The effect of sorafenib in postoperative adhesion formation in a rat uterine horn model

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Summary

Objective: Postoperative adhesions are a serious problem. In this study, we aimed to observe the effects of sorafenib in postoperative adhesions and, to examine the effects of sorafenib on tissue levels of vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF). *Material and Methods:* Twenty female Wistar albino rats were randomized into two equal groups; sorafenib group (sorafenib treated) and control group; then all rats underwent laparotomy. Adhesions were developed by scalping on the anti-mesenteric surfaces of the right uterine horns. After 14 days, adhesions were investigated by using macroscopic, histopathological and immunohistochemical (for VEGF and PDGF) methods. *Results:* The sorafenib group had lower scores of total adhesions [1 (0-2.5) vs 1.5 (1-4); p: 0.037], staining of VEGF [1 (0-1) vs 1 (1-3); p: 0.029] and PDGF [1 (0-2) vs 2 (1-3); p: 0.006], and vascular proliferation [1 (0-2) vs 2 (1-3); p: 0.038] than the control group. *Conclusion:* The findings of the present study show that sorafenib, a tyrosine kinase inhibitor, significantly reduced postoperative adhesion formation. This effect may be explained by inhibition of VEGF, PDGF, and thus vascular proliferation.

Key words: Rat; Uterus; Adhesion; Sorafenib; VEGF; PDGF; Angiogenesis.

Introduction

Intraabdominal adhesions after surgery lead to substantial problems. Infertility can be added to these in women, unlike men. It was reported that early formation of adhesions is associated with size of angiogenesis [1]. Regulation of new vessel formation provided by growth factors and angiogenesis inhibitors is the basis of angiogenesis. Fortunately infertility due to adhesions can be solved with assisted reproductive techniques today, but abdominal pain and bowel obstruction still continue to be a problem.

Sorafenib, known as a tyrosine kinase inhibitor, inhibits a wide range of kinase targets in addition to the vascular endothelial growth factor receptors (VEGFR-1, -2, and -3) [2]. The VEGF family consists of five proteins (VEGF-A, -B, -C, -D, and placental growth factor) and signaling is mediated by the binding of these growth factors to three receptors. The binding with receptor affects some biological functions such as proliferation, migration, and morphogenesis of endothelial cells [3, 4].

After sorafenib (30 mg/kg) administration, expression of VEGF significantly decreased on experimental choroidal neovascularization in the rat [5]. The role of VEGF has been demonstrated in wound repair and tissue remodeling through its effect on fibroblast function [6]. Most adhesions containing unwanted fibrous bands occurred between the two deperitonealized surfaces, as wound healing. The effect of sorafenib is not confined only to a potent inhibition of angiogenesis, but also to tyrosine kinase inhibition which could have multiple effects on several crucial mechanisms in adhesion formation. The aim of this study was to evaluate the effects of sorafenib on adhesions in a uterine horn model.

Materials and Methods

Twenty female Wistar albino rats (10-12 weeks old; weight 230-290 g) were used. They were housed five animals per cage with the appropriate diet and water ad libitum. All rats were observed for several days to ascertain health before operations. All procedures were approved by the local animal care and use committee and performed under their guidelines.

One day prior to surgery, the rats were randomly treated with sorafenib (30 mg/kg in 0.5 ml saline) or 0.5 ml saline only. The dose of sorafenib was selected based on a previous study [5]. Rats were anaesthetized by intraperitoneal administration of 60 mg/kg ketamine hydrochloric acid (Ketalar; Eczacibasi Warner-Lambert Ilac Sanayi, Levent, Istanbul, Turkey) and 7 mg/kg xylazine hydrochloric acid (Rompun, Bayer Sisli, Istanbul, Turkey). Before surgery, the abdominal skin was shaved and antisepsis was obtained with 10% povidone iodine solution. Using a sterile technique, a 3 cm ventral vertical incision was made to expose the reproductive organs. Punctuate serosal hemorrhage was generated by scraping with a scalpel blade (No: 15) until petechial bleeding emerged at the abdominal sidewall and antimesenteric surface of the right uterine horn to create adhesions (Figure 1A). This model was based on a previous postoperative adhesion formation of a rat uterine horn study [7]. The midline incision was closed after completion of the procedure. Both groups were treated daily by orogastric gavage at the same dose for ten days. On postoperative day 14 animals were sacrified by a high dose of anesthesia. A transverse subcostal incision was made above the cephaled extent of the midline laparo-

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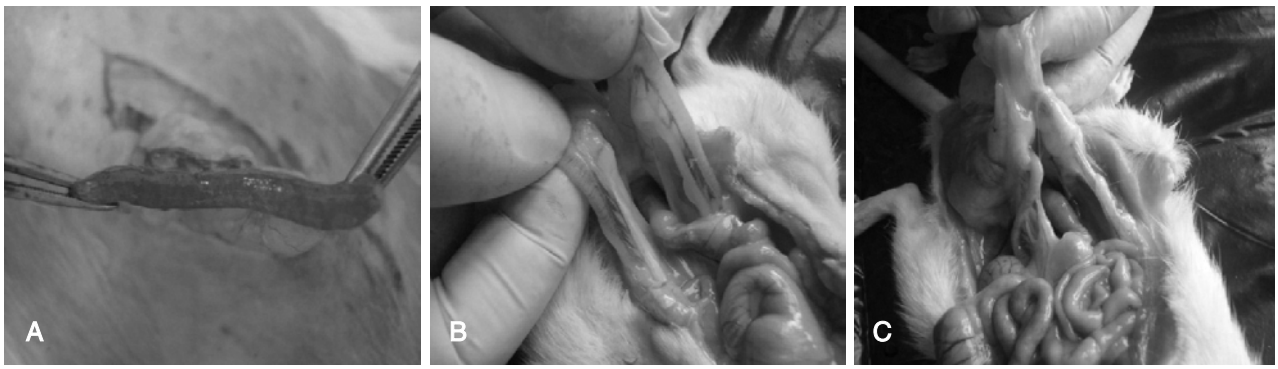

Figure 1. — A) Petechial bleeding of uterine horn to create adhesions. B) Adhesion free rat in the sorafenib group. C) Total adhesion score of 3 in the control group.

tomy site, and the abdominal cavity was inspected for the presence of adhesions.

The extent and severity of adhesions in the operation site for each uterine horn were evaluated according to the criteria of Linsky *et al*. [8]. The extent of adhesions was graded as follows: 0; no adhesion, 1; 25% of traumatized area, 2; 50% of traumatized area, 3; total involvement. Scores were recorded by an investigator blinded to the treatment group. The severity of adhesions was graded as follows: 0; no resistance to separation, 0.5: some resistance (moderate force required), 1; sharp dissection needed. Total adhesion score (TAS) was recorded as arithmetic sum of severity and extent of adhesions. The investigators scoring the adhesions were blinded to which group the rats belonged.

Tissue samples were taken from all serosal surfaces where adhesion had developed. All tissues were evaluated by the same pathologist, who was blinded to the origin of the samples. Biopsy materials of all four groups were fixed by using 10% formaldehyde solution to perform histological evaluation and histochemical and immunohistochemical staining. Hematoxylin-eosin stained slides obtained from paraffin blocks, prepared by routine tissue management, were assessed in a blind manner to macroscopic adhesion scores. The method proposed by Kanbour-Shakir *et al*. [9] was used to semi-quantitatively grade (grade 0 to 4) inflammation on the serosal surface, fibroblastic activity, foreign body reaction, collagen formation and severity of vascular proliferation (Table 1). Results were scored as 0, 1, 2 and 3. In addition to immunohistochemical analysis 4 μm thick sections were obtained to interpret angiogenesis, a step in the formation of adhesions. The sections were deparaffinized in xylene and dehydrated through graded concentrations of ethanol. After blocking of endogenous peroxidase activity with 3% hydrogen peroxide for 15 min, the sections were heated in 0.01 mol/l citrate buffer in a microwave pressure cooker for 20 min. The slides were allowed to cool to room temperature, and non-specific binding was blocked with normal horse serum for 20 min at room temperature. The sections were further incubated with the primary antibody against VEGF (Neomarkers, USA) and PDGF (Neomarkers, USA) for 30 min were applied. The sections were then stained using the avidin-biotin complex (ABC) immunoperoxidase technique employing commercially available reagent (ABC kit, Labvision, USA); for demonstration of binding sites AEC chromogen was applied. Phosphate-buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer's hematoxylin.

For VEGF and PDGF antibodies, staining density and severity were evaluated in the areas where the stained cells were mostly found in both adhesion positive and normal tissues. Results were evaluated as follows: 0 = no staining, 1 = mild, 2 = moderate and 3 = strongly positive (Figure 2C-F).

Table 1. — *Histological adhesion score* [23].

	Inflammation	Fibroblastic activity	Foreign body reaction	Collagen formation	Vascular proliferation
Grade 0	None	None	None	None	None
Grade 1	25% mixed	Mild	Mild	Mild	Mild
Grade 2	50% mixed	Moderate	Moderate	Moderate	Moderate
Grade 3	75% mixed	Marked	Marked	Marked	Marked
Grade 4	Massive	Massive	Massive	Massive	Massive

Table 2. — *Adhesion scores and staining with VEGF and PDGF scores of control versus sorafenib treated rats.*

Group	Severity score	Extent score	Total adhesion score	PDGF staining score	VEGF staining score
Control (n = 10)	0.5 (0-1)	1 (1-3)	1.5 (1-4)	2 (1-3)	1 (1-3)
Sorafenib (n = 10)	0 (0-0.5)	1 (0-2)	1 (0-2.5)	1 (0-2)	1 (0-1)
*p**	0.054	0.055	0.037	0.006	0.029

VEGF: vascular endothelial growth factor; PDGF: platelet derived growth factor.
* Statistical analysis comparing adhesion scores using Mann-Whitney rank sum test; $p < 0.05$ is considered statistically significant.

Table 3. — *Histological scores of control versus sorafenib treated rats.*

Group	Severity score	Extent score	Total adhesion score	PDGF staining score	VEGF staining score
Control (n = 10)	1 (1-1)	1 (0-3)	0 (0-2)	0 (0-1)	2 (1-3)
Sorafenib (n = 10)	1 (0-1)	1 (0-3)	0 (0-2)	0 (0-2)	1 (0-2)
*p**	0.146	0.813	0.687	0.861	0.038

* Statistical analysis comparing adhesion scores using Mann-Whitney rank sum test; $p < 0.05$ is considered statistically significant.

Statistical analysis

Data are presented as median (min-max). Mann-Whitney U test was used to compare scores of adhesion extent, adhesion severity, total adhesions, inflammation, fibroblastic activity, foreign body reaction, collagen formation, vascular proliferation, and staining of VEGF and PDGF between groups. Spearman's test was used for correlation analyses; a p value < 0.05 was considered as significant.

Fig. A

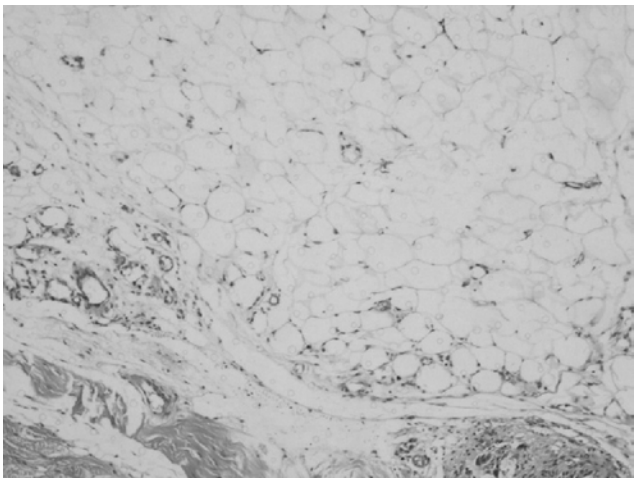

Fig. B

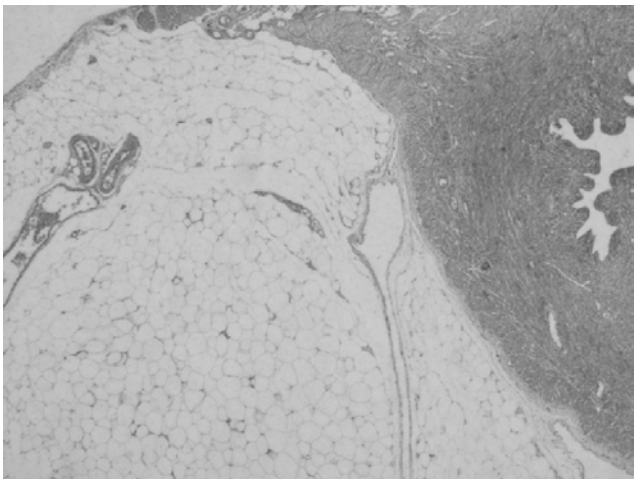

Fig. C

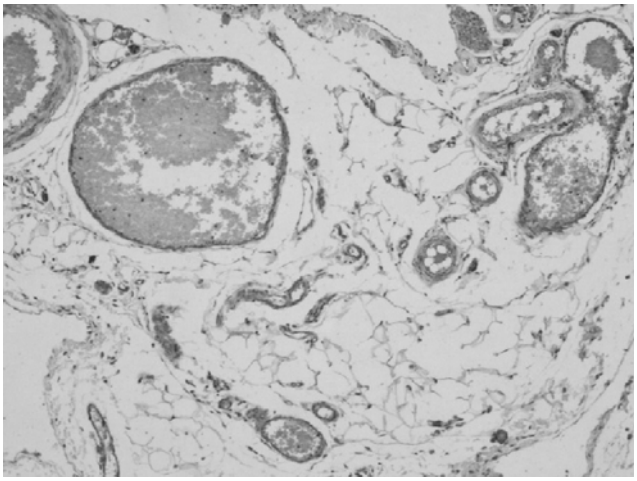

Fig. D

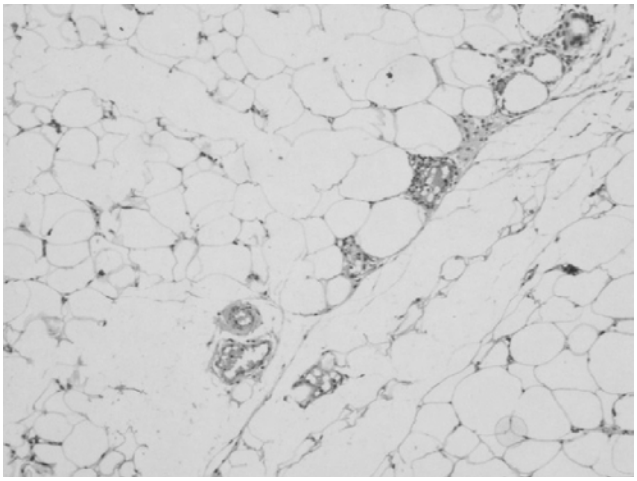

Fig. E

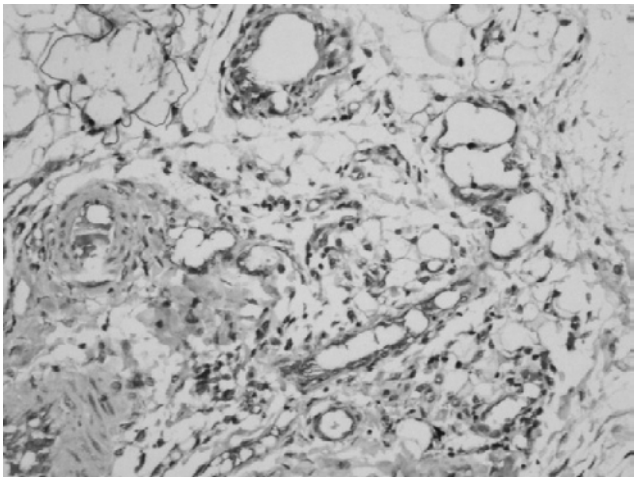

Fig. F

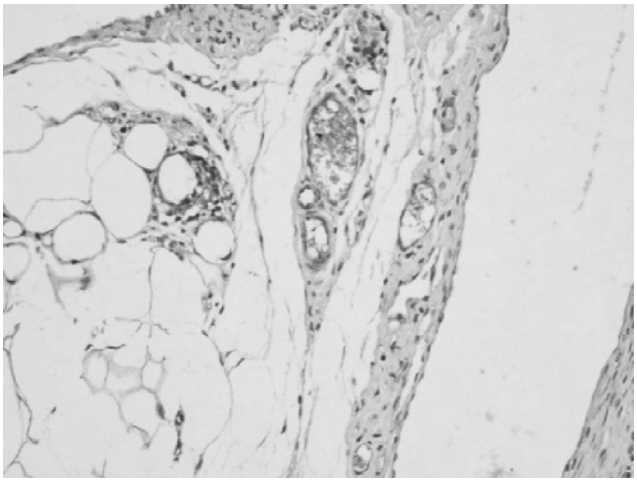

Figure 2. — Microscopic appearance of vascular proliferation and staining with VEGF and PDGF. A) Marked vascular proliferation in the control group (H&E; X100). B) Mild vascular proliferation in the sorafenib-treated group (H&E, x 40). C) Strong staining for PDGF in control group PDGF, x 100). D) Mild staining for PDGF in the group of sorafenib-treated rats PDGF, x 100). E) Strong staining for VEGF in the control group (VEGF, x 200). F) Mild positivity for VEGF in the sorafenib-treated group (VEGF, x 200).

Results

The adhesion model was performed on 20 rats. There was no intraoperative or postoperative mortality. Wound infection developed in one of the control group animals. Two of ten (20%) rats in the sorafenib group were completely adhesion-free (Figure 1B). All control rats developed intraabdominal adhesions (Figure 1C). There was no significant difference between the two groups for adhesion severity score and extent score. However total

adhesion score was statistically significantly lower in the sorafenib group than in the control group ($p < 0.05$) (Table 2). Staining with VEGF and PDGF of the sorafenib treated group was significantly lower than the control group ($p < 0.05$) (Table 2).

When inflammation, fibroblastic activity, foreign body reaction and collagen formation scores of the controls were compared to the sorafenib group, no significant difference was found between groups (Table 3). However the vascular proliferation score was statistically significantly lower in the sorafenib-treated group than in the control group ($p < 0.05$) (Table 2 and Figure 2A,B).

Remarkable results of the correlation analysis are mentioned below. There was a highly significant and positive correlation between vascular proliferation and total adhesion scores in both the control group and sorafenib group (r: 0.789; p: 0.007, r: 0.805; p: 0.005, respectively). There was a highly significant and positive correlation between vascular proliferation and staining of VEGF and PDGF in the sorafenib group (r: 0.791; p: 0.006, r: 0.750; p: 0.012). There was a highly significant and positive correlation between VEGF staining and PDGF staining in the sorafenib group (r: 0.791; p: 0.006).

Discussion

In this randomized prospective double-blind experimental study, the effects of sorafenib on the formation of intraperitoneal adhesions were assessed in a rat uterine horn model by both visual scores and histological analyses of adhesion. Findings of the study have shown that sorafenib is effective in preventing adhesion formation in an animal model.

In the past 30 years, after the discovery of vascular proliferation factors, angiogenesis has become one of the most intensively studied fields. Inflammation, fibrinolysis, angiogenesis and tissue remodeling are central to peritoneal wound repair and adhesion formation. Growth factors have a major role in adhesion formation and angiogenesis [10]. Peritoneal sclerosis rat models studied by an immunohistochemical method showed that VEGF is increased in thickened peritoneum [11]. Similar to this study, Arıtaş *et al*. demonstrated that VEGF staining in normal areas was significantly low compared with the adhesion-positive areas in the control and sham groups of a cecal abrasion model study [12].

Park *et al*. found that after sorafenib (30 mg/kg) administration, expression of VEGF was significantly decreased, however, they could not find any statistically significant difference in PDGF levels between treated groups and controls in a choroidal neovascularization study [5]. However VEGFR and, PDGF receptors (PDGFR) are major targets of sorafenib [13]. On the other hand, in our study both VEGF and PDGF were significantly less stained in the sorafenib group. In addition a highly significant and positive correlation was demonstrated between VEGF staining and PDGF staining.

The PDGF family (PDGF-A,-B,-C, and -D) bind to two different receptors, known as PDGFR-a and -b [14]. PDGFs are important for maturation and stability of the vasculature [15]. In our study, we also found a positive correlation between vascular proliferation and staining of VEGF and PDGF in the sorafenib group. The small-molecule tyrosine kinase inhibitors sunitinib and sorafenib target the VEGFR and PDGFR (primarily VEGFR), and have shown clinical efficacy in diverse cancer types [13, 16, 17].

Jonathan *et al*. found that the mean microvessel density was slightly lower in the sunitinib-treated animals compared with the control animals, although this difference was not significant [18]. We did not study microvessel density, but the vascular proliferation score was significantly lower in the sorafenib-treated group than in the control group. There were statistically significant fewer adhesions in the sorafenib group than the control group in our study. The results of our study are similar to the study of Jonathan *et al*. [18] and Kim *et al*. [19], where they studied the effect of sunitinib on postoperative adhesions in rabbits and mice, respectively.

Bevacizumab is the first angiogenesis inhibitor that was clinically approved. In experimental studies that investigated the effect of bevacizumab on intraperitoneal adhesions, it was demonstrated that intraabdominal administration of bevacizumab diminished intra-peritoneal adhesions [20-22]. In addition, significantly lower staining with VEGF was demonstrated by studies [20, 21]. The results of our study are similar to these studies.

In conclusion, our results demonstrated that sorafenib had a positive effect on prevention of postoperative adhesions. This effect could be through inhibition of vascular proliferation via VEGF and PDFG.

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