Infliximab "TNF-alpha antagonist" decreases intraabdominal adhesions

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ABSTRACT

Objectives: To evaluate the effect of infliximab on adhesion formation and it's associated morbidity and complications.

Methods: This study was performed in the Faculty of Medicine, Gazi University, Turkey between July 2005 and October 2005. Thirty-five rats were randomly divided into 4 groups. Laparotomy was performed in the Sham group (n=5), whereas cecal abrasion was carried out in all other groups. After cecal abrasion 0.9% sodium chloride was administered in the saline group (n=10), infliximab was administered to the study group (n=10) and nothing was administered to the last group (n=10). Adhesion formation was evaluated with macroscopic and microscopic adhesion scoring systems. Peritoneal fluid samples and mesenteric lymph node biopsies were taken to rule out bacterial peritonitis. Blood and peritoneal irrigation fluid samples were taken to measure the Tumor necrosis factor-alpha (TNF-α) levels.

Results: Macroscopic adhesion scores showed fewer adhesions in the infliximab group. The infliximab group had significantly fewer adhesions than the abrasion control and saline groups. According to the histological findings, there were no statistically significant differences between the groups.

Conclusion: Early blocking of the activity of TNF-α after cecal abrasion resulted in lower rates of adhesion formation, macroscopically. The TNF-α, a proinflammatory cytokine appears to be an important mediator for postoperative adhesion formation.

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Adhesion formation after abdominal and pelvic operation is an extremely common problem, and still a source of considerable morbidity such as small bowel obstruction, female infertility, chronic pelvic pain, and increased risk of visceral injury at subsequent laparotomies.[1] Adhesions result in a large surgical workload and high cost to healthcare systems.[2] Adhesions are abnormal deposits of fibrous tissue formed in the peritoneal cavity. Peritoneal injury, from a variety of causes, leads to peritoneal inflammation and production of plasminogen activator inhibitors, which result in the loss of normal mesothelial fibrinolytic activity and this allows the organization of fibrinous adhesions into permanent fibrous adhesions. The incidence of intraperitoneal adhesions ranges from 67-93% after general surgical abdominal operations, and up to 97% after open gynecologic pelvic procedures.[3] In autopsy studies of the patients who had prior laparotomies, the incidence of intra-abdominal adhesions was 70-90%.[4] Factors associated with the formation of post surgical adhesions include trauma, thermal injury, infection, ischemia and foreign bodies. Various other factors including tight suturing, which cause ischemia within the sutured peritoneum, abrasions, talc and powder from the gloves, intestinal contents, overheating by lamps, or irrigation fluid, may contribute to postoperative adhesion formation. Besides, individual factors such as nutritional status, disease states like diabetes, presence of concurrent infectious processes, which alter leukocyte and fibroblast function may affect adhesion formation. Although optimal surgical techniques are designed to minimize mesothelial injury, peritoneal trauma is unavoidable. Therefore, adjuvant therapy is necessary in preventing adhesion formation. Various agents have been tested experimentally and clinically to prevent postoperative adhesions including adjusting surgical techniques, limiting trauma to intra-abdominal structures and applying adjuvants to decrease adhesion formation such as

nonsteroidal anti-inflammatory drugs, glucocorticoids, and antihistamine therapy, anticoagulants, fibrinolytics, antibiotics, barrier solutions, and solid barriers with limited success rates.[5-7] Tumor necrosis factor-alpha (TNF-α), a polypeptide hormone, plays a major role in the immunological cascade leading to inflammatory responses to injury. Secreted early in the inflammatory cascade from activated macrophages, it plays a major role in stimulating the release of a variety of mediators including interleukin-2, interleukin-6, and platelet activating factor. It also increases the expression of adhesion molecules in mesothelial cells and appears to be a good biological marker for postoperative intra-abdominal adhesion formation.[8,9] It was demonstrated that higher levels of locally excreted (peritoneal exudates) and systemic TNF-α in the immediate postoperative period significantly correlated with higher rates of adhesion formation.[10] Modulating the immunological response by antagonist therapies against key inflammatory mediators may have an important role in reducing postoperative peritoneal adhesions. In this study, we investigate the role of antibodies against TNF-α, which plays a role in the early inflammatory reaction after intraperitoneal injury. The TNF-α antagonist was administered intraperitoneally in previous studies, however, the effect of local administration of this agent was observed in the current study.

Methods. Thirty-five male Wistar - Albino rats weighing between 200-250 g were acclimated for one week before the experiment. Animals were housed at 23 ± 3ºC and allowed free access to water and standard rodent chow. This study was performed in the Faculty of Medicine, Gazi University, Turkey between July 2005 and October 2005. Gazi University Ethical Council, Ankara, Turkey, approved the study and the experiments were conducted in accordance with animal protection laws. The 35 rats were randomly divided into 4 groups as follows: Sham group (n=5): Only laparotomy was performed, with no adhesion induction method or intraperitoneal (ip) administration of any substance. Saline group (n=10): Adhesion induction by cecal abrasion method and ip administration of 2 ml/rat 0.9% sodium chloride (NaCl). Abrasion control group (n=10): Adhesion induction but no ip administration of any substance. Infliximab group (n=10): Adhesion induction and ip administration of infliximab 5 mg/kg (Remicade®, Shering-Plough Co., Innishannon, County Cork, Ireland). Each rat was anesthetized with intramuscular ketamine hydrochloride 40 mg/kg (Sanofi-Ceva GmbH, Dusseldorf, Germany). The abdomen was shaved and prepared with povidone iodine solution. Powder free gloves were used for the procedures to avoid adhesions due to starch peritonitis. Before the surgical procedure, 1 ml of blood was drawn from the tail vein to determine baseline TNF-α level in rats. Using a sterile technique, a 4 cm vertical midline incision was made and abrasions on the cecal serosa layer were made using sterile gauze scraping until a punctuate hemorrhage on the 3 x 3 cm cecal serosal surface, but not perforation, were seen. Before this, peritoneal irrigation fluid samples were taken from all rats for calculating baseline peritoneal TNF-α concentrations. Handling of other tissues was minimized. Before the abdominal closure, no adjuvant therapy was given to the rats in the abrasion only group, immediately after the injury 2 ml of 0.9% NaCl was instilled onto the cecum of each rat in the saline group, and 5 mg/kg infliximab was given intraperitoneally in the infliximab group just after the cecal serosal injury. Only laparotomy was performed in the Sham group, with no adhesion induction method or intraperitoneally administration of any substance. The incision was closed in a single layer, excluding the peritoneum, with simple continuous sutures of 3/0 polypropylene. The animals were allowed to resume their diets until the 10th postoperative day when they were sacrificed with ether. The abdomen was opened via a "U" shaped incision for complete exploration. The previous midline incision and abdominal cavity were inspected. The extent and severity of adhesions were graded by an independent surgeon and veterinarian, who were blinded with respect to the groups, using the classification reported by Nair et al[11] (**Table 1**).

Microbiological examination. Peritoneal fluid samples and mesenteric lymph node biopsies were taken from all animals on revision laparotomy to rule out bacterial peritonitis. For peritoneal fluid samples, first 2 ml of 0.9% NaCl was given in the abdominal cavity and then aspirated for microbiological examination. The samples were immediately processed into medium and cultured semiquantitatively. Blood and eosin methylene blue agar along with thioglycolate medium was inoculated

Table 1 - Macroscopic adhesion scores were determined according to Adhesion Scoring System which was defined by Nair et al.[11]

Description of Adhesive Bands	Score
Complete absence of adhesions	0
Single band of adhesions, between viscera, or from one viscus to abdominal wall	1
Two bands: between viscera or from viscera to abdominal wall	2
More than two bands: between viscera, or viscera to abdominal wall, or whole of intestines forming a mass without being adherent to abdominal wall	3
Viscera directly adherent to abdominal wall, irrespective of number and extent of adhesive bands	4

for aerobic culture, and Schaedler agar incubated in a Gas-Pak jar was used for anaerobic culture. After 48 hours of incubation at 37°C growing colonies were identified with standard bacteriological techniques. For screening of bacteremia, 1 ml of blood was taken from the heart during sacrification and injected into pediatric blood culture bottles (Bact/ALERT PF: bioMerieux, Durham, NC, England) and cultivated for 10 days using an automated blood culture system. At the end of the incubation period, all blood culture bottles were subcultured on blood agar plates, to demonstrate any undetected bacterial growth.

Biochemical study. Blood samples (2 mL/rat) and peritoneal exudates, taken from all animals during sacrification, were processed. Serum TNF-α levels were measured with a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) using rat TNF-α kit (Biosource International, Camarillo, CA, USA). The TNF-α levels determined from a standard curve for recombinant TNF-α; and concentration were expressed as pg/mL.

Table 2 - Microscopic adhesion scores were determined according to Microscopic Adhesion Scoring System which was defined by Quesada et al.[12]

Description of Adhesive Bands	Score
Presence of suture material (present or absent)	1
Continuity of mucosal epithelium (present or absent)	2
Abnormal proliferation of mucosal epithelium (present or absent)	3
Inclusion of mucosal tissue in the muscle layer (present or absent)	4
Presence of foreign body granulation tissue Grade 1: absent Grade 2: mild Grade 3: moderate Grade 4: severe	5
Inflammatory reaction (grades 1-4)	6
Neovascularization (grades 1-4)	7

Histopathological examinations. The biopsies from the adhesive bands were fixed in 10% formalin solution. After routine tissue processing, serial sections (5μm) were stained with hematoxylin and eosin. The inflammatory reaction was assessed for each group by light microscopy using a scoring system described by Quesada et al,[12] (**Table 2**).

Statistical analysis. All data were entered into and processed by Statistical Package for Social Sciences SPSS version 7.5 for Windows statistical package. Differences between groups were evaluated by paired samples t-test. *P*-values of <0.05 were considered statistically significant.

Results. There were no deaths during the postoperative period. The degrees of adhesions in all groups were calculated according to the "Macroscopic adhesion scoring system" by Nair et al.[11] The adhesive band formation scores according to the groups are shown in **Table 3**. The difference between the infliximab group and the abrasion control group was statistically significant (p=0.0001), as was the infliximab group and the saline group (p=0.001). No significant difference was found between the Sham group and the infliximab group in terms of macroscopic adhesion scores (p>0.05). The microscopic adhesion parameters were scored using the scheme by Quesada et al.[12] The microscopic adhesion scores of groups are shown in **Table 3**. The difference of microscopic adhesion scores between the abrasion control group and the infliximab group were statistically significant (p=0.005) (**Figures 1a & 1b**). The scores of the Sham group and the infliximab group were similar, and the difference was not significant (**Figures 1a & 1c**). Although the saline group had a higher score than the infliximab group, the difference was insignificant. Cultures of the samples taken from the peritoneal irrigation fluid and mesenteric lymph nodes during the second laparotomy did not reveal any intra-abdominal infection. Also no growing colonies of bacteria typical for enteral flora were shown in blood cultures. The baseline serum TNF-α levels were determined as 2.5 ± 1.71 pg/ml. Serum TNF-α levels

Table 3 - Serum and peritoneal irrigation fluid TNF-α levels, macroscopic and microscopic adhesion scores according to the groups.

Parameters	Sham group	Saline group	Abrasion control group	Infliximab group
Serum TNF-α (pg/ml)	7.66 ± 0.93*	8.57 ± 4.25*	5.61 ± 4.49	2.90 ± 1.74*
Peritoneal TNF-α (pg/ml)	5.16 ± 2.09	3.43 ± 1.84	5.51 ± 3.90	3.03 ± 1.39
Macroscopic adhesion score	0.2 ± 0.44	2.1 ± 1.1†	2.2 ± 0.78†	0.5 ± 0.52†
Microscopic adhesion score	5.00 ± 2.7	7.00 ± 0.94	8.3 ± 0.76‡	6.2 ± 0.78‡

* Sham group - abrasion plus infliximab group, p=0.0001;
abrasion plus 0.9% NaCl administered group - abrasion plus infliximab group p=0.002
† abrasion plus 0.9% NaCl administered group - abrasion plus infliximab group, p=0.001;
abrasion only group - abrasion plus infliximab group p=0.0001
‡ abrasion only group - abrasion plus infliximab group, p=0.005

in the Sham group, saline group, abrasion control, and infliximab group are shown in **Table 3**. The difference between the saline group and the infliximab group was statistically significant (p=0.002) as was the infliximab group and the sham group (p=0.0001). There was a slight difference between serum TNF-α levels of the infliximab group and the abrasion control group, but it was not statistically significant (p=0.10). Baseline TNF-α levels in the peritoneal irrigation fluid were determined as 3.99 ± 1.66 pg/ml. **Table 3** shows the levels for the other groups, and the difference between groups was not statistically significant. The increases in the TNF-α level in serum and peritoneal fluid samples were found to have a relation with surgical procedure and not with any intra-abdominal infection.

Discussion. In this study, it was observed that the macroscopic and microscopic adhesion scores of the abrasion control group and saline groups were higher than infliximab group. According to the statistical analysis, the differences of macroscopic adhesion scores between the saline group and the infliximab group, and between the abrasion control group and the infliximab group were statistically significant, whereas the difference of microscopic adhesion scores of the abrasion control group and the infliximab group were found to be statistically significant. Although the microscopic adhesion score of the saline group was higher than the infliximab group, the difference did not reach statistical significance (p=0.055). The serum TNF-α level of the infliximab group was similar to the baseline value, and the difference between these 2 values was statistically insignificant (p=0.69). The TNF-α levels of sham, saline, and abrasion control groups were higher than the infliximab group. The difference between the sham group and the infliximab group (p=0.0001), and that between the saline group and the infliximab group (p=0.002) were statistically significant. Despite the higher serum TNF-α level in the abrasion control group, the difference between the abrasion control group and the infliximab group was not statistically significant (p=0.10). Finally, the differences of the peritoneal TNF-α levels between the groups were not statistically significant. The effects of preoperative systemic administration of antibodies against TNF-α and IL-1 were determined previously.[10] Kaidi AA et al[10] reported

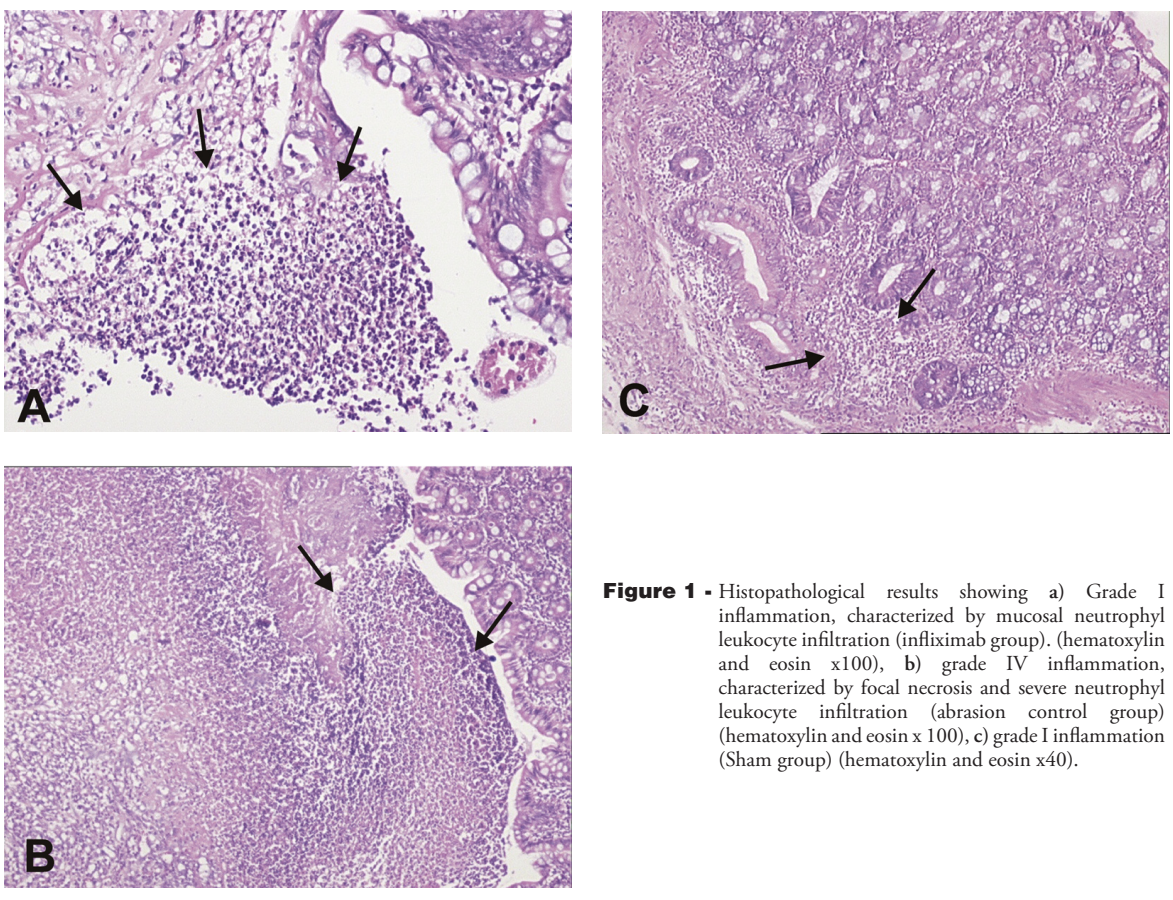

Figure 1 - Histopathological results showing **a**) Grade I inflammation, characterized by mucosal neutrophyl leukocyte infiltration (infliximab group). (hematoxylin and eosin x100), **b**) grade IV inflammation, characterized by focal necrosis and severe neutrophyl leukocyte infiltration (abrasion control group) (hematoxylin and eosin x 100), **c**) grade I inflammation (Sham group) (hematoxylin and eosin x40).

that the systemic administration of these antibodies preoperatively reduces adhesion formation. Modulation of proinflammatory cytokines alters adhesion formation after laparotomies. In our study, we aimed to investigate whether the local administration of TNF-α antagonist reduced adhesion formation. Our results showed that the local administration of this agent is effective, since serum and peritoneal TNF-α levels, and the macroscopic adhesion score were found to be lower in the study group. Peritoneal adhesion is a result of injury to the peritoneal surface. Despite elaborate efforts to develop effective strategies to reduce or prevent adhesions, their formation remains a frequently encountered problem after abdominal surgery. It is well known that good surgical technique alone does not prevent adhesion formation.[4] Post surgical adhesions severely affect the quality of life of millions of people worldwide, causing small bowel obstructions, difficult reoperative surgery, chronic abdominal and pelvic pain and female infertility. The problem of post surgical adhesions to the health care system increases with the patient's age, the number of laparotomies, and the complexity of operative procedures.[13] The most common cause of peritoneal adhesions is prior surgery.[14] Perry et al[15] found that 79% of 388 patients operated for intestinal obstruction had a history of surgery. Bowel obstruction from adhesions is most prevalent in the pediatric age group, in which 8% of neonates undergoing abdominal surgery require a future laparotomy for this complication.[16] Although it is not clear how often adhesive obstruction recurs after conservative or surgical treatment, it is well known that adhesions create a lifetime risk of intestinal obstruction.

Intra-abdominal adhesions are fibrous bands formed between serosal surfaces as a result of an inflammatory reaction. Damage to serosal surfaces is followed by secretion of histamine and other permeability increasing factors from the mast cells. All these factors increase vascular permeability and cause plasma exudation into the peritoneum, resulting in fibrin formation. The key step in adhesiogenesis is the deposition of fibrin, triggered by peritoneal trauma. Stimulation of the intraperitoneal fibrinolytic system is required for the degradation of this fibrin, and this fibrinolytic activity predominantly results from the production of tissue plasminogen activator.[4] Adhesion formation follows the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation with the formation of adhesions. Although several substances have been developed to prevent postoperative adhesions no reliable agent has achieved widespread acceptance for prophylaxis of postoperative adhesions.

The TNF-α is a well-known proinflammatory cytokine. Secretion of TNF-α increases early in the inflammatory cascade from stimulated monocytes and macrophages. In vitro experiments have shown that TNF-α is likely to have many functions in the abdominal cavity. It induces the production of interleukins from mesothelial cell cultures, which suggests that it may modulate the inflammatory response. It also increases the expression of adhesion molecules in mesothelial cells, which are functionally relevant for interaction with leucocytes and promoted coagulation by the induction of tissue factors, and the persistence of fibrin by reduction of tissue plasminogen activator production as well as increasing plasminogen activator inhibitor type-1 synthesis.[17] In humans the relevance of TNF-α in peritoneal tissue repair has been explored to some extent. TNF-α increases postoperatively when assayed in abdominal drain fluid.[18] Interestingly, experiments in rats showed a significant correlation between severity of adhesion formation and levels of TNF-α in serum and peritoneal fluid after operation.[10] Although suggested as a potential marker of adhesion formation and theoretically a potential modulator of adhesion formation by modulating inflammation, coagulation, and fibrinolysis, the importance of TNF-α in adhesion development in humans is yet to be elucidated. In our study, the peritoneal TNF-α levels were similar in groups. Rats were sacrificed on the 10th day of the operation. TNF-α levels increase early in the inflammatory cascade and return to normal levels in the latter term, as discussed before. This may explain our observation that the peritoneal TNF-α levels were similar between the groups.

This study investigates the effect of intraperitoneally administered infliximab, specific monoclonal antibody of TNF-α, on the development of intra abdominal adhesions in an experimental rat model. As demonstrated, lower levels of locally secreted and systemic TNF-α in the postoperative period after the administration of intraperitoneal infliximab, correlate with the lower rates of adhesion formation. Modulating the immunological response by antagonist therapies against key inflammatory mediators may play an important role in reduction of intra abdominal adhesion formation. In this study, we selectively inhibited the activity of TNF-α, at a molecular level, and studied its effect on postoperative adhesion formation. Blocking the activity of TNF-α early after bowel injury resulted in lower rates of adhesion formation and reduced amounts of collagen deposition and fibroblasts were seen in treated animals. TNF-α, a proinflammatory cytokine appears to be an important target for modulating the postoperative adhesion formation. Our results warrant further studies to determine the role of antibodies against TNF-α in the prevention of peritoneal adhesions in humans.

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