

Histamine iontophoresis on the viability of random skin flap in rats¹

Iontoforese de histamina na viabilidade do retalho cutâneo randômico em ratos

Ivaldo Esteves Júnior^I, Pascale Mutti Tacani^{II}, Victor Cicone Liggieri^{III}, Bruno Grinman Ruggi^{III}, Lydia Masako Ferreira^{IV}, Richard Eloin Liebano^V

^I Full Professor, Physical Therapy Department, UNIP and Mackenzie Presbyterian University, Sao Paulo, Brazil.

^{II} Fellow Master degree, Plastic Surgery, Federal University of Sao Paulo (UNIFESP). Full Professor, Physical Therapy Department, City University of Sao Caetano do Sul (USCS) and Sao Camilo University Center, Sao Paulo, Brazil.

^{III} Graduated, Physical Therapy, UNIP, Sao Paulo, Brazil.

^{IV} PhD, Full Professor, Department of Surgery, Plastic Division, Coordinator of Post Graduate Program in Plastic Surgery, UNIFESP, Sao Paulo, Brazil.

^V PhD, Full Professor, Physical Therapy Department, City of Sao Paulo University (UNICID), Sao Paulo, Brazil.

ABSTRACT

Purpose: To evaluate the effects of the histamine iontophoresis on the random skin flap viability in rats. **Methods:** Sixty adult male Wistar rats were used. A cranially-based dorsal skin flap measuring 10 x 4 cm was raised and a plastic barrier was placed between the flap and its bed. After the surgical procedure, the animals were randomized into four groups (G1-G4) (n=15 each group) as follows: G1 (control) – sham electrical stimulation, G2 (electrical stimulation) – direct current electrical stimulation, G3 (histamine) – histamine and sham electrical stimulation and G4 (histamine iontophoresis) – transdermal iontophoresis of histamine. In all groups the procedures were performed immediately after the surgery and on the two subsequent days. The percentage of flap necrosis was measured on the seventh postoperative day. **Results:** The mean and the respective standard deviation of the percentage of flap necrosis areas were as follows: G1 (control) – 47.87 ± 9.13%, G2 – 51.49 ± 8.19%, G3 – 46.33 ± 8.32% and G4 – 30.82 ± 11.25%. The G4 group presented a significantly smaller amount of flap necrosis when compared to the other groups ($p < 0.001$). **Conclusion:** The topical administration of the histamine by iontophoresis was effective to increase the viability of the random skin flaps in rats.

Key words: Histamine. Iontophoresis. Surgical Flaps. Necrosis. Rats.

RESUMO

Objetivo: Avaliar o efeito da iontoforese de histamina na viabilidade do retalho cutâneo randômico em ratos. **Métodos:** Foram utilizados 60 ratos adultos e machos da linhagem Wistar. O retalho cutâneo de base cranial, medindo 10x4 cm, foi elevado no dorso dos animais e uma barreira plástica foi interposta entre o retalho e a área doadora. Após o procedimento operatório, os animais foram distribuídos aleatoriamente em 4 grupos (G1-G4) (n=15 em cada grupo) a saber: G1 (controle) – simulação da estimulação elétrica, G2 (estimulação elétrica) – estimulação elétrica com corrente direta, G3 (histamina) – histamina e simulação da estimulação elétrica e G4 (iontoforese de histamina) – iontoforese transdérmica de histamina. Em todos os grupos os procedimentos foram realizados imediatamente após a operação e nos 2 dias subsequentes. A porcentagem de área de necrose foi avaliada no 7º dia pós-operatório. **Resultados:** As médias e respectivos desvios-padrão das porcentagens de área de necrose foram: G1 (controle) – 47,87 ± 9,13%, G2 – 51,49 ± 8,19%, G3 – 46,33 ± 8,32% and G4 – 30,82 ± 11,25%. O grupo G4 apresentou menor média de área de necrose quando comparado aos demais grupos ($p < 0,001$). **Conclusão:** A administração tópica de histamina por iontoforese aumentou a viabilidade do retalho cutâneo randômico em ratos.

Descritores: Histamina. Iontoforese. Retalhos Cirúrgicos. Necrose. Ratos.

¹Research performed at Department of Physical Therapy, Paulista University (UNIP), Sao Paulo, Brazil.

Introduction

In the field of plastic surgery, the skin flaps have fundamental importance in reconstructive procedures and new methods have been developed to minimize ischemic effects of the skin flap in postoperative period¹. According to Kerrigan¹ local arterial insufficiency, in contrast to the extrinsic factors plurality, is the only intrinsic factor responsible for the unwished tissue necrosis.

Searching for a solution for the inadequate blood flow, many studies using pharmacological^{2,3} and non-pharmacological^{4,5,6,7} resources have been developed.

In pharmacological way there are many vasoactive substances that may cause deleterious effects when systematically administrated. However, Abranson⁸ showed the possibility of topical use of some vasoactive agents as histamine, without undesirable effects of systemic application. Histamine has as primordial action vasodilation mediated by H1 and H2 receptors, distributed in vessels that produce vasodilation with simultaneous activation.

Among non-pharmacological resources low-frequency electric currents have been used. These currents have as vascular effects the inhibition of the sympathetic vasoconstrictor fibers and the release of some vasodilating neuropeptides, such as substance P (SP) and calcitonin gene-related peptide (CGRP), which are found in free nerve endings of A-delta and C fibers, and would be released by antidromic depolarization (axon reflex)^{5,9,10}.

Iontophoresis can be defined as the introduction of ions into the skin by means of electricity, being the direct current the most frequently utilized. It is a non-invasive method and has as advantage the localized action of the introduced substance without systemic side effects^{2,5,11}.

Analyzing the pharmacological and non-pharmacological resources with the aim of increasing blood flow in ischemic skin flaps, there are common vasoactive effects between the electrical currents and histamine. These evidences suggest the opportunity to integrate both effects through the iontophoresis^{2,9}. Based on these findings, the aim of this study was to evaluate the effects of histamine iontophoresis on the viability of random skin flap in rats.

Methods

Sixty adult male Wistar rats (*Rattus norvegicus*) weighing 290 to 380 g were used in this study. There was no statistically significant difference in weight among these animals. The animals were housed in individual cages with controlled temperature environment (between 23° and 25°C), on a 12:12 hour light-dark cycle (lights on at 7:00 am), and fed standard rat chow and water *ad libitum*. This research was approved by the Research Ethics Committee (CEP) at the UNIP, São Paulo, Brazil.

All animals were anesthetized with tiletamine hydrochloride and zolazepam hydrochloride (50 mg/kg, intraperitoneally) during surgery and treatments. Following anesthesia, all rats were placed on a flat surface with legs extended and their backs were shaved. The cranially based dorsal random skin flap, measuring 10x4 cm, following experimental model proposed by Mcfarlane, DeYoung,

Henry¹³ was raised from the deep fascia, including the superficial fascia, panniculus carnosus, subcutaneous tissue, and skin. After flap elevation, a plastic barrier (polyester/polyethylene), with the same dimensions (10x4 cm) was placed between the skin flap and its bed. The flap was then sutured back in place with simple 4-0 nylon sutures.

After the surgical procedure, the rats (n=60) were randomized into four groups (n=15 each group) and kept anesthetized for 20 minutes. All animals had electrodes (3x5 cm) placed on the base of the flap (anode) and on the abdominal region (cathode) and received the assigned treatment, as follows: G1 (control) – sham electrical stimulation, G2 (electrical stimulation) – received direct current electrical stimulation, G3 (histamine) – received sham electrical stimulation and topical application of 3 ml of histamine (0.01%) under anode electrode and G4 (histamine iontophoresis) – received direct current stimulation with histamine (3ml, 0.01%) under the anode electrode. Between the cathode electrode and the animal's skin, a gauze wetted by 3ml of a saline solution (0.9%) was used. In groups 1 and 2 the gauze containing the saline solution was also placed under the anode electrode. In groups 3 and 4, the 3ml of histamine solution (0.01%) was uniformly applied to a gauze that was interposed between electrode and the animal's skin. In all groups the procedures were performed immediately after the surgery and on the two subsequent days.

In the animals submitted to the electrical stimulation (G2 and G4) the current amplitude was set at 4mA and the application time was of 20 minutes, resulting in a total 80mAmin dose.

The direct current generator used in this experiment was the EGF® (Carci, São Paulo, Brazil).

The percentage of skin flap necrosis was measured on the seventh postoperative day, using the paper template method described by Sasaki and Pang¹⁴. The viable tissue limit has been characterized by soft skin, pink, warm and haired, and necrotic tissue by stiff, dark cool, and hairless skin, was demarcated on the rats.

Data were analyzed blind using GraphPad Instat® version 3.05 (GraphPad Software, Inc). Preliminary tests for normal distribution were carried out (Kolmogorov and Smirnov) and subsequent one-way analysis of variance (ANOVA), followed by post hoc Tukey-Kramer multiple comparisons test were performed. The significance level was fixed at 0.05 ($p \leq 0.05$). Data are presented as mean \pm standard deviation (SD).

Results

The mean \pm SD of the percentage of flap necrosis area was as follows: G1 (control) 47.87 \pm 9.13%, G2 (electrical stimulation) 51.49 \pm 8.19%, G3 (histamine) 46.33 \pm 8.32% and G4 (histamine iontophoresis) 30.82 \pm 11.25%. The smallest necrotic area was observed in G4 that received histamine iontophoresis. The statistical analysis presented significant difference when comparing G4 to other groups ($p < 0.001$). There were no statistically significant differences comparing other groups (G1xG2, G1xG3 and G2xG3) ($p > 0.05$), as shown in Figure 1.

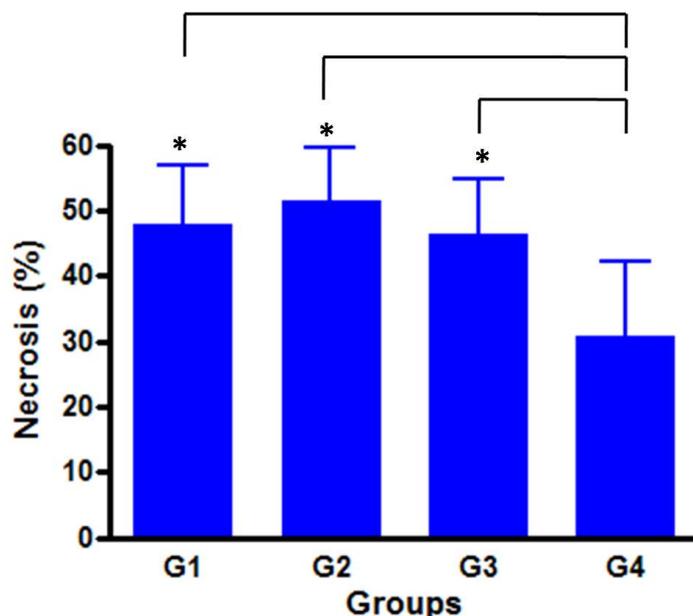


FIGURE 1 - Means \pm SD percentage of necrosis in studied groups. There were statistically significant differences between G4 (histamine iontophoresis) and other groups ($p < 0.001$ ANOVA – Tukey-Kramer)

Discussion

Decreasing tissue necrosis after reconstructive plastic surgery is an emerging study field for different health care researchers.

Comparative studies between vasoactive agents that are systemically applied were carried out by many authors, like Suarez Nieto *et al.*³ and Finseth¹⁵ that evaluated the effects of different vasoactive drugs on the survival of skin flaps in rats, showing a significant improvement in all groups when compared to the control group. However the study of Toomey *et al.*¹⁶ didn't present the same results, demonstrating the necessity of more researches with vasoactives drugs such histamine.

Electrophysical agents have been also investigated and among them transcutaneous electrical stimulation has being studied as a possible method to improve the viability of ischemic skin flaps^{4,7,17-20}. Although histamine iontophoresis have been researched before by other authors^{8,21} it wasn't found studies addressing the effects of this procedure for increasing skin flap viability.

The presented study has investigated the efficacy of topic administration of histamine by iontophoresis on the skin flap viability, presenting significant improvement when compared to control group and other groups. These results show that only the direct current with the anode electrode over the flap or the application of histamine directly on the skin without direct current application were not effective.

Similar studies have been performed with the vasoactive drugs applied by iontophoresis and they also have shown decreased of skin flap necrosis in rats, according with the results found in this work. Prostaglandin E1 has been used by Asai *et al.*² under the negatively charged electrode (cathode) and it has proved to

improve the viability of the skin flap, as well the CGRP applied under the anode electrode by Esteves Júnior *et al.*⁵.

The administration of hydralazine chloridrate by iontophoresis was also researched¹² but it has not shown satisfactory results on the viability of skin flaps, unlike the results found with histamine administration in this study.

The parameters of iontophoresis used in the present study (4mA for 20 minutes) were based on other published works^{2,5}. When these parameters of direct current were used without the histamine association (G2) it wasn't observed any beneficial effect on skin flap viability confirming the results of Branco *et al.*⁶ that did not observe any reduction of necrosis in the group submitted to the application of direct current with the anode electrode applied to skin flap.

Moreover, the used dose (80mAmin) is within the range recommended by literature (20 to 100mAmin)¹¹ and, according to the Food and Drug Administration (FDA), this should be the maximum correlation between amplitude and time for iontophoresis applications¹¹.

Thus, it can be observed that iontophoresis is a non-invasive physical method and when performed with vasoactive drugs as histamine reduces the skin flap necrosis in rats. Future comparative studies using different pharmacological agents and also various doses of iontophoresis are required for knowing new ways to prevent the tissue necrosis in skin flaps, improving this procedure.

Conclusion

The topical administration of the histamine by iontophoresis was effective to increase the viability of the random skin flaps in rats.

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Conflict of interest: none

Financial source: UNIP

Correspondence:

Ivaldo Esteves Júnior.
Richard Eloin Liebano
Instituto de Ciências da Saúde - Curso de Fisioterapia
Rua Dr. Bacelar, 1212
04026-002 Sao Paulo – SP Brazil
Phone: (55 11)5586-4000
Fax: (55 11)2275-1541
ivaldofisio@ig.com.br
liebano@gmail.com

Received: August 12, 2008
Review: October 14, 2008
Accepted: November 11, 2008

How to cite this article

Esteves Júnior I, Tacani PM, Liggieri VC, Ruggi BG, Ferreira LM, Liebano RE. Histamine iontophoresis on the viability of random skin flap in rats. *Acta Cir Bras.* [serial on the Internet] 2009 Jan-Feb;24(1). Available from URL: <http://www.scielo.br/acb>

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