

Analysis of effect isoxsuprine hydrochloride and nicotine in the *Transverse Rectus Abdominis Myocutaneous flap (TRAM)* in rats¹

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DOI: <http://dx.doi.org/10.1590/S0102-865020150070000008>

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ABSTRACT

PURPOSE: To evaluate the effects of isoxsuprine and nicotine on TRAM.

METHODS: Forty eight 48 Wistar rats distributed into four Groups (n=12). All rats received medication managed daily for 20 days: saline solution (SA), nicotine solution (NI), isoxsuprine solution (IS) and nicotine solution (NI) + isoxsuprine solution (IS). On day 21st the rats were submitted to the caudally based, right unipedicled TRAM flap and after 48 hours, made the macroscopic evaluation of the surface of the flap, photographic documentation and collection of material for histology. Data from macroscopic evaluation were analyzed by ANOVA and microscopic evaluation by Kruskal-Wallis test, with significance level of 5%.

RESULTS: In the macroscopic evaluation of isoxsuprine Group retail presented absolute numbers: final area (p=0.001*) and viable area (p=0.006*) with the highest values; necrosis (p=0.001*) had the lowest value. Microscopic examination revealed no significant findings in the study of TRAM under the action of isoxsuprine and nicotine to the percentage of necrosis in the left and right cranial and caudal regions.

CONCLUSIONS: There was significant improvement in viability of TRAM using the isoxsuprine solution alone. No influence using nicotine alone and in association with isoxsuprine.

Key words: Surgical Flaps. Rectus Abdominis. Surgery, Plastic. Isoxsuprine. Nicotine. Rats, Wistar.

Introduction

Surgical flaps are of fundamental importance for the programming of several Plastic Surgery procedures. The most serious complication of a surgical flap is partial or total necrosis, and Literature demonstrate several experimental Works in which nicotine increases in this series¹⁻³. Among the various techniques proposed for breast reconstruction, the Transverse Rectus Abdominis Myocutaneous Flap (TRAM) has been the preferential option for multiple departments working in this field of reconstructive surgery, by virtue of using only autogenous tissue^{4,5}.

In smoking patients, TRAM complications are frequent, especially for partial or total necrosis of the flap^{5,6}.

In experimental studies, the rat proved to be adequate for the study of TRAM flap, due to its anatomical similarity to humans⁷. Nicotine has detrimental effects on the viability of skin flaps and there are few studies showing its effects in musculocutaneous flaps⁸. The isoxsuprine hydrochloride produces peripheral vasodilation by a direct effect on vascular smooth muscle, primarily within skeletal muscle, with little effect on cutaneous blood flow⁹⁻¹³. In an attempt to neutralize the harmful effects of nicotine and reduce the areas of flap necrosis isoxsuprine hydrochloride was used^{19,20}.

Methods

Approved by the Animal Ethics Committee (CETEA - UFMG), on 10/03/2010, Protocol-209/2009. At all stages of the experiment, ethics of animal experimentation of the guide were observed¹⁴⁻¹⁶. Prospective study in 48 female rats, Wistar strain, with three months of age, weighing between 250g and 265g.

The animals were distributed by lot into four groups of 12 animals each (n=12) receiving medications daily for 20 days: SA group received 0.9% saline solution injected into the subcutaneous tissue in the posterior midline caudal by volume of 1 ml / kg. NI group received nicotine solution (L-1 Nicotine Sulfate Methyl-2- (3-pyridyl) -pyrrolidine Sulfate; II grid; MW 422-6; Sigma) was injected into the subcutaneous tissue in the posterior midline caudal, at a dose of 2 mg / kg. IS Group received isoxsuprine hydrochloride solution (Inibina[®]) injected into the subcutaneous tissue in the posterior midline cranial in 1 mg / kg doses. NI+IS group received nicotine solution injected into the subcutaneous tissue in the posterior midline caudal, at a dose of 2 mg / kg, and isoxsuprine hydrochloride (Inhibina[®]) injected into the subcutaneous tissue in the posterior midline cranial in 1 mg / kg doses. Intramuscular anesthesia on the lateral side of the root of

the left thigh, associating to 50 mg / kg of ketamine hydrochloride (Ketalar[®]) with 12 mg / kg of 2- (2.6 xylidine) -5.6di-dihydro-4H-1.3-thiazine (Rompun[®]). On day 21th all animals were subjected to TRAM flap desvascularization of unilateral right caudal base (vascular pedicle nondominant), 3.5 cm wide by 3.5 cm long, and its upper limit determined by a line transverse tangential to the xiphoid process, marked with the letter V (Figures 1 to 5).



FIGURE 1 – Marking of incisions with 3.5 cm wide and length.

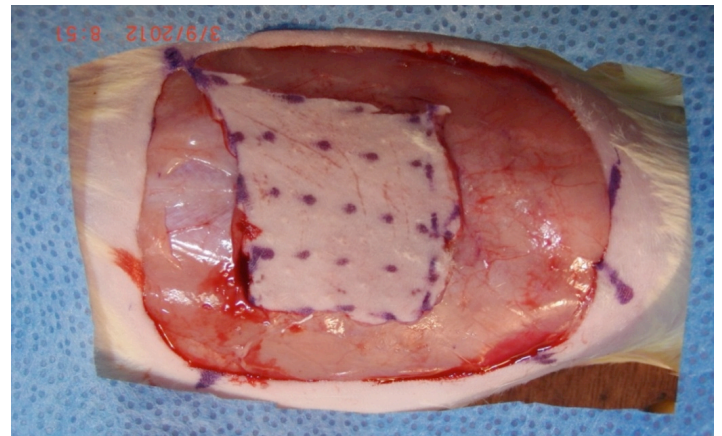


FIGURE 2 – Incision of the skin and subcutaneous tissue.

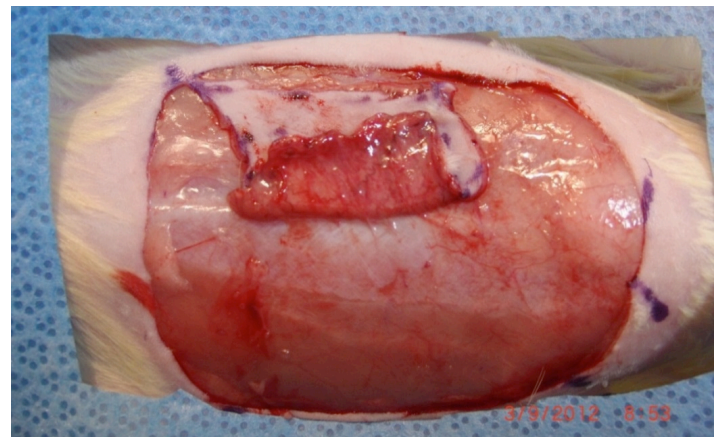


FIGURE 3 – Detachment of the skin and left subcutaneous tissue until alba linea, the skin and the right subcutaneous tissue the lateral border of the right rectus abdominis.

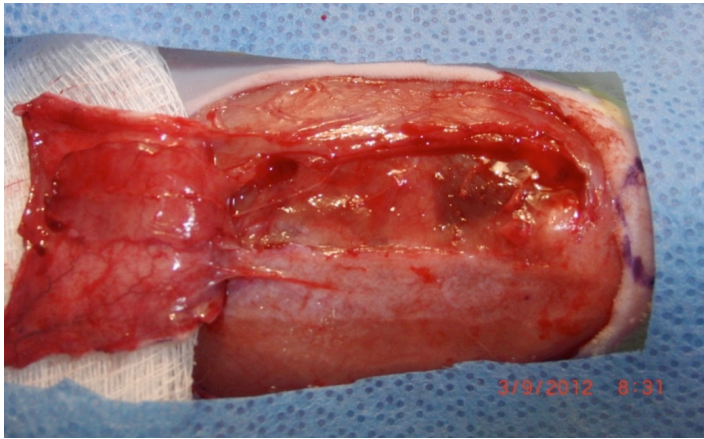


FIGURE 4 – TRAM flap folded from its bed towards cranial-caudal direction.

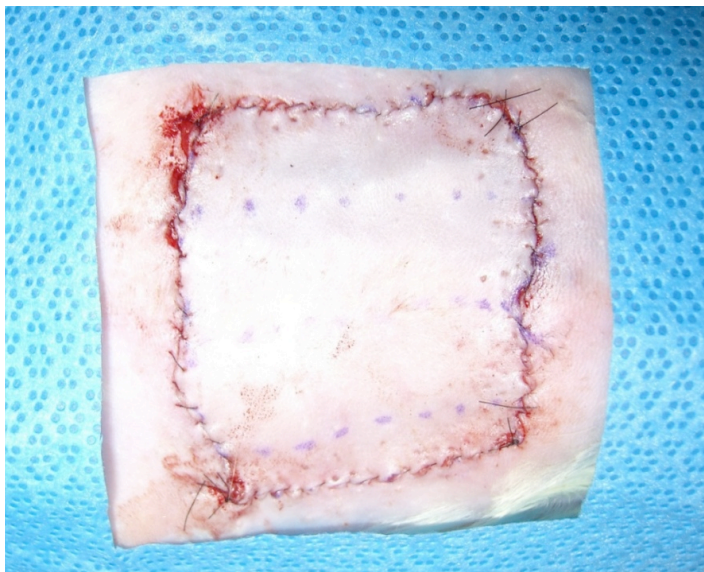


FIGURE 5 – Desvascularization of the TRAM flap completed after suture of the aponeurosis and skin in its bed.

On the second postoperative day (POD), each animal was anesthetized by the same technique used to empower the TRAM for macroscopic evaluation, conducting photographic documentation and collection of samples for histology. After these procedures, still anesthetized, the euthanasia of all animals was performed, with the section of abdominal aorta.

Photographic documentation

On the second postoperative day, each animal was positioned supine, with the setting of upper and lower limbs. On the abdomen was placed one fenestrated field that allowed display only the abdomen where the empowerment of the TRAM was performed, the cephalic and caudal segments were excluded. At the bottom of the operating field was a rectangular adhered label 16 mm x 9 mm, with the identification of the animal. We used a digital camera, 9.1 MP, Casio Exilim-FC100. The photos were taken at a distance of 16 cm, with automatic adjustment and without illuminator (Figure 6A).

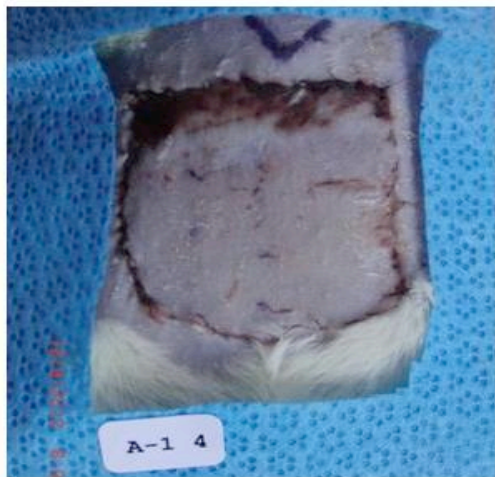
Graphical representation

The flaps were plotted on transparent acetate sheets, where the contours of photos with retail delimitation of areas of viability, suffering and necrosis have been designed. The criteria for classification were based on inspection and palpation. The flaps were delimited according to their characteristics in:

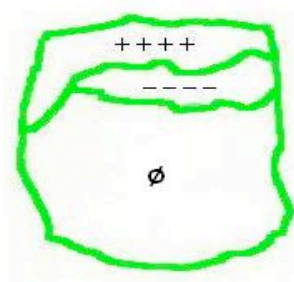
- a) Area of viability: normal coloration and texture.
- b) Area of suffering: purplish color and malleability during palpating.
- c) Area of necrosis: dark color and hard consistency.

Macroscopic analysis and calculation of areas

Figures plotted on transparent acetate sheets were scanned. The digital document has been working in Autocad 2009 version program - Autodesk, Inc. Areas of each region of the flap were delimited with the red colors to necrosis, yellow for suffering and green for viable skin. The absolute values refer to those calculated in square centimeters to the “AutoCAD” program. The percentage values mean the percentage that each area presented in relation to the final size of the flap (Figure 6A to C).



A



B



C

FIGURE 6 – (A) Animal Photography from SF Group on the second postoperative day. (B) Scanned image of blade acetate transparent, in which the signal “+” represents the necrosis area, signal “-” represents the distress area and signal “∅” represents the viable area of flap. (C) Image of Autocad program in red representing the area of necrosis, yellow representing the distress area and green representing de viable area of flap.

Collection of samples

For histological evaluation, the TRAM flap with the rectus muscles adhered of the right and left abdomen was removed in a monobloc at 0.5 cm from its cranial, caudal and lateral edges. After sectioning the linea alba was removed on each side, 0.8 cm length a segment of cranial and other from caudal region. The four fragments were placed in flasks of 10% formaldehyde and identified.

Microscopic evaluation

Regarding the necrosis of the epithelium the histological criteria was evaluated in a percentage of the cranial and caudal regions right and left, observing all the histological slides, and did not showed relevant results.

Statistic method

Data from macroscopic evaluation were analyzed by

Analysis of Variance (ANOVA), with significance level of 5%. We used the software - Statistical Package for Social Sciences (SPSS) 18 for comparison of means between the four Groups regarding the variables \pm the standard deviation. Histological evaluation of the data were analyzed using the Kruskal-Wallis test, with significance level of 5%.

Results

Macroscopic evaluation

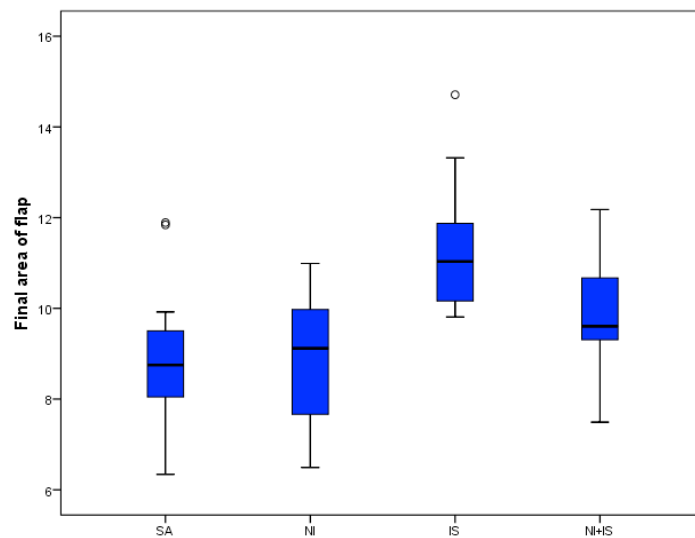


FIGURE 7 – Final area of flap, in absolute numbers, on SA, NI, IS and NI+IS groups (p-0.001*).

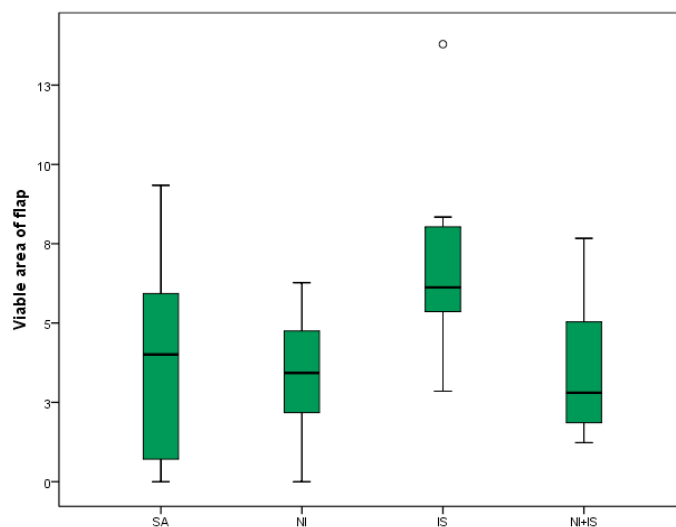


FIGURE 8 – Viable area of flap, in absolute numbers, on SA, NI, IS and NI+IS groups (p-0.006*).

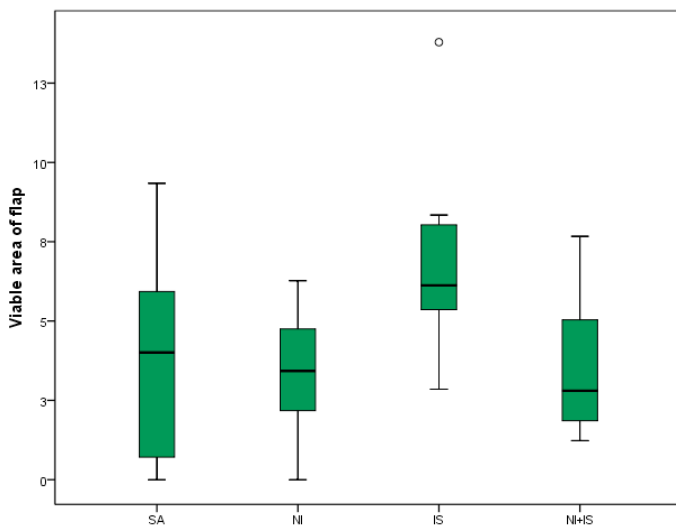


FIGURE 9 – Necrosis area of flap, in absolute numbers, on SA, NI, IS and NI+IS groups (p-0.001*).

Macroscopic findings of TRAM study, under the action of isoxsuprine hydrochloride and nicotine showed significant results for the Final area of flap, Viable area of flap, % Viable area of flap, Necrosis area of the flap and % Necrosis area of the flap (Table 1).

TABLE 1 – Action of nicotine and isoxsuprine hydrochloride in TRAM study. Mean (± standard deviation) of the Final area of flap, Viable area of flap, % of viable area of flap, Suffering area of the flap, % of Suffering area of the flap, Necrosis area of the flap and the % percentual Necrosis of the area flap and P value in the Group’s SA, NI, IS and NI+IS.

Variable	Group SA	Group NI	Group IS	Group NI+IS	P valor
Final area of flap (cm²)	8.98 (±1.69)	8.83 (±1.44)	11.30 (±1.50)	9.90 (±1.26)	0.001*
Viable area of the flap (cm²)	3.77 (±3.40)	3.42 (±1.82)	6.83 (±2.72)	3.45 (±2.08)	0.006*
% Viable area of the flap	.40 (± .35)	.38 (±.18)	.60 (±.20)	.33 (±.17)	0.047*
Suffering area of the flap (cm²)	1.52 (±1.70)	1.66 (±1.12)	2.87 (±1.84)	2.22 (±.62)	0.108
% Suffering area of the flap (cm²)	.18 (±.21)	.19 (±.12)	.26 (±.17)	.23 (±.07)	0.551
Necrosis area of the flap (cm²)	3.69 (±1.93)	3.75 (±1.25)	1.60 (±1.41)	4.23 (±1.77)	0.001*
% Necrosis area of the flap	.42 (±.25)	.43 (±.13)	.14 (± .12)	.44 (±.19)	0.000*

TRAM study results were significant, over the action of nicotine and isoxsuprine hydrochloride in the multiple comparisons (Bonferroni) of Final area of flap, viable area of flap and Necrosis area of flap.

Discussion

Nicotine has a detrimental effect on the viability of skin flaps and there are few experimental studies supporting this effect in musculocutaneous flap⁸. The harmful effects of nicotine have been discussed in clinical studies. Rees *et al.*¹⁷ reported a significant number of skin injury after surgical face rejuvenation due to smoking. Campos *et al.*¹ and Forrest *et al.*² demonstrated, in rats, a large area of necrosis in skin flap with the use of nicotine. Craig and Rees¹⁸ detected in hamsters.

In an attempt to neutralize the harmful effects of nicotine and reduce the areas of flap necrosis several medications were used^{19,20}. Desvascularization cranial of the *Transverse Rectus Abdominis Myocutaneous flap (TRAM)* in rats was a surgical technique used to induce a programmed and intentional tissue ischemia in flaps, making them survive with less blood flow than usual, causing a stimulus of increased internal vascularity, leading to reduced areas of necrosis flap were also used²¹⁻²³.

The isoxsuprine hydrochloride causes peripheral vasodilation by a direct effect on vascular smooth muscle, primarily within skeletal muscle, with little effect on cutaneous blood flow.

Neligan *et al.*¹³, evaluated the therapeutic effect of isoxsuprine on skin capillary blood flow and its viability in studies carried out on pigs with arterial buttock flap, flap musculocutaneous latissimus dorsi flap and random skin. They emphasized that isoxsuprine hydrochloride parenteral increased capillary blood flow in the skin flap with maximum three vascular effect using a dose of 1.0 mg / kg / day. However this effect was not significant in the viability of the cutaneous and musculocutaneous flap when compared to control group. They concluded that isoxsuprine hydrochloride alone is not effective in increasing viability of the skin and in skin musculocutaneous flap. The present study showed divergent results, with significant improvement in the TRAM flap in rats with isolated use of isoxsuprine hydrochloride solution.

To van Adrichem *et al.*²⁴ the effect of nicotine on the survival of free vascularized flap and the epigastric flap in rats caused large necrotic area in random epigastric pedicle flap. They concluded that nicotine was detrimental to the survival of epigastric free flap and did not affect the viability of the epigastric flap. The present study showed no significant influence on the

TRAM flap in rats using of nicotine alone and in association with isoxsuprine hydrochloride.

There was a significant reduction in the final size of the flaps in the four Groups. The IS Group showed significant results for the most final area of flap, higher viable area of flap and the lower necrosis area of the flap.

Isoxsuprine Hydrochloride had a beneficial effect in TRAM flap's survival. This result herein presented would support the clinical use in mammary reconstruction with the TRAM flap. However, further studies are required to support this suggestion.

Conclusions

There was a significant improvement in TRAM flap in rats using the solution of isoxsuprine alone. There was no influence in the use of nicotine alone and in association with isoxsuprine.

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Received: March 5, 2015

Review: May 10, 2015

Accepted: June 9, 2015

Conflict of interest: none

Financial source: none

¹Research performed at Department of Surgery, School of Medicine, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte-MG, Brazil. Part of PhD degree thesis, Postgraduate Program in Science Applied to Surgery and Ophthalmology. Tutor: Prof. Alcino Lázaro da Silva.