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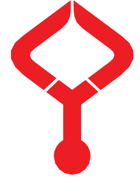

Research Article

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Investigation of the effect of sildenafil citrate on flap survival and SOD GPx antioxidant enzymes in random pattern skin flaps

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Abstract

In this study, the effect of sildenafil citrate, a phosphodiesterase type 5 (PDE 5) enzyme inhibitor, on the survival area of random pattern skin flaps and the effect of superoxide dismutase (SOD) and glutathione peoxidase (GPx) enzyme activities were investigated. Sildenafil citrate increases the level of cyclic guanosine monophosphate (cGMP) by inhibiting PDE 5. This causes dilation of the vessels and increased blood flow. In the study, 42 Wistar albino female rats weighing between 200-250 g were used. Rats were divided into 3 groups as sham group (S), experimental group (E) and control group (C). Subgroups were formed as day 1, day 4 and day 7 in groups E and C. In group E, 9 mg/kg/day sildenafil citrate was given intraperitoneally. Tissue samples were taken from the base, centre and the farthest living area of the flap of the sacrificed rats. SOD and GPx enzyme activity values were determined in the tissue samples. When SOD and GPx activities were compared between the groups, the difference was not significant ($p>0.05$). When the surviving flap areas were compared between the groups, an increase in necrotic area in the C7 group flaps was remarkable. However, there was no statistical difference between the groups in terms of flap survival area percentages ($p>0.05$).

Keywords: flap, sildenafil citrate, superoxide dismutase, glutathione peroxidase

1. Introduction

A flap refers to a section of tissue that can be repositioned from the donor area to the recipient area while maintaining its own blood supply. Flaps may be simple advances of skin and subcutaneous tissues or composite flaps consisting of any combination of skin, muscle, bone, fat, or fascia (1-5). The main reasons for flap necrosis are insufficient arterial blood flow and venous congestion, both of which decrease blood circulation through the flap (6). The flap elevation process disrupts the blood flow balance. Physical interruption of the internal flow vessels leads to acute ischemia of the peripheral parts of the flap (2). Increasing skin flap survival may depend on the preservation of the integrity of the circulatory system (7). Although blood flow continues in the basal part of the pedicled flap after flap elevation, blood flow at the tip of the flap decreases (8). When the length-to-width ratio of a flap surpasses 2:1, it can impair blood flow to the distal region. It results in extended ischemia, oxidative stress in tissues, and deficiencies in nutrition. These conditions increase the susceptibility to necrosis (9). The random pattern skin flap is susceptible to necrosis at its distal end because it lacks a defined arteriovenous system and blood supply, which restricts the size of the flap (10). Necrosis occurring in the distal regions of the flaps jeopardises flap use (11). In addition, reactive oxygen species (ROS) also trigger different cell death models in flap necrosis (12).

ROS consists of different types of reduced oxygen molecules, including superoxide anions, hydrogen peroxide, and hydroxyl radicals (13). ROS are produced by all cells (14). These reactive species originate from normal cellular processes and by-products of oxidative metabolism (15). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are essential antioxidant enzymes required to scavenge ROS in various cell compartments and to respond to stressful conditions (16). SOD is the enzyme that directly scavenges free radicals (17). SOD converts superoxide to oxygen (O_2) and hydrogen peroxide (H_2O_2) (18). The H_2O_2 component can easily cross cell membranes (19). Intracellular H_2O_2 concentration balance is important for cell viability and cell function. H_2O_2 is converted to H_2O and O_2 by GPx and CAT enzymes (20). GPx, as an important enzyme in the cell, converts H_2O_2 into water and lipid peroxides into alcohols (21). GPx protects cellular components from oxidative stress caused by ROS (22). This enzyme uses glutathione as a reducing agent (23).

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Sildenafil citrate is a PDE-5 enzyme inhibitor (24, 25). PDE-5 inhibitors often show strong vasodilatory properties (26). Sildenafil citrate promotes cyclic guanosine monophosphate accumulation, smooth muscle relaxation, and thus increased blood flow in target organs (27). Prevents thrombus formation and causes dilatation of arteries and veins (28). Flap necrosis can occur as a result of insufficient blood supply to the tissue, ischaemia-reperfusion injury, and inflammatory responses (29). In this study, the effect of sildenafil citrate application on the survival area of random patterned skin flaps and the effect of SOD and GPx enzyme activities were investigated.

2. Materials and Methods

All animals were obtained from Ondokuz Mayıs University Experimental Animals Application and Research Centre. Surgical procedures were also performed in this centre. The studies were carried out with permission numbered 2006/52, approved by the Ondokuz Mayıs University Animal Ethics Committee. In the experimental study, 42 Wistar albino female rats weighing between 200-250 g were used. Rats were kept at 22 ± 2 °C for 12 hours on a light/dark cycle. Rats were fed ad libitum with standard pellet rat chow and water.

2.1. Experimental groups

Rats were divided into 3 groups control group (C), experimental group (E), and sham group (S). Group E was subdivided into day 1 (E1), day 4 (E4) and day 7 (E7); and group C was subdivided into day 1 (C1), day 4 (C4) and day 7 (C7). A total of 42 rats were studied, six rats in each subgroup and S group. In group E, sildenafil citrate (Degra®) dissolved in saline was injected intraperitoneally at a dose of 9 mg/kg/day. In group E, a sildenafil dose was administered daily until the animals were sacrificed. Rats in group C were not administered any substance. In rats in groups E and C, flaps were sutured after elevation. Rats were sacrificed on day 1, day 4, and day 7, and tissue samples were taken. In the S group, rats were sacrificed after the flaps were sutured and tissue samples were taken.

2.2. Surgical Procedures

Surgical procedures were performed under sterile conditions and general anesthesia with adequate precautions to minimize pain or discomfort. General anesthesia was induced with 100 mg/kg Ketamine HCl (Ketalar®) intraperitoneally and 3 mg/kg Xylazine (Rompun®) intramuscularly. Rats were placed in the prone position, and all dorsal hair was shaved. Rats were sterilised with povidone-iodine before and after the surgical procedure. A 2.5x8 cm dorsal Mc Farlane (30) rat skin flap with a caudal pedicle was drawn with a skin pen. Following the drawing, the caudal pedicled flap was removed, including the panniculus carnosus, by taking the posterior iliac crest as the anatomical landmark (Fig. 1) and sutured with 4/0 silk.

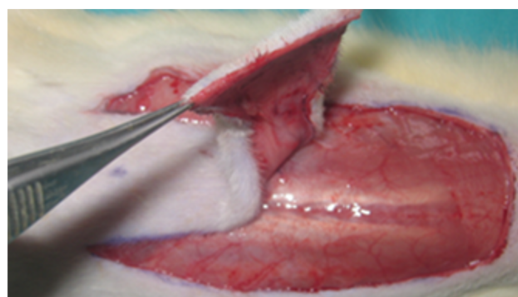

Fig. 1. Elevation of Mc Farlane rat skin flap with dorsal caudal pedicle

In the flaps of all groups, full-layer tissue samples were taken from the base, the middle, and the farthest living area of the flap, with the line assumed to pass through the midline as the center along the length of the flap after sacrifice. The samples were placed in labeled Ependorf tubes containing 0.25 M sucrose and stored in a -80ºC deep freezer.

2.3. Preparation of Tissues for Biochemical Analysis

Tissue samples were removed from -80 ºC and thawed at room temperature. Their weights were determined by weighing on a precision balance. 0.25 M sucrose solution (10 mg/ml) was added to the tissue samples taken in Eppendorf. Homogenisation + sonication was performed 6 times as a 20 s process - 10 s wait in ice medium. 15000 rpm 15 min. centrifugation was performed at + 4 ºC. Enzyme activity measurements were performed in the supernatants obtained after centrifugation.

2.4. SOD and GPx Activity Determination

SOD activity was determined using the methods of Mc Cord and Fridovich (31) and Flohe and Otting (32). A sample supernatant was added to the reaction solution containing xanthine and cytochrome c. The reaction was initiated by the addition of xanthine oxidase solution. The absorbance change was read at 550 nm after rapid stirring. Lawrence and Burk's method (33) was used to determine GPx activity. The reaction mixture and sample supernatant were mixed and incubated at 37 ºC for 5 min. Then H_2O_2 was added to the solution and absorbance change was read at 340 nm.

2.5. Calculation of flap survival areas

Digital photographs of the flaps were taken after suturing and before sacrifice in all groups. The living and necrosis areas of the flaps were calculated with the Image Tool programme. Measurement calibration was made on the ruler in the image. The total flap area was measured to include both living and necrotic areas. The necrotic tissue area was specifically measured, and the living tissue area was determined by subtracting the necrotic portion from the total flap area.

2.6. Statistical Analysis

For each animal, statistical analyses were performed by averaging the enzyme activity values obtained from tissue samples taken from the base, middle, and farthest living area of the flap. Statistical data were analysed with SPSS 14 software. Data were evaluated by ANOVA both in terms of

groups and days. The significance level was accepted as $p< 0.05$.

3. Results

When the antioxidant activities of the random pattern flaps were analysed, SOD activity was lower in the S group than in the E group and C group. However, the difference between the groups was not statistically significant ($p>0.05$). In group C, SOD activity was at its peak in group C1, decreased rapidly in group C4 and increased slightly in group C7. When SOD activity was compared between E1, E4, E7 groups and between C1, C4, C7 groups, the difference was not significant ($p>0.05$). GPx activity was measured as high in the S group compared to the E group and low compared to the C group. However, the difference between the groups was not statistically significant ($p>0.05$). In group E, GPx activity was below group C values on all days. When GPx activity was compared between groups E1, E4, and E7 and between groups C1, C4, and C7, the difference was also not significant ($p>0.05$) (Fig. 2).

When the living area measurements of the random pattern flaps were analysed, the living area was evaluated as 100% in the sham group because a digital photograph was taken immediately after the flap was lifted and sutured. In group E, which received intraperitoneal sildenafil citrate at a dose of 9 mg/kg/day, E1 was 92.8%, E4 85.9%, and E7 81.6%. In group C, C1 was 88.8%, C4 78.2%, C7 69.6%. (Fig. 3,4). Surviving flap area ratios of the E group were higher than those of the control group. This difference was especially evident in the E4 and E7 groups. Especially in the C7 group flaps, the increase in necrotic area was remarkable. However, there was no statistically significant difference between the E and C groups in terms of the percentages of survival flap area ($p>0.05$) (Fig. 5).

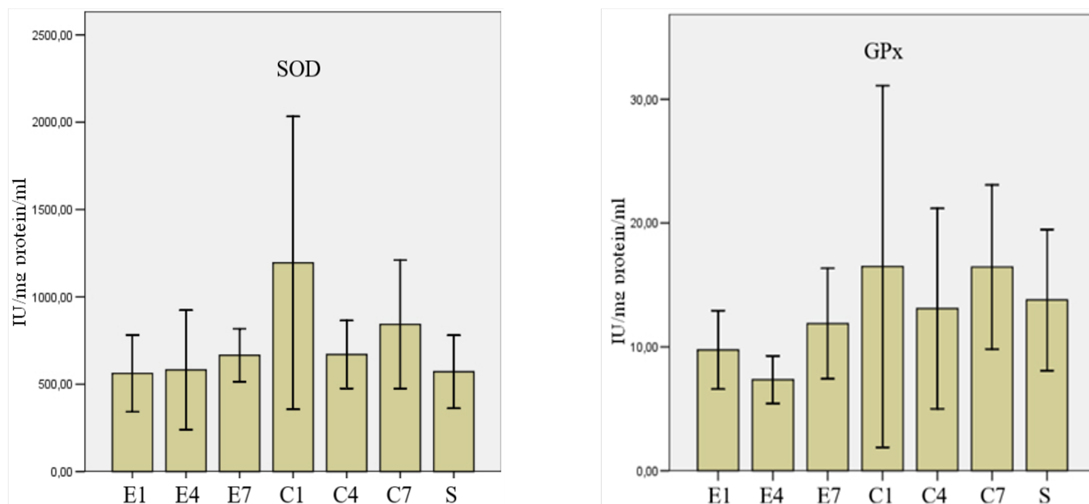

Fig. 2. The difference between SOD and GPx enzyme activity values in E, C and S groups was not statistically significant ($p>0.05$)

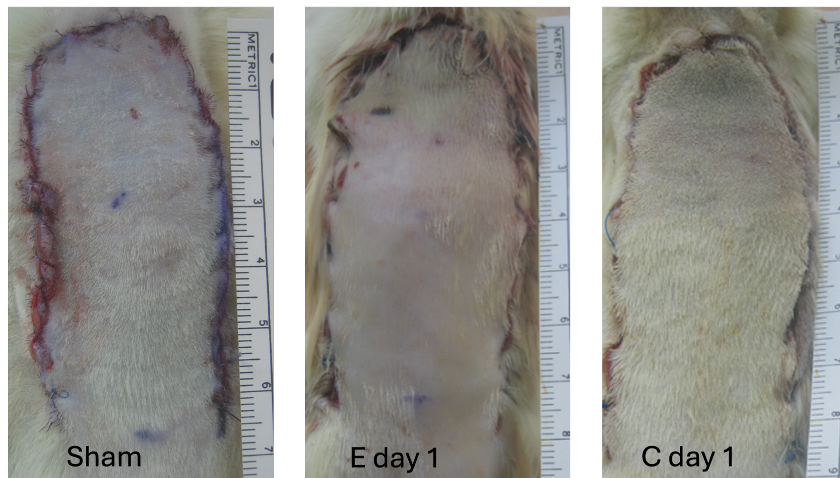

Fig. 3. Appearance of flaps in the S, E and C groups

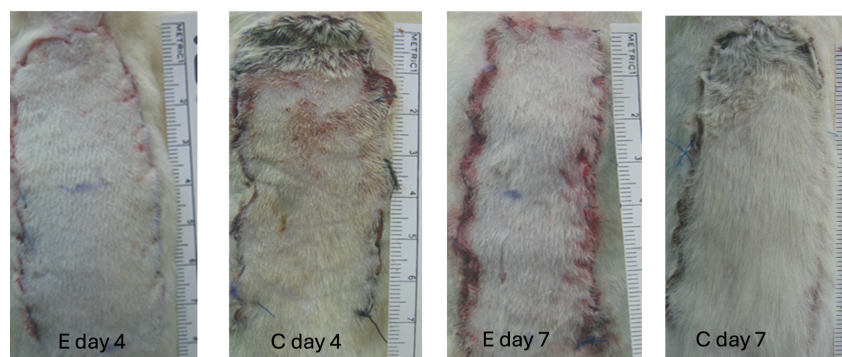

Fig. 4. Significant necrotic areas are seen in group C flaps

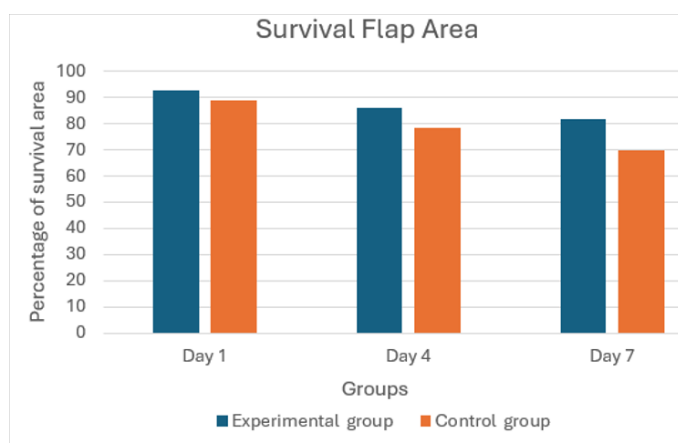

Fig. 5. Percentage of survival flap area in groups E and C (p>0.05)

4. Discussion

A flap is a tissue unit that can be transferred from donor to recipient sites and maintains blood supply during the procedure (27). It is widely used because flap elevation and transfer is simple and the colour is compatible with the tissues of the recipient site (34). These flaps are widely used for wound closure. Since the flap length is limited, flap viability is closely related to the aspect ratio (35). Complete or partial loss of tissue may be observed after flap application (36). Various pharmacological agents have been used to increase flap viability and prevent ischaemia. Sympatholytics, vasodilators, calcium channel blockers, antihemorrhagic agents, prostaglandin inhibitors, anticoagulants, glucocorticoids, and free oxygen radical inhibitors have been tried, and successful results have been obtained to varying degrees (8, 28, 36).

After flap elevation, there is a significant increase in superoxide radicals due to anaerobic metabolism conversion (2). When the length-to-width ratio of a flap exceeds 2:1, distal perfusion is compromised, leading to prolonged ischaemia. Tissue oxidative stress and nutritional deficiencies increase susceptibility to necrosis (9). SOD is the primary scavenger of free radicals and can catalyse the rapid conversion of superoxide to hydrogen peroxide (11,13). GPx can reduce hydrogen peroxide to water (23).

Sarıfakioğlu et al. (35) reported that sildenafil citrate had an effect on the survival of flaps and 10 mg/kg dose (20mg/kg/day) given orally twice was as effective as 9mg/kg/day dose given intraperitoneally. Ayyıldız et al. (28) reported that locally applied sildenafil citrate increased flap viability and the most effective dose range was between 0.3 and 0.5 mg/kg. Ulusoy et al. (36) used sildenafil citrate in combination with fibrin glue to increase the flap survival rate. They reported that topical application of sildenafil favourably contributed to the survival of random patterned skin flaps.

Hart et al. (37) reported a significant decrease in flap necrosis and oedema on day 1 and day 3 in 3 × 10 cm flaps treated with 9 mg/kg intraperitoneal sildenafil daily. Similar to our study, 9 mg/kg intraperitoneal sildenafil citrate was given to the animals. In our study, no significant difference was found between the groups in flap surviving areas. Hart et al. (37) worked with 3x10 cm sized flaps in their study. In our study, the flap size was 2.5x8 cm. These results suggest that flap size is also an important factor in flap viability. In our study, antioxidant enzyme activities in flap tissue were also investigated.

Serin et al. (38), in their study, rats in the sildenafil group received daily subcutaneous injections of sildenafil for seven days before the 9x3 cm dorsal skin flap was removed. Similar to our study, they did not administer any substance to the control group. They reported that sildenafil sitrate provided a significant increase in the flap living area compared to the C group. Baykan et al.(39) evaluated the effect of sildenafil citrate on the viability of skin exposed to nicotine-induced ischaemia in rats. They formed 7 x 3 cm McFarlane flaps and applied 20 mg/kg/day sildenafil citrate subdermally for 7 days and reported that a significant improvement was observed in the skin vitality of the group. Kaya et al. (40), in their study with 3x9 cm flaps, gave saline to the control group 2 hours before flap removal and for 2 days after the operation. The sildenafil, tadalafil, and vardenafil groups received the respective medication. They indicated that although the flap necrosis area was lower in these groups compared to group C, there was no significant difference. Barral et al. (41) subdermally administered 0.5 mg/kg dosage and 5 ml/kg volume of sildenafil, citrate in the experimental group in 3x7 cm flaps in Wistar rats. In the control group, 0.9% saline

solution was applied subdermal. Macroscopically, there was no significant difference in the percentage of necrosis, ischaemia, and tissue viability areas when both groups were compared.

Hafez and El-Kazazaz (42) divided rats into 3 groups in their study. The groups received 0.5 ml 0.9% NaCl, 5 mg/kg, and 10 mg/kg sildanafil citrate intraperitoneally. In the 10 mg/kg sildenafil citrate treated group, hippocampal SOD concentration was significantly decreased compared to the other groups In our study, 9 mg/kg/day sildenafil citrate administration did not cause a significant difference between the groups in terms of SOD activities in skin tissue.

In conclusion, the application of sildenafil citrate has been investigated in various experimental designs to enhance the viability of random pattern flaps and to prevent necrosis or ischemia. We believe that the effect of sildenafil citrate application on flap viability varies depending on the daily dosage of sildenafil administered, the method of application, and the size of the flap.

Conflict of interest

The authors declared no conflict of interest.

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Authors' contributions

Concept: R.D., Design: R.D., E.D., Data Collection or Processing: R.D., E.D., Analysis or Interpretation: R.D., E.D., Literature Search: R.D., E.D., Writing: R.D.

Ethical statement

The studies were carried out with permission numbered 2006/52, approved by the Ondokuz Mayıs University Animal Ethics Committee.

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