ORIGINAL ARTICLE

Protective effect of nitroglycerin ointment and dimethyl sulfoxide on necrosis of skin flaps in rats

Efecto protector del unguento de nitroglicerina y el dimetilsulfoxido en la necrosis de colgajos cutáneos en ratas

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Abstract

Objective: *To compare the protective effect of nitroglycerin ointment 2% and Dimethylsulfoxide (DMSO) in dorsal flaps of the rat.* **Methods:** *A blind, experimental study was conducted in 24 male Wistar rats, with a mean weight of 320 (286-376) grams. Group 1: Control. Petrolatum jelly (Vaseline), n = 8, Group 2: Nitroglycerin (NTG) ointment 2% (Nitro-Bid, Altana Co.) n = 8, and Group 3: DMSO gel 90% (Neogen corp. Lexington KY, 40611), n = 8.* **Results:** *A total of 24 rats were operated on in the 6-month period of this study. Using a non-parametric Mann–Whitney U-test analysis, a statistically significant p was obtained between the control group and 2% NTG ointment, both in the area of necrosis and in the healthy area (p = 0.026). In contrast, the comparison between DMSO [CH3) 2SO] and the control group (p = 0.180) and between both study groups, with a p = 0.18, was not significant.* **Conclusions:** *Our study concluded that there is a protective effect of 2% NTG ointment for flap survival in relation to the control group (petrolatum). DMSO administered topically did not show a protective effect, compared to the control group.*

Keywords: *Dimethyl sulfoxide. Nitroglycerin ointment. Flap necrosis.*

Resumen

Objetivo: *Comparar el efecto protector del ungüento de nitroglicerina 2% y el dimetilsulfoxido 90% en colgajos dorsales en ratas.* **Métodos:** *Se realizó un estudio experimental ciego en 24 ratas Wistar macho, con un peso medio de 320 gramos. Grupo 1: Control. Petrolato n = 8, Grupo 2: Nitroglicerina unguento al 2 % (Nitro-Bid, Altana Co.), n = 8, Grupo 3. Dimetilsulfóxido al 90% (Neogen corp. Lexington KY.), n = 8.* **Resultados:** *Un total de 24 ratas fueron operadas en el período de 6 meses de este estudio. Mediante un análisis no paramétrico de la prueba U de Mann Whitney, se obtuvo una p estadísticamente significativa entre el grupo control y la pomada de nitroglicerina al 2%, tanto en el área de necrosis como en el área sana (p = 0.026). Por el contrario, la comparación entre DMSO y el grupo control (p = 0.180) y entre ambos grupos de estudio, con una p = 0.18, no fue significativa.* **Conclusiones:** *Nuestro estudio concluyó que existe un efecto protector de la pomada de nitroglicerina al 2% para la supervivencia del colgajo en relación al grupo control (vaselina). El DMSO administrado por vía tópica no mostró un efecto protector, en comparación con el grupo de control.*

Palabras clave: *Dimetilsulfóxido. Pomada de nitroglicerina. Necrosis del colgajo.*

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Introduction

The survival of the fasciocutaneous flaps is dependent on the adequate supply of oxygen from blood flow, throughout the entirety of the flap. The main challenge is in the distal areas of the flap, which suffer from a decreased supply, generating tissue ischemia[1]. Nitric oxide acts as a tissue protector by inhibiting aggregation of thrombocytes, leukocyte adhesion to the endothelium, eradicating free radicals, maintaining vascular permeability, regulating vascular tone, and stimulating the regeneration of vascular endothelium, among others[2-7].

Dimethyl sulfoxide [CH3) 2SO] (DMSO) is a small polar molecule that contains sulfur at its center, 2 methyl groups, and oxygen at its tips. Due to its characteristics, it is one of the most common solvents, having the ability to penetrate membranes in skin, cells, and organelles. It can associate with various components including water, proteins, carbohydrates, nucleic acids, and ionic substances. Absorption capacity in humans has been demonstrated, and 5 min after topical application, it is detectable in blood[8]. With a peak at 4-8 h in serum after topical application or oral intake, elimination is registered 120 h after application. Its elimination route is through the kidneys[9,10].

Various investigations have been carried out in rats regarding the attributes and different dosages of DMSO, seeking to quantify the optimal doses to obtain maximum benefit and minimum toxicity[11]. Among its qualities, it has been shown that, at low doses, it inhibits platelet aggregation, decreases pathological deposition of collagen in fibrotic tissue (without affecting the normal balance of collagen in healthy tissue)[12], accelerates wound healing through the activation of fibroblast proliferation, mediated Akt/mTOR activation, leading to translation of proteins, such as collagen, and the secretion of TGF-β1 by fibroblasts, indirectly increases keratinocyte migration, stimulated by the increase imTGF-β1[13]. It has a vasodilator effect by releasing a response similar to the release of histamine in the area of application. It is anti-inflammatory, by reducing the production of lymphocytes, neutrophils, IFN-γ, TNF-α, NF-KB, IL-8, IL-2, and prostaglandin E2[14]. Analgesic, due to its ability to block C fibers of peripheral nerves[15]. It was identified to be effective when applied to pressure ulcers at an early stage, allowing a reduction in their torpid evolution, as well as analgesia of the affected area, reducing inflammatory signs such as redness, pain, heat, and increase in volume.

Nitroglycerin (NTG) increases prostacyclin synthesis by endothelial cells and acts as nitric oxide, whose main function is as a vasodilator and antithrombotic at the microvascular level. It acts both at the arterial and venous levels, its effect being more accentuated at the venous level[16-18]. It generates vascular dilation by relaxing smooth muscle, without altering pre- and post-capillary resistance[19]. In ointment form, it is safe, inexpensive, easy to apply, and fast-acting. In topical use, various studies have observed improvement of flaps that present ischemic signs. It has been used for multiple pathologies including coronary syndromes and anal fissures[20,21].

Rohrich, in 1984, reported its topical use and effect on flaps in animal models, where he observed increased survival of the flap after the application of NTG[22]. Research has been carried out on its use in the area of breast reconstruction, taking into account that cutaneous necrosis after mastectomy is relatively frequent, occurring in between 2.5% and 60% of all patients. These studies showed improvement in the evolution and/or prevention of tissue ischemia. The latter presenting as a partial and full-thickness necrosis. Its use showed better effectiveness with serial application compared to a single dose. According to research in breast reconstruction, the dose that has proven to be safe and significant is 5.5 mg applied once a day every 48 h.

Methods

This project was approved by the Mexican Institute of Social Security animal care and ethical committee. Based on some articles published previously[4,22,23], a blind, experimental study was conducted in 24 male Wistar rats, with a mean weight of 320 (286-376) grams.

The population was divided into 3 groups with 8 rats each one (n = 24):

- Group 1: control: Petrolatum jelly (Vaseline), n = 8 (Fig. 1)
- Group 2: NTG ointment 2% (Nitro-Bid, Fougera, Altana Co, Melville, NY, USA), n = 8 (Fig. 2)
- Group 3: DMSO gel 90% (Neogen corp. Lexington KY, 40611), n = 8 (Fig. 3).

Surgical technique

Anesthesia: 6.5% sodium pentobarbital was used, injected directly, intraperitoneally using a 1 mL syringe with a 25 Gg needle, at a dose of 50 mg/kg of weight.

Table 1. Flap survival and necrosis (in mm) by groups

Group	n	Lower (mm^2)	Higher (mm^2)	Mean (mm^2)	SD
Control	8				
Necrosis		600	1400	1041.67	304.001
Healthy tissue		1400	2400	1958.33	304.001
Nitroglycerin	8				
Necrosis		350	850	616.67	183.485
Healthy tissue		2150	2650	2383.33	183.485
DMSO*	8				
Necrosis		450	1100	800	221.359
Healthy tissue		1900	2550	2200	221.359

*DMSO: Dimethyl sulfoxide; SD: Standard deviation.

Table 2. Statistical significance by groups

Group	Necrosis (p)	Healthy tissue (p)
Control/Nitroglycerin	0.026	0.026
Control/DMSO	0.180	0.180
Nitroglycerin/DMSO	0.180	0.180

*DMSO: Dimethyl sulfoxide.

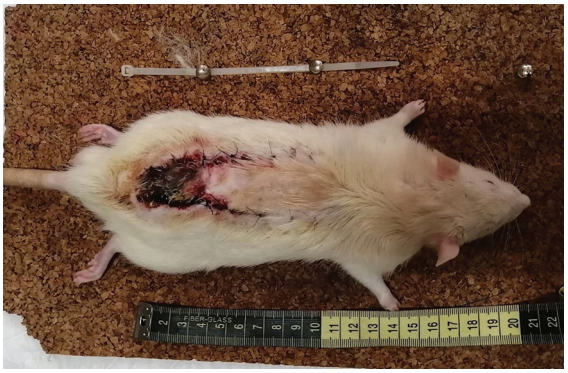

Figure 1. *Control group.*

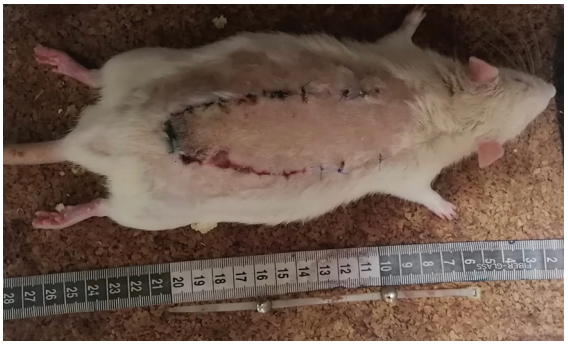

Figure 2. *Nitroglycerin ointment group.*

Once deep anesthesia had been verified by the absence of painful stimuli, a modified McFarlane flap[23] for Wistar rats was designed with an indelible marker to be 3 cm × 10 cm with a cephalic base. Trichotomy, asepsis, and antisepsis of the area were performed with povidone-iodine.

Incision was made with a No. 15 scalpel blade and dissected with scissors until the entire flap was completed suprafascially. Subsequently, it was sutured with simple stitches using 4-0 nylon.

The ointment was applied by a researcher other than the main author, who carried out the statistical analysis and review of the results (blind). Topical application was carried out immediately after surgery and subsequently every 12 h for 5 days.

The survival and necrosis of the flaps were evaluated at 7 days using a transparent acetate sheet squared every 5 mm, to complete a total area of 3000 mm^2, for subsequent analysis.

For the statistical analysis, measures of central tendency and dispersion were used, as well as the non-parametric Mann-Whitney's U-test, using the Statistical Package for the Social Sciences 10.0 program for windows (IBM; Armonk NY), taking as significant a $p \leq 0.05$.

Results

A total of 24 rats were operated on in the 6-month period of this study, in the microsurgery laboratory of the UMAE 21 (High Specialty Medical Unit 21) of the IMSS (Mexican Institute of Social Security), in Monterrey NL.

The average weight was 320 g (limits 286-376 gr).

All the rats that participated in the study survived the observation period (7 days). The total area of the flap was 3000 mm^2 in all experimental animals.

In the control group (petroleum jelly), mean necrosis of 1041.67 mm^2 was observed, in Group I (NTG ointment) 616.67 mm^2 and in Group 2 (DMSO) of 800 mm^2 table 1.

Using a non-parametric Mann–Whitney U-test analysis, a statistically significant p was obtained between the control group and 2% NTG ointment, both in the area of necrosis and in the healthy area ($p = 0.026$). In contrast, the comparison between DMSO and the control group ($p = 0.180$) and between both study groups (DMSO- NTG), with a $p = 0.18$, was not significant table 2.

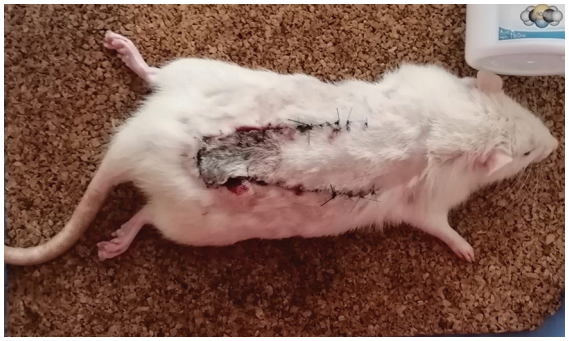

Figure 3. *Dimethyl sulfoxide group.*

Discussion

The vascularity of fasciocutaneous flaps has been extensively studied in recent decades; however, interest in them has increased exponentially since the 1987 work of Ian Taylor on skin territories and angiosomes[24]. Simultaneously, with the evolution of reconstructive microsurgery, multiple studies have been added about improvement in the survival of flaps. It is well known that the surgical technique at the time of flap dissection and the microvascular anastomosis are the most important prognostic factors for its success. However, there are many studies that look at adjuvant medications that can be used to increase local and systemic capillary vascularity[1,2,4,6,11,15,17,19,20,22,23].

Two drugs widely known to be local and systemic vasodilators were used in our study: DMSO and NTG.

We found a difference in the means of necrosis between the control group (1041.67 mm^2) and the NTG group (616.67 mm^2). The results were favorable for 2% NTG ointment, with a statistically significant p compared to the control group ($p = 0.026$).

Since 1984, Rohrich et al.[22] had already used NTG in experimental animals, also obtaining favorable results; however, unlike our study, he used axial flaps with a wider base (8 cm × 8 cm) based on the ventral inguinal vessels. In addition, some clinical studies in mastectomized patients have also shown a favorable response to NTG ointment[17,19,20]. Conversely, a study published by Nichter et al.[21] concluded that NTG does not increase the survival of flaps in rats. However, this study used prolonged-release NTG skin patches (transderm-nitro 5), their main indication being for systemic absorption rather than local effect.

Unlike 2% NTG ointment, dimethyl sulfoxide did not reduce the risk of flap necrosis ($p = 0.18$), behaving similar to the animals in the control group, despite showing a decrease in the mean necrosis in relation to the control group (800 mm^2 vs. 1041.67 mm^2). We also found no statistically significant difference between the DMSO and NTG ointment groups ($p = 0.18$) (mean 800 mm^2 vs. 616.67 mm^2).

There are many studies in animal models that demonstrate a protective effect of DMSO in random flaps, based on the decrease in systemic pro-inflammatory factors (IFNγ, TNF-α, NF-KB, IL-8, IL-2, and E2 prostaglandin). However, in many of them, DMSO is administered orally[2,4] or injected subcutaneously[11]. In our study, we found that there is no such protective effect using topically applied DMSO ointment. Although flap survival improved in some of the rats, the difference compared to the control group and the NTG group was not statistically significant.

In clinical practice, the use of NTG ointment has been found useful to improve the survival of flaps[5,8,17,19,20], these studies looked at flaps based on random circulation, where flap circulation is from capillaries and not from a main pedicle. In the case of axial microvascular flaps, where distress is mainly due to problems with the vascular pedicle, either at the level of the anastomosis or farther away due to torsion or kinking, the use of NTG ointment will not be useful, and the indication to reexamine the flap is the best salvage option.

Conclusions

Our study concluded that there is a protective effect of 2% NTG ointment for flap survival in relation to the control group (petrolatum). DMSO administered topically did not show a protective effect, compared to the control group.

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Conflicts of interest

The authors declare no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code

of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Use of artificial intelligence for generating text. The authors declare that they have not used any type of generative artificial intelligence for the writing of this manuscript, nor for the creation of images, graphics, tables, or their corresponding captions.

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