PHARMACOLOGY AND TOXICOLOGY

Effect of Richlocaine Alone or in Combination with Energostim on the Severity of Endotoxemia and Survival of the Skin under Conditions of Reduced Blood Flow

A. V. Zadorozhnyi, V. L. Popkov, V. P. Galenko-Yaroshevskii, A. V. Antelava*, E. A. Chikobava*, G. V. Sukoyan*, and V. N. Meladze

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 9, pp. 290-293, September, 2003
Original article submitted June 24, 2003

The local anesthetic richlocaine decreased the area of necrosis in the skin flap under conditions of reduced blood flow by 29.5%. Improved survival of skin flap after richlocaine treatment alleviated endogenous intoxication, reduced secondary inflammatory reaction, improved liver function, and normalized the ratio between vasoconstricting and vasodilating prostaglandins. This effect was most pronounced after combination therapy with richlocaine and direct-action antihypoxant energostim.

Key Words: *skin flap; rihlocain; energostim; endotoxemia; hydroxyproline*

Changes reflecting necrobiotic processes, appearance of the inflammatory exudate, impairment of membrane stability, and development of endotoxemia dominate in the focus of injury at the early stage of blood flow reduction during trauma. Previous studies showed that the local anesthetic richlocaine possesses immunomodulatory properties and decreases total peroxidase activity and extraerythrocytic hemoglobin content [1]. Here we evaluated the effects of richlocaine alone or in combination with the antihypoxant energostim [2,3] on survival of skin flap and endotoxemia under conditions of reduced blood flow in the skin flap (SF).

Krasnodar Research Center, Russian Academy of Medical Sciences, Krasnodar Krai Administration; *N. V. Karsanov Republican Research Center for Medical Biophysics and Implementation of New Biomedical Technologies, Tbilisi. ***Address for correspondence:*** galinasukoian@mail.ru. Sukoyan G. V.

MATERIALS AND METHODS

Experiments were performed on 32 male albino rats weighing 175-190 g in autumn and winter. The animals were randomly divided into 4 groups (control, groups 1 and 2; treatment, groups 3 and 4, 8 rats per group and were kept in a vivarium under standard conditions with adequate food supply. Surgeries were performed under sterile conditions. The rats were intraperitoneally narcotized with 60 mg/kg hexenal and SF (12×52 mm) was dissected [6,8]. Group 1 animals were intraperitoneally injected with 0.2 ml physiological saline 15 min before the experiment. Group 2 rats received physiological saline for 3 days. Group 3 animals received 0.2 ml richlocaine in a dose of 5 mg/kg. Group 4 rats received 0.2 ml richlocaine and 0.2 ml energostim in doses of 5 and 115 mg/kg, respectively, for 3 days. Keratinocytes of SF were isolated 1 h (group 1) or 3 days after surgery (groups 2,

0007-4888/03/1363-0253$25.00

3, and 4). The count of viable cells was determined using trypan blue exclusion test [8]. The severity of endotoxemia was estimated by the formation of nonenzymatic proteolytic products in the plasma and erythrocytes using integral parameters for toxicity (express test proposed by M. Ya. Malakhova) [4]. Changes in the form of spectrograms reflected the metabolic response of the organism to adverse factors. The contents of histamine and serotonin and activities of alanine transaminase (ALT) and aspartate transaminase (AST) in suspensions of SF and rat blood were measured daily by the fluorometric method using Bio-La-Test reagents (Lachema). The concentrations of prostaglandins E and F were estimated using radioimmunological kits (Clinical Assays). Lactate content was evaluated using Boehringer Mannheim kits. Lipids were removed with a chloroform-methanol mixture in the 1:20 ratio (wet weight) to determine the amount of hydroxyproline and hexosamines [5,7]. The results were analyzed by Student's *t* test. The differences were significant at $p<0.05$.

RESULTS

After 72 h the necrotic area of SF in group 3 and 4 rats decreased by 29.5 and 36.3%, respectively, compared to that in group 2 animals (control, Table 1). The number of dead keratinocytes per 1 cm^2 skin on the back in rats of groups 3 and 4 decreased by 29 and 35%, respectively (Table 1). These data indicate that in animals with ischemic SF combination therapy produced a greater antinecrotic and protective effect compared to monotherapy with richlocaine. We revealed that energostim possesses high antinecrotic activity and sharply increases the resistance of keratinocytes to ischemic and hypoxic factors.

On the spectrogram of rat blood plasma the relative peak of optical density at 282 nm tended to increase compared to normal 2 h after dissection of SF (0.21±0.03 optical density units). Changes in the spectrogram of erythrocytes were most pronounced at this term. The erythrocyte extinction maximum at 258 nm increased by 2.2 times compared to group 1 rats (normal 0.56± 0.05 optical density units). The total toxicity index (area under the curve) 2.4-fold surpassed that in group 1 animals. Medium molecules were released from the focus of aggression and sorbed on the glycocalyx or in erythrocytes.

Twelve hours after surgery peak optical density of erythrocytes increased to a lesser extent than after 2 h (by 1.3 times). These changes were accompanied by a significant increase in extinction of the plasma (by 1.8 times). The plasma was rich in biochemical substrates of endogenous intoxication, catabolic substances, and products of lipid peroxidation and cell disintegration. Inflammatory and focal dystrophic necrotic injury and secondary hyperergic allergic reaction of the skin probably developed 2-12 h after surgery. Changes in the spectrogram of the plasma and erythrocytes reflected stage I of endogenous intoxication (compensation).

Two hours after dissection of SF optical density of erythrocytes increased by 2.1, 1.6, and 1.28 times in rats of groups 2, 3, and 4, respectively. It should be emphasized that optical density of the plasma remained practically unchanged. The number of medium molecules in the plasma and erythrocytes considerably increased by the 24th hour after dissection of SF. The index of plasma extinction at 282 nm reached a maximum, 4-fold surpassed normal, and remained high over the next 2 days. Erythrocyte extinction reached a maximum 12 h after surgery and was 2.8 times higher than in the control (Table 2). The first 3 days after dissection of SF were characterized by severe toxemia and accumulation of toxic products of proteolytic degradation of necrotized skin, hepatocytes, and damaged blood cells. The rapid release of these substances reflects normal functioning of lysosomal enzymes. Stage II of toxemia was characterized by a sharp increase in optical density of the plasma and correlated with manifestation of necrotic changes in SF.

After 3 days the erythrocyte peak on the spectrogram progressively decreased and was below normal

TABLE 1. Effect of Richlocaine Alone or in Combination with Energostim on the Number of Viable Keratinocytes and Survival of SF (*M*±*m*)

Group	Average area of SF, cm^2	Keratinocyte count, 10^5		Ratio between the count of dead keratinocytes and total number of keratinocytes, %
		living	dead	
1	(8) 6.25±0.22	5.8±0.4	0.45±0.12	7.2±1.0
2	(8) 6.40±0.28	4.6±0.2**	1.92±0.14*	29.5±2.4*
3	(8) 6.36±0.26	5.0±0.2^{++}	1.36±0.13^{*+}	21±2^{*++}
4	(8) 6.82±0.10	5.55±0.23$^{***+\circ}$	1.28±0.11^{*+}	18.8±1.0$^{*+\circ}$

Note. Number of SF is shown in brackets. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to group 1; $^{+}p<0.01$ and $^{++}p<0.05$ compared to group 2; $^{\circ}p<0.05$ compared to group 3.

by 18%, which reflected structural abnormalities in these cells. However, the amount of optically active substances in the plasma continued to increase over 3 days. These results indicate that the severity of endogenous intoxication increased under conditions of reduced blood flow in SF. It was not followed by the development of homeostatic system failure and polyorgan insufficiency syndrome. We did not reveal changes in activity of ALT and AST. Richlocaine decreased the contents of serotonin and histamine and attenuated the systemic inflammatory reaction on day 3 (Table 2). It was most pronounced in group 4 rats. A sharp increase in blood lactate content reflected the development of lactacidosis and impairment of aerobic and anaerobic glycolysis.

Spectrograms of blood plasma and erythrocytes were analyzed during the development and progression of endotoxemia under conditions of reduced blood flow in SF. Endogenous intoxication proceeded in 3 initial stages: increase in the erythrocyte peak and normal spectrogram of the plasma (compensatory stage I); rise in the content of metabolites in the plasma and erythrocytes (accumulation of products from the focus of aggression, stage II); and stabilization of erythrocyte spectrograms (complete saturation) and further increase in the peak of plasma on spectrograms (reversible decompensation of organs, stage III). By the 72nd hour of endotoxemia irreversible decompensation of organs was followed by the development of homeostatic failure and irreversible decompensation (stages IV and V). Phase IV was characterized by a decrease in the erythrocyte peak below normal due to structural changes in the erythrocyte membrane and further accumulation of optically active substances in the plasma. Damage to membranes was observed in terminal stage V and accompanied by a decrease in the

TABLE 2. Changes in Optical Density of the Plasma and Erythrocytes, Activities of ALT and AST, and Contents of Serotonin, Histamine, and Lactate under Conditions of Reduced Blood Flow in SF ($M \pm m$)

Sample, time of investigation, h		Normal	SF		
			control (group 2)	richlocaine	richlocaine and energostim
Plasma, λ=258 nm	2	0.25±0.05	0.38±0.04	0.28±0.12	0.28±0.08
	12		0.78±0.12*	0.59±0.11	0.48±0.12***
	24		0.98±0.04*	0.98±0.12	0.78±0.18*
	72		1.14±0.21*	0.68±0.12**	0.48±0.07*+++
Erythrocytes, λ=258 nm	2	0.60±0.08	1.31±0.08*	1.03±0.19**	0.77±0.05++oo
	12		1.68±0.24*	1.28±0.11***	0.88±0.09+o
	24		1.38±0.14*	0.98±0.10**++	0.82±0.08***++
	72		0.45±0.05***	0.55±0.12*+	0.68±0.06+++
ALT, μcat/liter	24	0.70±0.06	0.82±0.06	0.78±0.04	0.72±0.05
	72		0.91±0.05	0.77±0.06***	0.73±0.04
AST, μcat/liter	24	0.30±0.06	0.39±0.05	0.44±0.03	0.38±0.03
	72		0.42±0.04***	0.35±0.04	0.32±0.05
Serotonin, nmol/ml	24	39±5	60±8***	57±9***	49±6+++
	72		57±6***	48±4+++	46±4+++
Histamine, nmol/ml	24	0.24±0.05	0.66±0.08*	0.60±0.06*	0.45±0.05**+oo
	72		0.62±0.04*	0.49±0.04**++	0.35±0.04***+oo
Prostaglandin E, pg/ml	24	60±4	57±7	57±7	62±5
	72		50±7	70±8	80±10++
Prostaglandin $F_{2\alpha}$, pg/ml	24	115±15	105±8	160±18**++	91±9
	72		123±10	106±0.08	134±18
$E/F_{2\alpha}$ ratio	24	0.52±0.08	0.64±0.05	0.35±0.04*+	0.68±0.04***
	72		0.41±0.08	0.66±0.03**	0.60±0.08+++
Lactate, mmol/ml	24	72±8	60±8	65±9	78±7
	72		167±28*	120±18**	88±12+oo

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to normal; +p<0.001, ++p<0.01, and +++p<0.05 compared to the control; op<0.001 and oo$p$<0.01 compared to richlocaine.

content of metabolites in erythrocytes and plasma. These changes were insignificant during ischemia of SF. Production of medium molecules was suppressed in the plasma and erythrocytes from group 3 and 4 rats. These changes reflected a decrease in the degree of endotoxemia and were most pronounced in group 3 animals. The decrease in the contents of histamine and serotonin indicates that richlocaine blocked the development of endotoxemia (Table 2). Energostim produced an antihypoxic effect and normalized transaminase activities in the plasma and AST/ALT ratio on day 3 after treatment. It should be emphasized that the increase in transaminase activities in the plasma reflects the influence of endotoxins released during necrosis of SF and damage to the liver and heart. Moreover, the AST/ALT ratio in ischemic SF increased from 1.1±0.2 to 1.36±0.13 (normal 1.4±0.2). These data indicate that energostim decreases the severity of tissue edema and secondary inflammatory reaction, which contributes to attenuation of necrotic changes.

As differentiated from processes in SF under conditions of reduced blood flow, prostaglandin F content did not increase after combination therapy. It improves microcirculation and platelet aggregation. Combination treatment with energostim and richlocaine shifted the ratio between prostaglandins toward vasoconstricting substances. It should be emphasized that blood content of histamine decreased, but not increased (in contrast to serotonin). Prostaglandin E concentration decreased compared to that observed 2 h after surgery. The content of prostaglandin $F_{2\alpha}$ remained practically unchanged. These variations contribute to a sharp increase in the content of vasoconstricting prostaglandins.

Changes reflecting necrobiotic processes and appearance of the inflammatory exudate were primarily observed in the focus of injury at the early stage after trauma. It was accompanied by the development of endotoxemia and increase in the content of hexosamines in the focus of inflammation to 27.1±1.3 mg/g wet tissue (*vs.* 17.8±1.2 mg/g wet tissue in the control). Hydroxyproline content decreased from 15.9±0.9 to 12.3±0.8 mg/g wet tissue ($p<0.05$). Accumulation of fibroblasts with high functional activity determined collagen synthesis and was followed by a decrease in the contents of hexosamines and collagen on day 3 (23.8±0.9 and 21.2±1.1 mg/g wet tissue, respectively). Treatment with richlocaine alone and, especially, in combination with energostim increased the ratio between the amount of collagen and hexosamine (acute fibrosis). On day 3 we observed a decrease in hexosamine content (21.5±0.9 and 18.0±1.1 mg/g wet tissue, respectively) and increase in hydroxyproline concentration (23.8±1.0 and 25.1±1.1 mg/g wet tissue, respectively). Variations in the content of hexosamines and hydroxyproline and the dynamics of endotoxemia suggest that treatment with richlocaine alone and in combination with energostim markedly reduces the severity of inflammation, promotes changes in the amount of soluble glyco- and mucoproteins in the skin, accelerates collagen biosynthesis, and relieves endotoxemia.

REFERENCES

1. Yu. N. Bordyushkov, L. I. Malyutina, I. A. Romanova, *et al*., *Byull. Eksp. Biol. Med*., Suppl. 2, 96-97 (2002).
2. S. V. Vasil'eva, V. P. Galenko-Yaroshevskii, Yu. Yu. Fedchenko, *et al*., *Ibid*., Suppl. 3, 97-101 (2002).
3. N. V. Karsanov, G. V. Sukoyan, E. A. Chikobava, *et al*., *Ibid*., **134**, No. 9, 338-345 (2002).
4. M. Ya. Malakhova, *Efferent. Ter*., No. 1, 61-64 (1995).
5. L. I. Slutskii, *Biochemistry of Normal and Pathologically Changed Connective Tissue* [in Russian], Leningrad (1969).
6. V. P. Tumanov, K. Sh. Matevosyan, L. S. Basagin, *et al.*, *Byull. Eksp. Biol. Med.*, **104**, No. 9, 110-113 (1987).
7. P. N. Sharaev, *Lab. Delo*, No. 5, 283-285 (1981).
8. V. M. Sholokhov, B. I. Lyubimov, N. N. Samoilov, *et al*., *Byull. Eksp. Biol. Med.*, **101**, No. 3, 375-376 (1986).