Experimental model to study the effect of nicotine in a random skin flap, in the rat[1]

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ABSTRACT - Presentation of an experimental model to study the effect of nicotine in a random skin flap, in the rat. Adult rats of the same lineage are exposed to nicotine for seven days in the preoperative period. After the preoperative period, the animals are anesthetized and a dorsal random pattern skin flap, cranially based is elevated. On the seventh postoperative day, the rats are anesthetized and the percentages of distal necrosis in the flaps are determined. After that, excision of skin fragments for biochemical analysis is possible. Blood samples may be collected by decapitation.

KEY WORDS – Surgical flaps. Nicotine. Tobacco. Rats

Introduction

The importance of random skin flaps in Plastic Surgery is unquestionable, both in reparative and aesthetic areas. Nevertheless, the elevation of a random flap is not trouble-free. The most feared complication is necrosis.

The main cause of necrosis in a random skin flap is inadequate blood flow to the flap[1], what makes this kind of flap particularly susceptible to the action of vessel-constrictive drugs, as nicotine.

Proposition

The present report describes the model utilized by the authors to study the effect of nicotine in a random pattern skin flap, in the rat, including comments about via of administration and dose.

Method description

This experimental model utilizes rats (*Rattus norvegicus: var. albinus, Rodentia, Mammalia)*, adults, of the same lineage, male or female, weighting around 300g. Nicotine used in the experiment [Nicotine Sulphate L-1 Methil-2 (3-Piridyl)-Pirrolidyne Sulphate; grade II; MW 422-6; SIGMA], is

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diluted with saline to the concentration of 1 mg/ml and injected in the rats subcutaneous tissue, in the preoperative period, for seven days (minimum). Control animals receive saline, only. When this preoperative administration of nicotine is interrupted five days before the operative procedure, the results are similar to the control[2].

After the preoperative period, the rats are anesthetized with Pentobarbital (40 mg/kg), intraperitoneally or Ether, by inalation. Then, the animals are positioned in ventral decubit over a flat surface, immobilized with extended limbs and have their dorsum manually depilated. Delineation of the flap is done by means of a flexible plastic template, cut in the standard dimensions. The operative procedure follows.

The present experimental model uses a dorsal random pattern skin flap, cranially based[3], with 4 x 10 cm (FIGURE 1). After flap elevation, an impermeable plastic barrier is interposed between the flap and its donor bed[4] (FIGURE 2). Synthesis is done with simple stitches of monofilamentar naylon 4-0.

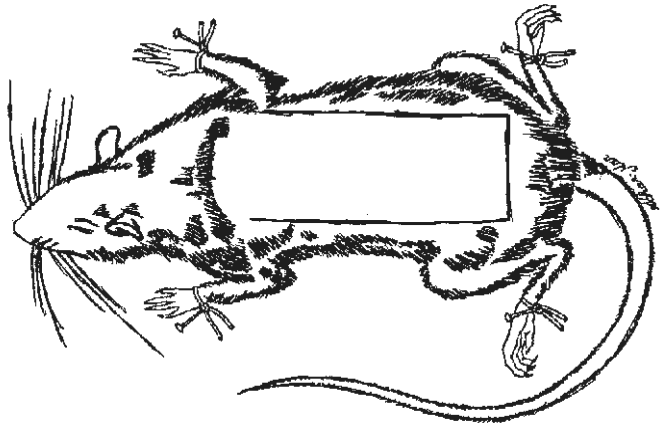

FIGURE 1 – Delineation of the dorsal random pattern skin flap, in the rat.

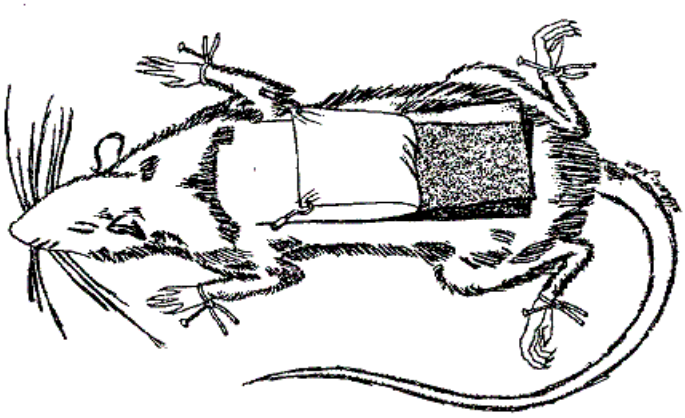

FIGURE 2 – Interposition of a plastic barrier between the flap and its donor bed.

After the operative procedure, the animals return to their specific cages, receiving food and water *ad libitum*. The programmed treatment follows, according to stablished research protocol.

On the seventh postoperative day, the rats are anesthetized and the percentages of distal necrosis in the flaps are determined via the "paper template method"[5]. A template of each flap is drawn and cut in vegetable paper, being weighted in a precision scale. Then, the area that represents necrosis is cut and weighted. Dividing the value obtained for the necrosis by that of the total flap and multiplying the result by 100, the percentage of necrosis is obtained.

After that, the animals may be submitted to the excision of skin fragments for biochemical analysis. These skin samples may be collected from different regions of the flap, like the proximal, middle and distal portions and, from a region external to the flap that represents the normal skin. Blood samples may be collected by decapitation.

Perspectives

The random pattern skin flap, cranially based, in the rat, was proposed as an experimental model to study cutaneous necrosis and its prevention[3]. It is an easy to elevate flap that, on the seventh postoperative day presents a percentage of distal necrosis of, at least, around 25 %. The maximum amount of necrosis is usually around 50 %. Notwithstanding, not always a significant necrosis occurs, perhaps due to a possible nutrition of the distal extremity of the flap by its donor bed[6].

In order to guarantee the dermo-subdermic plexus the exclusivity of flap nutrition, an impermeable plastic barrier is interposed between the flap and its donor bed[4]. This procedure keeps the minimum percentages of distal necrosis in the flap around 25 %.

The physiological responses to nicotine include cutaneous vasoconstriction associated to a reduction in the skin temperature[7], elevation of the platelet adhesion[8] and reduction in the coagulation time[9], all highly deleterious to the random pattern skin flap.

As regards via of administration and dose of nicotine, the subcutaneous injection is the choice, in a dose of 1.2 mg/kg/day, that allows plasmatic levels similar to those found in the heavy smoker[10] or 0.6 mg/kg/day, equivalent to the light smoker[11]. This drug should be administered for, at least, seven days in the preoperative period[12], its use in the postoperative period being optional, according to the purpose of the study. Interruption in the administration of nicotine in the preoperative period, when included in the study, must occur five days before the operative procedure to be effective or, in other words, in order to keep the percentages of distal necrosis in levels similar to those of the control animals[2].

The use of smoking chambers differs essentially from the subcutaneous administration of nicotine. Besides the presence of other constituents of the cigarette smoke (impeding the identification of the main nocious agent), the frequency of exposure does not correspond to that of the smokers and the confinement of the animals in chambers may result in hypoxia, another difference in relation to the human. As regards dosage of nicotine, there are experiments with very high doses (4 mg/kg/day)[13] that, in spite of leading to the expected deleterious effect, are not adequate in the comparison with the human.

The present experimental model allows the elaboration of many studies, evaluating the effect of different drugs or physical methods in the viability of this random pattern skin flap, in the rat, in the presence of nicotine. Being a standardized model, it also permits the comparison between results of different studies.

References

1. Kerrigan CL - Skin flap failure: pathophysiology. Plast Reconstr Surg. 1983; 72: 766-77.
2. Campos JHO - Interrupção do uso da nicotina nos retalhos cutâneos randomizados em ratos [Tese de Doutorado] São Paulo: Universidade Federal de São Paulo – Escola Paulista de Medicina; 2001.
3. Mc Farlane RM, De Young G, Henry RA - The design of a pedicle flap in the rat to study necrosis and its prevention. Plast Reconstr Surg. 1965; 35: 177-82.

4. Ugland O - Flaps and flaps necrosis. Acta Chir Scand. 1966; 131: 408-12.
5. Sasaki GH, Pang CY - Hemodynamics and viability of acute neurovascular island skin flaps in rats. Plast Reconstr Surg. 1980; 65:152-8.
6. Nolan J, Jenkins RA, Kurihara K, Schultz RC - The acute effects of cigarette smoke exposure on experimental skin flaps. Plast Reconstr Surg. 1985;75:544-9.
7. Van Adrichem LNA, Hovius SER, Van Strik R, Van Der Meulen JC - Acute effects of cigarette smoking on microcirculation of the thumb. Br J Plast Surg. 1992; 45: 9.
8. Ambrus JL, Mink IB - Effect of cigarette smoking on blood coagulation. Clin Pharmacol Ther. 1964; 5:428-31.
9. Dintenfass L - Elevation of blood viscosity, aggregation of red cells, haematocrit values and fibrinogen levels in cigarette smokers. Med J Aust. 1975;1: 617-20.
10. Forrest CR, Pang CY, Lindsay WK - Dose and time effects of nicotine treatment on the capillary blood flow and viability of random pattern skin flaps in the rat. Br J Plast Surg. 1987;40:295-9.
11. Gomes HC, Bueno PCS, Scardoeli CAC, Percario S, Landman MRL, Ferreira LM - Effect of N-acetylcysteine in a random skin flap after administration of nicotine, in the rat. F Méd (BR). 1998; 117: 209-11.
12. Fonseca AV, Gomes HC, Ferreira LM, Percario S, Aoki MM - Interação terazosina-propranolol na necrose de retalho cutâneo randomizado, após a administração de nicotina, no rato. IX Congresso de Iniciação Científica - Anais PIBIC 2001:48.
13. Mosely LH, Finseth F, Goody M - Nicotine and its effect on wound healing. Plast Reconstr Surg. 1978; 61: 570-4.

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RESUMO – Apresentação de um modelo experimental para estudar o efeito da nicotina em um retalho cutâneo randômico, no rato. Ratos adultos da mesma linhagem são expostos à nicotina por sete dias no período pre´-operatório. Após este período os animais são anestesiados e um retalho cutâneo randômico dorsal, de base cranial é elevado. No sétimo dia de pós-operatório, os ratos são anestesiados e as porcentagens de necrose distal são determinadas. Isto feito, é possível colher fragmentos de pele para análise bioquímica. Amostras de sangue podem ser colhidas por decapitação.

DESCRITORES: Retalhos cirúrgicos. Nicotina. Tabaco. Ratos.

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