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Effect of Topically Applied Minoxidil on the Survival of Rat Dorsal Skin Flap

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Abstract
Background Flap necrosis still is a challenging problem in reconstructive surgery that results in irreversible tissue loss. This study evaluated the effect of topically applied minoxidil on angiogenesis and survival of a caudally based dorsal rat skin flap.
Methods For this study, 24 male Wistar rats were randomly divided into three groups of eight each. A caudally based dorsal skin flap with the dimensions of 9 × 3 cm was raised. After elevation of the flaps, they were sutured back into their initial positions. In group 1 (control group), 1 ml of isotonic saline was applied topically to the flaps of all the animals for 14 days. In group 2, minoxidil solution was spread uniformly over the flap surface for 7 days after the flap elevation. In group 3, minoxidil solution was applied topically to the flap surface during a 14-day period. On day 7 after the flap elevation, the rats were killed. The average area of flap survival was determined for each rat. Subdermal vascular architecture and angiogenesis were evaluated under a light microscope after two full-thickness skin biopsy specimens had been obtained from the midline of the flaps.
Results The lowest flap survival rate was observed in group 1, and no difference was observed between groups 1 and 2. Compared with groups 1 and 2, group 3 had a significantly increased percentage of flap survival ($P < 0.05$). Intense and moderate angiogenesis also was observed respectively at the proximal and distal areas of the flaps in group 3.
Conclusions The results of this experiment seem to show that the early effect of minoxidil is vasodilation and that prolonged use before flap elevation leads to angiogenesis, increasing flap viability.
Level of Evidence III This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Angiogenesis · Flap survival · Minoxidil · Topical application

Flap necrosis still is a challenging problem in reconstructive surgery, resulting in irreversible tissue loss, which leads to undesirable results in a planned surgical intervention. Many factors are related to flap necrosis, involving some local and systemic causes, but in most cases, the main reason is arterial insufficiency, venous congestion, or both.

Although many surgical methods and pharmacologic agents have been described to increase cutaneous flap viability, those capable of increasing flap blood perfusion may be considered more effective and useful in daily clinical practice. Currently, surgical methods continue to be the most preferred procedures for increasing flap blood perfusion and viability compared with pharmacologic agents, which have different action mechanisms. Because all surgical procedures have some disadvantages related to surgery itself, researchers have focused on pharmacologic agents to find an easily administered substance that has high therapeutic action, reproducible results, a well-known mechanism of action, low cost, and suitability for clinical use at the same time [1].

N. Gümüş (✉) · Y. Ödemiş · S. Yılmaz · E. Tuncer
Department of Plastic, Reconstructive, and Aesthetic Surgery, Cumhuriyet University Medical Faculty, Sivas, Turkey
e-mail: gumus1970@hotmail.com

Minoxidil (3-oxido-2.4-diamino-6-piperidinopyrimidine) is a vasoactive agent known as a strong peripheral vasodilator drug. It causes dilation of arterioles by means of opening the potassium channels [2]. Topically, it is used widely for the treatment of baldness, and it also is used orally for the treatment of hypertension.

In this study, we aimed to investigate whether the vasodilator effect of minoxidil leads to angiogenesis in the rat dorsal flap and whether it is effective in improving flap survival.

Materials and Methods

This study used 24 male Wistar rats, 110–120 days old, weighing between 270 and 300 g. The animals were randomly divided into three groups of eight each. This experiment was approved by the Ethical Committee of the University for Animal Researches. The rats were housed in individual cages and fed standard rat chow and water ad libitum at completion of the experiments.

After the rats had been anesthetized with intramuscular injections of ketamine 10 mg/kg and subcutaneous injections of xylazine hydrochloride 3 mg/kg, the dorsal skin was shaved using an electric clipper. A supplemental anesthetic dose was given if needed during the experiment.

A caudally based dorsal skin flap consisting of skin and panniculus carnosus with dimensions of 9 × 3 cm was raised. This flap design was a modified version of the McFarlane flap model described by Khouri et al. [3, 4]. After their elevation, the flaps were sutured back into their initial positions.

In the group 1 (control group), 1 ml of isotonic saline was applied topically twice a day to the flap for 14 days. Application to the previously marked areas of the flap surface was started 7 days before flap elevation and continued until postoperative day 7. In group 2, 20 mg of 5 % minoxidil solution was spread uniformly over the flap surface twice a day for 7 days after the flap elevation.

In group 3, 20 mg of 5 % minoxidil solution was applied topically to the flap surface twice a day for 14-days, starting 7 days before flap elevation and continuing 7 days afterward (Fig. 1). After the minoxidil solution had been sprayed over the flap surface via its commercial applicator, reproducible distribution of the minoxidil solution on the flaps was provided by spreading it with fingertip massages.

On day 7 after flap elevation, the rats were killed using an intraperitoneal injection of sodium thiopental (150 mg/kg). The total flap area and necrotic regions then were marked on clear acetate templates. After these had been electronically scanned, the average area of flap survival was determined for each rat. For the histologic assessments, two full-thickness skin biopsy specimens, 0.5 × 0.5 cm in size, were obtained from the midline of the flaps 1 cm away from the proximal side and 1 cm proximal to necrotic area on the distal side.

Biopsy samples were fixed in 10 % formaldehyde solution for 24 h, embedded in paraffin, sectioned, and stained with routine hematoxylin and eosin (H&E) stain. The effects of the minoxidil on the subdermal vascular architecture and angiogenesis were evaluated under a light microscope.

The slides were examined in a blind fashion by a pathologist who was unaware of the groups. Angiogenesis (newly formed capillaries) was evaluated semiquantitatively, so intensity of neovascularization was scored in areas that had the highest neovascularization using ×200 magnification. The scores were 0 (no angiogenesis), 1 (minimal angiogenesis), 2 (moderate angiogenesis), and 3 (intense angiogenesis) (Table 1).

For the statistical analysis, a P value lower than 0.05 was regarded as indicating statistical significance, and data were expressed as the mean ± standard deviation. The SPSS 16.0 software package (SPSS, Chicago, IL, USA) for Windows was used for the statistical analyses of the data. The analysis of variance (ANOVA) (post hoc Tukey) test was used to detect differences between the groups.

Results

All the animals survived throughout the study, and no sign of infection or hematoma was observed in the flaps. No systemic

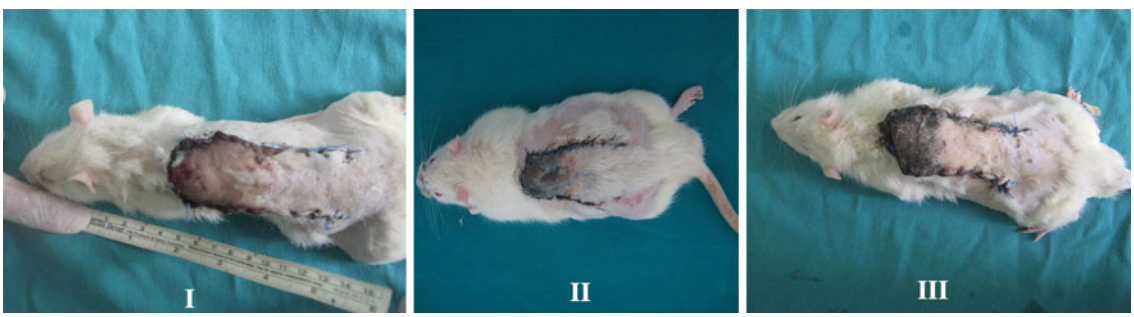

Fig. 1 Appearance of viable and necrotic skin regions in rat dorsal skin flaps on day 7 in groups 1, 2, and 3

Table 1 Angiogenesis scores in the experimental groups

Angiogenesis site of the flap	Group 1	Group 2	Group 3
Proximal zone	1	1	3
Distal zone	0	1	2

Scoring was evaluated as 0 (no angiogenesis), 1 (minimal angiogenesis), 2 (moderate angiogenesis), 3 (intense angiogenesis)

Table 2 Survival areas of the flaps in the three groups area of survival (%)

Rat no.	Group 1	Group 2	Group 3
1	57	60	76
2	61	67	68
3	58	59	70
4	54	61	75
5	62	65	69
6	65	68	72
7	63	63	69
8	59	66	77
Mean	59.8 ± 3.56	63.6 ± 3.37	72.3 ± 3.33[a]

[a] Significant difference versus groups 1 and 2 ($P < 0.05$)

reaction to the vasodilating agent appeared in the animals. The mean ± standard deviation percentages of surviving flap areas were determined for each group (Table 2).

In group 1 (control group), the average survival area was 59.8 ± 3.56 %. In group 2, the percentage of flap survival was 63.6 ± 3.37 %, and in group 3, this percentage was 72.3 ± 3.33. The lowest flap survival rate was observed in group 1, which was statistically different from group 3. Groups 1 and 2 showed no significant difference (Fig. 2). Compared with groups 1 and 2, group 3 had a significantly increased percentage of flap survival ($P < 0.05$).

After the histologic assessment, minimal angiogenesis appeared in the proximal area of the flaps in the control group, which was scored as 1. No angiogenesis was evident in the distal area, which was scored as 0. Minimal angiogenesis also was found in group 2, which was scored as 1 for both sides. Intense angiogenesis was observed in the proximal area of the flaps in group 3, which was scored as 3, and moderate angiogenesis appear in the distal area, which was scored as 2 (Figs. 2, 3, 4, and 5).

Discussion

Minoxidil has been used as an antihypertensive agent, causing direct vasodilation of vascular smooth muscles induced by the active metabolite, minoxidil sulfate ($MxSO_4$). It acts as a K+ channel agonist to enhance K+ permeability, leading to relaxation in smooth muscle [2, 5].

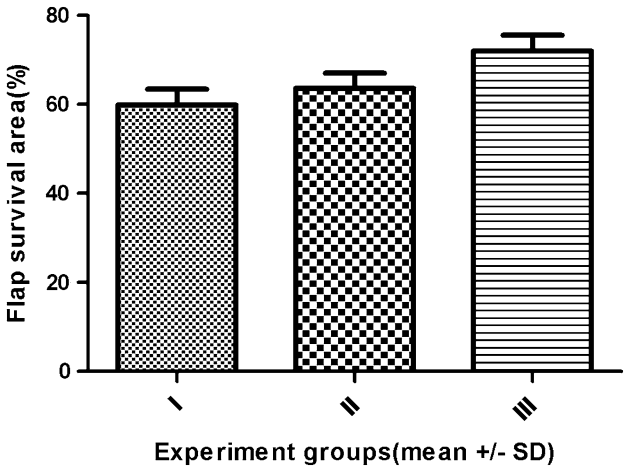

Fig. 2 Percentages of surviving areas among the three groups

In addition to its antihypertensive properties, minoxidil also has a specific effect on hair follicles, stimulating hair growth [6]. The mechanism by which it affects hair growth remains undetermined. However, it currently is widely used topically for its hair-growth-promoting effects in the treatment of androgenic alopecia.

Minoxidil, as a potent vasodilator, causes significant local cutaneous vasodilation, so it has been used clinically to increase local blood flow. In one study, the acute effect of topical minoxidil on digital blood flow was investigated in patients with Raynaud's phenomenon. A single application of topical minoxidil 5 % solution to the subject's fingers on two separate occasions was done, and measurements of digital skin temperature, systolic blood pressure, and laser Doppler flow were obtained. The results showed that minoxidil was not effective in improving digital blood flow [7].

Another study investigating human balding scalps found that minoxidil stimulated cutaneous blood flow measured by laser Doppler velocimetry and photopulse plethysmography [8]. On two consecutive days, a 0.25-ml volume of minoxidil was spread uniformly over a 100-cm^2 area of the bald scalp, with cutaneous blood flow recorded for the following 4 h. The increase in blood flow occurred within 15 min after application of the 5 % solution of minoxidil and was maintained at least through 1 h.

Pavlovitch et al. [9] presented two cases with angiogenetic lesions of the scalp that developed 2 and 3 months after the topical application of minoxidil, suggesting that it induced angiogenesis in long-term use. It seems that the effect of minoxidil on cutaneous blood flow and angiogenesis depends on application doses, concentration, and duration so that its single application, especially at a concentration lower than 5 %, and short-term use are not sufficient to show its effect on cutaneous vascular structures.

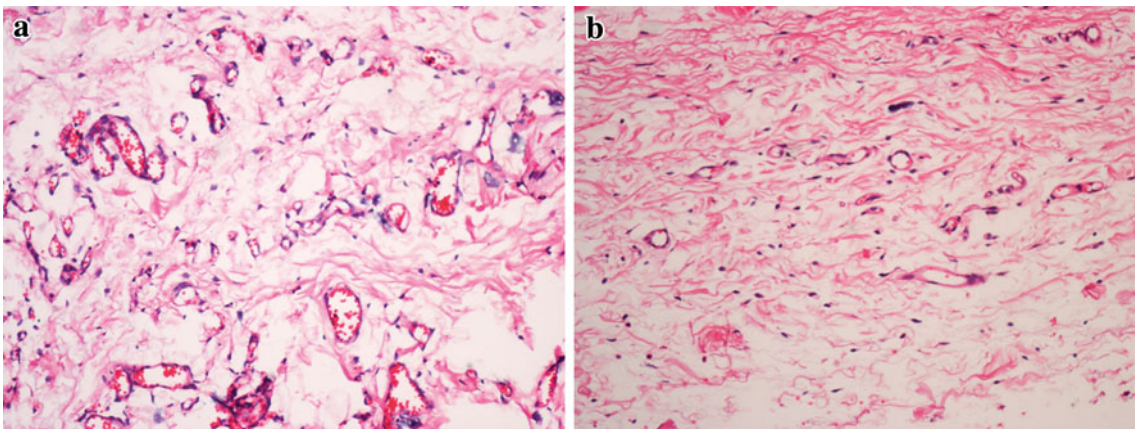

Fig. 3 **a** Minimal angiogenesis in the proximal zone of the flaps in group 1, which was scored as 1 (H&E, ×200). **b** Appearance of vascularity in the distal site of the flaps in group 1, which was scored as 0 (H&E, ×200). Note that no increase in vascularization occurred

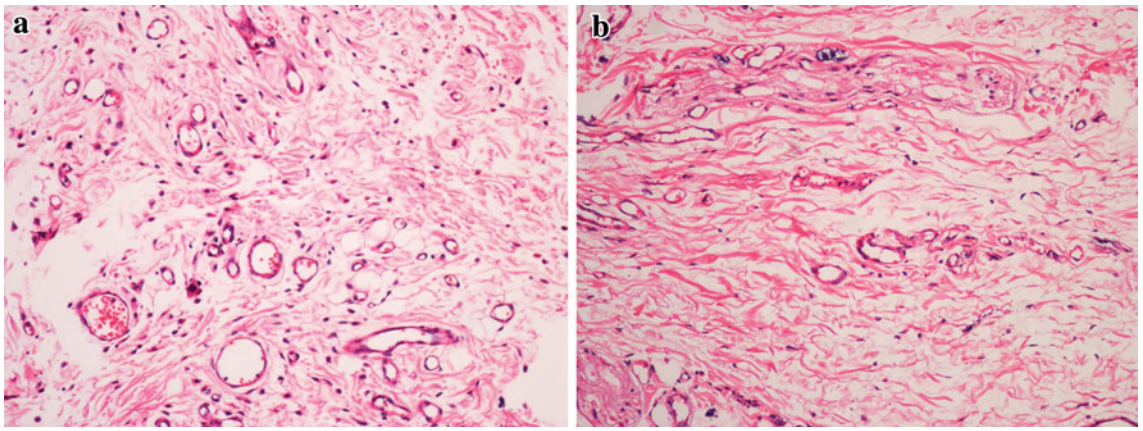

Fig. 4 Minimal angiogenesis in the proximal (**a**) and distal (**b**) sites of the flaps in group 2, which was scored as 1 for both sides (H&E, ×200)

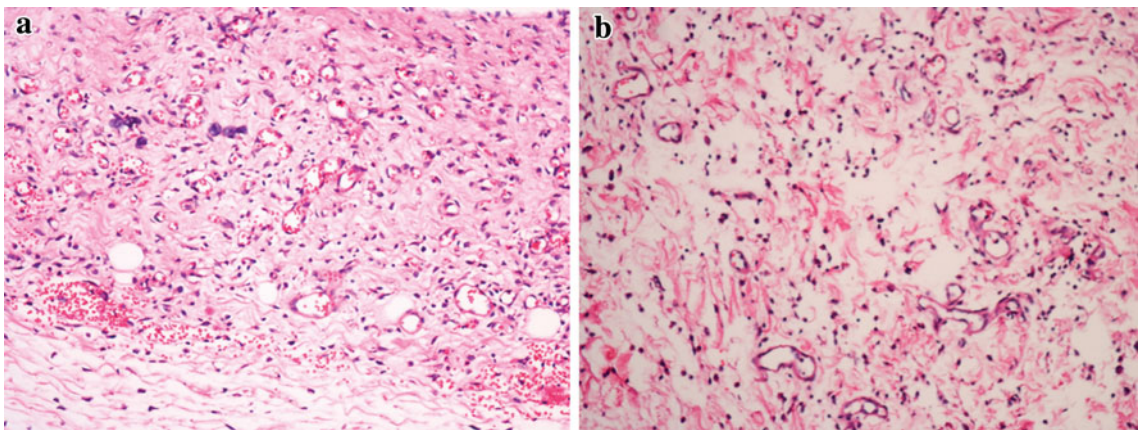

Fig. 5 **a** Intense vascularity in the proximal part of the flaps in group 3, which was scored as 3 (H&E, ×200). **b** Moderate vascularity was scored as 2 in the distal part of the flaps in group 3 (H&E, ×200)

In our study, a 5 % concentration of minoxidil twice a day during a 7- or 14-day period was used to show its effect on cutaneous blood perfusion and flap viabilty. This duration also was thought sufficient to allow angiogenesis, being capable of increasing the flap viabilty.

Smith and Dolan [10] studied three vasoactive topical agents for their ability to improve the survival of random skin flaps in rats. After the application of prostaglandin E2, minoxidil, or nitroglycerin to rat dorsal flaps, their effects on the viability of the flaps were investigated in 35 adult

Sprague-Dawley rats. The average areas of flap survival in the nitroglycerin and minoxidil groups were not found to be significantly different from those of the control group [10]. In contrast to our study, this experiment had minoxidil 2 % applied topically to the flaps during a 72 h period after flap elevation. However, our outcomes obtained from group 2 were similar to their findings.

It seems that minoxidil is not efficient in increasing the flap survival area if it is applied to the flap surface after flap elevation. Although its vasodilator effect results in significant local cutaneous vasodilation after the application, this seems not to be permenant, having a temporary effect on vascular muscles. Histologic assessments in group 2 proved that minoxidil stimulated a little more angiogenesis in the distal flap than in the control group, showing insufficient neoangiogenesis to increase flap survival and suggesting that the effect of minoxidil in this use was transient for cutaneous vascular structures.

In an experimental study, rat ventral cutaneous flaps were raised to evaluate the influence of minoxidil on ischemic flap necrosis prevention. In the experiment group, 50 mg/kg/day of minoxidil sulfate was administered by an orogastric tube once a day. This was initiated 24 h before the surgery and continued until postoperative day 7. Although laser fluxometry showed an increased blood flow at some points of the flaps, necrotic areas did not differ significantly between the experiment and control groups [11]. Our results in group 2 were similar to the findings of this study, suggesting that the vasodilator effect of minoxidil was temporary when used after the flap elevation.

When minoxidil application was started 7 days before flap elevation and continued until postoperative day 7 in group 3, flap viability increased significantly compared with the results in groups 1 and 2. Also in the histologic evaluation, significant angiogenesis scored as 3 was found in the subcutaneous tissue, possibly leading to an increase in flap blood perfusion and viability. Increased angiogenesis can make its effect on flap blood perfusion permanent, so the application should be long enough to allow angiogenesis to provide better flap survival in this model. It seems that the early effect of minoxidil after the cutaneous application is vasodilation and that with prolonged use before flap elevation, it leads to angiogenesis capable of increasing flap viability.

In this experimental study, we did not observe any systemic reaction to minoxidil. However, it is known to have some systemic effects as a vasodilator agent. Although topical use of minoxidil is considered sufficiently safe, it may cross biologic barriers and accumulate in lipids. Therefore, its concentrations in some tissues such as the brain may be significantly higher than its concentrations in plasma [12, 13]. Its chronic use on the skin may cause hypotension and tachycardia, suggesting that when topically applied, minoxidil is capable of rising to pharmacologically active concentrations in the blood [13, 14].

The results of this experiment showed that minoxidil, as an effective vasoactive agent, not only causes vasodilation but also stimulates angiogenesis in rat cutaneous flaps. However it requires sufficient time and a suitable dose to act as an angiogenetic factor for increasing flap vascularity and viability before flap elevation.

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