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Effect of Topical Nitroglycerin on the Survival of Random Pattern Vascular Skin Flaps in a Rat Model: Pilot Study

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Abstract

Background: The use of flaps is an important procedure in Plastic Surgery for the coverage of traumatic wounds, oncological resections, and burn reconstruction, among others. Drugs such as vasodilators (isoxsuprine, nitroglycerin), could increase tissue perfusion to optimize flap viability. In this study, the local and systemic effects of the use of topical nitroglycerin to increase the survival area of skin flaps were analyzed in a murine model. **Methods:** In our pilot study, we included twenty male Wistar rats, which underwent random pattern vascular flap formation surgery. Groups were designated as control (vaseline), 12-h local nitroglycerin, 24-h local nitroglycerin, and 12-h systemic nitroglycerin. The results were recorded with digitalized photographs of the flap to obtain the viability, necrotic, and total area measured after ten days of surgery. **Results:** The group of animals that received local nitroglycerin for 24-h had significantly higher survival and viability compared to the control group (P = 0.008). We did not observe differences in flap viability between the 12-h local nitroglycerin group and the control group, however, in the group with systemic nitroglycerin we observed a trend in reduction of viability. **Conclusion:** We demonstrate the increased viability of the random vascular pattern skin flap with the application of topical nitroglycerin. However, this beneficial effect was only observed in the schedule of local applications every 24 hours.

Subject Areas

Dermatology

Keywords

Flap Survival, Nitroglycerin, Topical Nitroglycerin, Skin Flaps, Necrosis

1. Introduction

Random pattern skin flaps (RPSF) are an important procedure in plastic surgery to cover various defects in several acquired or congenital defects situations such as traumatic wounds, oncological resections, ulcers, surgical excision, burn reconstruction, or malformations [1]. The irrigation of RPSFs does not depend on direct blood vessels but rather on a microvascular plexus that supplies their metabolic needs [2]. Depending on the area and also the characteristic of the tissue is the kind of flap that is suggested [3]. Unfortunately, their susceptibility to ischemic necrosis (often distal) is difficult to predict due to variations in their design and the intrinsic vascularity of their tissues, despite using the best techniques and novel flap designs [4] [5]. In addition, it has been shown that due to the uncertain vascularity of this type of flaps, vascular supply decreases in its distal part due to venous congestion after the elevation of the flap [6]. Although some other experts suggest that stretching the cell in the flap and changing its shape improves the process of mitosis [7].

Vasoactive substances produced by endothelial cells are important in controlling blood flow to the tissue since endothelium-mediated nitric oxide (NO) is relaxation-dependent factor and plays an important role in the physiology of microcirculation [8]. NO synthase produced by the endothelium helps maintain blood pressure and blood flow by acting on vascular smooth muscle, promoting angiogenesis and survival of the ischemic flap [9].

Many pharmacological agents have been used to prevent or reverse skin flap ischemia without good efficacy [10]. Thus, nowadays the ideal agent for flap rescue still does not exist. Recently, vasodilators such as nitroglycerin [11] or sildenafil [1] showed improvement of flap survival. Furthermore, pro-angiogenic compounds such as pravastatin [4] or even antihypertensives [12] have been tested to improve blood perfusion locally to increase flap survival [13].

Nowadays, nitroglycerin is used as a powerful topical vasodilator that increases local blood flow through vein and artery dilation without altering pre and post-capillary resistance [14]. It has been assumed that nitroglycerin will postoperatively help improve skin flap survival by increasing local blood flow [8]. Although its mechanism of action is not fully understood, topical nitroglycerin (glyceryl trinitrate) is an attractive agent for improving flap survival for several reasons, such as its anti-thrombotic effect and enhancement of vasculature width, which reduce the necrotic effects [15]. Also, nitroglycerin releases NO into the vascular smooth muscle cells, releasing cyclic guanosine monophosphate (cGMP), causing relaxation of these muscle cells and vasodilatation [16].

The potential use of topical nitroglycerin anti-ischemic therapy offers numerous

advantages over conventional oral or parenteral therapy, such as continuous administration despite postoperative nausea, oral intake restrictions, or patient discharge, making it ideal for postoperative outpatient use [17]. The first *in vivo* studies performed comparing and analyzing nitroglycerin in flaps were carried out in murine [18] and pigs models [19]. However, in those studies, no improvement in flap survival was observed when applying topical nitroglycerin pre and postoperatively. Moreover, previous studies have shown that the pre-conditioning effect of spermine/nitric oxide on flaps depends on both the selected dose and the type of application [14]. Also, nitroglycerin enhances the blood perfusion in a dose-dependent manner in patients with severe heart failure [20].

Since the effect of nitroglycerin as a vasodilator is short-lived (less than 24 hours) an efficient dosage to protect the full-thickness skin grafts is difficult to achieve [8]. Appropriate doses and ranges are crucial for patient safety when applying topical nitroglycerin and the comprehensive monitoring of the patient after the administration of this agent is essential [21]. Although the effectiveness of nitroglycerin has been demonstrated for compromised flaps [15], its mechanism of action is not well defined. Therefore, there is no established treatment guide for clinical application. In this sense, the use of topical nitroglycerin has proven to have the potential to improve flap survival, however, its use has side effects such as dizziness and hypotension [21]. Due to its vasodilator and antithrombotic effect, it has been shown that nitroglycerin helps to preserve the survival of skin flaps; however, it could cause an adverse effect on the studied site with high doses of application. Therefore, it is necessary to define a dosage protocol to measure the effectiveness to increase the viability of the flap and define the presentation of side or adverse effects depending on the applied dose.

In this work, we aim to demonstrate a first approach to the dosage of topical nitroglycerin, testing the frequency and region of application with a defined concentration and measuring its impact on the viability of the flap.

2. Methods

This prospective, analytical, experimental, and cross-sectional study evaluated the effect of flap survival with topical nitroglycerin administered in different schemes. Our study was approved by our institutional Ethics Committee in a regional hospital with the decision reference number CP19 - 00005 and complied with the ethical principles established in the World Medical Association Statement on the use of research animals.

2.1. Murine Model Experimental Design

Twenty male Wistar rats of approximately 250 grams of weight were included. The animals were randomized into four groups with five animals each that were treated as follows: Group 1 received post-operative application of vaseline directly to the flap every 12 hours (control group); Group 2 received post-operative application of 5 cm (30 mg) of 2% nitroglycerin ointment (Nitro-BID®) every 12

hours on the flap (systemic and local effect); Group 3 received a postoperative application of 5 cm (30 mg) of 2% nitroglycerin ointment(Nitro-BID®) every 24 hours on the flap (local effect); Group 4 received postoperative application of 5 cm (30 mg) of 2% nitroglycerin ointment(Nitro-BID®) every 12 hours outside the flap (systemic effect).

2.2. Surgical Procedure for Flap Formation

The general objective was to evaluate the effect of nitroglycerin ointment on the survival of skin flaps with a random vascular pattern. For this, we determined nitroglycerin ointment's local and systemic effect on flap survivaland defined the percentage of necrotic skin in each group.

For the flap formation surgery, ketamine 40 mg/kg and xylazine 1 - 3 mg/kg, were applied in a single dose intraperitoneally to provide preoperative anesthesia. Moreover, tramadol 1 mg/kg every 12 hours subcutaneously and meloxicam 1 mg/kg every 12 hours subcutaneously were administered for preoperative analgesia Subsequently, the back was shaved, and a flap measuring 8 × 1 cm (**Figure 1(a)** and **Figure 1(b)**) was made with the cranial base composed of skin and subcutaneous tissue. Once the flaps were dissected, they were immediately sutured to their original position with a continuous 4-0 nylon suture. After performing the skin flaps, the established analgesia management was continued (tramadol 1 mg/kg every 12 hours and meloxicam 1 mg/kg every 12 hours).If any indicator of pain was detected (release of porphyrins, shaggy hair, apathy, anorexia, dehydration, and wound inflammation), a single dose of meloxicam 2 mg/kg was applied subcutaneously as an analgesic agent.Antibiotics were not used as a prophylactic measure.

2.3. Flap Follow–up and Evaluation of the Viability

Nitroglycerintreatment was administered during ten days in all the groups with

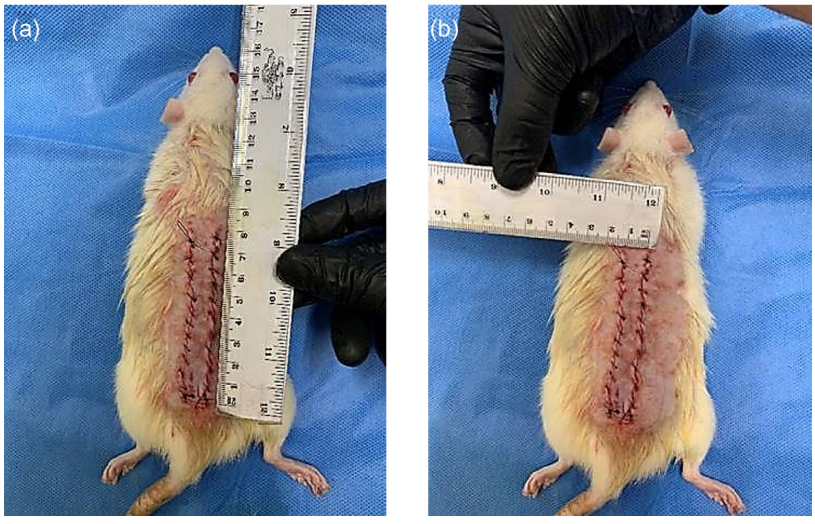


Figure 1. Surgical intervention to generate the random vascular pattern flap measuring 8 cm long by 1 cm wide.

the previously established frequency. During this period signs of pain and stress were checked in the mice and at the end of the tenth day, they were euthanized by an overdose of sodium pentobarbital (120 mg/kg) to proceed to the final analysis.

For viability evaluation, measurements were taken with a vernier instrument to distinguish necrotic areas (hardening, color change, epidermolysis) in the flap total area. In addition, photographs were taken and digitized to perform a detailed analysis of the number of pixels and areas corresponding to the viable and necrotic areas using Adobe Photoshop image software.

2.4. Statistical Analysis

In our study, we evaluated and compared the number of pixels (area) of the mice with the different treatments. To make these comparisons, we used the Mann-Whitney U test by obtaining a nonparametric distribution in our measurements across all groups.

3. Results

To achieve the determination of viability as well as the necrotic areas in the flap we use image digitalization and measured these zones in pixels ten days after the flap formation surgery same time at which the treatments were administered.

For the analysis of the images, the areas with characteristics of necrotic tissue and viable tissue were delimited. Figure 2 shows representative images of the

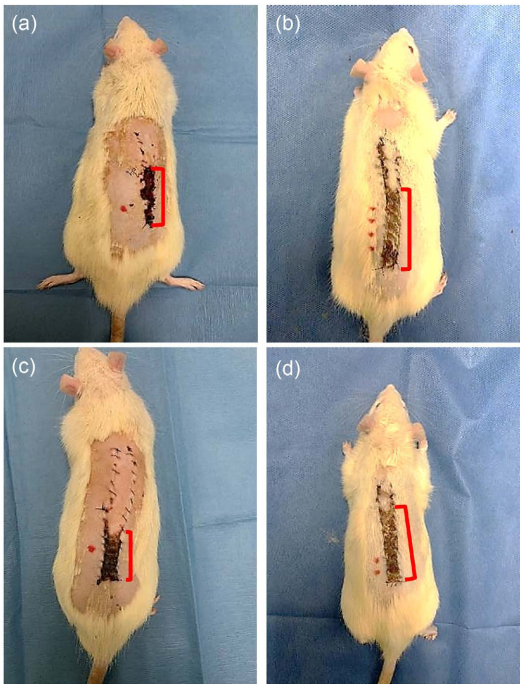


Figure 2. Evolution of viability of random vascular pattern flaps after 10 days of treatment. (a) Control group (Vaseline). (b) Local Nitroglycerin 12 hours group. (c) Local Nitroglycerin 24 hours group. (d) Systemic Nitroglycerin 12 hours. The viable area of the flaps is indicated with red brackets.

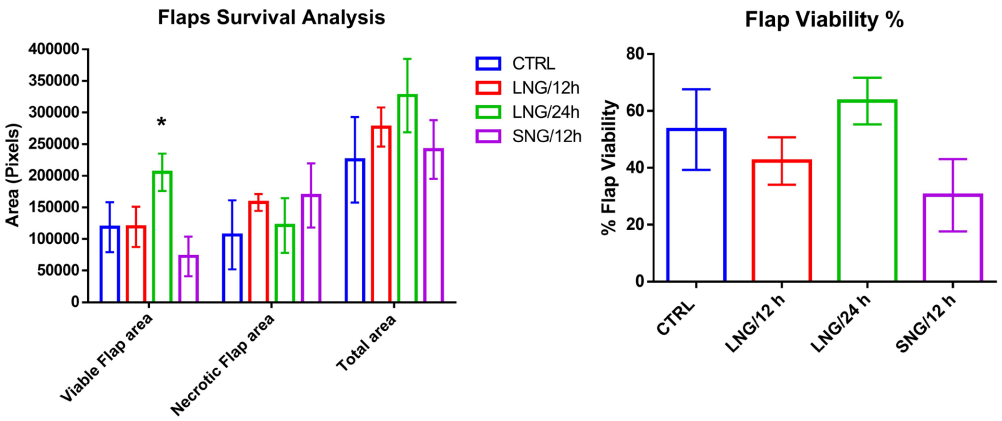
animals in each of the groups, where the group of animals treated with the nitroglycerin scheme every 24 hours locally (**Figure 2(c)**) has a smaller area of affected tissue with color changes and epidermolysis than the control and the other groups.

The viability percentage of the flap (**Figure 3(a)**), viable flap area, necrotic flap area, and total flap area (**Figure 3(b)**) were measured using the area function of the software. In the analysis of the percentage of the viable area of the flap, we were able to observe how the application of nitroglycerin every 24 hours turned out to be the most beneficial, while the groups administered with both the local 12-hour scheme and the systemic 12-hour scheme showed a trend towards decrease (without reaching statistical significance) of the viability of the flap compared to the control.

On the other hand, when analyzing the absolute values of the viable, necrotic, and total areas, we were able to observe a significant difference (P = 0.008, Mann-Whitney U-Test) in the increase of the viable area of the flap in the animals treated with nitroglycerin locally every 24 hours compared against the control group (**Figure 3(b)**). In addition, in the absolute values, we also observed a trend in the reduction of the viable area in the animals treated with the systemic nitroglycerin scheme every 12 hours (P = 0.095, Mann-Whitney U-Test) (**Figure 3(b)**).

4. Discussion

Nitroglycerin ointment increases the perfusion of the traumatized area in skin flaps, in consequence decreasing the occurrence of flap necrosis [22]. In this study, we demonstrate the effectiveness of topical administration of 2% nitroglycerin in increasing the viability of a random vascular pattern flap. Importantly,



(a) (b)

Figure 3. Quantification of flap survival measurement analyses. (a) Percentage of flap survival according to the study group. (b) Analysis of flap survival by viable flap area, necrotic flap area, and total area. * P < 0.05 vs control group, U-Mann-Whitney Test. LNG/12h: Local nitroglycerin every 12 hours, LNG/24h: Local Nitroglycerin every 24 hours. SNG/12h: Systemic Nitroglycerin every 12 hours.

we defined the survival area as tissue with normal appearance and without necrotic compromise or partial epidermolysis.

In recent studies of rat models with flaps subjected to nitroglycerin rescue, resources such as near-infrared spectroscopy have been used to detect the oxygenation of tissues to measureviability [11], other studies have also performed histopathological studies to determine apoptosis and inflammation [1]. Our study focuses on the effects at the macroscopic level of the tissue, so ithas certain limitations to detect more subtle necrotic changes.

The maximum suggested ratio for a skin flap is 2:1 and it has been observed that exceeding this ratio subjects the flap to necrosis [23]. Based on this, the random vascular pattern flap model with an 8 cm length by 1 cm base used in this study was designed to ensure necrosis in the flaps inat least half of the flap area. Other previous studies used this technique of extending the length of the flap and narrowing the base to force necrosis in the distal areas of the flap in their models [2] [5].

The use of other vasodilator drugs requires extensive preoperative treatment or direct intravascular administration. We decided to use the easy-to-apply topical nitroglycerin doses emulating a previously described study, which demonstrated with repeated monitoring of blood samples that a 2-inch/5 cm/30 mg dose of 2% topical nitroglycerin applied to rodents maintained levels of 3 mg/dL for more than 2 hours after application, triplicating the value needed for a systemic effect in humans [19]. Based on this, we decided to carry out the study design in divided schemes with doses every 24 hours to have a local effect and every 12 hours to keep blood levels above these values, resulting in a systemic effect.

In this study, we found that nitroglycerin administered locally every 24 hours significantly increased flap viability compared to vaseline; however, this same effect was not observed with the local administration scheduled every 12 hours. As mentioned, nitroglycerin can generate muscle relaxation in blood vessels causing its relaxation and the formation of prostacyclin, which can inhibit platelet activation. High blood nitroglycerin levels can produce hypotension which can lead to secondary hypoperfusion as an adverse effect, increasing tissue ischemia and reducing the perfusion pressure of the already vascularly compromised flap [10].

On the other hand, we observed that nitroglycerin applied outside the flap every 12 hours resulted in a marked decrease in its viability, confirming that the systemic effect is not sufficient to reverse this necrotic process. Although this result was not significant, there is a trend that could probably cross this limit if more study subjects are included in further analysis.

5. Conclusion

In conclusion, in this work, we were able to demonstrate the increased viability of the random vascular pattern skin flap. However, this beneficial effect was only

observed in the schedule of local applications every 24 hours. This will serve as the basis for optimizing the dosage of topical nitroglycerin to achieve a higher percentage of survival in these flaps in future studies.

Conflicts of Interest

The authors declare no conflicts of interest.

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