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EXPERIMENTAL STUDY

The effect of ovariectomy on the skin flap viability and myeloperoxidase levels

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Abstract: *Objectives:* Estrogen could affect the rate and quality of wound healing in skin. We aimed to investigate the effects of ovariectomy on skin flap viability and myeloperoxidase (MPO) levels in a rat model.
Background: Estrogens have many important beneficial and protective roles in skin that they improve collagen content and quality, maintain skin thickness and enhance vascularization. It has been shown that estrogen supplementation accelerates cutaneous wound healing in elderly patients.
Methods: Forty-eight cycling female Wistar albino rats were randomly divided into three groups (n = 16); ovariectomy (Group 1), sham (Group 2), and control (Group 3). Rats were subjected to bilateral ovariectomy in the Group 1, and only laparotomy in the Group 2. Twenty-one days later in the Group 1 and 2, a dorsal caudally based skin flap elevation was done. In the Group 3, the rats had a dorsal skin flap without any surgical intervention. Ten days later, the flaps were harvested for histopathologic examination and biochemical analyses.
Results: The rats in the Group 1 had significantly larger necrotic area and lower flap viability than in the Group 2 and 3 ($p<0.05$). Histopathologic examination showed that necrotic flap regions contained muscle necrosis with an abundant neutrophil infiltration, and severe edema in the Group 1. The MPO activity in the distal of skin flaps was significantly higher in the Group 1 compared to the Group 2 and 3 ($p<0.05$).
Conclusion: This study shows that ovariectomy has deleterious effects on skin flap viability in a rat model *(Tab. 1, Fig. 6, Ref. 44)*. Text in PDF *www.elis.sk.*
Key words: ovariectomy, skin flap viability, myeloperoxidase, rat.

In the field of plastic surgery, skin flaps are frequently used especially in reconstructive surgery. However, ischemia complications represents a major concern that may require secondary surgical interventions, generate multiple infections, and delay future treatments due to the presence of tissue necrosis (1, 2). Although the total loss rate of microsurgically transferred flaps 1–5 % (3, 4), partial flap necrosis may occur in 7–20 % of free flaps (5) and even 20–33 % of pedicled flaps (6). It is believed that severe ischemia which results from local arterial insufficiency causes the necrosis especially in the distal part of the flap (7). Thus, a considerable amount of research has been done to find ways to improve blood flow to the flap reducing ischemic conditions and preventing necrosis of skin flaps (2, 8, 9).

A number of studies have shown that estrogens have many important beneficial and protective roles in skin, that they improve collagen content and quality, maintain skin thickness and enhance vascularization (10, 11). Recently, it has also been shown that the rate and quality of wound healing in skin is estrogen dependent (12), while the delay in wound healing in elderly patients of both sexes can be significantly reduced by topical estrogen (13).

Current literature supports the thesis that estrogen acts as a free radical scavenger and this effect extends beyond its hormonal role in biological systems. Estradiol has been shown to increase the threshold in cerebral subcortex against transient cerebral ischemia in ovariectomized rats (14). Estrogen deficiency has also been shown to decrease ischemic tolerance in the aged rat (15). Clinically, estrogen replacement therapy has also been shown to have an anti-ischemic action in postmenapausal women (16). Estrogens have been recognised as a regulator of vascular tone and structure particularly in the skin. The skin microcirculation has been shown to be impaired if estrogen deficiency is present (17). Also, it has been shown that estrogen supplementation accelerates cutaneous wound healing in elderly patients (13). Estrogens exerts this anti-ischemic actions by favoring angiogenesis, limiting endothelial dysfunction, and exerting inflammatory and antiapoptotic effects (18, 19).

It is known that neutrophils likely serve as a significant source of free radicals which contribute to flap failure by lipid peroxidation and tissue degradation (20). In several studies it has been shown that antioxidants and neutrophil inhibitors are independently capable of counteracting some of the damaging conditions leading to the necrosis of distal flap tissue (21, 22). Myeloperoxi-

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Ovariectomy group		
↓	⇓	↓
0 day	21 days	31 days

Sham group		
↓	⇓	↓
0 day	21 days	31 days

Control group	
⇓	↓
0 day	10 days

Figure 1. Experimental groups and protocol. (Ovariectomy(↓); laparotomy (↓); flap constitution (⇓); flap removal (↓)).

dase (MPO) is a major neutrophil protein and has been found to be a reliable marker for detection of neutrophil accumulation in inflammed skin in vivo (23). Decreased tissue MPO content suggests a decreased neutrophil recruitment (24). Gurlek et al (25) reported a statistically higher MPO activity in the ischemia/reperfusion (I/R) group when compared to the sham group in their study. Also they demonstrated that administration of melatonin reduced the flap necrosis area and MPO activity in I/R injury of rat epigastric (axial pattern) flaps (25).

In the present study, we aimed to investigate the effects of ovariectomy (leading to endogenous estrogen deficiency) on skin flap viability and MPO levels in a rat model.

Methods

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes

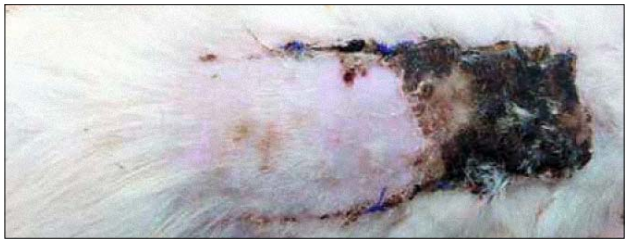

Fig. 2. Flap necrosis on the 10th postoperative day in ovariectomy group.

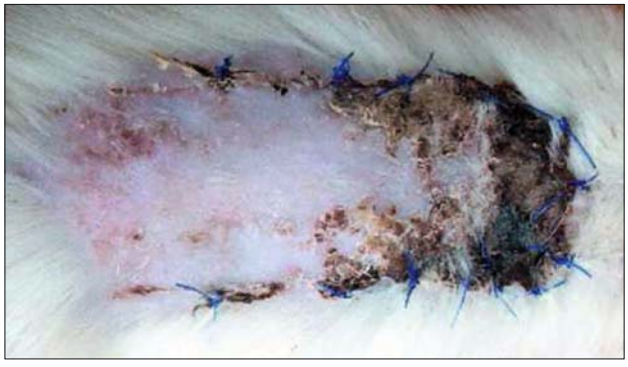

Fig. 3. Flap necrosis on the 10th postoperative day in sham group.

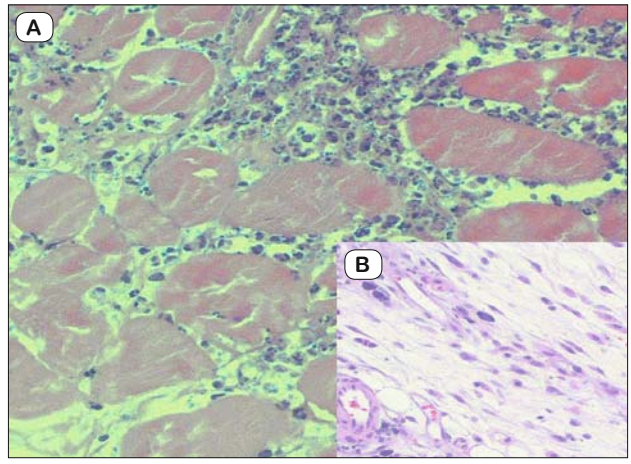

Fig. 4. Histopathology of muscle necrosis with heavy neutrophil infiltration (A) and severe edema (B) in ovariectomy group (H&E, original magnification ×20).

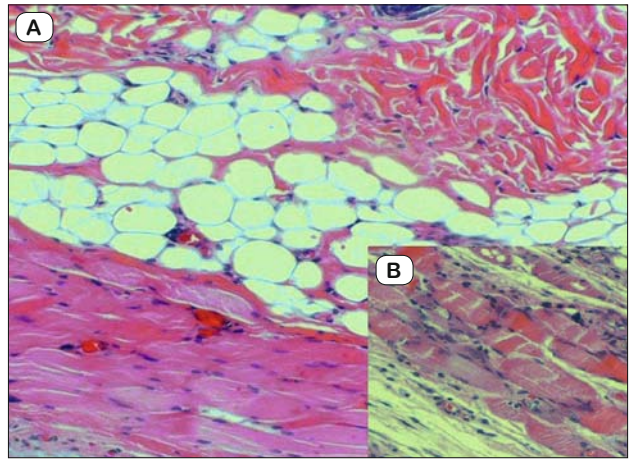

Fig. 5. Histopathology of normal muscle, adipose tissue (A) and mild edema (B) in sham group (H&E, A; original magnification ×20, B; original magnification ×40).

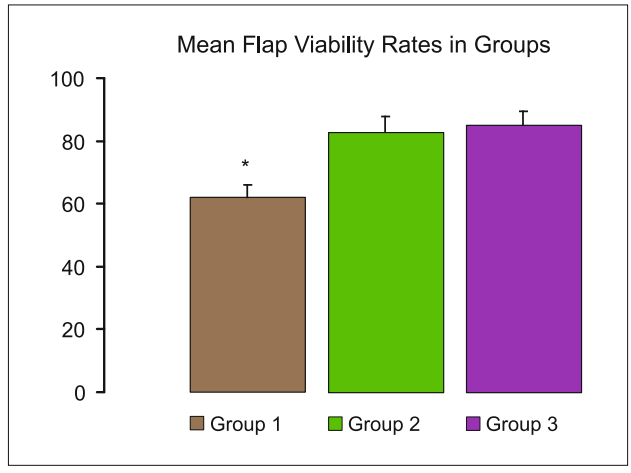

Fig. 6. Graphic represantation of mean flap viability rates in groups. Values are mean ± standart error (SE).

of Health and were approved by our Animal Ethics Committee (Approval no. 2007/6-2).

Animals

Forty-eight, 70-day-old, cycling female Wistar albino rats (weight range, 240–260 g) were obtained from KSU Animal Research Laboratory and all the rats were housed in individual cages under standard conditions and fed with rat-chow diet. All female Wistar albino rats were randomly divided into the three groups each with 16 animals as follows (Fig. 1):

Group 1 (Ovariectomy group): Bilateral ovariectomy was done and three weeks later (we waited 3 weeks to see the effect of ovariectomy on hormone levels as reported in studies (7, 26, 27)), a dorsal caudally skin flaps were constituted. Ten days later, skin flaps were removed.

Group 2 (Sham group): The rats had laparotomy without ovariectomy and three weeks later, dorsal skin flaps were constituted. Ten days later, skin flaps were removed.

Group 3 (Control group): The rats had a dorsal skin flap without any surgical intervention. Ten days later, skin flaps were removed.

Each group, which consisted of 16 rats, were further divided into 2 subgroups as following Group 1A, 1B; 2A, 2B; 3A, 3B. The skin flaps in A-subdivision were used for histopathological examination and the others (B-subdivision) were used for biochemical analysis.

Rats were anesthetized with 60 mg/kg of i.p. ketamine hydrochloride (Ketalar; Eczacibasi AS, Istanbul, Turkey), and anesthesia was maintained by additional injections of the same anesthetic.

Flap model

Mc farlane dorsal caudal-based flaps were used in the study. The dorsal skin was shaved and marked according to 3 x 8 cm of diameters. Then, the area was sterilized with povidone iodine (Biokadine, Kansuk, Turkey). The skin flaps including panniculus carnosus were elevated and sutured to the original bed with 3/0 prolene (Dogsan, Turkey).

All flaps were allowed to live for 10 days. At the end of the 10th day, the rats were sacrificed and the flaps were excised for pathologic and biochemical examination.

Flap viability measurement

The necrotic pattern of dorsal flaps were evaluated on the 10th day. Black eschar was clinically evident in all flap distal regions (Figs 2 and 3). These areas were traced to an acetate paper and calculation was done according to the measurements. Flap viability was represented as the following formula:

Skin flap viability = (Total flap area - Necrotic flap area) / Total flap area x 100

Histopathologic assessment

On the flap viability measurement day, the dorsal skin flaps were harvested for histopathologic examination. All skin flaps included skin, subcutaneous fat and muscle. The specimens were fixed in 10% neutral buffered formalin solution and then embedded in paraffin. Serial sections were cut using a microtome at a thickness of 4 mm and the sections were stained with hematoxylin & eosine (H&E). The histopathologic sections were examined for the presence of necrosis, inflammation (neutrophil infiltration) and edema in the flap tissues under a microscope (Olympus BX50F4, Tokyo, Japan) and were photographed (Figs 4 and 5). Five microscopy fields were used to determine the presence or severity of tissue damage. Examination and scoring of the flap sections was performed in a blinded fashion by the same pathologist.

Measurement of Skin Flap Myeloperoxidase Content

To determine flap neutrophil recruitment, the distal halves of the flaps were collected, weighted and homogenized in 10 mL of 0.5 % hexodecyltrimetyl ammonium bromide. Homogenate was centrifuged at 4000 rpm. Supernatant was collected and then the MPO activity of the supernatant was determined by a modification of the O-dianisidine method with spectrophotometer (10). Values expressed as MPO units g^{-1} tissue.

Statistical analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 15.0. Data were analyzed by the Kruskal–Wallis test followed by the Mann–Whitney U test as a post hoc test. The data were expressed as the means ± standard of deviation (SD), and median (minimum-maximum). Statistical significance was defined as $p < 0.05$.

Results

All animals survived and no complication was seen during the test protocol. At the 10th postoperative day after the flap construction, there was an evident black eschar at the most distal regions in all of the flaps (Figs 2 and 3). Skin flap viability rates in the Groups 1, 2 and 3 were 62 ± 4.1 %, 83 ± 4.9 % and 85 ± 4.6 %, respectively (Fig. 6).

The rats in the Group 1 had significantly larger necrotic area and lower flap viability than in the Group 2 and 3, as shown in Figure 2 and 3 ($p < 0.05$). Histopathologic examination showed that necrotic flap regions contained muscle necrosis with an abundant neutrophil infiltration, and severe edema at the distal flap regions in the Group 1 (Fig. 4 A,B). The Group 2 and 3 had minimal edema without inflammatory reaction in the distal flap regions nearby to necrotic flap areas and the muscle necrosis was not detected (Fig. 5 A,B).

Flap tissue MPO activity (U/g wet tissue) of all groups is shown in the Table 1. The MPO activity in the distal of dorsal skin flaps was significantly higher in ovariectomy group compared to sham and control groups ($p < 0.05$). There was no difference between the sham and control groups ($p > 0.05$).

Discussion

The present study has shown that ovariectomy (as a result estrogen deficiency) could have a deleterious effect on skin flap in a rat model. In 1997, Ashcroft et al (28) demonstrated a link betwe-

Table 1. Myeloperoxidase values in groups.

	Ovariectomy group (Group 1) n=8		Sham group (Group 2) n=8		Control group (Group 3) n=8	
	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)	Mean±SD	Median (min-max)
MPO (U/ wet tissue)	16.96±3.18*	16.00 (13.60–21.40)	8.13±1.56	8.09 (6.12–11.20)	7.56±1.91	7.05 (5.60–10.80)

Data are presented as mean ± SD, median (min-max) (minimum-maximum) in Table 1. $p<0.05$ was considered to be statistically significant, * $p<0.05$ vs sham and control group.

en the menopause and delayed healing of acute wounds in elderly women, after that there has been a concerted effort to describe and explain the roles of estrogens in wound inflammation. Recently Toutain et al (7) demonstrated that estrogen has preventive effect on skin flap necrosis through a prevention of ischemic-induced skin lesions. Estrogens increase collagen content and skin thickness and improve skin moisture. Declining estrogen levels are associated with a variety of cutaneous changes, many of which can be reversed or improved by estrogen supplementation (29). Decreased estrogen shows itself with thinned and decreased elasticity of dermis. Estrogens have been shown to exert a systemic anti-ischemic effect (30).

Previous studies demonstrated that the protective effect of estrogen is not confined to skin (31). Estradiol treatment given immediately at the time of ovariectomy attenuated central and peripheral production of proinflammatory cytokines after ischemic stroke (32). Interestingly, the protective effect of estradiol has been shown to occur in male rats in a trauma-hemorrhage experimental model (33). Not only estrogen, but also its analogues have been shown to be effective in producing a systemic anti-ischemic effect (34). The protection afforded by selective estrogen receptor agonists has been shown to be the result of up-regulation of heat shock proteins (35). Heat shock proteins improve musculocutaneous flap survival as shown by Wang et al. (36). The observed enhanced skin viability in this study may partially be due to estrogens' effect on skin microcirculation. Stojanovic et al (17) showed that endogeneous estrogens increases postischemic hyperemia in the skin microcirculation. Sex steroid hormones are known to have significant contributions on the regulation of cutaneous repair processes (37). Physiological studies on estrogen and wound healing suggested that hormone replacement therapy might play a beneficial role in cutaneous injury repair. Also estrogen has antiapoptotic effect as shown in ischemic brain (38) and heart (26, 39). In several studies it has been shown that estrogen promoted skin survival by enhancing the anti-apoptotic Bcl-2 expression in keratinocytes (7, 40).

In the present study for the first time, to our knowledge, we demonstrated increased tissue MPO activity in distal skin flap of ovariectomized rats. The protective effect of endogenous estrogens has been shown by Cuzzocrea et al. (41). This protective feature has been explained by antioxidative effects of estradiol (41). Estrogens trigger nitric oxide bioavailability through activation of endothelial nitric oxide synthase activity (42) and reduce production of reactive oxygen species (43). As mentioned previously, neutrophils are the significant source of free radicals (20) and it is well established that MPO-derived oxidants damage cells and tissues (44). So, increased tissue MPO activity, which is a sign of neutrophil accumulation could lead to flap necrosis in the abscence of estrogen in our ovariectomy group. Similarly, Tyner et al (20), demonstrated a correlation between increased flap viability and a decrease in myeloperoxidase content in a rat model. They attributed the beneficial effects of propofol to the reduction in neutrophil activity within the flap (20).

In conclusion, our results showed that ovariectomy has unfavorable effects on skin flap viability in a rat model. There is a significant increase in tissue MPO levels, which is supported by abundant neutrophil infiltration in skin flaps of ovariectomized rats. The importance of endogenous estrogens is possibly reducing inflammatory reactions within the distal regions of skin flaps. Therefore, in combination with skin flap operations in natural or surgical menopausal women, prior estrogen replacement therapy may enhance the success of the operation. However, the lack of estrogen levels is a limitation of our study. Further studies that substantiate our results should be performed in ovariectomized models to show the protective effect of estrogen, which could be used to help healing of any incision on skin in women with estrogen deficiency.

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