

Acetylcysteine in random skin flap in rats¹

Acetilcisteína em retalho cutâneo randômico em ratos

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ABSTRACT

Purpose: Analyze the ability of Acetylcysteine to reduce distal necrosis in a random skin flap, in the rat. **Methods:** The present study utilized 28 adult male Wistar-EPM rats distributed, at random, in two groups of 14 animals. Control group rats (CG) received distilled water and Acetylcysteine group animals (NACG) received NAC (300 mg/kg) by oral infusion, 15 minutes before flap elevation. On the seventh postoperative day, percentage of distal necrosis was determined and skin samples collected in order to allow determination of MDA levels. **Results:** The mean necrotic area in CG group (control) was 66 % and in NACG group (Acetylcysteine) 52 %, a statistically significant difference according to the Mann-Whitney test (U calc = 25; U crit = 45). MDA levels were lower in the CG flap skin samples than in the NACG samples (U calc = 24; U crit = 45), the opposite being true in the normal skin samples (U calc = 10; U crit = 45). **Conclusion:** Acetylcysteine was effective, according to the model used, reducing the percentage of distal necrosis in NACG rats.

Key words: Surgical Flaps. Lipid peroxidation. Acetylcysteine. Rats.

RESUMO

Objetivo: Analisar a capacidade da Acetilcisteína em reduzir a necrose distal em um retalho cutâneo randômico, no rato. **Método:** O presente trabalho utilizou 28 ratos machos adultos Wistar-EPM divididos, ao acaso, em dois grupos de 14 animais. Os ratos do grupo controle (CG) receberam água destilada e os animais do grupo Acetilcisteína (NACG) receberam NAC (300 mg/kg) por infusão oral, 15 minutos antes da elevação do retalho. No sétimo dia de pós-operatório, a porcentagem de necrose distal foi determinada e amostras de pele colhidas para permitir a determinação dos níveis de MDA. **Resultados:** A área média de necrose no grupo CG (controle) foi 66 % e no grupo NACG (Acetilcisteína) 52 %, uma diferença estatisticamente significativa de acordo com o teste de Mann-Whitney (U calc = 25; U crit = 45). Os níveis de MDA foram menores nas amostras de pele do retalho no grupo CG do que nas amostras do grupo NACG (U calc = 24; U crit = 45), o oposto sendo verdadeiro nas amostras de pele normal (U calc = 10; U crit = 45). **Conclusão:** A Acetilcisteína foi eficaz, de acordo com o modelo usado, reduzindo a porcentagem de necrose distal nos ratos NACG.

Descritores: Retalhos Cirúrgicos. Peroxidação de Lipídeos. Acetilcisteína. Ratos.

Introduction

The risk of skin slough after elevation of a random flap is a matter of preoccupation in Plastic Surgery^{1,2}. Experimental works focussing on the reduction of skin necrosis have been done and the benefic role of antioxidant drugs discussed^{3,4}. Acetylcysteine, a well known antioxidant substance, had been used before with favorable results^{3,5}. Therefore, the role of Acetylcysteine in the protection of random skin flaps in rats was studied.

Methods

Twenty-eight adult male Wistar-EPM rats were divided, at random, into two groups of 14 animals. Animals in the control group (CG) received distilled water and rats in group NACG received Acetylcysteine (300 mg/kg) by oral infusion, 15 minutes before flap elevation. Pentobarbital (40 mg/kg)

was used intraperitoneally to provide anesthesia. After that, the rats were shaved and a cranially based random pattern skin flap was elevated (10x4 cm) on the back of the animals⁶. A plastic barrier was then interposed between the flap and its bed⁷. The wound was closed with simple nylon 4-0 stitches.

On the seventh postoperative day, necrosis on the distal portion of the flaps was estimated by means of the paper template method⁸. Skin samples were obtained to permit analysis of malondyaldehyde (MDA) levels⁹. One sample was collected from the transition area between viable and necrotic tissue and the other, distant from the flap, from a normal skin area.

Results

Necrotic area in CG group (control) ranged between 51 % and 77 % (average 66 %), while that in NACG group

(Acetylcysteine) ranged between 38 % and 70 % (average 52 %). MDA concentrations in normal skin samples ranged between 1255 and 3345 ng/ml (average 2038 ng/ml) in CG and between 177 and 1889 ng/ml (average 935 ng/ml) in NACG. MDA levels in the viable to necrotic transition skin samples ranged between 417 and 1915 ng/ml (average 1235 ng/ml) in CG and between 1051 and 4465 ng/ml (average 2346 ng/ml) in NACG. Mann-Whitney test for independent groups showed statistically significant differences (Tables 1-3).

TABLE 1 - Percentage of necrotic area on the seventh postoperative day. Mann-Whitney test (statistically significant)

Animal	CG	NACG
1	66	70
2	51	57
3	63	45
4	77	62
5	67	*
6	68	55
7	67	60
8	71	42
9	59	44
10	51	42
11	75	60
12	59	38
13	72	45
14	72	*
Average	66	52

* two rats died in the postoperative period.

TABLE 2 - Concentration of malondyaldehyde (ng/ml) from the normal skin samples on the seventh postoperative day.

Animal	CG	NACG
1	2049	1889
2	2012	874
3	1458	862
4	1255	1287
5	3345	*
6	2393	1169
7	1784	1417
8	2116	519
9	1612	578
10	2329	354
11	2332	1582
12	2339	177
13	1614	519
14	1885	*
Average	2038	935

Mann-Whitney test (statistically significant)

* two rats died in the postoperative period.

TABLE 3 - Concentration of malondyaldehyde (ng/ml) from the viable to necrotic transition skin samples on the seventh postoperative day.

Animal	CG	NACG
1	1683	4465
2	786	2456
3	884	1944
4	759	4240
5	1683	*
6	1915	1523
7	1477	3201
8	697	1700
9	417	1850
10	1005	1252
11	1299	2657
12	1661	1051
13	1545	1815
14	1485	*
Average	1235	2346

Mann-Whitney test (statistically significant)

* two rats died in the postoperative period.

Discussion

Random pattern skin flaps represent valuable and common procedures in Plastic Surgery. Nevertheless, the risk of ischemia and necrosis is always present, justifying the need of research for protective measures. Free radicals produce deleterious effects on skin flaps and continue to be intensively studied. Recent works have focussed on the antioxidant drugs.

Acetylcysteine (NAC) is a very well known antioxidant compound, used extensively in Pneumology. Easily obtainable and manipulated, it has low toxicity and the advantage of oral administration^{3,5}. The dosis herein utilized (300 mg/kg/day) was two times higher than that previously presented⁵ and was chosen in view of the low toxicity of this drug. It must be stressed that this is a high dose, not readily applicable to human beings, but considered necessary in order to promote adequate antioxidant effect in this experimental model.

The herein revisited McFarlane-like skin flap represents a practical experimental tool to study distal necrosis. The plastic barrier interposed between the flap and the donor bed (a latter contribution), prevents flap revascularization through the bed vessels. The percentage of necrosis was determined via the paper template method, another very usefull procedure.

In CG group (control) rats, the average percentage of necrosis was 66 %; in NACG group (Acetylcysteine) it was 52 %. Acetylcysteine was effective, according to the model used, reducing the percentage of distal necrosis on the flap in NACG animals in relation to CG rats.

The antioxidant role of Acetylcysteine was studied through the determination of MDA levels in skin samples. MDA was measured from two skin samples of each animal from both groups, using normal and viable to necrotic transition skin.

In order to allow proper study of the antioxidant action of Acetylcysteine, this animal model had to promote both ischemia and necrosis in the flaps, with consequent lipid peroxidation and liberation of its products and byproducts. Normal skin samples in CG group (control) revealed an average MDA level of 2038 ng/ml, as opposed to NACG group (Acetylcysteine), with 935 ng/ml, corroborating the antioxidant action of this substance.

Notwithstanding, MDA values obtained from samples of skin from the viable to necrotic transition area were lower in CG (1235 ng/ml) than in NACG (2346 ng/ml), an unexpected finding, but previously presented¹⁰. In that occasion, the author stated that high MDA levels do not necessarily lead to cellular death, provided that the proteic thiols remain unharmed.

The present study contributed to the understanding of the oxidative processes that occur after elevation of a random skin flap, as well as the benefic role of the antioxidants.

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