Araştırma

Effects of locally applied clinoptilolite (Froximun®) on seroma formation in a rat mastectomy and axillary lymph node dissection model

RATLARDA MASTEKTOMİ VE AKSİLLER DİSSEKSİYON SONRASI KLİNOPTİLOLİT (FROKSİMUN®) KULLANIMININ SEROMA OLUŞUMUNA ETKİSİ

Süleyman Özkan AKSOY[1], Cevdet Onur TUNK[1], Ali İbrahim SEVİNÇ[1], Merih GÜRAY DURAK[2]

[1]Dokuz Eylül Üniversitesi Tıp Fakültesi, Genel Cerrahi Anabilim Dalı, İzmir
[2]Dokuz Eylül Üniversitesi Tıp Fakültesi,Patoloji Anabilim Dalı, İzmir

ABSTRACT

Objective: Seroma formation is one of the most frequent complications following breast cancer surgery. Although many techniques and drugs have been investigated, no definitive data are available concerning how to prevent this complication. The present study aims to investigate impact of local clinoptilolite application on seroma formation in an experimental mastectomy model created in rats.

Material and Method: Two groups with seven rats in each were randomised. Under general anesthesia, all rats underwent right mastectomy and axillary dissection. Clinoptilolite was locally applied to surgical sites in the study group, whereas no application was performed in the control group. Ten days after the surgery, seroma formation was evaluated and tissue samples were obtained from the surgical sites, for both biochemical and histopathological examination

Results: There was statistically significant decrease in seroma formation after clinoptilolite application (p=0.002). In addition, granulation tissue formation was significantly higher in the study group (p=0.006). No statistical difference was found between groups in terms of biochemical parameters.

Conclusion: The reducing effect of seroma formation by clinoptilolite application may be due to its crystal cage structure. It collects seroma with its sero-absorbent features and creates granuloma formation that reduces dead- space, therefore accelarating wound healing.

Keywords: seroma, mastectomy, clinoptilolite, wound healing

ÖZ

Amaç: Seroma oluşumu, meme kanseri cerrahisini takiben en sık görülen komplikasyonlardan biridir. Birçok teknik ve ilaç araştırılmasına rağmen bu komplikasyonun nasıl önleneceğine dair kesin bir veri bulunmamaktadır. Bu çalışmada, sıçanlarda oluşturulan deneysel mastektomi modelinde, lokal klinoptilolit uygulamasının seroma formasyonu üzerindeki etkisinin araştırılması amaçlanmıştır.

Süleyman Özkan AKSOY
Dokuz Eylül Üniversitesi Tıp Fakültesi,
Genel Cerrahi Anabilim Dalı, İzmir
https://orcid.org/0000-0032-2217-6819

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Gereç ve Yöntem: Çalışmamızda, sıçanlar çalışma grubu (n=7) ve kontrol grubu (n=7) olarak iki gruba randomize edildi. Genel anestezi altında tüm sıçanlara sağ mastektomi ve aksiller disseksiyon yapıldı. Çalışma grubuna lokal olarak klinoptilolit uygulanırken, kontrol grubuna herhangi bir uygulama yapılmadı. Ameliyattan on gün sonra, seroma formasyonu değerlendirildi ve hem biyokimyasal hem de histopatolojik incelemeler için cerrahi alanlardan doku örnekleri alındı.
Bulgular: Klinoptilolit uygulanan çalışma grubunda, kontrol grubuna göre seroma oluşumunda istatistiksel olarak anlamlı azalma saptandı (p = 0,002). Ek olarak, çalışma grubunda granülasyon dokusu oluşumu anlamlı olarak daha yüksekti (p = 0,006). Biyokimyasal parametreler açısından gruplar arasında istatistiksel bir fark bulunmadı.
Sonuç: Seroma oluşumunun klinoptilolit uygulaması sonrası azalması, kristal kafes yapısına bağlı olabilir. Sero-emici özellikleriyle seromayı toplar ve ölü boşluğu azaltan, dolayısıyla yara iyileşmesini hızlandıran granülom yapılarını oluşturur.
Anahtar Sözcükler: seroma, mastektomi, klinoptilolit, yara iyileşmesi

Seroma formation is the most prevalent complication encountered secondary to mastectomy and axillary dissection (15-81%) (1). Seroma causes significant discomfort and morbidity in patients, with possible delays in adjuvant therapies and increase in treatment costs. Although the pathophysiological mechanism of seroma formation is ambiguous, it has traditionally been assumed that blood and lymphatic oozing in the residual dead space contributes to its development. However, up to date studies have revealed that loculation of the inflammatory exudate leads to delayed wound healing (2-5).

Several surgical procedures and sclerosing agents have been tried to prevent seroma formation, but none of these methods could accomplish a result (6, 7). Up to date, various studies have been performed to prevent formation of seroma, in terms of removing the dead-space. Various agents, such as cattle thrombin, fibrin adhesives, talc, tranexamic acid, *Corynebacterium parvum*, tetracyclines and many antineoplastic agents have been tried to increase local fibrosis, but none of them provided significant efficiency (8).

Zeolites are solid crystals with micropores that contain aluminum, silica and oxygen in their structure, and cation and water in their pores. Clinoptilolite (Froksimun®) is a natural zeolite from silicate family that is obtained from volcanic tuffs (9).

It consists of a three dimensional framework with silicate and aluminum tetrahedron. Its hydrated natural microporous crystals have 8-10 rings. After clinoptilolite covers the surface of the open wound area, it prevents vascular leakage and provides wound healing by its hemostatic, absorbent, antiviral, antifungal and antibacterial features (10, 11).

The aim of this study was to investigate the effects of topical application of clinoptilolite on seroma formation in an experimental mastectomy and axillary dissection models in rats.

MATERIALS AND METHODS

Fourteen male Wistar rats with a median weight of 213 g (range, 200-250 g) were used in this study. The approval of Dokuz Eylul University Medical Faculty Ethical Committee for Laboratory Animal Research was obtained (2011-17). All of the animals were provided with a free access to food and water as much as they wanted, and were approached in accordance with guidelines for the care and use of laboratory animals established by the Ethics Committee of Dokuz Eylül University, and the National Institute of Health (U.S. publication no 86-23, revised 1985). Surgical interventions were performed at Dokuz Eylul University Medical Faculty Laboratory Animals Research Center under non-sterile but clean conditions. The rats were anesthetized by injecting 5 mg/kg xylazine and 50 mg/kg ketamine plus, intraperitoneally. Animals were left to breathe spontaneously during the course of the trials.

The rats were randomized into two groups: Group I was the control group (n=7). Group II was the study group that were applied local clinoptilolite after surgery (n=7).

Operative procedure

Anterior thoracic wall and axillary region were shaved and cleaned by 10% povidone-iodine. Right breast mastectomy and axillary dissection was implemented based on the definition by Harada et al (11). A vertical incision starting from the jugular notch to the xyphoid process was performed. Skin and the subcutaneous tissue were detached from the chest wall while the flaps were prepared. Major pectoral muscle was dissected up until the level of the latissimuss dorsi muscle, and then it was excised from the thoracic wall (Figure 1a). Preserving the integrity of the tissues, axillary artery, vein and nerve, axillary lymph nodes were excised. Unilateral mastectomy and axillary dissection were performed (Figure 1b).

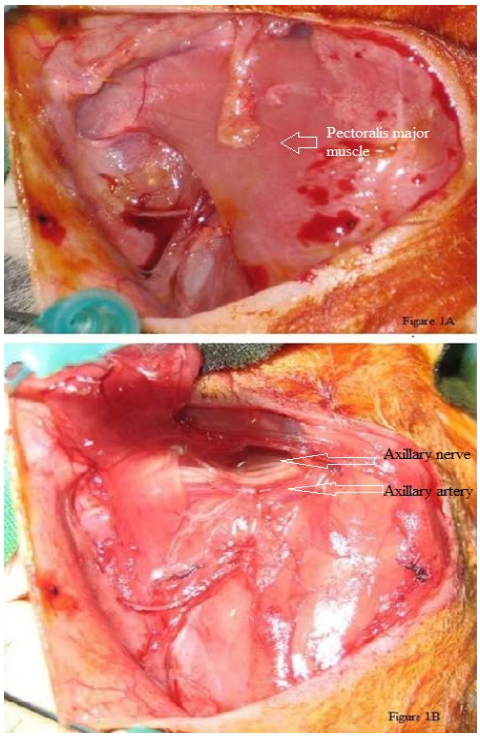

Figure 1. Pectoralis major muscle was dissected and the fatty cellular tissue was exposed **(a)**, pectoral muscle was completely resected, lymph nodes in the axillary fossa were excised. Axillary artery, vein and nerve were preserved **(b)**.

After the hemostatic control, the skin was closed with continued suturing. Clinoptilolite was locally applied to the surgical site in the study group, whereas no additional application was performed in the control group. Rats were monitored for 10 days after the surgery.

Analytical procedures

On the postoperative 10th day, mastectomised area was explored under ketamine anesthesia. The seroma was aspirated with a needle, and the total volume was recorded for each rat. These aspirates were analyzed for total protein, albumin, lactate dehydrogenase (LDH), and white blood cell (WBC) counts. Tissue samples were obtained from axilla and thoracic wall for histopathological examination. The rats were sacrificed with high dose ether anesthesia at the end of all procedures.

Histopathological examination

Tissue samples were transferred to pathology laboratory in 10% formaldehyde solution. After fixation, the tissues were embedded in paraffin blocks and 5 mm sections were obtained. The slides were stained with both hematoxylin-eosin (H&E) and Masson's trichrome to evaluate fibrosis. Presence of vascular proliferation, fibrin deposition, hemorrhage, edema, necrosis, congestion, microorganisms, polymorphonuclear leukocytes, fibroblasts, lymphocytes, macrophages and increase in the fibrous tissue were examined and graded semiquantitatively, under the light microscope and scored on a 0 to 3+ scale, as 0=absent, 1=mild, 2=moderate and 3=marked (MGD).

Statistical analyses

Statistical analyses were performed using Statistical Package for the Social Sciences (version 15.0; SPSS Inc. Chicago, IL, USA). Seroma volumes and biochemical parameters were evaluated using the Mann-Whitney U test, whereas histopathological grades were evaluated using the Fischer's exact test. A p-value < 0.05 was considered statistically significant.

RESULTS

There was no flap necrosis, wound dehiscence or wound infection during the course of the experiment. None

of the rats died or developed a problem that required exclusion from the experiment. All rats in the control group developed seroma formation. The mean seroma volume was measured as 1.9 ±0.44 mL in the control group, while it was 0.8 ±0.13 mL in the study group (p=0.002, Figure 2).

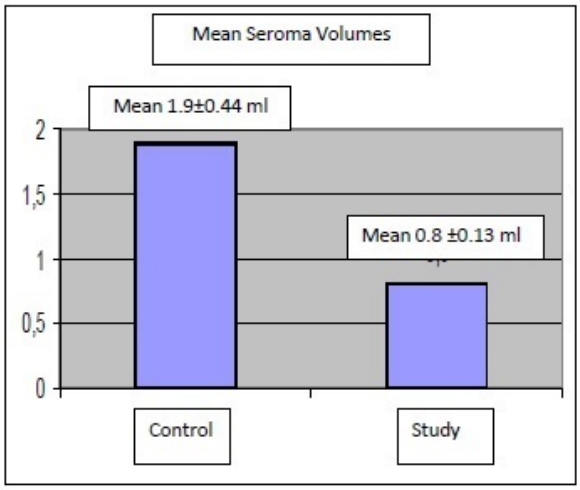

Figure 2. The mean seroma volumes in groups on postoperative day 10. Clinoptilolite significantly decreased seroma formation (p= 0.002).

No microorganisms, edema, hemorrhage or necrosis was seen in the samples that we examined. There was no difference between the groups in terms of vascular proliferation, fibrin deposition, congestion, polymorphonuclear leukocytes, fibroblasts, lymphocytes, macrophages and increase in the fibrous tissue (Table I). The increase in the formation of granulation tissue was greater in the study group, that was statistically significant (p=0.006, Figure 3).

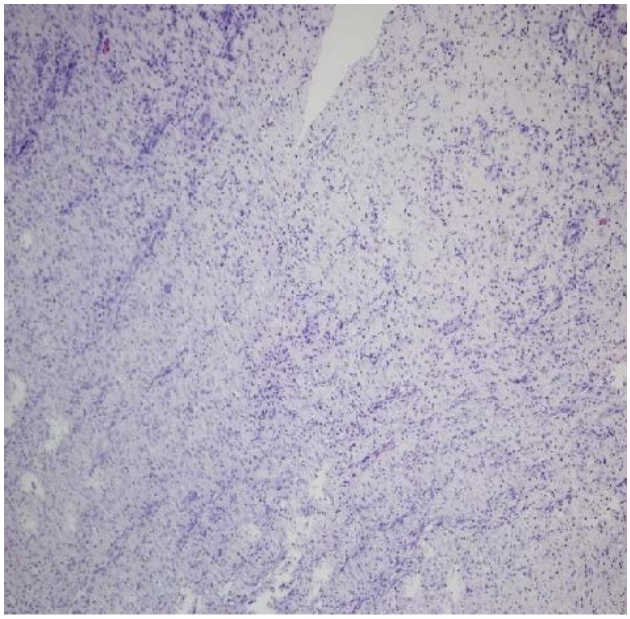

Figure 3A

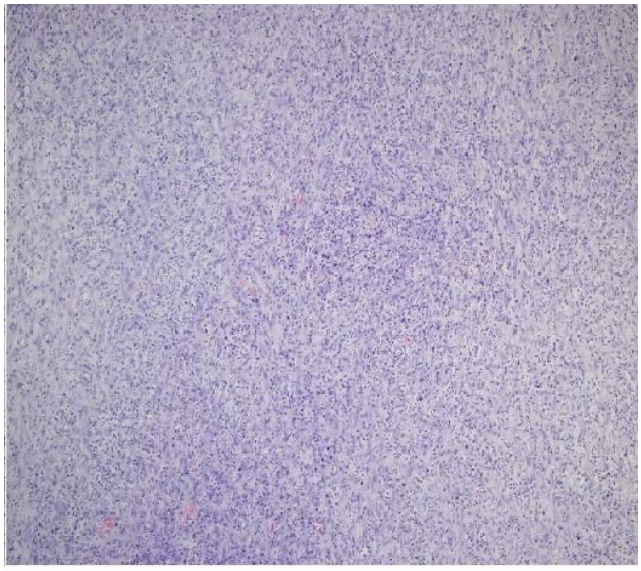

Figure 3B

Figure 3. Two microscopic images from the study (a) and the control (b) groups. Granulation tissue formation was more prominent in the study group (H&E, x 100).

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

Parameters	Control group (mean± SD)	Study group (mean± SD)	p
Vascular proliferation	2.14±0.69	2.00±0.69	0.68
Fibrin deposition	0.28±0.48	0.25±0.48	0.62
Congestion	1.57±1.27	1.42±0.50	0.87
PNL	1.85±0.37	2.14±0.69	0.33
Fibroblast	1.14±0.37	1.00±0.00	0.35
Lymphocyte	1.00±0.00	1.2±0.48	0.17
Macrophage	1.14±0.37	1.0±0.81	0.68
Fibrosis	1.00±0.00	1.00±0.00	1
Granulation tissue	0.42±0.53	1.71±0.48	0.006
SD: standard deviation			

There was no axillary seroma development in three of the rats from the study group, with only a soft mass of clinoptilolite granuloma formation filling the space. The seroma aspirates that were obtained from the control and the study groups were analyzed in terms of total protein, albumin, LDH and WBC counts. As there was no observed seroma formation in three rats in the study group, only four aspirates were processed.

There was no significant difference between the groups in terms of mean albumin, LDH levels and WBC counts. The mean total protein level was higher in the study group, but the difference was not significant (p=0.19, Table II).

Table II. Levels of biochemical parameters of the groups

Parameters	Control group (mean± SD)	Study group (mean± SD)	P
Total protein (g/dL)	2.53±2.0	4.65±1.25	0.19
Albumin (g/dL)	2.03±1.0	2.09±1.01	0.83
Lactate dehydrogenase (U/L)	808.9±106	1318.9±144	0.67
White blood cell count (x 1000/mm^3)	37.6±3.9	37.5±4.5	0.67
SD: standard deviation			

DISCUSSION

Seroma is defined as fluid collection under flaps or in dead-spaces in the axillary region, because of a large area of vascular and lymphatic structure injury after surgery. Its formation has traditionally been attributed to blood and lymphatic leakage in the mentioned spaces. It is the most frequent complication after breast surgery that can cause many problems such as prolonged hospitalization time, wound infection and delayed adjuvant treatment.

The pathogenesis of seroma formation is still unclear (3,4). However, it has been indicated that post mastectomy fluid includes cellular components of inflammatory exudate (12). During the early inflammatory course of the healing process, leukocytes and macrophages start to migrate to this area, leading to an uncontrolled exudate release rich in inflammatory cells, structural molecules, cytokines and growth factors under the influence of histamine and bradykinin, which causes the persistence of seroma (13, 14). Apart from this, it has been shown that seroma fluid includes significantly greater levels of high-molecular-weight proteins, such as albumin and globulin, in contrast to plasma or lymph. These findings support the idea that seroma fluid exhibits the characteristics of inflammatory exudate (2).

Several studies including surgical techniques (15-17), the efficacy of immobilization of upper extremity movement (18, 19), pressure dressings (20), many types of tissue adhesives and local sclerosing agents (6, 7, 21-23) have been investigated in the treatment of seroma. Various agents such as absorbent materials, cattle thrombin, fibrin adhesive, talc, tranexamic acid, *Corynebacterium parvum*, tetracycline and a number of antineoplastic agents as well as alternating surgical methods were used to decrease seroma formation. However, none of these was found to be efficient enough (24, 25).

Zeolite material that we used in this study has extraordinary and unique physical and chemical features, which enables its use in various fields such as agriculture, ecology, manufacture and industrial processes. Recently, more specific application of a natural forming zeolite material, clinoptilolite, has been analyzed in detail in veterinary and human medicine. Due to its positive effects on health issues including detoxification, in vivo use of clinoptilolite-based products have increased considerably (26).

Recent studies have shown a high potential of use for clinoptilolite in various in vitro and in vivo medical applications. A number of documented positive medical effects of clinoptilolite has been attributed to basic clinoptilolite material features, particularly the reversible ion change and adsorption capacity (27). The pivotal clinoptilolite feature related to elimination of toxic agents, that seem to support body homeostasis can be used extensively in various medical applications.

Clinoptilolite is a hemostatic, absorbent, antiseptic agent and its efficacy has been proven by a number of clinical studies (9, 28). It is obtained from volcanic rocks and consists of a three dimensional framework with silicate and aluminum tetrahedron. Its hydrated natural microporous crystals have 8-10 rings. It is a powder form and completely non-toxic. It can be hydrated or dehydrated (26).

Clinoptilolite acts as a cage, and fills the surgical area with its microporous structure that can prevent leakage of the lymphatic channels mechanically. Similarly, vascular leakage may also be prevented by this mechanical effect (29).

It has been shown that wound healing is accelerated by clinoptilolite, due to its effects on cytokines. After the injury, cytokines activate complement formation, platelet aggregation, blood coagulation and hemostasis, as well as release of growth factors, neovascularization, granulation tissue formation, and finally wound healing occurs (30).

In this study, it is suggested that clinoptilolite can prevent formation of seroma via two mechanisms. According to the first mechanism, it covers the surface that it has been used, and prevents lymphovascular leakage due to its absorbent structure, which also has micropores, and therefore decreases the fluid volume significantly that fills the dead-space. The second mechanism includes its accelerating effect in wound healing, due to its effects in both rapid granulation tissue formation and asepsis. In this respect, it is thought that clinoptilolite can decrease prolonged inflammatory response and delay wound

healing, the two factors that are accused in seroma formation.

To the best of our knowledge, this is the first study in assessing the effectiveness of topical application of clinoptilolite on seroma formation. It is also a cost effective agent, and is easy to apply with no potential side effects.

In this study, we found that clinoptilolite significantly reduces the amount of seroma formation (p=0.002). Clinoptilolite absorbes the lymphovascular fluid collection due to its absorbent effect. The pressure that creates a mechanical effect also blocks seroma formation. Total protein, albumin, LDH levels, and WBC counts were higher in the study group when compared to the control group as expected, though it was not statistically significant. These findings led us think that clinoptilolite application in the study group did not have an effect on formation of inflammatory seroma. Studies about clinoptilolite in the literature emphasize its effects in hemostatic control, that further gives rise to efficiency in seroma control (31).

Histopathological examination revealed that granulation tissue formation was significantly higher in the group that used clinoptilolite (p=0.006). The number of macrophages and fibroblasts, which play an essential role in wound healing, were higher in the control group when compared to the study group, although not statistically significant. These cells gradually increase in number in the early phase of the healing process, reach their peak levels, and gradually reduce in number as the healing process progresses (23).

Clinoptilolite powder does not have an effect to prevent formation of inflammatory exudate. Due to its crystal cage structure it collects seroma with its sero-absorbent features, and creates granuloma formation in dead-spaces, therefore accelerates wound healing. On the other hand, some materials such as Bovine Collagen Sponge that leads to fibroblast growth and neovascularization, increase granulation tissue formation and adhesion, and so reduces seroma formation (32).

Our findings indicate that local application of clinoptilolite may prevent seroma formation in this experimental model. The most important limitation of this study is that is is an animal study, and therefore has limited capacity to make any assumptions. Another aspect that needs to be clarified is elimination of granuloma formation. Although there was no infection in the postop examination period, the absence of granuloma formation and development of granulation tissue needs to be examined in a longer treatment period.

Author's Statement

There is no conflict of interest.

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