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Effect of local bleomycin sulfate application on seroma formation in a rat mastectomy and axillary lymph node dissection model

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ABSTRACT

Seroma formation is one of the most common complications following breast cancer surgery. It may lead to delay of adjuvant therapies and increasement of therapy costs. Bleomycin sulfate is a sclerosing antibiotic with antineoplastic efficacy. It is locally used in the treatment of pleural effusion. The present study aimed to investigate seroma-reducing effect of local bleomycin application after mastectomy. Sixteen female Wistar Albino rats were used in this study. The rats were divided into two equal groups. Under general anesthesia all rats underwent unilateral mastectomy as definition by Harada. Serum physiologic was applied to animals in Group 1 (control group) and bleomycin to Group 2. Mastectomized localization was explored on the 10th day postoperatively. Seroma and tissue samples were obtained from axilla and thoracic wall for histopathological examination. The amount of seroma was significantly lower in the bleomycin group as compared to the control group ($P=0.002$). Fibrosis, PNL infiltration and the number of fibroblasts were significantly higher in the bleomycin group. No difference was identified between the groups in terms of angiogenesis, edema, congestion, and monocyte, lymphocyte and macrophage infiltration. Local bleomycin sulfate application might be a therapeutic option in patients with seroma formation, as well as in the patients with malignant pleural effusion. Nonetheless, further studies that compare the efficacy and adverse effects (benefit-to-harm ratio) of bleomycin sulfate are needed.

1. Introduction

Seroma formation is one of the most common complications after breast cancer surgery (15–81%) (Woodworth et al., 2000). Seroma occurs following mastectomy as a fluid collection causing fluctuation and tension in the surgical area. Seroma formation might be tedious for the patient because of prolonged therapy period and frequent recurrent aspirations. Moreover, it is an important complication leading to delay in adjuvant therapies and increase in therapy cost.

Although its pathophysiology remains unclear, it is well known that the dead space, which occurs between the tissues after the surgical procedure, contributes to seroma formation. Therefore, various surgical procedures and sclerosing agents are being tried to remove this postoperative dead space. Bleomycin sulfate is a sclerosing antibiotic with antineoplastic efficacy. One of the adverse effects of bleomycin is lung fibrosis, which is a potentially fatal complication that can be developed during systemic use (Sleijfer, 2001). Local sclerosing adhesive effect of bleomycin results from extreme inflammatory stimulation and fibrosis, which occurs locally in the application area. For this purpose, intrapleurally application in the treatment of pleural effusion was reported with 31% and 85% success rate (Paladine et al., 1976; Ruckdeschel et al., 1991; Kessinger and Wigton, 1987; Bitran et al., 1981).

Some sclerosing agents have been used to treat seroma formation after breast cancer surgery and it was reported that they reduced seroma formation to some extent (Burak et al., 1997; Oertli et al., 1994; Tekin et al., 2001; Rice et al., 2000). However, as far as we know, there is no study on this issue performed with bleomycin sulfate. Thus, the present study aimed to investigate seroma-reducing effect of local bleomycin application.

2. Material and method

Sixteen female Wistar Albino rats (mean weight was 220 g) were used in this study. Approval of Yeditepe University Medical Faculty Ethical Committee for Laboratory Animal Research was obtained (02.05.2011 decree no.: 183). Rats were obtained from

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Yeditepe University Medical Faculty Laboratory Animals Research Center (YULARC). Rats were fed with standard laboratory feed (rodent chow) and water (ad libitum), and were monitored under 12-h dark/light cycles in an isolated room provided with heating control (22 ± 2 °C). Surgical interventions were performed at YULARC laboratory under non-sterile but clean conditions. In order to prevent drug interaction, neither prophylactic antibiotic nor analgesic and anti-inflammatory drug were given during the preoperative period. Rats were weighed prior to the surgical procedure. Exclusion criteria were specified to flap necrosis, infection and death. Bleomycin sulfate (Bleocin-S® Nippon Co. Ltd.) solution was prepared to 10 IU/ml (1000% IU) dissolving in normal saline. Under general anesthesia by injecting 50 mg/kg ketamine (Ketalar®, Parke Davis and Co. Inc.) and 5 mg/kg xylazine (Rompun® Bayer) via intramuscular route, anterior thoracic wall and axillary region were shaved; cleaned by 10% povidone–iodine; and waited for a fine film layer to occur. In accordance with the definition of Harada et al., an incision was made beginning from the sternal notch extending to the xyphoid (11). Skin and the subdermal tissue were detached from thoracic wall and flaps were prepared. The right major pectoral muscle was excised from the thoracic wall. Axillary artery and vein were exposed in the axillary region and preserved. Axillary adipose tissue was excised and unilateral mastectomy and axillary dissection was performed. Neither cautery nor chemical agents were used for hemostasis. Bleeding was stopped by compressing. Cautery use and suturing were avoided. Surgical area was dried with sterile hydrophilic gauze. The rats were divided into two equal groups.

1st Group (control group): The skin was closed by continue suturing; before the last two suture, 0.1 ml normal saline was injected into the surgical space. After the skin was completely closed, gentle massage was applied for a short time for the fluid to disperse within the whole surgical area.

2nd Group (bleomycin group): The skin was closed by continue suturing; before the last two sutures, 0.1 ml bleomycin solution was injected into the surgical space. After the skin was completely closed, gentle massage was applied for a short time for the fluid to disperse within the whole surgical area.

The rats were postoperatively monitored for 10 days in terms of anterior extremity movements, gait, vitality, wound infection,

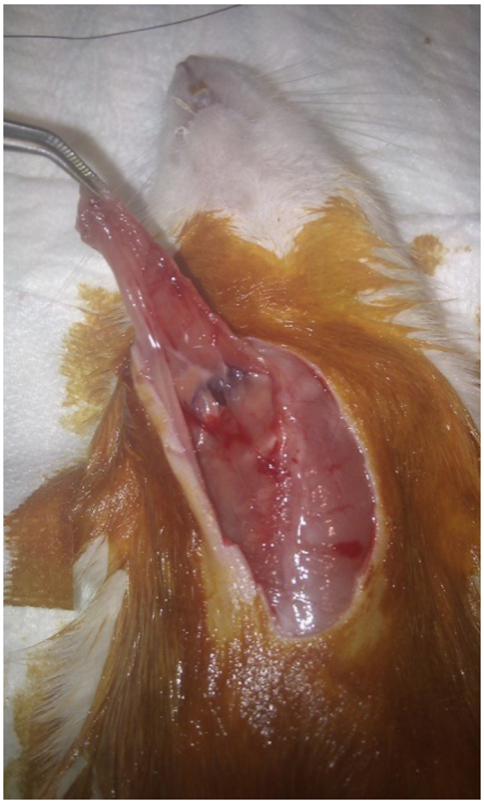

Fig. 1. Dissection of pectoral muscle and exposing axillary structures.

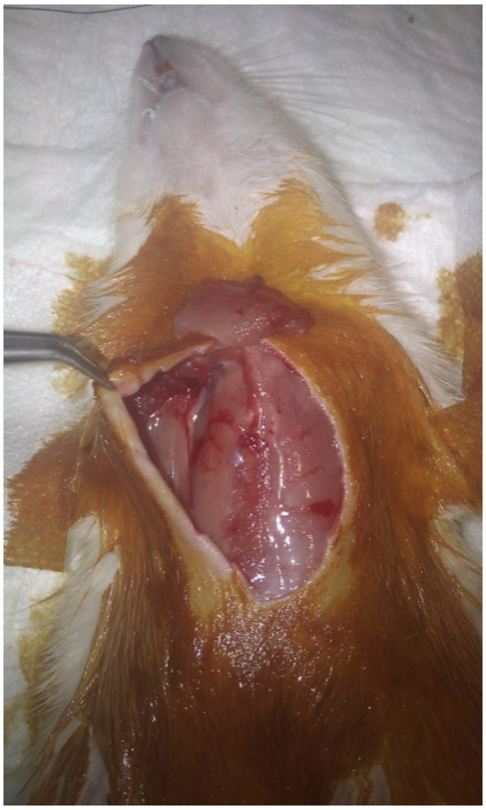

Fig. 2. Removing pectoral muscle (pectoral muscle is demonstrative).

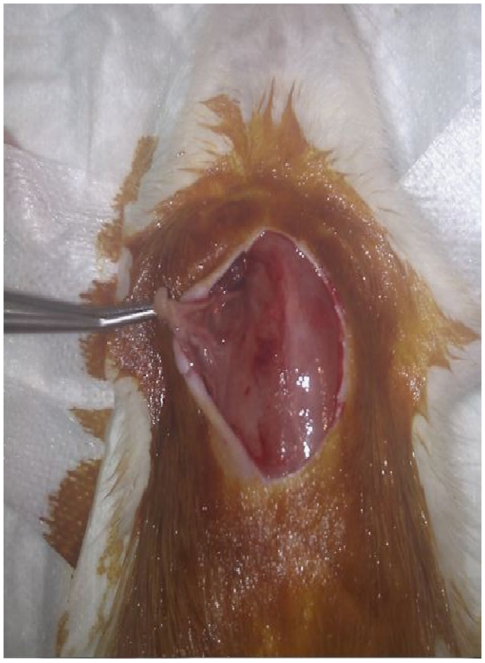

Fig. 3. Dissection of the lymph nodes.

wound healing and seroma formation, flap necrosis, and potential additional complications; the changes were recorded. Mastectomized area was explored on the postoperative 10th day under general anesthesia. Macroscopic view was recorded; existing seroma was aspirated by injectors and their amounts were recorded. Tissue samples were obtained from axillae and thoracic wall for histopathological examination, and animals were sacrified by decapitation.

Histopathological examination: Tissue samples, which were transferred to the pathology laboratory in 10% formaldehyde solution, were embedded in paraffin blocks and 5 μm sections were obtained. Preparations were stained with hematoxylen–eosin (HE) and with Masson's Trichrome to evaluate fibrosis better. Light microscope was used to investigate angiogenesis, edema, congestion and fibrosis, as well as polymorphonuclear leukocyte (PNL), monocyte, fibroblast, lymphocyte and macrophage infiltration; they were semi-quantitatively scored as follows: Grade 1: no change (infiltration) or minimal findings, Grade 2: moderate changes (infiltration), Grade 3: manifest (increased) changes (infiltration), and Grade 4: intensive (dense) changes (infiltration) (Figs. 1–14).

Statistical analyses were done using 'SPSS Data Editor for Windows version 17.0' program. Seroma volume and histopathological parameters were evaluated by the Mann–Whitney *U* test. A '*P*' value of < 0.05 was considered significant.

3. Results

Limitation was observed in the movement of anterior extremities of the same side with mastectomy for the first 2 days of surgery. Neither of the rats developed flap necrosis, wound dehiscence or wound infection. None of the rats died or developed a problem that required exclusion from the study. Although seroma formation was observed in all rats in the control group, it was observed that flaps completely adhered in two rats and

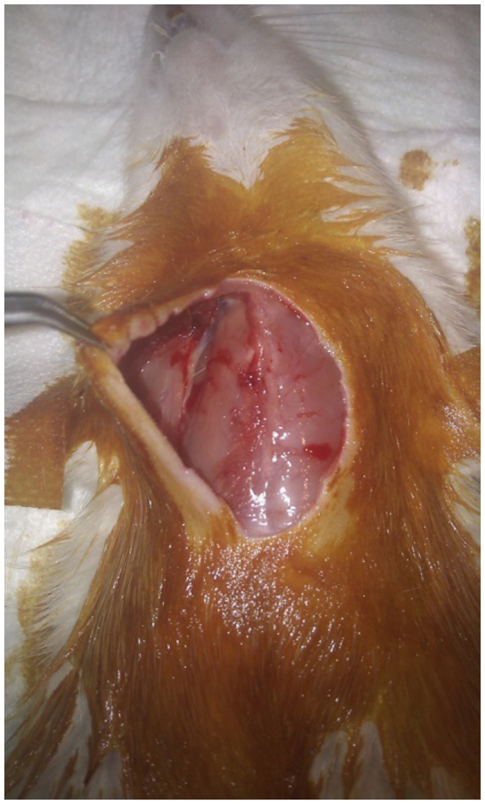

Fig. 4. Postoperative view.

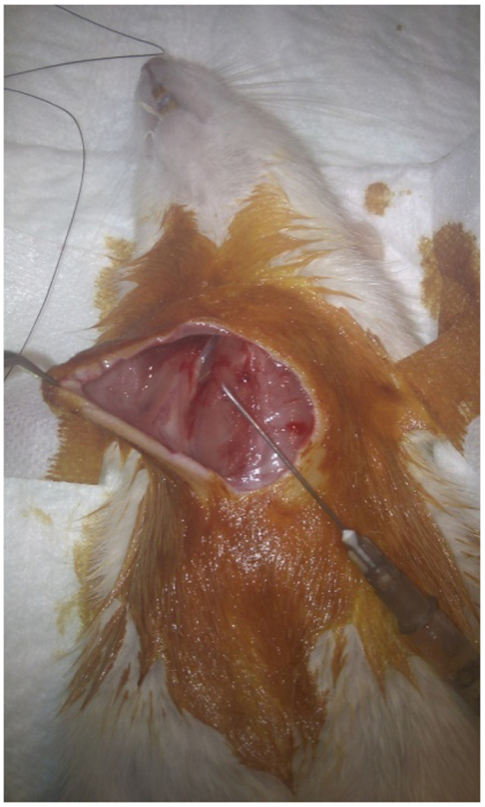

Fig. 5. Normal saline and bleomycin injection into the cavity.

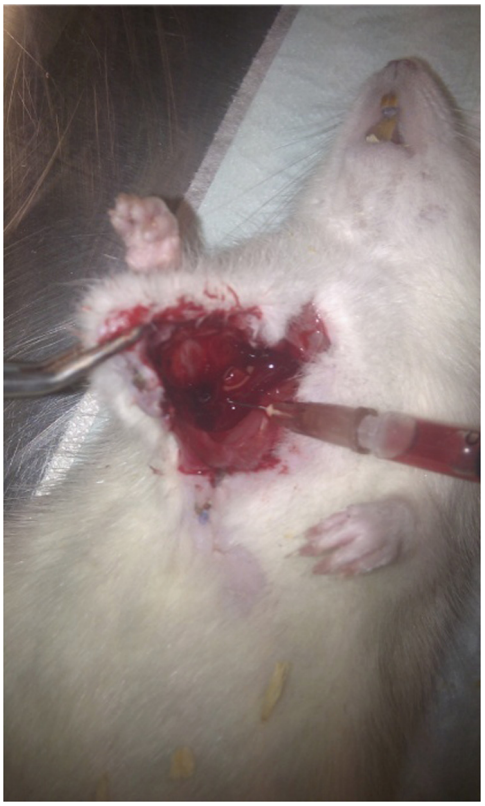

Fig. 6. Aspirating seroma on the postoperative 10th day.

there was no fluid collection in the surgical area in the bleomycin group. The amount of seroma was 0.975 ml ($\pm$0.328 ml) in the control group, whereas it was 0.313 ($\pm$0.217) in the bleomycin

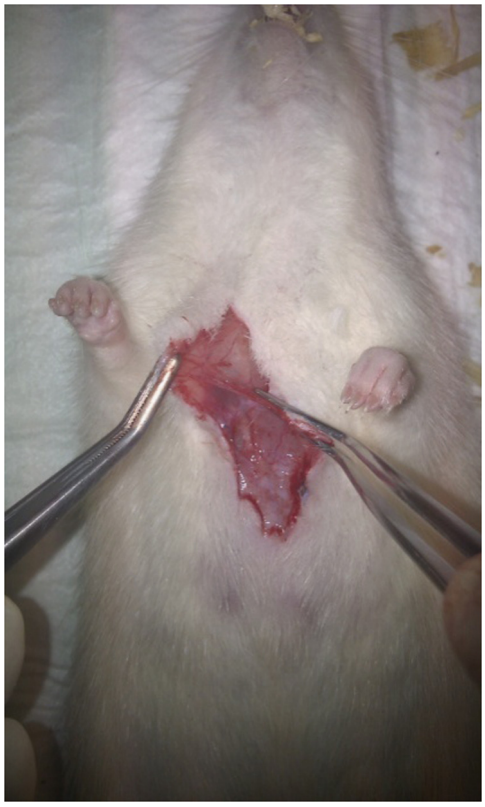

Fig. 7. Experimental animal B2 with completely closed cavity after bleomycin.

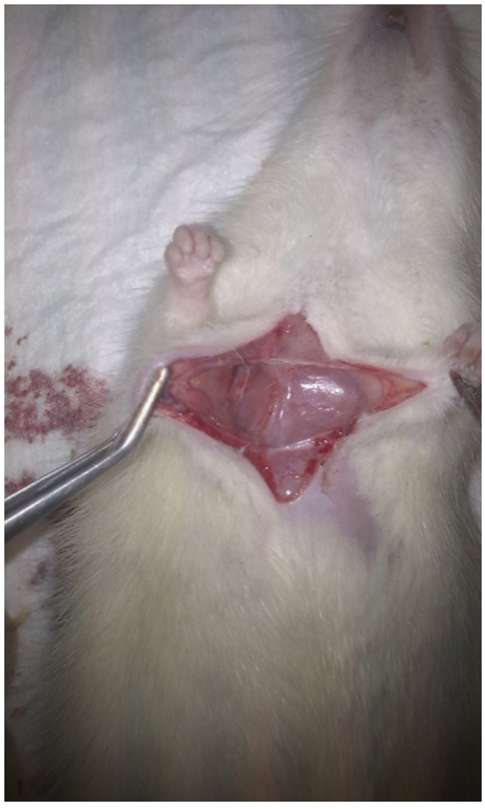

Fig. 8. Experimental animal B6 with completely closed cavity after bleomycin.

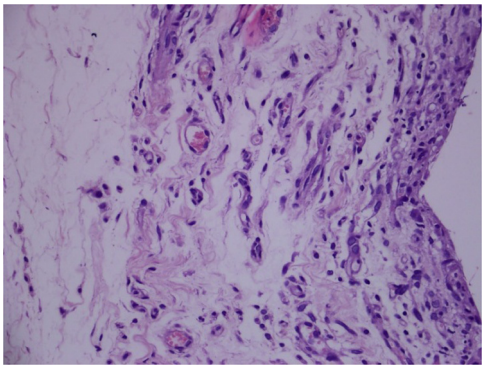

Fig. 9. Control group's edema and congestion dense areas.

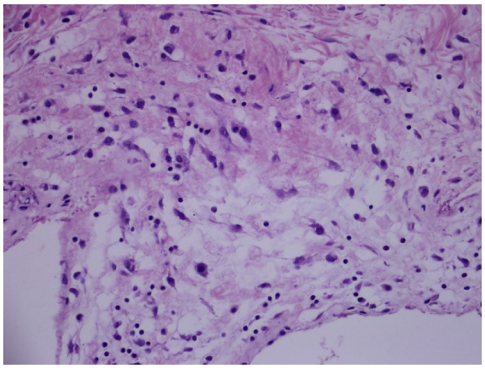

Fig. 10. Control group's edema dens areas.

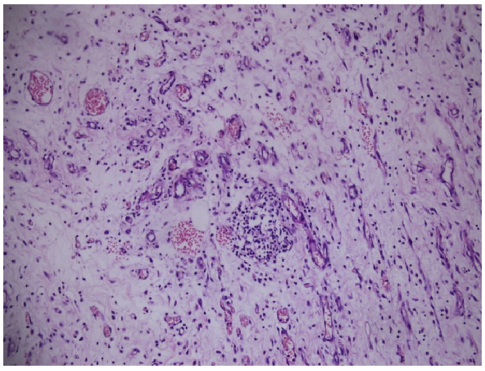

Fig. 11. Control group's edema and dense bleeding dense areas.

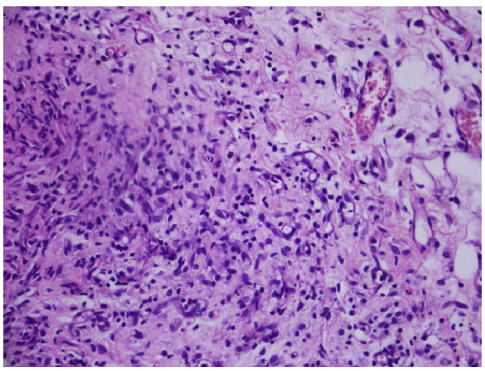

Fig. 12. Bleomycin group's fibroblast area.

group (Tables 1 and 2). Accordingly, the mean seroma volume was significantly lower in the bleomycin group as compared to the control group ($P=0.002$).

Histopathological findings: Semi-quantitative scores of histopathological findings are demonstrated in Table 3. Fibrosis, PNL infiltration and the number of fibroblasts were significantly higher

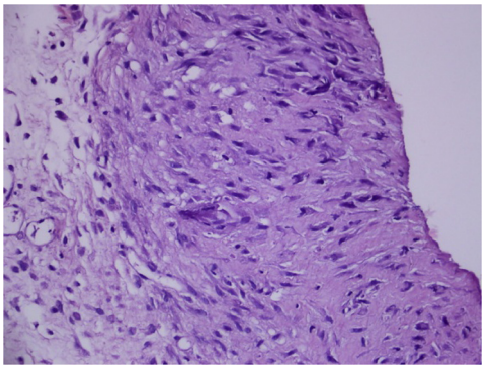

Fig. 13. Dense fibrosis and fibroblast in in bleomycin group.

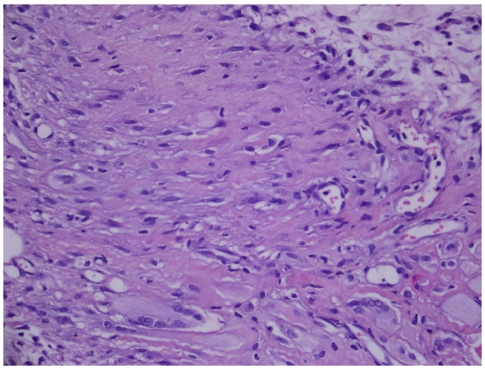

Fig. 14. More dense fibrosis and in bleomycin group.

Table 1
Seroma volumes in the control (C) group.

Experimental animal no.	Seroma volume (ml)
1st Experimental animal (C1)	1.20
2nd Experimental animal (C2)	1.10
3rd Experimental animal (C3)	0.90
4th Experimental animal (C4)	1.30
5th Experimental animal (C5)	1.40
6th Experimental animal (C6)	0.50
7th Experimental animal (C7)	0.80
8th Experimental animal (C8)	0.60

Table 2
Seroma volumes in the bleomycin (B) group.

Experimental animal no.	Seroma volume (ml)
1st Experimental animal (B1)	0.40
2nd Experimental animal (B2)	0.00
3rd Experimental animal (B3)	0.30
4th Experimental animal (B4)	0.60
5th Experimental animal (B5)	0.40
6th Experimental animal (B6)	0.00
7th Experimental animal (B7)	0.50
8th Experimental animal (B8)	0.30

in the bleomycin group. No difference was identified between the groups in terms of angiogenesis, edema, congestion, and monocyte, lymphocyte and macrophage infiltration.

4. Discussion

Seroma is the collection of acute inflammatory exudative fluid in the surgical area in response to the surgical trauma.

Table 3
Semi-quantitative values of histopathological findings of the groups.

	Control (mean ± S.D.)	Bleomycin (mean ± S.D.)	*P* value
Angiogenesis	3.38 ± 0.517	2.88 ± 0.64	0.11
Edema	2.25 ± 0.71	1.75 ± 0.46	0.11
Congestion	2.13 ± 0.35	1.75 ± 0.46	0.09
Fibrosis	2.25 ± 0.74	3.13 ± 0.35	0.02
PNL	1.50 ± 0.53	2.00 ± 0.00	0.03
Fibroblast	2.76 ± 0.46	3.25 ± 0.46	0.05
Lymphocyte	2.13 ± 0.35	2.38 ± 0.52	0.26
Macrophage	2.38 ± 0.74	1.75 ± 0.71	0.10

Pathophysiology of seroma formation is unclear. However, blood and lymph fluid, which are collected under the flaps in early postoperative period, plays an important role in seroma formation by detaching the flaps, which are prepared for mastectomy, from the chest wall and inhibiting contact and adherence of flaps to the chest wall (Watt-Boolsen et al., 1989).

Kuroi et al. investigated the factors thought to be associated with seroma formation. They found that there is no strong evidence (Grade A) for any of the factors associated with seroma formation, whereas there is moderate evidence (Grade B) supporting that seroma is likely to occur in case of higher amount of drainage volume in the postoperative first 3 days in obese patients that underwent radical mastectomy as compared to simple mastectomy. And finally there is weak evidence (Grade C) for the factors including type of skin incision, hypertension, whether drainage is applied or not, type of drain, time of removal of the drain, obesity, duration of surgery, whether the flaps prepared by electrocautery or cold dissection (Katsumasa et al., 2006). There are numbers of studies, the outcomes of which conflict with each other, investigating the efficacy of restriction of upper extremity movement (Dawson et al., 1989), application of external pressure over surgical area (O'Hea et al., 1999), and local sclerosing agents (Burak et al., 1997; Oertli et al., 1994; Tekin et al., 2001; Rice et al., 2000; Roberto et al., 2008) in the treatment of seroma.

Some studies particularly aimed to enhance local fibrosis. Local sclerosing agents including bovine thrombin (Burak et al., 1997), tranexamic acid (Oertli et al., 1994), *Corynebacterium parvum* (Tekin et al., 2001) and tetracycline (Rice et al., 2000), which have been used for this purpose, failed to come into routine use despite their seroma reducing effects. Results of numerous studies that investigated seroma reducing effects of fibrin glue conflict with each other by means of reporting that it reduces (Harada et al., 1992; Lindsey et al., 1990; Mustonen et al., 2004; Langer et al., 2003; Segura-Castillo et al., 2005), or has no effect on (Udén et al., 1993), or even enhances (Vaxman et al., 1995) seroma formation.

Bleomycin sulfate is a sclerosing antibiotic with antineoplastic efficacy. It shows antineoplastic efficacy by inhibiting deoxyribonucleic acid (DNA) synthesis and, less frequently, RNA and protein synthesis. It is used for chemotherapeutic purpose in Hodgkin lymphoma, squamous cell carcinoma, lymphoma, and germ cell tumors. When administered via systemic route, bleomycin sulfate causes secretion of tumor necrosis factor (TNF)-α in particular, as well as interleukin (IL)-1β, IL-8, and transforming growth factor (TGF)-β, from inflammatory cells in human and animal lungs (Sleijfer et al., 1998). Although pathogenesis of bleomycin-induced pulmonary fibrosis is not understood exactly, TGF-β is considered to be an important cytokine associated with fibroblast proliferation and collagen synthesis (Yamamoto et al., 1999), and TNF-α is considered to be a central mediator in bleomycin-induced pulmonary fibrosis (Yamamoto et al., 1999; Ortiz et al., 1999; Zhang et al., 1997; Khalil et al., 1993). When applied locally, bleomycin acts as an irritating agent and initiates inflammatory process. It activates inflammatory cells by stimulating cytokine

synthesis; it also stimulates fibroblast proliferation, and enhances collagen synthesis (Khalil et al., 1993; Scheule et al., 1992). We did not evaluate tumor necrosis factor (TNF)-α and transforming growth factor (TGF)-β values. It would be helpful to measure these levels in further studies.

In the present study, we found that bleomycin significantly reduced the amount of seroma. Histopathological examination revealed that the number of PNL, fibroblast and the amount of fibrosis were significantly higher in the group applied bleomycin. These findings raise the thought that seroma reducing effect of bleomycin might result from rapid adherence of flaps and rapidly filled dead space with fibrosis due to locally induced fibrosis.

In conclusion, local bleomycin sulfate might be a therapeutic option in the patients that undergo surgical procedure due to breast carcinoma and developed chronic seroma. However our data is too preliminary to make any assumption. It has also limited capacity, since it is an animal study. Nonetheless, further studies that compare the efficacy and adverse effects (benefit-to-harm ratio) of bleomycin sulfate are needed.

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