The Effects of Pentoxifylline and Buflomedil Hydrochloride in Random Flaps in Rats under the Influence of Nicotine

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ABSTRACT

Many reports in the literature show the harmful effects of nicotine on microcirculation, leading to increased risk of skin flap ischemia. In an attempt to reverse these effects, an experimental trial was carried out to test two drugs: pentoxifylline, whose action changes blood viscosity, and buflomedil, which is a vasoactive substance that acts on microcirculation. After a 6-week treatment with nicotine, 40 rats were subdivided into 4 groups and operated on to prepare a random dorsal skin flap. Group A was not treated with any of the drugs. Groups B and C were treated for 7 postoperative days with pentoxifylline and buflomedil, respectively. Group D, the control group, received only saline solution. When compared, there was a significant difference between groups A and D ($p<0.05$), thus proving that nicotine effectively contributed to the occurrence of necrosis. The groups treated with the drugs showed an important clinical response in the improvement of flap survival; however, those values were not statistically significant ($p>0.05$). We concluded that these drugs showed some effectiveness in the reversal of the effects caused by nicotine, but it would be necessary to have larger samples to achieve statistically significant results.

INTRODUCTION

Clinical and experimental evidence shows an increased risk of skin flap necrosis in smokers. The increase in morbidity due to vascular hypoperfusion implies that nicotine metabolites significantly affect microcirculation[1]. Mosley and Finseth were the first to report the negative effects on healing, observing the reduction in blood flow in distal regions in the fingers, and changes in healing in the hands of smokers[2]. Another study, by Rees et al., showed that smoking patients are twice more likely to develop skin necrosis after rhytidectomy when compared to non-smoking patients[3].

Cigarette smoke has more than 3,800 components, but only carbon monoxide, nitric acid, hydrogen, cyanide, and nicotine have been widely identified as leading causes of adverse effects, both in the cardiovascular system and in skin microcirculation [1]. Nicotine reduces skin blood flow by 30 to 40%, because it stimulates the release of catecholamines and norepinephrine, which cause vasoconstriction. It also reduces the synthesis of vasodilating prostacyclin and increases the release of A_2 thromboxane, a vasoconstritor. In microcirculation it induces platelet activation and aggregation, producing microthromboses[2]. Moreover, it changes the healing process of wounds by reducing proliferation fibroblasts and macrophages and stimulating catecholamines, which are co-factors in the formation of chalones, which, in turn, inhibit the epithelialization process.

There have been some studies with vasodilating drugs, like nifedipine and nitroglicerine in rats exposed to cigarette smoke, with significant clinical results in the survival of random flaps[4].

Pentoxifylline is a byproduct of methylxanthines that is used in the treatment of intermittent claudication. It improves blood viscosity, as it increases the capacity of deformation of erythrocytes and granulocytes, reduces fibrinogen levels and thromboxane release, inhibits platelet aggregation, and increases prostacyclin levels. These effects were not seen in people with normal blood coagulation, only changes in blood viscosity[2].

Buflomedil hydrochloride is a vasoactive substance that acts on cerebral and peripheral microcirculation, working by blocking the passage of calcium to vascular smooth muscles. It does not have an adrenergic blocking action nor does it change hemodynamic constants [5]. Due to these effects, it has been used empirically in plastic surgery to improve flap perfusion, although no studies yet show its effectiveness.

OBJECTIVE

The first objective was to establish the negative effects of nicotine in the survival of random flaps in rats. The second objective was to check the influence of pentoxifylline (TrentalÒ or PentoxÒ) and buflomedil hydrochloride (BufedilÒ) in the survival of random flaps in rats exposed to nicotine at doses that produce levels comparable to human smoking.

MATERIALS AND METHODS

Forty female rats of the race LOU / M, weighing between 250 g and 350 g and aged between 6 and 8 months were used in this study. This group was randomly divided into 4 groups, with 10 animals each:

A: rats exposed to nicotine

B and C: rats exposed to nicotine and drugs

D: control group

GROUP A: NICOTINE + SALINE SOLUTION

Nicotine: 0.5 mg/kg in 0.1 ml of saline solution injected subcutaneously, twice a day, for 6 weeks in the preoperative and 1 week in the postoperative.

Saline solution: 1 ml of saline solution, intraperitoneally, for 1 week in the postoperative.

GROUP B: NICOTINE + PENTOXIFYLLINE

Nicotine: 0.5 mg/kg in 0.1 ml of saline solution injected subcutaneously, twice a day, for 6 weeks in the preoperative and 1 week in the postoperative.

Pentoxifylline: 20 mg/kg in 1.0 ml of saline solution, intraperitoneally, twice a day, for 1 week in the postoperative.

GROUP C: NICOTINE + BUFLOMEDIL

Nicotine: 0.5 mg/kg in 0.1 ml of saline solution, injected subcutaneously, twice a day, for 6 weeks in the preoperative and 1 week in the postoperative.

Buflomedil: 34.2 mg/kg in 1.0 ml of saline solution, intraperitoneally, twice a day, for 1 week in the postoperative.

GROUP D: CONTROL GROUP: SALINE SOLUTION + SALINE SOLUTION

Saline solution: 0.1 ml of saline solution, subcutaneously, twice a day, for 6 weeks, and 1.0 ml, intraperitoneally, twice a day, for 1 week in the postoperative.

TECHNIQUE

We initially conducted a pilot test to adjust the dose of nicotine. For one week, the 30 rats were submitted to the application of intradermal nicotine at the initial dose of 2 mg/kg, which was slowly reduced until it reached a non-convulsant dose. The stan-

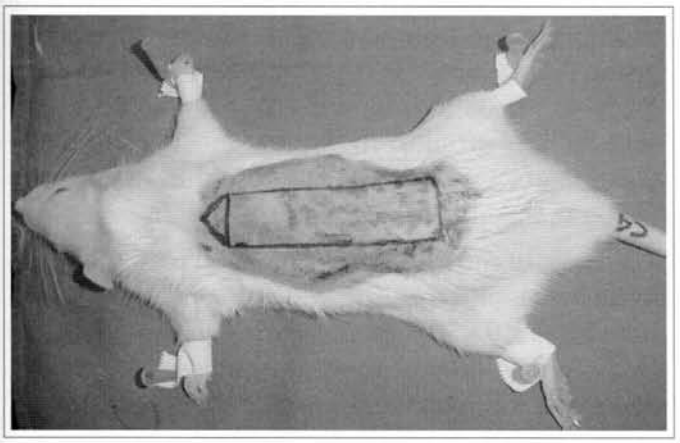

Fig. 1 – Marking of flap on the dorsal region of the rat.

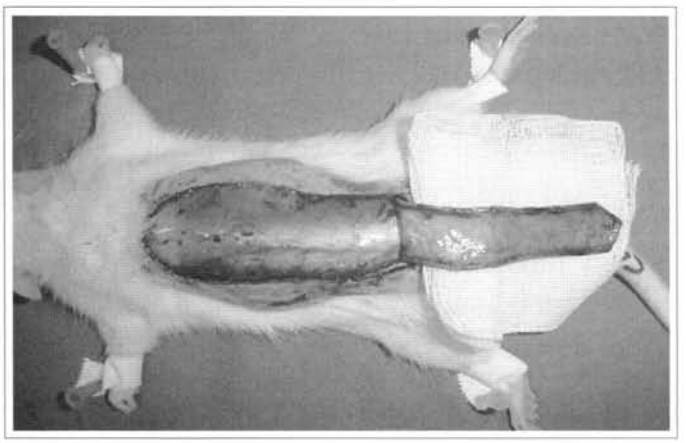

Fig. 2 – Construction of dorsal flap.

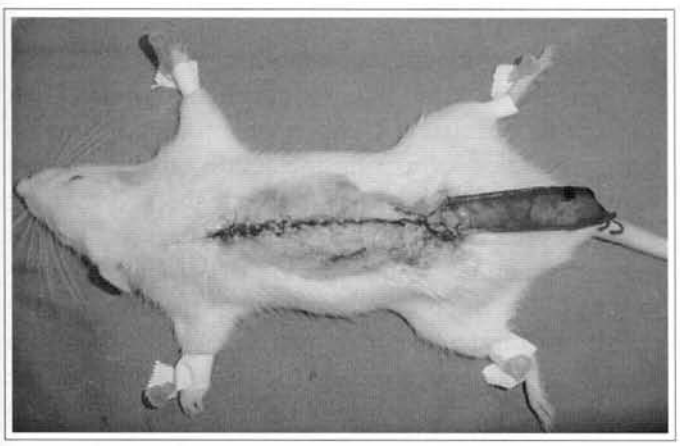

Fig. 3 – Primary synthesis of donor site with fixation of graft at the base of the flap.

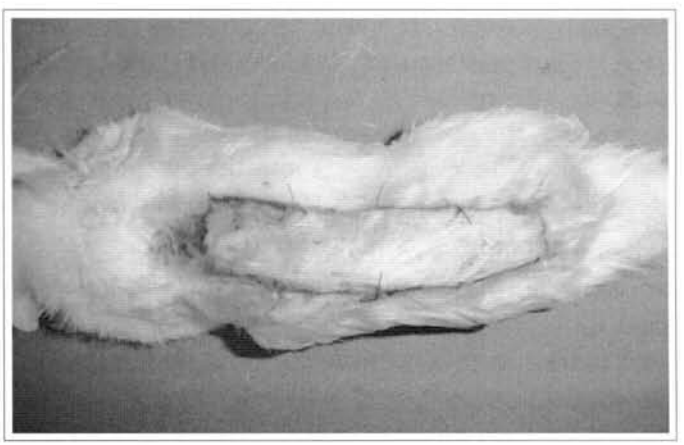

Fig. 4 – Fixation of flap on the dorsal region after suture of the donor site.

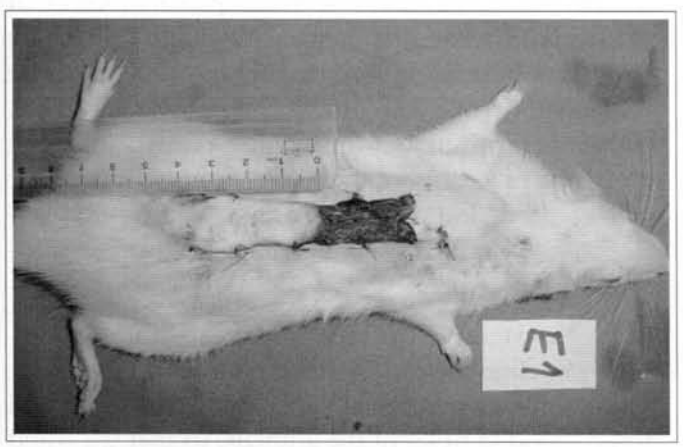

Fig. 5 – Measurement of viable areas in centimeters.

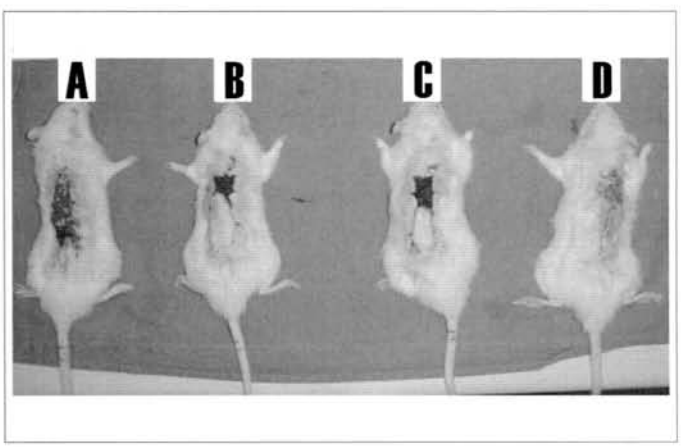

Fig. 6 – Comparison of groups: A= nearly total necrosis of flap, B and C = partial necrosis and D =minimum necrosis.

dard dose was established at 0.5 mg/kg, compatible with many reports in the literature[1, 2].

After 6 weeks of treatment with nicotine for groups A, B, and C, and saline solution for group D, the rats underwent a surgical procedure. Ketamine + Clorpromazine were used for anesthesia at the intramuscular doses of 2.5 ml/kg and 0.5 ml/kg, respectively. Dorsal flaps were obtained according to McFarlane's caudal base technique[6] modified by Hammond[7], based on the level of the posterior iliac crest, formed by skin and the panniculus carnosus (Figs. 1 and 2).

2 x 7 cm molds were used to design these flaps, with a compensation triangle on their distal end (2 x 1.5 x 1.5), and they were used as total skin grafts (Fig. 1).

After dissection and elevation of the flap, first the donor site was closed with continuous suture (Fig. 3). Then the graft was sutured to the base of the lifted flap to prevent any contact with the vascularized bed where attachment of the donor site is not normally possible. The flap was attached over the dorsal skin and sutured with 4 equidistant lateral stitches, and one on each angle (Fig. 4). After the procedure, the dorsal region of the rats was covered with a BioclusiveÒ dressing and they were put in separate cages, receiving water and food *ad libitum*.

The rats of groups A, B and C continued receiving nicotine during the postoperative, and groups B and C received pentoxifylline and buflomedil, respectively. Group D continued receiving intramuscular and intraperitoneal saline solution.

The percentage of skin necrosis in the flaps was assessed on the 5th and 7th postoperative day and observed for another day to confirm these data. Measures were taken in millimeters and the area of viable tissue was calculated in square centimeters (Fig. 5).

STATISTICAL ANALYSIS

The Anova F Test was used in this study to analyze the four groups and the T Test was applied to for the paired analysis of groups A x B, A x C, A x D, B x D and C x D. Averages and standard deviations were calculated. The level of significance adopted was "p" smaller than or equal to 0.05.

RESULTS

The results of each group are shown in Table I.

In the entire experiment there were a total of 4 deaths without an identifiable cause. These deaths occurred either during the anesthetic procedure or in the immediate postoperative. Some rats were excluded from the study because, despite care to isolate the operated area with an occlusive dressing, autophagia of the flap occurred, hindering appropriate evaluation.

Averages and standard deviations were calculated for each group for these results. They were respectively: group A = 6.83 ± 0.70; group B = 7.71 ± 1.73; group C = 7.68 ± 1.42; and group D = 8.91 ± 2.67 (Fig. 8). The group with the smallest standard deviation was group A, and the one with the largest deviation was D.

The result of the F Test was 1.65, with $p>0.05$, therefore, not significant. The T Test paired analyses resulted in $p<0.05$ when comparing groups A and D (Fig. 7). The other groups, in spite of the considerable clinical difference, did not have a significant "p" at 5% (Fig. 6).

DISCUSSION

The pharmacokinetics of nicotine has been widely studied and there are many experimental models that attempt to simulate the effects of smoking in human beings. Studies comparing the action of nicotine as a base with the action of tartarate salt showed that the first has greater and longer plasmatic levels. In this manner, when one chooses using the base, it is necessary to titrate the previously defined dose to administer the tartarate. For this reason, we conducted a weeklong pilot test, to study the clinical effects of the drug and define the non-lethal dose for the chosen rat strain.

Another difficulty in studying the effects of nicotine in experimental models is to reach plasma levels that effectively simulate that of smoking adults. There are many variables, such as type of exposure (cigarette smoke, subdermal injections, slow-release capsules), amount of exposure/day for keeping a constant plasma level, time of treatment, type of nicotine, strain of the animals used, and all of them influence results considerably. Therefore, these variables need other studies to be better defined.

Based on extensive research of the literature, we decided to define the time of treatment with nicotine at 6 weeks. During surgical procedures, we drew and stored blood samples of the animals, so that we could continue the studies with the plasmatic levels of

cotinine, a metabolite of nicotine. However, we may regard the treatment as effective, considering that when comparing groups A (nicotine) and D (saline solution), we found a significant difference ($p<0.05$) in the survival of flaps of non-smoking rats.

The action of pentoxifylline on the survival of random flaps has been fairly well explored. Its hematological effects include increased deformation capacity of erythrocytes and increased capillary blood flow[10]. Other results have been described nonetheless. Pentoxifylline increases hypercoagulability, decreases platelet aggregation, by increasing plasmin, plasminogen activating factor, antithrombin III, and by decreasing fibrinogen, alpha-2-antiplasmin, alpha-1-antitrypsin and alpha-2-macroglobulin[11].

Despite all the effects described, the drug's clinical applicability is still controversial. Experimental studies have shown conflicting results. Some authors such as Karacaoglan et al. have demonstrated an increase in flap survival with pentoxifylline[12,16]. Others[13] have not correlated microscopic changes with increased flap survival. Another major aspect is the initial time of treatment. For Williams et al.[14] preoperative treatment of at least 14 days is necessary in order to reach desired results. On the other hand, the work carried out by Hayden[15], that tested 3 different utilization regimens including preoperative administration, did not confirm previous findings.

In the present study we began using the drug on the same day of surgery, based on studies performed previously[15], and maintained its use for 7 days. Results showed an improvement in flap survival, as shown in Table I and Fig. 8, although values were not significant ($p>0.05$).

Buflomedil hydrochloride has an inhibitory effect on platelet aggregation and improves the deformation capacity of red blood cells with abnormal flowability. *In vitro* studies suggest that the drug has an unspecific antagonist effect on the calcium ion and an unspecific alpha receptor blocking effect[5].

Experimental studies have shown adequate results in reverting flap ischemia. Uhl et al.[18] showed that the drug can be used therapeutically in skin flaps and that preoperative treatment for an additional 5 days did not change previous results. Another study, in which treatment was given 4 hours before the operation and 5 minutes after, attained significant values for both groups[19].

In our study, buflomedil and pentoxifylline were used in the same way. Results were also very similar. There was no significant result in the group treated with buflomedil in comparison to the remaining groups, despite marked clinical improvement. This finding, like the results found for the pentoxifylline group, may be due to the small sample size, although it is an experimental study. Contrasting results would probably be diluted in a higher number of events, as with rat D1, whose results are completely different from those in the remainder of the group. Thus, we may infer that the present study would tend to become statistically significant, with a higher number of observations in each group.

Table I

Rat	Grup A	Grup B	Grup C	Grup D
1	†	8.2	7.6	4.2
2	7	6.6	6	11.6
3	7	†	5.6	*
4	5.8	6.6	8.6	*
5	7.2	6.4	8.2	*
6	6.4	9.4	9	9
7	*	7.8	11	7.2
8	†	10	7.4	10.6
9	8	6.4	6	11.6
10	6.4	†	†	8.2
Mean	**6.83**	**7.68**	**7.71**	**8.91**

Measurements of viable flap areas of each group in cm^2.
*Impossible to measure the viable area adequately. †Death.

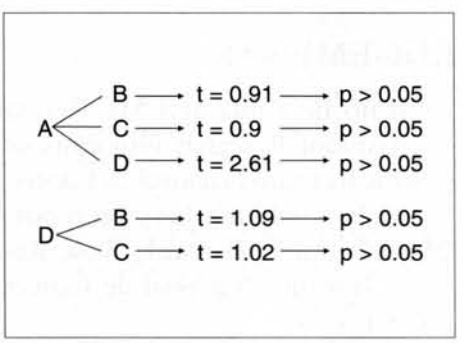

Fig. 7 – Results of T test for each pair and sequencee.

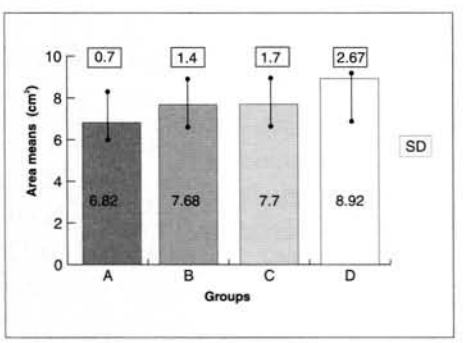

Fig. 8 – Area means of each group and standard deviations.

CONCLUSION

Various drugs have been tested for

reverting the deleterious effects of nicotine, although findings are still unclear. No definitive results have been found, but there is clinical evidence that some drugs exert favorable effects, and improve the survival of skin flaps. The present study has confirmed the effects of nicotine described elsewhere, by showing greater damage to flaps constructed in rats exposed to the drug. Although buflomedil and pentoxifylline improve vascularization of flaps, no statistically significant results have been recorded. We therefore need to expand our research, by increasing the number of cases in each group to create significant data.

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