Viability of a random pattern dorsal skin flap, in diabetic rats[1]

Viabilidade do retalho cutâneo randômico dorsal em ratos diabéticos

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ABSTRACT

Purpose: Evaluation of the viability of a random pattern dorsal skin flap, in rats with experimentally induced *diabetes mellitus*. **Methods:** Thirty adult, male, Wistar EPM rats were distributed, at random, in two groups: I - Control (flap elevation) and II - Experimental (flap elevation ten days after alloxan induced diabetes). **Results:** The mean areas of necrosis in the different groups were 36.4% (Control) and 52.1% (Experimental). **Conclusion:** The random pattern dorsal skin flap was less viable in the diabetic rats.

Key words: Diabetes mellitus. Cutaneous flap. Aloxane. Rats.

RESUMO

Objetivo: Avaliar a viabilidade do retalho cutâneo randômico dorsal em ratos experimentalmente induzidos ao *diabetes mellitus*. **Métodos:** Foram utilizados 30 ratos Wistar, adultos, machos, distribuídos em 2 grupos: I – controle – submetidos somente ao retalho cutâneo e II – experimental – submetidos ao retalho cutâneo após 10 dias da indução do *diabetes mellitus* com aloxano. **Resultados:** O grupo controle teve 36,39% e o grupo experimental 52,06% de média de área de necrose. **Conclusão:** A viabilidade do retalho cutâneo randômico dorsal foi menor no rato diabético do que no rato normal.

Descritores: Diabetes mellitus. Retalho cutâneo. Aloxano. Ratos.

Introduction

Due to its high prevalence and potential deleterious effects on a patient´s physical and psychological state, diabetes mellitus, which can result in a morbid condition, is a major medical concern[1, 2]. According to the World Health Organization (WHO) the number of diabetics has doubled in the past few years and is expected to double once again by the year 2025. Today, there are 160,000 diabetics worldwide, 10,000 only in Brazil, which makes the country the sixth in the world rank[3]. In humans, *diabetes mellitus* is one of the most prevalent conditions with spontaneous manifestation. In animals, it can be induced by partial pancreatectomy or by the administration of diabetogenic drugs such as alloxan[4].

These agents selectively destroy the Langerhans islet â-cell. The best known drug-induced diabetes model is the alloxan diabetes. Alloxan, a derivative of uric acid, as well as of other substance of different chemical groups, cases â-cells to degranulate and consequently degenerate[1,5,6,7,8,9,10]. Skin flaps are largely used in all fields of plastic surgery, especially in reconstructives[11]. They have been utilized for centuries and, during this time, one concerning has been to develop techniques to provide more assurance in skin flap realization. The research of skin flaps survival mechanisms and your possible curate factors have been issue for publications[12, 13, 14].

The purpose of this experimental study was to evaluate the viability of a random pattern dorsal skin flap, in rats with alloxan induced diabetes.

Methods

This study was approved by Commission of ethics in Research of Federal University of Sao Paulo. All the procedures followed, rigorously, the existent regulations about animal experimentation. Thirty adult, male, Wistar EPM rats, weighting around 300g, were distributed, at random, in two groups of 15 animals: I – Control, exposed to elevation of a skin flap; II – Experimental, exposed to flap elevation ten days after alloxan induced diabetes. The animals were individually allocated in cages, receiving standard food and water *ad libitum*. Rats in Group II were kept in 48 hours fast before diabetes induction. They were weighted and anesthetized by her inhalation in glass dome. A solution of alloxan at 2 % diluted in saline at 0,9 % was administered to the animals in a single dose corresponding to 40 mg of

alloxan per Kg of animal weight injected into their penial vein[15] (Figure 1).

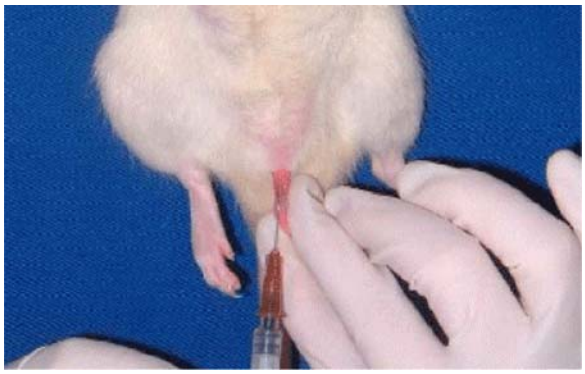

FIGURE 1 - Administration of a lloxan in the animal's penial vein.

In order to assess the effect of alloxan and to chemically establish the diabetic condition, an incision was done in any of the four veins in the tail of the rat using a 15 scalpel blade 10 days after the diabetes induction. A sample of the rat's venous blood was collected on a reagent strip 10 days after the diabetes induction procedure for blood glucose level determination using a portable glucose analyzer[15] (Figure 2).

FIGURE 2 - Portable blood glucose test device.

The level of serum glucose considered to be normal in *rattus novergicus* ranges from 50 to 135 mg/100ml[15]. In this study, rats with glucose levels above 200 mg/dl were considered as having severe diabetes. After determination of the glicemic state, the rats were weighted again and after 24 hours, anesthetized with Tiletamin Chloridrate (25 mg/kg) and Zolazepam Chloridrate (25 mg/kg), intraperitoneally. Then, the animals were positioned over a flat surface, with extended limbs, their backs were shaved and a random pattern, cranially based dorsal skin flap was elevated. Delineation of the flap was done in the dorsum of the rats by means of a transparent plastic pattern, cut in the standard dimensions (4 x 10 cm). The flap was then incised with scalpel, being elevated in a plane superficial to the deep muscular fascia, including the superficial fascia, *paniculum carnosum*, subcutaneous tissue and skin[16]. After flap elevation, an impermeable plastic barrier, cut in the same dimensions, was placed between the flap and its donor bed[17,18](Figures3,4). After that, the rats were placed in individual cages, receiving food and water *ad libitum*. The percentage of skin flap necrosis area was calculated on the seventh postoperative day via the paper template method.

FIGURE 3 – Random skin flap raised.

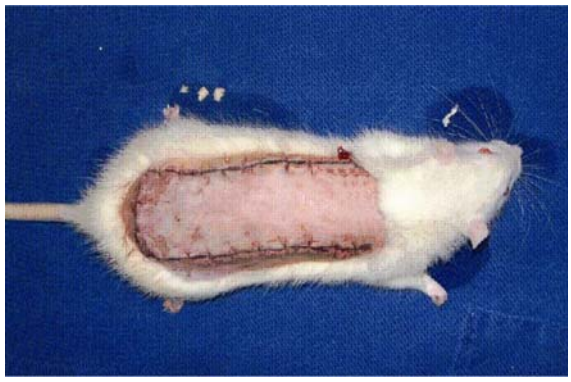

FIGURE 4 – Suture with 4-0 nylon stitches after the flap was raised and a plastic barrier was interpositioned.

The limit between viable tissue characterized by soft skin, rosy, warm and with hair and necrotic tissue by stiff skin, dark, cool and without hair was demarcated in the animals[16] (Figures 5,6). A mould of entire flap was drawn and cut in transparent paper, being checked in a precision balance (+/ - 0,001g error). It was cut from this fragment just the correspondent area to flap necrosis that was also checked.

After that, it was used the following formula:

$$\text{Percentage of necrosis area of the flap} = \frac{\text{weight of paper template of flap necrosis}}{\text{weight of paper template of total area of flap}} \times 100$$

Considering the nature of the data involved in this study, like percentage of necrosis, non parametric tests were used in the statistical analysis. The Mann-Whitney test (non parametric, independent groups) was used to compare groups 1 and 2 as regards the percentages of necrosis in the flaps. The significance level was fixed in 0.05 or 5% (<0.05). Significant values were marked (*); non significant values were identified as N.S.. The weights of the animals in the immediate preoperative period were compared using the "t" Student's test for independent groups (parametric, non paired).

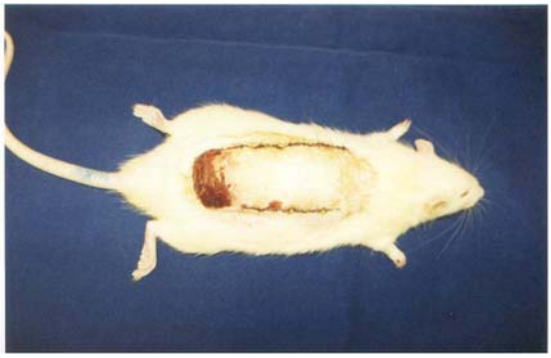

FIGURE 5 - Necrotic tissue (control animal).

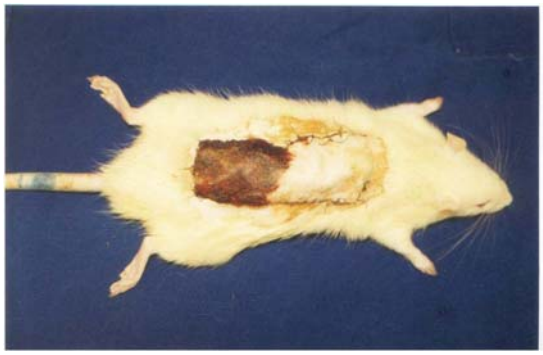

FIGURE 6 - Necrotic tissue (experimental animal).

Results

All the alterations caracteristic of a diabetic rat were observed, like polydipsia, polyuria, weight loss, asthenia, dehydration and weight loss. Alongside with all these signs, the percentages of necrosis in the control animals varied from 24.2 to 46.9% (mean 36.4%) and in the diabetic animals ranged between 35.5 and 54.9% (mean 52.1%) (Table 1, Figure 7).

TABLE 1 - Percentages of necrosis in the seventh postoperative day (%).

	Control Group	Experimental Group
	48,43	58,42
	34,71	66,95
	48,79	61,24
	20,80	53,32
	48,15	51,85
	31,06	39,72
	33,14	35,50
	46,82	54,00
	43,10	48,87
	24,25	60,69
	25,52	54,90
	40,42	48,31
	30,15	65,21
	37,78	40,17
	32,85	41,80
Mean	36,39	52,06
SD	9,29	9,65

"t" Test for independent groups / t calculated + 4.53 ($p<0.001$)* / G1 < G2*

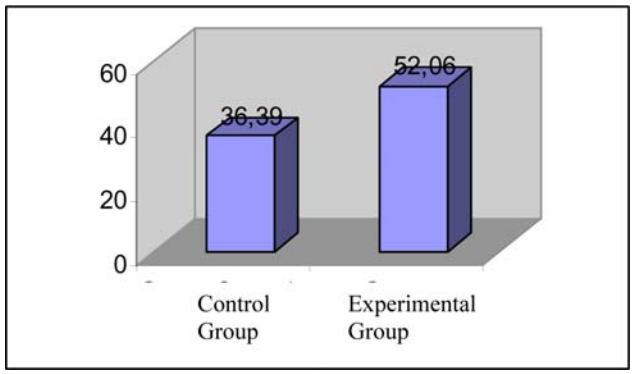

FIGURE 7 - Mean percentages of necrosis in the seventh postoperative day (%).

Discussion

Experimental diabetes induced by chemical agents, such as alloxan, causes kidney lesions that are similar to human diabetic nephropathy. It is a derivative of uric acid, able to induce permanent diabetes in animals after 24 hours, due to its selective toxic effect on beta pancreatic cells, leading to a primary insulin insufficiency of the pancreas[21, 22]. Alloxan may be administered intravenously, intraperitoneally or subcutaneously, but in the present study the intravenous route was chosen, based on the rapidity of the effect and the possibility of using a lower dose of this toxic substance. Alloxan, once administered intravenously, leads to diabetes by means of destruction of the pancreatic insular tissue, including the beta cells that produce insulin, which suffer degenerative alterations and necrosis. The result is a diabetic animal, an excellent experimental model to study organic alterations developed by insulin deficiency[19].

Among the complications related to diabetes is tissular healing impairment. In diabetic patients, protein synthesis is reduced while catabolism is increased, with changes in the tissues' growing process, regeneration and reparation. Healing is altered due to problems in the proteic metabolism and fibroblasts depression. Skin flaps represent, often, the only adequate option for surgical repair after removal of skin lesions and tumors, specially when larger reconstructions are necessary, demanding adequate functional and aesthetic results.

The most common complication after reconstructions with flaps is partial necrosis[3, 4]. This undesirable event demands more operative procedures, increasing hospitalization period and delaying the patients return to normal activities. Failure in the operation may make new reparation very difficult and mine the surgeon-patient relationship[17]. The most important aspect that rules the viability of a skin flap is adequate nutritive blood flow in the microcirculation.

In the present study, Control animals (Group I) had blood glucose levels between 60 and 94 mg/ml, what according to the literature indicate normal rats, while Experimental animals (Group II) had glucose levels between 212 and 418 mg/ml, evidencing an uncontrolled diabetic state. Besides hyperglycemia, it was observed that rats that received alloxan presented clinic signs of the disease, similar

to those described for humans, polydipsia (abnormal thirst), polyuria (increased urine volume), weight loss (due to lean mass loss), asthenia (weakness due to the inability to use glucose as a source of energy), dehydration (due to the animal body's attempt to get rid of the excess blood glucose as the normal process of storing glucose in the body cells is impaired) and weight loss[1].

The dorsal random pattern skin flap, cranially based, in the rat, used in the present work, was described by McFarlane et al.[16], as an experimental flap model to study skin necrosis and its prevention. Dimensions are 10 x 4 cm. Usually, it is associated with a distal necrosis rate between 25 and 50%, but in 5.7% of the animals there is no significant necrosis. In the herein presented study, percentages of necrosis in control animals (Group I) ranged between 20.8 and 48.4% (mean 36.4%). In the experimental rats (Group II), the values ranged between 35.5 and 65.2% (mean 52.1%). Statistical analysis showed a significant difference between these groups. Uncontrolled diabetes increased the percentage of distal necrosis in this flap in relation to normal rats. Future perspectives involve the use of drugs able to reduce distal necrosis in this random skin flap in uncontrolled diabetic rats.

Conclusion

Uncontrolled diabetes reduced the viability of this random pattern skin flap in the rat.

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