

Uric acid as a monitor of oxidative stress in a random skin flap in rats¹

Sueli Teresinha Freire²
Lydia Masako Ferreira³
Heitor Carvalho Gomes⁴
Sandro Percario⁵

Freire ST, Ferreira LM, Gomes HC, Percario S. Uric acid as a monitor of oxidative stress in a random skin flap in rats. *Acta Cir Bras* [serial online] 2003 Nov-Dec;18(6). Available from URL: <http://www.scielo.br/acb>.

ABSTRACT – Purpose: To verify the possible role of uric acid as a monitor of oxidative stress in a random skin flap, in the rat. **Methods:** This study utilized 21 adult male Wistar-EPM rats divided, at random, in three groups of seven animals, to evaluate the behavior of uric acid levels during oxidative stress zero, 5 and 15 minutes after elevation of the flap. According to the time schedule, skin samples were collected in order to allow determination of uric acid and malondialdehyde (MDA) levels. **Results:** Uric acid levels in the skin samples did not change with time, but the values in normal skin were higher since the beginning. Malondialdehyde (MDA) levels in the skin samples remained stable throughout. **Conclusion:** Uric acid levels in the flap skin were lower than those in the normal skin, denoting local oxidative stress.

KEY WORDS – Surgical flaps. Lipid peroxidation. Uric acid. Rats.

Introduction

Skin flaps necrosis is a matter of great concern to the Plastic Surgeons. In order to reduce the risk of it, many experimental studies have been carried out, specially in the field of oxidative stress and antioxidants. Oxidative stress is a phenomenon that occurs after elevation of ischemic flaps, with excessive production of free radicals and damage to the cells. Antioxidants are substances that reduce or block the deleterious effects of these radicals^{1,2}. Uric acid is an endogenous antioxidant and represents part of the tissues' natural defense against oxidative stress. It is an important antioxidant in the serum, representing a final product in the metabolism of the purines and acts as a potent free radical scavenger, inhibiting lipid peroxidation³. Malondialdehyde (MDA) is a product of lipid peroxidation, helpful in studies that deal with oxidative stress, serving as a quantifier of the aggression suffered by the tissues⁴. This

study determined the levels of both uric acid and MDA in skin samples, at early stages after elevation of a random skin flap and it aims to elucidate the role of this acid in this model of oxidative stress.

Methods

Twenty-one adult male Wistar-EPM rats were divided into three groups of seven animals: an immediate postoperative (GPOI), a five-minute postoperative (GPO5) and a fifteen-minute postoperative group (GPO15). After anesthesia with pentobarbital (40 mg/kg) intraperitoneally, the rats were shaved and a random skin flap was elevated, with a cranial base, measuring 10x4 cm, on the back of the animals⁵. After that, and according to the time schedule, skin samples were collected in order to allow determination of uric acid and MDA levels. One sample was obtained from the middle of the flap (5 cm from the base), another from the distal end, and one more, distant from the flap, from a normal skin area.

Results

Uric acid levels in the skin samples in the middle of the flap ranged between 1.9 mg/dl and 8.6 mg/dl (average 4.9 mg/dl), 1 mg/dl and 13 mg/dl (average 4.6 mg/dl) and 0.9 mg/dl and 11.2 mg/dl (average 4.9 mg/dl), in groups POI, PO5 and PO15, respectively.

Distal end of the flap's uric acid values ranged between 1.5 mg/dl and 6.1 mg/dl (average 3.6 mg/dl), 1.2 mg/dl and 13.3 mg/dl (average 5.6 mg/dl) and 1.1 mg/dl and 11.2 mg/dl (average 5.6 mg/dl), in groups POI, PO5 and PO15, respectively.

Uric acid values in normal skin samples ranged between 3.6 mg/dl and 8.1 mg/dl (average 5.2 mg/dl), 2.5 mg/dl and 28.6 mg/dl (average 10.7 mg/dl) and 2.5 mg/dl and 13 mg/dl (average 6.6 mg/dl), in groups POI, PO5 and PO15, respectively.

Malondialdehyde (MDA) levels in the skin samples in the middle of the flap ranged between 1282 ng/ml and 21558 ng/ml

1. Trabalho da Disciplina de Cirurgia Plástica da Universidade Federal de São Paulo – Escola Paulista de Medicina (UNIFESP – EPM).
2. Mestre em Medicina pelo Programa de Pós-Graduação em Cirurgia Plástica Reparadora da UNIFESP – EPM.
3. Professora Titular da Disciplina de Cirurgia Plástica da UNIFESP – EPM.
4. Professor Colaborador Doutor da Disciplina de Cirurgia Plástica da UNIFESP – EPM.
5. Professor Colaborador Doutor da Disciplina de Cirurgia Vasculard da UNIFESP – EPM.

(average 8310 ng/ml), 2565 ng/ml and 13021 ng/ml (average 7062 ng/ml) and 1427 ng/ml and 13021 ng/ml (average 6083 ng/ml), in groups POI, PO5 and PO15, respectively.

Distal end of the flap's MDA levels ranged between 1220 ng/ml and 10425 ng/ml (average 5648 ng/ml), 2275 ng/ml and 9756 ng/ml (average 5831 ng/ml) and 1531 ng/ml and 11959 ng/ml (average 5731 ng/ml), in groups POI, PO5 and PO15, respectively.

MDA values in normal skin samples ranged between 3930 ng/ml and 14398 ng/ml (average 10464 ng/ml), 2795 ng/ml and 14398 ng/ml (average 6847 ng/ml) and 1800 ng/ml and 6530 ng/ml (average 4042 ng/ml), in groups POI, PO5 and PO15, respectively.

Variance Analyzis showed higher levels of uric acid in the normal skin samples than in the middle and distal end of the flap samples. No statistical differences were found as regards MDA levels in the skin samples (Tables 1-6).

Discussion

Ischemia and necrosis are important complications in Plastic Surgery. Random pattern skin flaps are particularly sensitive and measures to reduce this risk have been studied. The action of oxidative stress upon random surgical flaps has been pointed out but not completely elucidated yet.

The early events that take place after elevation of random skin flaps are particularly interesting, for an adequate understanding of this period may allow the surgeon to take control of this physiopathological situation and avoid the failure of the operative procedure.

The rat skin is similar to the human skin, with diferences as regards the tissue underneath the dermis. In the rat, the muscular layer (=panniculus carnosus) is more adhered to the skin while in the human, the subcutaneous tissue is more adhered to the deep fascia. Nevertheless, the blood supply to the skin is similarly associated with these layers, which contains the main nutrient vessels.

McFarlane's flap represents an efficient tool to work with when random skin flaps are studied in the rat. It is well suited to ischemia/reperfusion studies because after flap elevation a hyperadrenergic state takes place, due to noradrenalin accumulation after section of sympatic nerves, and the resultant vasoconstriction leads to a temporary, but complete, capillary occlusion. Reperfusion of the distal ischemic skin occurs after

noradrenalin removal, with resultant capillary dilation⁶.

As this particular study focused on the early events after elevation of the flap, there was no distal necrotic area to be quantified. Skin samples were collected from the middle of the flap and distal flap areas as well as from normal skin.

Uric acid is an endogen antioxidant and takes part in the tissues natural defense against oxidative stress. An important serum antioxidant, it represents a final product in the metabolism of the purines, acting as a potent free radical scavenger and inhibitor of lipid peroxidation³. Beyer is an antioxidant that

TABLE 1 – Concentration of Uric Acid (mg/dl) from the middle of the flap skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	1.9	3.4	2.2
2	3.6	13	9.1
3	3.2	4.5	7
4	8.1	6.6	11.2
5	8.6	1.5	2.5
6	4.1	2.4	1.8
7	4.9	1	0.9
Average	4.9	4.6	5

TABLE 2 – Concentration of Uric Acid (mg/dl) from the distal end of the flap skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	6.1	5.8	6.6
2	2.5	5.6	9.1
3	1.5	7.7	7
4	4.5	13.3	11.2
5	3.6	1.7	1.8
6	3.4	4.1	1.1
7	3.5	1.2	2.7
Average	3.6	5.6	5.6

TABLE 3 – Concentration of Uric Acid (mg/dl) from the normal skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	3.9	4.2	6.1
2	4	28.6	13
3	6.2	21.2	8.8
4	3.6	9.7	10
5	6.1	2.5	2.9
6	8.1	5.3	2.8
7	4.8	3.4	2.5
Average	5.2	10.7	6.6

TABLE 4 – Concentration of MDA (ng/ml) from the middle of the flap skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	1282	2565	1427
2	9481	13030	13021
3	1534	8179	8183
4	21558	7613	7711
5	3816	7974	4580
6	9914	6806	5290
7	10582	3174	2369
Average	8310	7062	6083

TABLE 5 – Concentration of MDA (ng/ml) in the distal end of the flap skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	1220	2275	1531
2	1730	9756	1888
3	2675	8694	10308
4	8536	3541	11959
5	5350	6394	4437
6	9599	7312	6206
7	10425	2842	3791
Average	5648	5831	5731

TABLE 6 – Concentration of MDA (ng/ml) in the normal skin samples at zero, 5 and 15 minutes PO.

Animal	POI (0 min)	PO5 (5 min)	PO15 (15 min)
1	3930	3640	2172
2	14398	14270	3698
3	10976	8222	6530
4	12707	7789	4603
5	6570	2795	5464
6	13375	6570	4027
7	11291	4516	1800
Average	10464	6847	4042

is present in the tissues, accompanying probable variations in its levels since the very early phases after flap elevation may allow us to use it as a monitor of oxidative stress. As no articles were found in the Literature involving uric acid levels in the early phases after elevation of a random pattern skin flap, the authors decided to study the subject in the rat.

The finding of higher levels of uric acid from the normal skin samples, when compared to those from the middle and from the distal end of the flap (Variance Analysis), since POI and extending into PO15, suggests that the oxidative reactions involving consumption of uric

acid were so early and fast, that even the POI period failed to identify their beginning. Indeed, the maintenance of uric acid levels in the skin samples obtained from the flap throughout all the study suggests that the uric acid in the flap was consumed early and fast (before POI), and that this consumption also stopped early.

Malondialdehyde (MDA), a product of lipid peroxidation, is often used in studies that deal with oxidative stress, acting as a quantifier of the aggression suffered by the tissues. Its levels were determined via a modified Kohn, Liversedge method⁴. MDA levels in the skin samples were high, corroborating the hypothesis of the local

occurrence of oxidative stress. Comparison among the different skin samples from different periods revealed no difference (Variance Analysis), indicating that, at least, the oxidative stress remained stable throughout.

This study unveiled a small part of the complex oxidative phenomenon that occurs after elevation of a random pattern skin flap in the rat, and even trying to identify the very early events as regards uric acid and malondialdehyde levels, the study faced extremely fast pathophysiological reactions.

Conclusion

Uric acid levels in the flap skin were lower than those in the normal skin, denoting local oxidative stress.

References

1. 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 Med* 1998; 117: 209-11.
2. Duarte IS, Gomes HC, Ferreira LM. Effect of dimethyl sulphoxide on necrosis of skin flaps in rats. *Can J Plast Surg* 1998; 6: 93-7.
3. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Clarendon Press; 1985.
4. Percario S, Vital ACC, Jablonka F. Dosagem do malonaldeído. *Newslab* 1994; 6: 46-50.
5. McFarlane RM, DeYoung G, Henry RA. The design of a pedicle in the rat to study necrosis and its prevention. *Plast Reconstr Surg* 1965; 35: 245-8.
6. Pearl RM. A unifying theory of the delay phenomenon: recovery from the hyperadrenergic state. *Ann Plast Surg* 1981; 7: 102-12.

Freire ST, Gomes HC, Ferreira LM, Percario S. Ácido úrico como monitor do estresse oxidativo em um retalho cutâneo randômico em ratos. *Acta Cir Bras* [serial online] 2003 Nov-Dez;18(6). Disponível em URL: <http://www.scielo.br/acb>.

RESUMO – **Objetivo:** Verificar o possível papel do ácido úrico como monitor do estresse oxidativo em um retalho cutâneo randômico, no rato. **Métodos:** Este estudo utilizou 21 ratos adultos Wistar-EPM, divididos, ao acaso, em três grupos de sete animais, para avaliar o comportamento dos níveis de ácido úrico durante o estresse oxidativo, aos zero, 5 e 15 minutos após a elevação do retalho. De acordo com os períodos de tempo estabelecidos, amostras de pele foram coletadas de modo a permitir a determinação dos níveis de ácido úrico e malondialdeído (MDA). **Resultados:** Os níveis de ácido úrico nas amostras de pele não variaram com o tempo, mas os valores na pele normal foram maiores desde o início. Os níveis de malondialdeído (MDA) nas amostras de pele permaneceram estáveis. **Conclusão:** Os níveis de ácido úrico na pele do retalho foram menores que aqueles da pele normal, denotando estresse oxidativo local.

DESCRIPTORES – Retalhos cirúrgicos. Peroxidação lipídica. Ácido úrico. Ratos.

Correspondence:

Heitor Carvalho Gomes
Rua Napoleão de Barros, 715/4 andar
04024-900 São Paulo - SP
Tel: (11)5576-4118 – Fax: (11)5571-6579
sandra.dcir@epm.br – heitorgomesmd@aol.com

Conflict of interest: none
Financial source: none

Data do recebimento: 22/08/2003
Data da revisão: 16/09/2003
Data da aprovação: 09/10/2003