material, a red Teckniplast tunnel from Scanbur BK, and a gnawing-stick in wood. They were given standard SDS feed from Scanbur BK and tap water (which is tested for microbes once a month) ad libitum. The light was on from 8:00 AM to 8:00 PM and the temperature was 21°C ± 2°C; the room had 20 air changes within 24 hours and the humidity was 45 ± 5%. The cages and bedding were changed 2 times per week and the water was changed once per day by a skilled technician. The rats were acclimatized to the environment at the unit for 2 weeks before surgery.

Anesthesia

The rats were fasted from the night before surgery. Anesthesia was obtained by isoflurane gas, given in a chamber using airflow 200 mL and 2.5% isoflurane. When anesthesia was achieved the rats were moved to a mask giving isoflurane and then they were given Temgesic (Buprenorfin 0.3 mg/mL, Schering-Plough, Brussels, Belgium) 0.5 mg/kg subcutaneously (SC), carprofen 5 mg/kg SC, and NaCl 30 mL/kg SC.

Surgery

All surgical procedures were performed under aseptic conditions by a single surgeon (D.I.). The adhesion formation in the cecum abrasion rat model was induced between the cecum and anterior abdominal wall through a mini-laparotomy. A standardized lesion was made on the wall of the cecum and on the corresponding parietal peritoneum through stroking and scraping with gauze and pinching of the serosal surface with tweezers until local hemorrhage was induced to the site of injury. The cecum was placed back into the abdomen. A catheter was placed through the abdominal wall, outside the laparotomy in the superior right abdominal quadrant, and then the peritoneum, fascia, and skin were closed with 2/0 absorbable sutures. The skin was sutured intracutaneously. After closing the abdomen, the test material was injected intra-abdominally through the catheter. Group A rats were given NaCl and represent the control group. Group B rats were given 2.5 mg/kg bevacizumab (Avastin, Roche 25 mg/mL, Basel, Switzerland) diluted to 5 mg/mL with .9% NaCl. After the injection, the catheters were removed and the abdomen massaged to achieve equal spreading of the injected material. The duration of the total procedure, and therefore the duration of the anesthesia, was standardized to no more than 30 minutes. Animals that died were immediately replaced and operated on the same day.

Postoperative care

The rats were allowed to awake from the anesthesia in a single cage with a towel and a warming bottle. All rats were given a second injection of Temgesic 6–8 hours after surgery, on the evening of the day of surgery, and a third injection on the morning of the day after surgery. The rats were observed closely by a skilled technician during the days after surgery to look for symptoms of pain and to check the wound for swelling and bleeding.

After surgery

Four weeks after surgery the rats were given isoflurane anesthesia and then humanely killed by cervical dislocation during anesthesia. They were opened through the excision of the anterior abdominal wall, and the adhesions were identified and graded. The abdominal organs were then excised from the posterior abdominal wall and placed in 4% buffered formaldehyde solution together with the anterior abdominal wall with undisturbed adhesions. Photographs of adhesions were taken during the procedure.

Adhesion assessment

Adhesions were assessed between organs and abdominal wall and between the organs themselves by 2 surgeons (M.P. and D.I.) independently for location, extent, and type. The procedure of adhesion assessments included photography, discussion, and staging through consensus. The number of adhesions between organs/abdominal wall was determined by counting individual adhesion bands. For assessment of the extent of the adhesions between organs and...
abdominal wall, the latter was divided into 4 quarters, and the presence of adhesions in these quarters was noted (<25%, 25%–50%, 50%–75%, >75%). The type of adhesions was determined according to the method of Zühlke et al., whereby grade 0 indicates no adhesions and grade IV indicates firm extensive adhesions that are only dissectible with sharp instruments, with organ damage almost unavoidable. If different adhesion types were present, the highest type was scored. The severity of adhesion formation was calculated by multiplying the extent and type of adhesions.

**Histology**

The en block removed and formaldehyde fixed specimens were then re-examined by 1 of the pathologists (Y.C.) for adhesion formation. Representative samples from adhesions were taken out after staining with dye and the tissue samples embedded in paraffin blocks. Sections for microscopy were cut at 4 μm and stained according to standard laboratory procedure with hematoxylin–eosin (HE) in an automated stainer (Leica, ST 5020, Nussloch, Germany). Two HE slides were prepared from each block, and deeper sections were taken in selected cases. All cases were examined by light microscopy by 2 of the authors (S.S. and Y.C.). Adherences were categorized as histological types I–IV based on the presence and extent of fibrosis. Grade I was defined as scarce fibrosis of localized extent, grade II implied scarce but extensive fibrosis (at least 2 fibrous bands), grade III indicated pronounced but localized fibrosis, and grade IV was pronounced and extensive fibrosis. Significant (extensive) fibrosis was defined as the presence of granulation tissue with distinct capillaries within the area of adhesion (Fig. 1A and B). The fibrotic areas often contained

Figure 1  (A) Fibrous band of adhesion. Granulation tissue with capillaries (arrows) and bundles of collagen (C). Infiltration of lymphocytes (arrowheads) and mast cells (asterices). Histological score 4 (severe fibrosis, more than 1 focus). Hematoxylin-eosin (HE), original magnification × 200. (B) Fibrous adhesion between outer layers of gut wall (arrows). Histological score 3 (1 focus of severe fibrosis). HE, original magnification × 40. (C) Scarce fibrous adhesions between gut wall and serosal-layered abdominal adipose tissue (arrows), without significant granulation tissue. Histological score 1 (scarce fibrosis in 1 focus). HE, original magnification × 200.
distinct collagen fibers and mild–moderate infiltration of lymphocytes; mast cells within the infiltrates were striking in some cases. Another common finding was foreign body granulomas with giant cells containing highly birefringent material in polarized light, probably derived from gauze fibers; these granulomas occurred sometimes within the fibrous adhesions and sometimes along the serosal surface, and were not used for lesion scoring. Cases of scarce fibrosis were characterized by subtle changes only, with interposition of abdominal fat tissue between serosal surfaces, with only small and discrete areas of fibrosis with no or minimal granulation tissue (Fig. 1C).

Histological scoring was performed independently of surgical grading. All cases were examined microscopically by each of the pathologists; thereafter, the series of slides were reviewed by both together. Consensus on the final score was achieved in all cases. If several types of adhesions were present in 1 animal, all were noted; however, the most severe was used for the statistical analysis.

Study design

The randomization method was block randomization using Random Allocation software v 1.00 (Isfahan University of Medical Sciences, Iran). The researchers were blinded to the treatment and control groups. The sample size was calculated with SSD v 7.3 software (Henrik Lehman, Haugesund, Norway). The sample size for a 2-sided test, study power 80%, to detect a 20% difference between the groups was 38 (19 animals in each arm).

Statistics

Analysis was done using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) software. Percentages were compared using Student t test and continuous variables with the Mann–Whitney test. Chi-square test was used for nonparametric values and Spearman test for correlation. The P values given are 2-sided; P = .05 was considered to be the limit of significance.

Results

A total of 42 rats were operated. Four animals died, 2 during anesthesia and 2 in the following 2 days after surgery. All were immediately replaced but not included in the study. There were no symptoms before death; all 4 underwent necropsy without significant findings. Serology for mycoplasma, carbacillus, Sendai, and pneumonia virus from mice (PVM) were negative. Deaths were not related to surgery. The remaining 38 animals recovered without incident and resumed preoperative physical activity and feeding patterns by postoperative day 2. There was no wound dehiscence; 2 animals developed an incision hernia (5.3%), 1 in each group. All animals, except 1, developed intra-abdominal adhesions (97.4%). Most animals developed adhesions between the cecum and small bowel (73.7%), omentum (47.3%), and abdominal wall (23.6%) and there was no statistical significant difference between the groups according to location of the adhesions (Figs. 2 and 3). However, adhesion grades were significantly different between the groups (Fig. 4) (mean grade 2.7:1.6; P = .018). There was no statistical difference in the area occupied by the adhesions (mean surface 27.7:25.0%; P = .1628), nor in the number of adhesions (mean number 2.1:1.3; P = .064), although this difference was nearly significant. The adhesion severity score gave a high significant difference (mean score × area 3.8:2.7; P = .0086) between the groups (Fig. 5).

All specimens were eligible for histopathology. The mean adhesion grade at histology was 3.55 (SD .69) and

![Figure 2](image2.png) Adhesions between the cecum and anterior abdominal wall, macroscopic grade 2 (arrow). The laparotomy can be seen free of adhesions.

![Figure 3](image3.png) Adhesions of the omentum to the anterior abdominal wall, macroscopic grade 3 (arrow). The adhesion is to the laparotomy site.
2.63 (SD 1.46) for control and test groups, respectively. The difference between the groups was statistically significantly different ($P = .049$). Moreover, a highly significant and positive correlation has been demonstrated ($r = .758; P = .0001$) between the macroscopic grading and the microscopic grading (Fig. 6). Mean histological grade was significantly higher ($P = .0001$) than macroscopic grading for 1.022 grades.

Granuloma formation was noted in 23 (60.5%) animals, 12 (31.5%) in the control group and 11 (28.9%) in the treatment group. Granulation tissue within the adhesions was noted in 31 (81.6%) animals, 15 (39.5%) and 16 (42.1%) in the control and test groups, respectively. There was no statistical difference between groups.

### Comments

The main finding of this study is that a single dose of bevacizumab, consisting of a half therapeutically administered dose for the treatment of patients with metastatic disease, seems to hinder the development of abdominal adhesions.

Although there was a statistically significant correlation between the surgeons’ adhesion scorings and the histopathological grading of fibrosis, there were cases of definite discrepancy between the 2. In general, sampling problems for histology can explain such cases. For example, a few very focal adhesions detected on the fixed organ block seem to have been overlooked by the surgeons on the fresh specimen, while some adhesions can be too thin to sample optimally.

The overall data collected in this study correlate well with data previously published. Two similar, previously published studies were performed with species specific antibodies to VEGF; in these studies, the test period was restricted to 7 and 14 days.$^{10,11}$ Our study was performed with a humanized antibody, in a species where abundant literature suggests a similarity in effect of bevacizumab in rats and humans.$^{12,13}$ Our follow-up period was 4 weeks, and the adhesion maturation process can be affected by the reabsorbed circulating bevacizumab since it remains in circulation up to 6 weeks. Compared with the previous studies,$^{10,11}$ our findings may imply a lesser area of adhesions and a lower adhesion maturation level. It is difficult to determine whether this is the effect of the reabsorbed bevacizumab, which remains to be tested in further studies.

The change in the perception of abdominal adhesions from inert to dynamic structures seems to have occurred at the shift of the century. Human peritoneal adhesions have been proved to be highly cellular, vascularized, and innervated.$^{14-17}$ The presence of blood vessels and nerves (both

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**Figure 4** Y axis: adhesion grade. X axis: $C =$ control group; $T =$ test group. The test group had a significantly lower adhesion grades, $P = .018$.

**Figure 5** Y axis: adhesion severity score. X axis: $C =$ control group; $T =$ test group. The test group had a significantly lower adhesion severity score, $P = .0086$.

**Figure 6** Y axis: macroscopic adhesion score. X axis: histopathological adhesion score. A highly significant positive correlation between the groups was found ($r = .758; P = .0001$).
It has been demonstrated that fewer adhesions develop after laparoscopic bowel resection and laparoscopic adhesiolysis than after conventional surgery. The type of trauma inflicted while performing adhesiolysis, where the surgeon usually does not use diathermy but rather blunt dissection and scissors. In this context, the addition of a single intraperitoneal dose of bevacizumab after performing a safe laparoscopic adhesiolysis could be attempted for the prevention of adhesions in patients suffering from chronic abdominal pain and/or in those suffering from recurrent small bowel obstructions. The fact that bevacizumab has unwanted side effects does not exclude its use but rather narrows the patient group that could benefit from a single intra-abdominal application, and can cause modifications in the operative technique (eg, use of muscle splitting trocar insertion). The clinical success and safety of VEGF neutralization in the treatment of malignant diseases adds further momentum to such a statement.

From our study, we conclude that a single intraperitoneal dose of bevacizumab diminishes both the grade and severity of abdominal adhesions in the rat cecum abrasion model. We consider this model of value for further studies on the mechanisms involved in adhesion prevention within the peritoneum.

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