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Influence of the Platelet-Activating Factor Receptor Antagonist BB-882 on Intra-Abdominal Adhesion Formation in Rats

S. Otcu[a] H. Ozturk[a] M. Aldemir[b] N. Kilinc[c] A.I. Dokucu[a]

Departments of [a]Pediatric Surgery, [b]General Surgery and [c]Pathology, Dicle University Medical School, Diyarbakır, Turkey

Key Words
BB-882 · Lexipafant · Postoperative intraperitoneal adhesions

Abstract
Postoperative intra-abdominal adhesion formation is a major clinical problem. We aimed to examine the preventive effect of treatment with the platelet-activating factor (PAF) antagonist (lexipafant, BB-882) on experimentally induced intra-abdominal adhesion formation in rats. Twenty male Sprague-Dawley rats weighing 250 and 290 g were studied. Generation of adhesions in rats by brushing a 1-cm² area of the cecum and the peritoneum on the right side of the abdominal wall was followed by intra-abdominal administration of saline and 5 mg/kg in a volume of 0.2 ml PAF receptor antagonist BB-882. After 45 days, formation of adhesions was graded and histological evaluation was processed. The severity of adhesions was significantly less in the BB-882 group than in the control group ($p < 0.001$, $p < 0.05$). The average adhesion scores in the control and BB-882 groups were 3.2 ± 0.6 and 0.6 ± 0.6, respectively, and the difference between both groups was found to be significant ($p < 0.0001$). The number of polymorphonuclear leukocytes and fibrotic areas was significantly decreased in the BB-882 group when compared to the control group ($p < 0.001$, $p < 0.002$). In conclusion, this study confirms the efficacy of BB-882 in the prevention of postoperative intra-abdominal adhesions in a rat model.

Introduction

Postoperative intra-abdominal adhesion formation is a major clinical problem. More than 90% of all abdominal surgical procedures are estimated to cause adhesions [1]. The complications of adhesion formation include bowel obstruction, chronic pelvic pain, and female infertility [2]. Intra-abdominal adhesion formation is a complex process that involves multiple factors that control inflammation, cellular proliferation and migration, collagen and matrix synthesis, and interactions between many different cell types, blood and matrix components [3]. To date, many animal and clinical studies have tested a variety of agents, such as interleukin-10, corticosteroids, nonsteroidal anti-inflammatory drugs, lactated Ringer's solution, dextran, hyaluronic acid, and physical barriers, to prevent intra-abdominal adhesion formation [3]. Some of these agents have been shown to reduce the number and quality of adhesions; unfortunately, however, the success rate of these trials has not been encouraging. Therefore, new approaches to this problem are highly warranted.

Platelet-activating factor (PAF) is an inflammatory chemical mediator and has various biologic actions such as stimulation of platelets and neutrophils, and an increase in response to various inflammatory stimuli [4]. PAF is a rapid stimulator of prostaglandins and oxygen radical synthesis. In addition, PAF can increase the toxicity of other proinflammatory cytokines, including tumor necrosis factor (TNF) and interleukin (IL)-2, and

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Selcuk Otcu, MD
Department of Pediatric Surgery
Dicle University Medical School
TR–21280 Diyarbakır (Turkey)
Fax +90 412 2488440, E-Mail selcukotcu@hotmail.com

may contribute to the induction of nitric oxide [5–8]. It is suggested that BB-882 (lexipafant) blocks PAF receptors on the surface of endothelial cells, and so downregulates the activation of leukocytes/macrophages [9]. Because of this property, we wanted to evaluate the effects of the PAF antagonist, BB-882 (lexipafant), on experimentally induced intra-abdominal adhesion formation in rats.

Materials and Methods

Animals and Experimental Design

Twenty male Sprague-Dawley rats (Dicle University Research Center) weighing between 250 and 290 g were housed in a climate-controlled animal care facility. The animals were fed with standard rodent chow and water ad libitum. All surgical procedures were performed under phenobarbital anesthesia (2.4 mg/100 g i.p.) employing a sterile technique. Sterile surgical techniques were used throughout the study. After being shaved, the skin was prepared with 1% povidone-iodine solution. A midline incision, 3 cm in length, was made and the cecum was exteriorized. A 1-cm^2 area of the cecum was brushed 10 times with a medium bristle brush until the serosal layer was denuded and petechial hemorrhages had developed. Then a 1-cm^2 peritoneal injury on the right side of the abdominal wall opposite the denuded cecum was created again by brushing [1]. Before abdominal closure, the first (control, n = 10) and second (BB-882, n = 10) groups were intraperitoneally administered 1 ml of normal saline and 5 mg/kg in a volume of 0.2 ml PAF receptor antagonist BB-882 (Lexipafant, British Biotech Pharmaceuticals Ltd., Oxford, UK), respectively. The abdomen was closed with continuous 4–0 silk sutures in two layers.

The rats were allowed to resume their diet until they were sacrificed on the 45th postoperative day by exposure to an overdose of ether. Before they were sacrificed, all rats were weighed. The abdomen was inspected through a U-shape incision, retracted to the right side to provide maximum exposure. The number of adhesions and their severity were recorded for every case, and the severity of the adhesions was graded. Grading was done by an investigator blinded to the study according to the system employed by Nair et al. [10] (table 1).

Histopathology

The adhesion tissue specimens were fixed in 10% formaldehyde, then dehydrated and embedded in paraffin wax. The samples were sectioned at 4 μm and stained with Masson's trichrome and assessed in a blinded fashion by pathologists.

The amount of fibrotic area (μm^2) and the number of polymorphonuclear leukocytes in 100 fields were separately counted using either an objective mounted micrometer (200× magnification, Olympus Eyepiece Micrometer®) or a light microscope (100× magnification, Olympus, BH4).

Statistical Analysis

Data were entered and analyzed on a personal computer using SPSS version 9.0. All values are expressed as the median ± SEM. Differences among groups were evaluated by the Mann-Whitney U test. p values of <0.05 were considered significant.

Table 1. Grading of adhesions in rats according to the criteria of Nair et al. [9]

Grade	Description	Classification
0	Complete absence of adhesions	Insubstantial adhesions
1	Single band of adhesions between viscera or from one viscus to the abdominal wall	Insubstantial adhesion
2	Two bands, either between viscera or from viscera to the abdominal wall	Substantial adhesions
3	More than two bands between viscera or from viscera to the abdominal wall, or whole of intestines forming a mass, without being adherent to the abdominal wall	Substantial adhesions
4	Viscera directly adherent to the abdominal wall, regardless of number and extent of adhesive bands	Substantial adhesions

Results

Table 2 presents the numbers of animals with different grades of adhesions in each group. The difference between groups was extremely significant ($p < 0.0001$). The severity of adhesions was significantly less in the BB-882 group than in the control group ($p < 0.001$ for BB-882 vs. control in grade 0, $p < 0.05$ for BB-882 vs. control in grade 1). In contrast, in the control group the severity of adhesions was found to be grade 3 and this was significantly different in comparison with the BB-882-treated group ($p < 0.002$ for BB-882 vs. control in grade 3; fig. 1). The average adhesion scores in the control and BB-882 groups were 3.2 ± 0.6 and 0.6 ± 0.6, respectively (table 3), and the difference between both was found to be significant ($p < 0.0001$).

The count of polymorphonuclear leukocytes was found to be 51.2 ± 8.1 and 8.4 ± 1.9 in the control and BB-882 groups, respectively. The number of polymorphonuclear leukocytes was significantly decreased in the BB-882 group when compared to the control group ($p < 0.001$; fig. 2).

The fibrotic area was 4,450 ± 280 and 850 ± 170 μm^2 in the control and BB-882 groups, respectively. The fibrotic area was significantly smaller in the BB-882 group when compared to the control group ($p < 0.002$; fig. 3, 4).

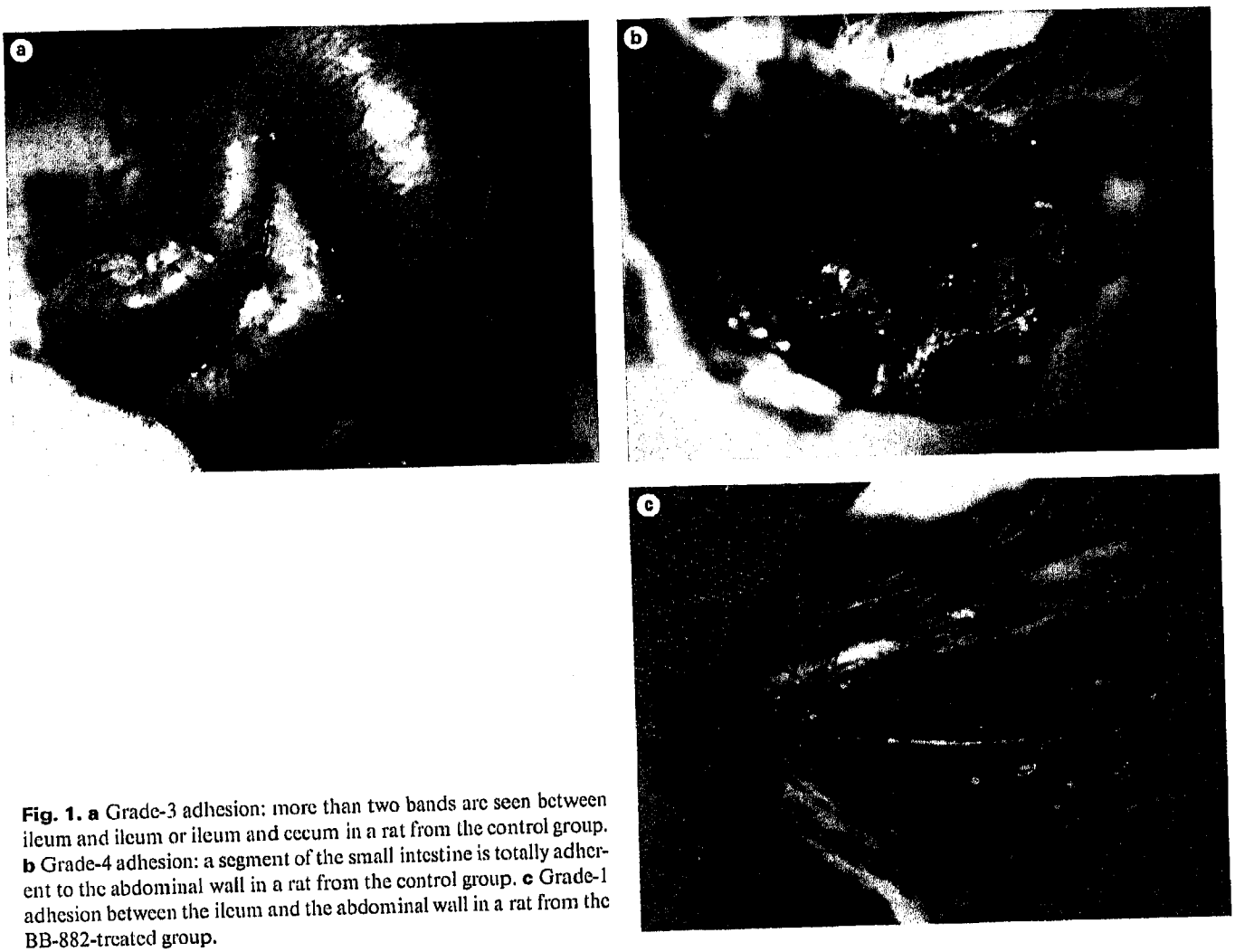

Fig. 1. a Grade-3 adhesion: more than two bands are seen between ileum and ileum or ileum and cecum in a rat from the control group. **b** Grade-4 adhesion: a segment of the small intestine is totally adherent to the abdominal wall in a rat from the control group. **c** Grade-1 adhesion between the ileum and the abdominal wall in a rat from the BB-882-treated group.

Table 2. The number of rats with different grades of adhesions in the groups

Groups	n	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Control	10	0	0	1	6[a]	3
BB-882	10	5[b]	4[c]	1	0	0

[a] $p < 0.002$ compared with BB-882.
[b] $p < 0.001$ compared with control.
[c] $p < 0.05$ compared with control.

Table 3. Adhesion scores of the groups

Groups	n	Histological score
Control	10	3.2 ± 0.6
BB-882	10	0.6 ± 0.6[a]

[a] $p < 0.0001$ for BB-882-treated vs. control group.

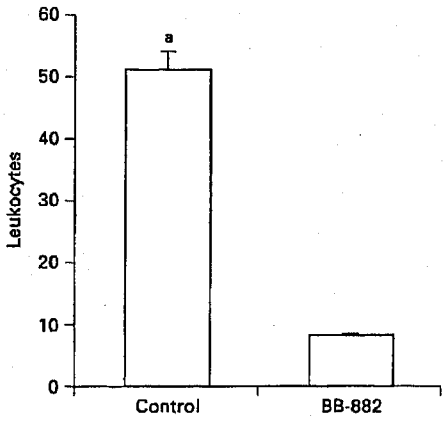

Fig. 2. Count of polymorphonuclear leukocytes in adhesion strands in both groups. [a] $p < 0.001$ compared with control.

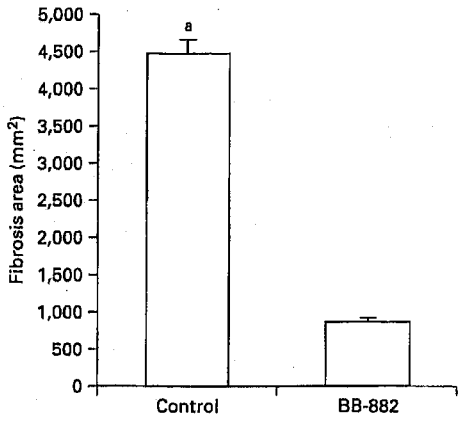

Fig. 3. Fibrosis area in the adhesion strands in both groups. [a] $p < 0.002$ compared with control.

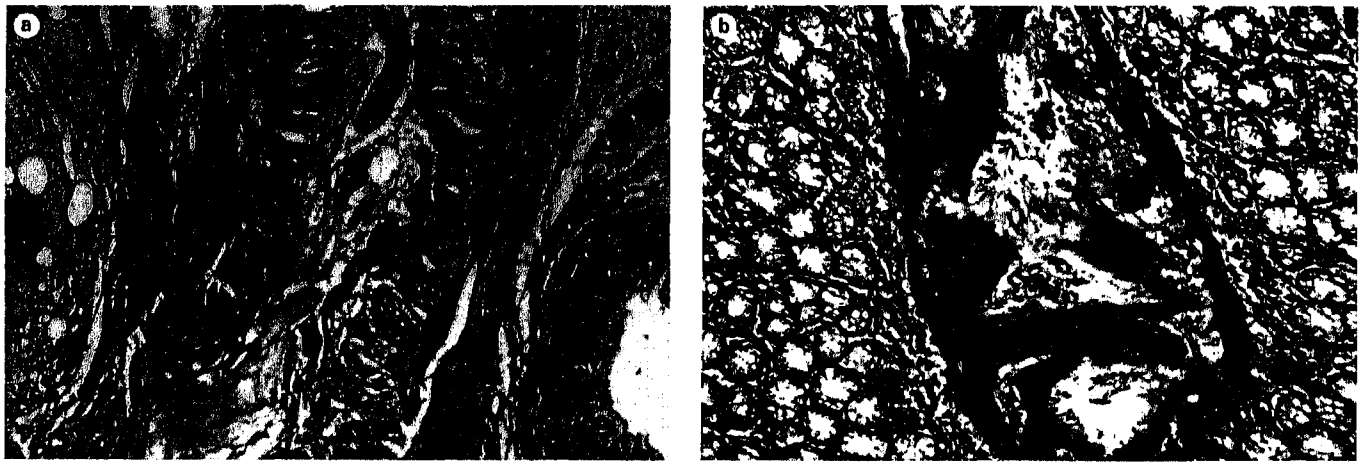

Fig. 4. a Fibrosis areas and polymorphonuclear leukocyte infiltration into the adhesion strands were significantly observed in the control group. Masson's trichrome. × 200. **b** Fibrosis areas and polymorphonuclear leukocyte infiltrates were not significant in the BB-882 group. Masson's trichrome. × 200.

Discussion

Intra-abdominal adhesions are believed to develop as a result of ischemia, foreign body reaction, and trauma to the serosal surface of the bowel or peritoneum [11–14]. These wounding events are associated with platelet deposition, release of kinin and histamine, increased vascular permeability, exudation of fluid into the peritoneal cavity, and coagulum formation [14]. An early coagulum forms at sites of peritoneal or visceral injury, much like a protective covering. It is invaded by a variety of cellular elements, including platelets, polymorphonuclear leukocytes, and macrophages. These cellular elements coordinate the early and transient deposition of fibrin, which has an adhesive quality that promotes visceral apposition. Macrophages play an early and important role in coordi-

nating wound healing as they synthesize and release growth factors that are chemotactic, mitogenic, and angiogenic [15, 16].

We have evaluated the protective effect of the PAF antagonist BB-882 in the evolution of experimentally induced intra-abdominal adhesions in the rats. Rats treated with BB-882 presented an important reduction in adhesion formation ($p < 0.001$ as opposed to nontreated rats). PAF is a phospholipid chemical mediator that has been shown to have a variety of biologic activities and to be produced by inflammatory cells, endothelial cell edema, tissue injury and an increase in vascular permeability [17]. It can act as an intercellular signal responsible for cell communication and an inflammatory mediator involved in the pathogenesis of inflammation. The major contributions of PAF during the inflammatory reaction include acting as an inflammatory stimulator on the leukocyte system, causing leukocyte rolling on the endothelium, adhesion and passage through inter-endothelial cells to the interstitium [18]. Leukocyte-endothelial cell interaction leads to excessive movement of leukocytes to the tissues, as well as endothelial barrier compromise, responsible for the initiation of the inflammatory reaction, serious tissue injury and organ dysfunction. Leukocyte recruitment involves transient tethering and rolling of leukocytes along endothelial cells, triggering of signals that activate upregulation of leukocyte function, tight adhesion of leukocytes to the vascular endothelium and transendothelial migration [19]. BB-882 is one of the most powerful PAF antagonists so far developed. It is suggested that BB-882 blocks PAF receptors on the surface of endothelial cells, thus reducing circulating PAF and so downregulating the activation of leukocytes/macrophages and the interaction between leukocytes and endothelial cells, and maintaining the integrity of the endothelial barrier [9]. We, here, observed a decreased polymorphonuclear leukocyte infiltrate and fibrosis in the adhesion strands in the BB-882-treated group in comparison with the control group.

We suggest that BB-882 (lexipafant) may be effectively used in the prevention of postoperative intraperitoneal adhesion. The protective effects were mediated by a reduction in inflammatory reaction following surgical trauma and a decreased deposition of fibrosis tissue into the adhesion strands.

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