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Effects of tamoxifen on survival of cutaneous and myocutaneous flap (experimental study)

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Abstract. – OBJECTIVES: Breast cancer is the most common non-skin malignancy in women. In breast cancer, the basic principle of endocrine therapy is to deprive tumor cell from the growth-stimulating effect of estrogens. The oldest, best-known and most widely used endocrine therapy medicine is tamoxifen, which is a selective estrogen receptor blocker. All factors that are known to have adverse effects on flap and pre-reconstruction flap survive should be eliminated 3 weeks before the surgery and started at the end of the 3rd week after the surgery. When the literature is reviewed, there are limited studies about that tamoxifen increases the risk of deep vein thrombosis and pulmonary embolism. The aim of this study was to research whether tamoxifen had adverse effects on the skin flap and muscle-skin flap survive.

MATERIALS AND METHODS: In this study, 32 female Sprague-Davley rats were used. Their weights ranged from 220 to 250 g. 4 groups, each consisting of 8 rats, were formed. In this experimental study, in which rat models were used caudal based rat dorsal skin flap and superior pedicle rectus abdominis musculocutaneous flaps were applied. Control groups were formed for both flap models. Study groups were treated with tamoxifen citrat and control groups were treated with placebo.

RESULTS: As a result of the statistical evaluation done by measuring the surviving flap areas, it was found out that tamoxifen had no negative effect on flap survive.

CONCLUTIONS: Based on these findings, it was concluded that there was no need to stop tamoxifen as long as 6-7 weeks in patients undergoing breast reconstruction with pedicle flap techniques.

Key Words:
Tamoxifen, Flap survival, Cutaneous flap, Myocutaneous flap, Rat.

Introduction

Breast cancer is the third most common cause of cancer deaths in women after lung and colorectal cancers[1]. The increase of opportunities for early diagnosis and the advances in post-surgical adjuvant treatment protocols are the main cause of the fall of breast cancer, which was in the first place in cancer-related deaths in women until 1980, to third place today.

In the breast cancer, decision of hormonal treatment depends on several factors. The most important indicator of getting response from hormonal treatment is the presence of estrogen and progesterone receptors in the tumor. The presence of estrogen receptor in breast cancer cells was first demonstrated in 1971[2], and the idea that the presence of receptor would respond better to endocrine therapy was put forward. After this date, with the aim of endocrine therapy, selective estrogen receptor blockers and aromatase inhibitors were developed and included to standard use.

The response rate to endocrine therapy in the advance stage of breast cancer is 33% in patients with a positive hormone-receptor, and 50-70% in patients whose both receptors are positive[3]. Endocrine therapy reduces the risk of metastasis and recurrence of breast cancer and prolongs the patient's survival[4,5,6]. While selective estrogen receptor blockers are preferred as endocrine therapy in premenopausal women, in postmenopausal women, there are several options of endocrine therapy such as antiestrogens, aromatase inhibitors or progestins.

Tamoxifen has non-steroidal structure and effects much rather through its active metabolites. N-desmethyl-tamoxifen, and 4-hydroxy tamoxifen, active metabolites of tamoxifen, are much more stronger than tamoxifen[7,8]. Since tamoxifen, which is the oldest and most commonly used medicine for breast cancer treatment, is rather a tumorostatic drug and there is a possibility of recurrence after short-term treatment when tamoxifen is stopped, the long-term treatment (at least 5 years) is said to be the best clinical strate-

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gy[8]. As a result of 5-10 years use, no tolerance develops against the use of tamoxifen; however after short-term uses the frequency of recurrence increases[9]. All the research conducted up to today indicates that tamoxifen increases the rate of survival at least 10% in patients with breast cancer[10,11].

Prevention of skin flap necrosis and minimizing the risks are the most important research topic of reconstructive plastic surgery. This is because reconstruction with flap is a repair option whose compensation is very difficult in case of flap necrosis. If the organ to be reconstructed is the breast, the most important flap is TRAM flap removed from the abdominal wall or its different pedicle versions. Cigarettes and medicines, which have adverse effects on flap, survive during flap surgery and all the medicines whose effects are known should be interrupted for 6 weeks, 3 weeks before and 3 weeks after surgery. Tamoxifen's *half-life* is 5-7 days; N-desmethyl tamoxifen and 4-hydroxy tamoxifen's, which are its active metabolites, *half-life* is 14 days. It is suggested that 4 weeks before, which is 2 times the *half-life* period, tamoxifen should be discontinued[12]. The studies about the effect of tamoxifen on flap survive in patients who used tamoxifen and would undergo breast reconstruction are limited. In a study, positive effects of tamoxifen on intestinal anastomosis and wound healing in rats were observed[13].

In a study in which the risk of retrospective thromboembolism of patients undergoing breast reconstruction with microsurgical methods is investigated, tamoxifen is recommended to be stopped for 4 weeks prior to surgery. It is initiated at the end of the third week which is the postoperative flap's completion period of vascularization from the surrounding tissue[12]. It is a serious problem to stop a medicine for a period of 7 week, which is required to be taken every day to prevent recurrence and metastasis. The fact that a vital drug will not be given for a period of 7 weeks to the person receiving treatment for cancer causes both the medical oncologist and the patient to have concern about the breast reconstruction. We planned this study to show the positive or negative effects of tamoxifen on flap survive.

Modified McFarlane flap

The cranial pedicle removal of rat dorsal skin flap including panniculus carnosus muscle was defined by McFarlane et al in 1965[14]. Khouri et al[15] removed the caudal pedicle by modifying the flap in 1986.

This flap, which is removed as caudal pedicle in a lot of sources, is called modified McFarlane flap (Figure 1a, b).

Superior Pedicle Rectus Abdominis Muscle-Skin Transposition Flap

Along with rectus abdominis muscle extending between the pelvis and the xiphoid, it is the muscle-skin flap which includes fascia surrounding the muscle, and 1.2 cm wide and 5 cm long skin, where inferior portion of the muscle is cut, and which only feeds with the superior epigastric artery[16] (Figure 1c, d).

In our study, we used the modified McFarlane skin flap and the superior pedicle rectus abdominis muscle-skin transposition flap.

Materials and Methods

In this study, 32 female Sprague-Dawley rats were used. Their weights ranged from 220 to 250 grams. The study started after the receipt of the approval of the Ethics Committee of Pamukkale University. The rats were obtained from Pamukkale University Experimental Research Laboratories and were subjected to research in the same laboratory. All rats were fed with standard rat food and drinking water. They were kept in standard laboratory animal rooms with a light pattern which is 12 hours light and 12 hours dark and with a temperature of about 21°C. For the study, tamoxifen citrate obtained from LKT lab. Minnesota SA and sesame oil and ethyl alcohol obtained from Pamukkale University School of Medicine Pharmacology Department were used.

4 groups, each consisting of 8 rats, were formed. The first group was named the experimental group in which modified McFarlane flap was removed and the third group was named the experimental group in which the superior rectus abdominis muscle-skin transposition flap was removed. At a dose of 0.1 mg/kg for each rat daily, a total 0.5 cc tamoxifen citrate that will be given to 16 rats in the study group was solved by ethyl alcohol and was mixed with 7.5 cc sesame oil so that the solution was created. Solution was prepared fresh each day and rats were injected 0.5 cc of it subcutaneously. Injection was continued for two weeks.

The second and the fourth group were the control groups of Modified McFarlane Flap and rec-

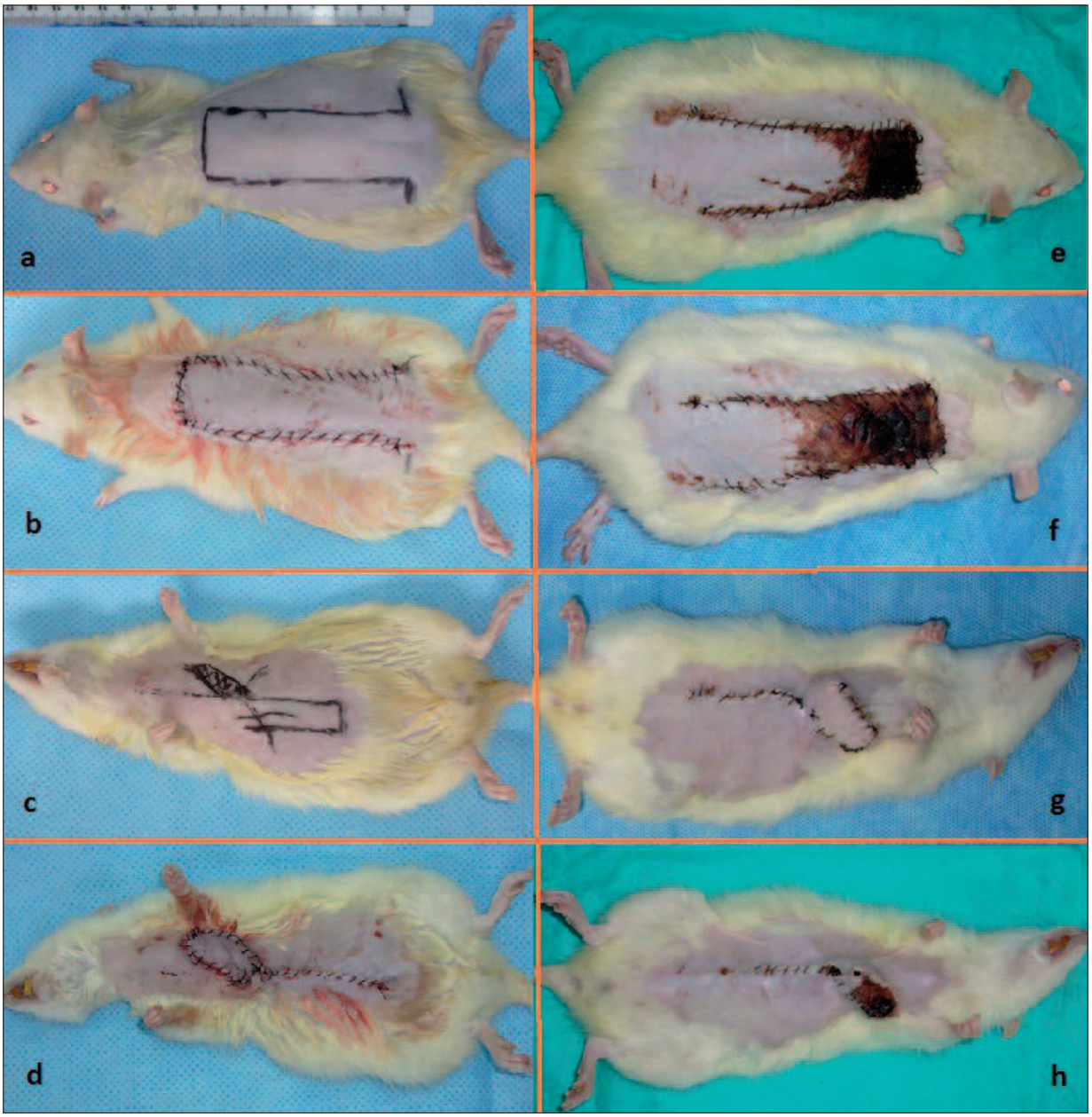

Figure 1. ***a, b,*** Views of caudal pedicle modified McFarlane flap. ***c, d,*** Views of superior pedicle rectus abdominis muscle-skin transposition flap. ***e,*** View of a rat in first group was observed 28% flap necrosis. ***f,*** View of a rat in second group was observed 60% flap necrosis. ***g,*** View of a rat in third group was not observed flap necrosis. ***h,*** View of a rat in fourth group was observed 68% flap necrosis.

tus abdominis muscle-skin flap transposition studies. For 14 days, 16 rats in the control group, 0.5 cc solution consisting of 0.5 cc ethyl alcohol and 7.5 cc sesame oil (placebo) was injected subcutaneously.

The rats in all groups, after 14 days of tamoxifen and placebo injection, were anesthetized with intramuscular 90 mg/kg ketamine hydrochloride and 10 mg/kg xylazine.

After the first group were injected tamoxifen solution daily for 14 days, 3 × 9 cm caudal based modified McFarlane dorsal skin flap with panniculus carnosus muscle was removed, and the same area was sutured with 4/0 silk.

In the second group, after the 14 day injection of alcohol and sesame oil solution as a placebo, modified McFarlane flap was removed and the previous one was sutured.

In the third group, after the 14 day injection of tamoxifen solution, 1.2 cm wide and 5 cm long superior pedicle right rectus abdominis muscle-skin flap that extends between the xiphoid and pelvis was removed.

Transposed on the defect area prepared in the left thoracic region, flap was sutured with 4/0 silk. The donor area was sutured as primarily.

In the fourth group, after the injection of 14 day placebo solution, the right rectus abdominis muscle-skin flap was removed, and sutured on the defect in the thoracic region after being transposed. Tamoxifen and placebo injections were not given on the day of surgery. In the following 6 days the injection was continued in all groups. On the 7th after the day flap surgery, rats in all groups were anesthetized with intramuscular 90 mg/kg ketamine hydrochloride and 10 mg/kg xylazine.

Statistical Analysis

Statistical analysis was performed using SPSS software (version 17, Chicago, IL, USA). Descriptive statistics were used for quantitative demographic data. Mann-Whitney U-test was used in the comparison of tamoxifen with placebo effect in subjects in which dorsal flap was removed and in comparison of tamoxifen with placebo effect in subjects in which muscle-skin flap was removed. Statistical significance level was accepted as $p < 0.05$.

Surviving flap areas were measured with graph paper.

Results

No subject was lost in all groups. In all groups, on the postoperative 7th day, surviving flap areas were measured planimetrically with graph paper. Since the necrosis developed on flap distal contracted and the rate of contraction differed among the rats, assessments were made based on the surviving flap areas[17].

In the rats of the first group, where tamoxifen was injected and modified McFarlane flap was removed, there was flap necrosis at rates ranging from 6% to 28% (Figure 1e). In the second group, in which the rats were injected sesame oil and alcohol solution as a placebo and modified McFarlane flap was removed, flap necrosis at rates ranging from 23% to 60% was found (Figure 1f). The average ratio of flap survive in the 1st group to the total flap area was found to be 82.59%. The average ratio of flap survive in the 2nd group to the total flap area was found to be 60.32%. Between the tamoxifen group and the control group in which modified McFarlane flap was removed, there was a significant difference according to measurement results of living flap area ($p = 0.001$). In the group that was given tamoxifen the average surviving flap area was more than 22% (Table I).

In the rats of third and fourth group, superior pedicle rectus abdominis muscle-skin flap was removed and transposed on the defect area that was prepared in the left thoracic area. On the 7th postoperative day, surviving flap areas were measured. The amount of surviving flap areas was very high in the rats of third group which were given tamoxifen before and after the flap transposition. There was 5% flap necrosis in one of the rats and 10% in another rat; flap necrosis was not observed in the transposed flaps of remaining 6 rats (Figure 1g).

In the fourth group, which was injected with sesame oil-alcohol solution without tamoxifen, there was flap necrosis ranging from the rates 8% to 67% in superior pedicle rectus abdominis muscle-skin transposition flaps (Figure 1h).

The average surviving flap area was 98.125% in the third group. The average surviving flap area was 75.875% in the forth group. The amount of surviving flap area in the groups receiving tamoxifen was found out to be more than 23% (Table II). The difference was found statistically significant ($p = 0.001$). The rate of difference in the third and fourth group in which superior pedicle muscle-skin flap has been removed was close to the rate in the first and second group in which modified McFarlane skin flap was removed.

Table I. Areas and percentages of flap survive in rats in tamoxifen group and control group in which modified McFarlane flap was removed.

	Tamoxifen group	Control group	p
Surviving flap area	2230.00 ± 211.11	1628.75 ± 306.47	0.001
Survive/total flap area (%)	82.59 ± 7.81	60.32 ± 11.35	0.001

Table II. Areas and percentages of flap survive in rats in tamoxifen group and control group. In which superior pedicle rectus abdominis muscle-skin flap was removed.

	Tamoxifen group	Control group	p
Surviving flap area	588.75 ± 22.32	455.00 ± 113.26	0.001
Survive/total flap area (%)	98.13 ± 3.72	75.88 ± 19.05	0.001

Discussion

In this study, we observed that tamoxifen increased pedicle muscle-skin flap survive over 20%. In a study, carried out by Toutain et al[18] in which the effect of 17-beta-estradiol (E2) and tamoxifen on skin flap necrosis was investigated, statistically significant rate of reduction in flap necrosis was also found in the rats which started to receive tamoxifen treatment 14 days before the flap surgery. In the analyses conducted on skin specimens taken histologically during skin flap surgery, mild vascular congestion and minor hemorrhage were observed in the rats receiving tamoxifen as it was in E2 group. In the flaps of rats treated with E2 and tamoxifen it was shown that flow of blood was reshaped and reperfusion was completed.

In the study of Kelley et al[12] in which tamoxifen complications were retrospectively investigated in patients undergoing microsurgical breast reconstruction methods, the risk of thromboembolism was found to be 1.7 times higher in the group using tamoxifen in preoperative period compared to one which did not use. However, in this study, the fact that arterial insufficiency was observed in 2 patients in the group which did not use tamoxifen but not in tamoxifen group, and that the 1.5% rate of flap thrombosis was the same in both groups were contradictory. In the study, the increase in complications in non-abdominal flap options using tamoxifen and particularly in Gluteal flaps may be explained by difficulties in surgical techniques. In the article, the hypothesis that thickening (inflammation) in vascular endothelium, vasoconstriction and platelet aggregation would occur with connection of tamoxifen to estrogen receptor was put forward.

But in the rat study, done by Pessoa et al[19] in which the effect of tamoxifen on arterial anastomosis was investigated, in histopathological examination, a significant rate of intimal hyperplasia was found in the group treated with moderate tamoxifen and vasculitis and it was higher in the group treated with tamoxifen. In macroscopic evaluation done in the following week, arterial occlusion and intraluminal thrombosis was found in all subjects of the both groups.

McNamara et al[13] investigated the effect of tamoxifen on intestinal anastomosis and wound healing in skin with rat study. In the study in which resistance of wound healing in skin and intestinal anastomosis to pressure was examined, nothing negative was observed in rats given tamoxifen compared to the control group. Rather, both improvements were affected positively.

Chemoendocrine therapy itself is better than endocrine therapy. In the majority of patients with breast cancer the estrogen receptor (ER) is positive. In patients with ER (-) there is no significant difference between chemoendocrine therapy and chemotherapy in terms of survival. In a study in which the effects of chemotherapy and endocrine therapy in early stage breast cancer on 15 year survival and recurrence are investigated it was revealed that tamoxifen could cause death due to thromboembolism and uterus Ca. However, researchers[20] indicate that a rate of mortality as low as 0.2% is not very significant in a 10 year process, and that because 5 year tamoxifen therapy significantly reduced the mortality rate of breast cancer patients whose lymph node is negative and positive in 10 year process, rare complications such as thromboembolism and uterus CA should not be taken into consideration and also these are influenced by the patient's age and other risk factors.

In the study, it is stated that death due to thromboembolism is the least among the other vascular diseases which result in death and it is meaningless[20].

According to the study, done by Hernandez et al[21] on 16,289 Danish patients who had breast cancer surgery, the risk of deep vein thrombosis and pulmonary embolism, at the end of a 5 year period, was found out to be 1.2% in the group treated with tamoxifen and 0.5% in the group which was not given tamoxifen. The risk was 3.5 times higher in the group which was given tamoxifen in the first 2 years of treatment com-

pared to the group which was not given tamoxifen. After the second year, these rates were found to be close to each other. Especially the group over the age of 50 was more at risk. Lee et al[22] determined the 5 year risk of DVT and PE in patients with breast cancer as 0.2% – 0.9% – 4.2% in the groups receiving placebo, tamoxifen only, and tamoxifen + chemoterapy, respectively. At this point, the addition of chemotherapy to treatment increased the risk fivefold.

Mousavi et al[23] gave tamoxifen to 138 patients for the treatment of hypertrophic scar after surgery at a dose of 20 mg daily for 2 months. At the end of this period, no significant complication including deep vein thrombosis and thromboembolic was encountered.

In a study, by Fisher et al[24] including 13,388 women, 6,681 of whom were given tamoxifen and 6,707 of whom received placebo, pulmonary embolism was diagnosed in 18 women in tamoxifen group and in 6 women in the group not given tamoxifen. The ratio of risk was 3.01. Deep vein thrombosis found in 35 patients in tamoxifen group and in 22 patients in placebo group. The risk rate of this was 1.6. In the same study, within 5 years 71 patients in placebo group and 57 patients in tamoxifen group died. In the placebo group, 42 patients died of various types of cancer, 22 patients died of cardiac and vascular, and 14 patients died of other causes. While in tamoxifen group, 23 patients died of various cancers, 22 patients died of cardiac and vascular, and 12 patients died of other causes. The results in this study are another indicator that tamoxifen with complications do not increase morbidity and mortality.

In our study we investigated the effects of tamoxifen on pedicle flaps. Further studies about the effect of tamoxifen on free reconstruction options with transfer tissue requiring vessel anastomosis are necessary. Tamoxifen's risk of causing deep vein thrombosis and pulmonary embolism is not only during flap surgery. Publications which show that deep vein thrombosis and pulmonary embolism form by 1-7% especially in the first years of 5 year therapy accepted as duration of tamoxifen's usage are available.

From flap surgery on, aspirin, known to have positive effects on blood flow, low molecular weighted dextran and heparin are often given to the patient. These drugs both improve the circulation of the flap and reduce the risk thrombosis and embolism that could be caused by immobilization to minimum.

In our study, the fact that tamoxifen does not negatively affect the survive of pedicle muscle and skin flaps has been revealed. That the risk of thrombosis for microsurgical methods requiring vascular anastomosis does not increase has been revealed in other studies, as well.

Conclusions

Breast reconstruction surgery, through which the patient feels herself/himself psychologically relieved and the integrity of the body is achieved, can be carried out without even a short interruption of tamoxifen therapy, which affect the survival of the patient positively.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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