4 - ORIGINAL ARTICLE

Terazosin and propranolol as blockers to the deleterious effect of nicotine in a random skin flap, in the rat[1]

Terazosina e propanolol como bloqueadores do efeito deletério da nicotina em um retalho cutâneo randômico, no rato

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ABSTRACT

PURPOSE: To evaluate the effect of Terazosin and Propranolol on the prevention of necrosis induced by nicotine, in a random skin flap.
METHODS: This study utilized 32 adult male Wistar-EPM rats divided, at random, into four groups of eight animals each. All the 32 animals received nicotine (2 mg/kg/day) subcutaneously, for one week before and one week after flap elevation. CG (Control) group received distilled water (0.2 ml/day) by gavage and saline (0.5 ml) intraperitoneally, for seven days in the postoperative period. TG (Terazosin) group received terazosin hydrochloride (3 mg/day) by gavage and saline, intraperitoneally, for seven days in the postoperative period. PG (Propranolol) group received propranolol (1.5 mg/day) intraperitoneally and distilled water, by gavage, following the stablished pattern. TPG (Terazosin + Propranolol) group received both drugs. On the seventh postoperative day, the distal necrotic area of the flaps was determined via the paper template method. Blood and skin samples were collected in order to allow determination of Malondialdehyde (MDA) levels
RESULTS: The control group had a mean value of 39.5 % of necrosis; the Terazosin group 25.1 %; the Propranolol group 34.5 % and the Terazosin + Propranolol group 26.2 % of necrosis. MDA levels in the serum and in the skin samples behave similarly, with an exception regarding Propranolol group in this case.
CONCLUSION: Terazosin is effective in the prevention of necrosis in this animal model and Propranolol is not effective in this case.

Key words: Surgical flaps. Nicotine. Lipid peroxidation. Terazosin. Propranolol. Rats.

RESUMO

OBJETIVO: O objetivo deste estudo experimental foi avaliar o efeito da Terazosina e do Propranolol na prevenção da necrose induzida pela nicotina, em um retalho cutâneo randômico.

MÉTODOS: Este estudo utilizou 32 ratos machos adultos Wistar-EPM divididos, ao acaso, em quatro grupos de oito animais. Todos os 32 animais receberam nicotina (2 mg/kg/dia), por via subcutânea, por uma semana antes e uma semana após a elevação do retalho. O grupo CG (Controle) recebeu água destilada (0,2 ml/dia), por gavagem e salina (0,5 ml), por via intraperitoneal, por sete dias no período pós-operatório. O grupo TG (Terazosina) recebeu terazosina (3 mg/dia), por gavagem, e salina, intraperitoneal, por sete dias no pós-operatório. O grupo PG (Propranolol) recebeu propranolol (1,5 mg/dia), intraperitoneal e água destilada, por gavagem, seguindo o padrão estabelecido. O grupo TPG (Terazosina + Propranolol) recebeu ambas as drogas. No sétimo dia de pós-operatório, a área de necrose distal dos retalhos foi determinada pelo método do gabarito de papel. Amostras de sangue e de pele foram coletadas de forma a permitir a determinação dos níveis de malondialdeído (MDA).
RESULTADOS: O grupo controle apresentou um valor médio de necrose de 39,5 %; o grupo Terazosina 25,1 %; o grupo Propranolol 34,5 % e o grupo Terazosina + Propranolol 26,2 % de necrose. Os níveis de MDA no soro e nas amostras de pele comportaram-se de maneira similar, com uma exceção no caso do grupo Propranolol neste caso.
CONCLUSÃO: A Terazosina foi eficaz na prevenção da necrose neste modelo animal e o Propranolol não foi eficaz neste caso.

Descritores: Retalhos cirúrgicos. Nicotina. Peroxidação lipídica. Terazosina. Propranolol. Ratos.

Introduction

Nicotine induced skin flap necrosis is a matter of great concern to the Plastic Surgeon[1, 2]. Even understanding that elective cosmetic surgery may be postponed until the patient quits smoking, there is an important percentage of this specific population that simply cannot function without cigarette smoking. In order to reduce the risk of postoperative necrosis in smokers, many experimental studies have been carried out[3, 4, 5, 6, 7], particularly in the field of antioxidants[3, 8]. Antioxidants are substances that prevent or reduce the deleterious effects of the oxidative stress that occurs after elevation of surgical flaps, at the moment of reperfusion. An effective block of the oxidative stress prevents or reduces necrosis in the flap. Malondialdehyde (MDA) is a product of lipid peroxidation (oxidative stress in cellular membranes) and is used as a monitor of the oxidative phenomenon.

Nicotine is associated with flap necrosis, mainly due to vasoconstriction[3, 4, 5, 6, 7]. The specific mechanism involves stimulation of norepinephrine release by nicotinic cholinergic receptors, with stimulation of a_1-adrenergic receptors of smooth muscle cells. Terazosin hydrochloride, an a_1-specific neuroreceptor antagonist, reduced nicotine induced skin flap necrosis in the rat[7], but a possible antioxidant action was not analyzed. Similarly, propranolol, a beta receptor blocker, proved effective in the acute

myocardial infarction[9] but, again, no antioxidant role was studied. In fact, theoretically, the association of both drugs may, indeed, be favorable in preventing flap necrosis.

Methods

Thirty-two adult male Wistar-EPM rats were divided, at random, into four groups of eight animals each. All the 32 animals received nicotine (2 mg/kg/day) subcutaneously, for one week before and one week after flap elevation. CG (Control) group received distilled water (0.2 ml/day) by gavage and saline (0.5 ml) intraperitoneally, for seven days in the postoperative period. TG (Terazosin) group, received terazosin (3 mg/day) by gavage and saline, in a similar way and period of time. PG (Propranolol) group, received propranolol (1.5 mg/day) intraperitoneally and distilled water following the stablished pattern. TPG (Terazosin + Propranolol) group received both drugs.

After anesthesia with pentobarbital (40 mg/kg) intraperitoneally, the back of the rat was shaved and a random cranial based, dorsal skin flap was elevated, measuring 10 x 4 cm[10]. A plastic barrier was placed between the flap and its bed[11]. Simple 4-0 naylon stitches were then used to close the wound. On the seventh postoperative day, the distal necrotic area of the flap was determined via the paper template method[12]. Blood and skin samples were collected in order to allow determination of MDA levels[13]. One sample was obtained 5 cm distal to the flap basis, in the middle of the flap and in the midline (flap sample) and the other, 1 cm lateral to the flap basis, on the right side, from a normal skin area (normal skin sample). The skin samples measured 1 cm^2.

The methods herein presented were approved by UNIFESP-EPM's Ethical Commitee.

Results

The necrotic area in CG (control) ranged between 23.9 % and 51.3 % (average 39.5 %); in group TG (Terazosin) ranged between 15.5 % and 49.7 % (average 25.2 %); in PG (Propranolol) ranged between 16.9 % and 52 % (average 34.5 %) and in group TPG (Terazosin + Propranolol) ranged between 20.7 % and 34 % (average 26.2 %). Statistical analysis showed difference between groups CG and TG; CG and TPG (Mann-Whitney's Test; $p < 0.02$) (Table 1, Figure 1).

TABLE 1 - Percentage of necrotic area on the seventh postoperative day.

	CG (control)	TG (terazosin)	PG (propranolol)	TPG (tera/prop)
Animal 1	23.9	20.6	48.1	29.1
2	43.3	18.7	25.3	34
3	34.3	25.4	52	25.3
4	51.3	21.8	16.9	23
5	50	16.7	40	28.4
6	49.9	15.5	Died	27.4
7	30.8	32.8	38.4	22
8	32.8	49.7	20.7	20.7
Average (%)	39.5	25.2	34.5	26.2
SD	10.4	11.3	13.7	4.4

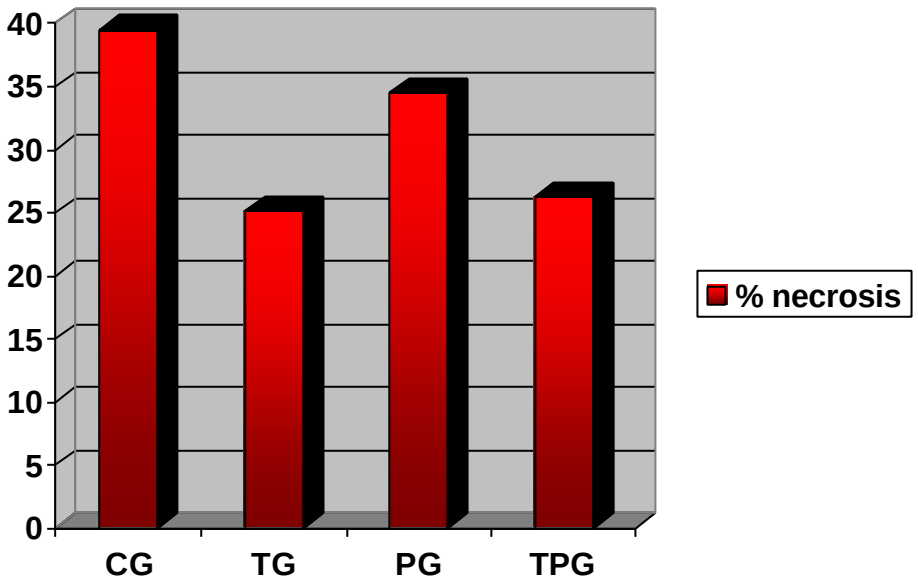

FIGURE 1 - Average percentage of necrotic area on the seventh postoperative day.

MDA serum concentrations in CG (control) ranged between 1634 ng/ml and 4716 ng/ml (average 3395 ng/ml); in TG (Terazosin) ranged between 2472 ng/ml and 7910 ng/ml (average 3774 ng/ml); in PG (Propranolol) ranged between 1702 ng/ml and

3826 ng/ml (average 2504 ng/ml) and in TPG (Terazosin + Propranolol) ranged between 2628 ng/ml and 3782 ng/ml (average 3202 ng/ml). Statistical analysis showed no difference between the groups (Variance Analysis; p = 0.19) (Table 2, Figure 2).

TABLE 2 - Average concentration of Malondialdehyde (ng/ml) in the serum collected on the seventh postoperative day.

	CG (control)	TG (terazosin)	PG (propranolol)	TPG (tera/prop)
Average(ng/ml)	3395	3774	2504	3202
SD	1001	1758	760	460

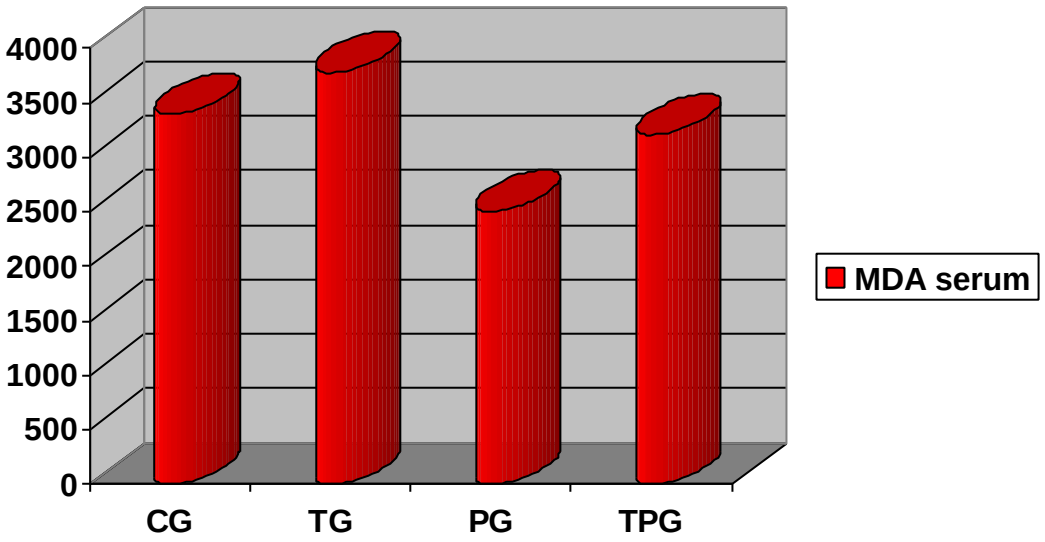

FIGURE 2 - Average concentration of malondialdehyde (ng/ml) in the serum collected on the seventh postoperative day.

MDA levels in the flap samples ranged between 21 ng/ml and 331 ng/ml (average 150 ng/ml) in CG (control); between 0 ng/ml and 694 ng/ml (average 130 ng/ml) in TG (Terazosin); between 64 ng/nl and 4677 ng/ml (average 2387 ng/ml) in PG (Propranolol) and between 464 ng/ml and 2845 ng/ml (average 1264 ng/ml) in TPG (Terazosin + Propranolol).

MDA levels in the normal skin samples ranged between 0 ng/ml and 331 ng/ml (average 106 ng/ml) in CG (control); between 0 ng/ml and 69 ng/ml (average 65 ng/ml)

in TG (Terazosin); between 111 ng/ml and 1718 ng/ml (average 717 ng/ml) in PG (Propranolol) and between 277 ng/ml and 3904 ng/ml (average 1378 ng/ml) in TPG (Terazosin + Propranolol) (Table 3, Figure 3).

TABLE 3 - Average concentration of malondialdehyde (ng/ml) measured from the flap and normal samples of skin on the seventh postoperative day.

	CG (control)		TG (terazosin)		PG (propranolol)		TPG (tera/prop)	
	flap	normal	Flap	normal	flap	normal	flap	Normal
Average	150	106	130	65	2387	717	1264	1378
SD	143	110	231	89	1820	544	798	1482

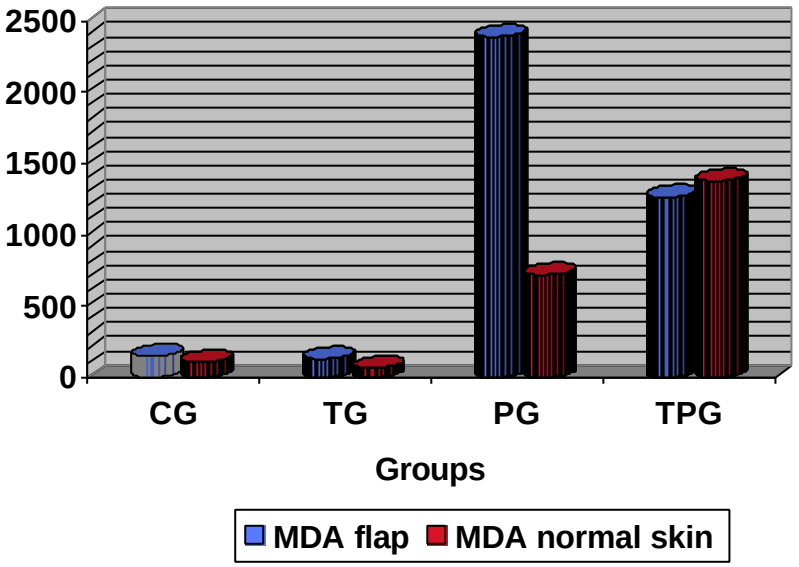

FIGURE 3 - Average concentration of malondialdehyde (ng/ml) measured from the flap and normal skin on the seventh postoperative day.

Statistical analysis of the MDA levels in the skin samples (flap x normal skin), inside the groups, showed difference in group PG (Propranolol), in which these levels were higher in the flap samples (Linear Paried Analysis; p = 0.12).

Comparisons between the groups showed, for the flap samples, that MDA levels in groups PG (Propranolol) and TPG (Terazosin + Propranolol) were higher than in

groups CG (control) and TG (Terazosin) (Paried Significance; p values varied between < 0.001 and 0.003).

Comparisons between the groups showed, for the normal skin samples, that MDA levels in groups PG (Propranolol) and TPG (Terazosin + Propranolol) were also higher than in groups CG (control) and TG (Terazosin) (Paried Significance; p values varied between < 0.001 and 0.006).

Discussion

Ischemia and necrosis are well known complications in Plastic Surgery. Random skin flaps are particularly risky and any measure that reduces this risk is welcome[3, 4, 5, 6, 7]. Recent knowledge of the undesirable effects of free radicals in skin flaps was followed by a myriad of clinical and laboratory studies and the importance of the antioxidant substances was stressed[3, 8].

Malondialdehyde (MDA), a product of lipid peroxidation, is useful in experimental studies that focus on antioxidant drugs, since its values reflect the oxidative stress to which the animal is exposed. Classically, MDA levels rise in the flap itself and the normal skin values are used as control. Serum levels were measured as a parameter of nicotine presence in the circulation, since they are elevated in rats exposed to nicotine effect[3].

The present rat model provoked both ischemia and necrosis in the flaps with local liberation of lipid peroxidation products. Also, malondialdehyde (MDA) levels in the serum were similar in all groups and, as expected, high. These elevated values are similar to those found in a previous study[3], and represent a direct result of the presence of nicotine in the circulation. These MDA levels do not reflect the events that occurred in the flap.

As expected, percentages of distal necrosis in the control (CG) rats flaps were high (average 39.5 %), indicating the deleterious effect of nicotine, as evidenced before[3, 4, 5, 6, 7]. It is important to notice that, according to Forrest et al[5,6], a four weeks preoperative period of nicotine injection was necessary to increase distal flap necrosis, what proved not true in this experiment, which used nicotine preoperatively for one week only and obtained similar results.

Terazosin, an a_1-specific neuroreceptor antagonist, reduced nicotine induced skin flap necrosis in the present experimental model, both alone (average 25.1 % of necrosis) and associated with Propranolol (average 26.2 % of necrosis), when compared to the control (average 39.5 % of necrosis). This result for Terazosin is in accordance to the work of Karlen and Maisel[7], which used the same doses of nicotine and Terazosin in a similar random-pattern flap, in the rat.

Regarding the possible role of Terazosin as an antioxidant substance, the data obtained showed that malondialdehyde (MDA) levels in both serum and skin samples were similar to those found in the control rats. Therefore, it seems that this drug does not act as an antioxidant, in spite of its necrosis reduction capability, probably explained by its vascular action.

Propranolol, a beta receptor blocker, did not reduce nicotine induced skin flap necrosis in the herein presented experimental model (average 34.5 % of necrosis), when compared to the control (average 39.5 % of necrosis). Indeed, the finding that the association with Terazosin reduced the percentage of necrosis (average 26.2 % necrosis), strongly suggests that this was the really efficient substance.

As regards the possible role of Propranolol as an antioxidant drug, the findings showed that malondialdehyde (MDA) levels in the serum were similar to those found in control and Terazosin groups, suggesting no systemic antioxidant action. Nevertheless, in the skin samples, MDA values were much higher than those found in the control and Terazosin groups, suggesting even a local oxidant effect (Figure 3).

Terazosin, the effective drug in this study, is currently used in the reduction of prostatic urethral smooth muscle contraction, with consequent improvement in urinary flow, in normotensive elderly men with benign prostatic hypertrophy[14]. This drug is well tolerated in this population, with minimal side effects[7], what justifies its clinical use to improve skin flap survival in smokers.

The herein presented data stresses the importance of Terazosin as a flap necrosis reduction drug, at least in this particular experimental model, and elicits the adequacy of the study of this drug in experiments involving different kinds of flaps.

Conclusion

Terazosin is effective in the prevention of necrosis in this animal model and Propranolol is not effective in this case.

References

1. Rees TD, Liverettt DM, Guy CL. The effect of cigarette smoking on skin-flap survival in the face lift patient. Plast Reconstr Surg 1984; 73: 911-5.
2. Riefkohl R, Wolfe JA, Cox EB, McCarty KS. Association between cutaneous occlusive vascular disease, cigarette smoking, and skin slough after rhytidectomy. Plast Reconstr Surg 1986; 77: 592-5.
3. 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 (BR) 1998; 117: 209-11.
4. Campos H, Ferreira LM, Santos WLC, Cardeal M, Araújo M. Efeitos da nicotina nos retalhos cutâneos em ratos. Acta Cir Bras 2001; 16: 206-10.
5. Forrest CR, Pang CY, Lindsay WK. Pathogenesis of ischemic necrosis in random-pattern skin flaps induced by long-term low-dose nicotine treatment in the rat. Plast Reconstr Surg 1991; 87: 518-28.
6. 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.
7. Karlen RG, Maisel RH. Terazosin blockade of nicotine induced skin flap necrosis in the rat. Arch Otolaryngol Head Neck Surg 1997; 123: 837-40.
8. 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.
9. Gottlieb SS, McCarter RJ. Comparative effects of three beta blockers (atenolol, metoprolol, and propranolol) on survival after acute myocardial infarction. Am J Cardiol 2001; 87: 823-6.
10. McFarlane 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: 245-8.
11. Ugland O. Flaps and flaps necrosis. Acta Chir Scand 1966;131: 408-12.
12. Sasaki GH, Pang CY. Hemodynamics and viability of acute neurovascular island skin flaps in rats. Plast Reconstr Surg 1980; 65: 152-8.

13. Percário S, Vital ACC, Jablonka F. Dosagem do Malonaldeido. Newslab 1994;6:46-50.

14. Lepor H, Meretyk S, Knapp-Maloney G. The safety, efficacy and compliance of terazosin therapy for benign prostatic hyperplasia. J Urol 1992; 147: 1554-7.

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