BIOPHYSICS AND BIOCHEMISTRY

Effect of Monotherapy and Combination Therapy with Richlocaine on Tissue Hypoxia and Activity of Keratinocyte Detoxifying Systems in Ischemic Skin Flap

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Treatment with richlocaine alone and, especially, in combination with antihypoxant energostim decreased the total content of hydroxyproline in the ischemic skin flap on day 3 after excision. Combination therapy with richlocaine and energostim normalized the redox potential in the energy supply system, improved antioxidant protection, and promoted the recovery of a balance between various components in the antioxidant system. These changes were not accompanied enhanced production of malonic dialdehyde. Our results suggest that combination therapy with richlocaine and energostim maintains the adaptive reserves of detoxifying systems in keratinocytes and prevents endotoxemia. Richlocaine primarily stimulates glycolytic synthesis of ATP, activates nonmitochondrial antioxidant enzymes, and increases RNase activity in lysosomes.

Key Words: *ischemic skin flap; energostim; rihlocain; tissue hypoxia; antioxidant protection; lysosomes*

Previous studies showed that local anesthetic richlocaine possesses antiperoxidase activity [3], improves survival of ischemic skin flap (SF), and prevents endotoxemia [6]. It was interesting to determine the mechanisms underlying keratinoprotective activity of richlocaine alone and in combination with direct-action antihypoxant energostim [7]. Attention was aimed at the influence of these preparations on the degree of endogenous intoxication. We evaluated the effects of richlocaine and energostim on the severity of tissue hypoxia, activity of detoxifying systems, antioxidant protection, and protective potential of lysosomes under conditions of reduced blood flow in SF.

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MATERIALS AND METHODS

Experiments were performed on 32 male albino rats weighing 175-190 g. The animals were randomly divided into 4 groups (intact, control, and two experimental groups). Each group consisted of 8 rats.

The animals were kept in a vivarium under standard conditions and received an adequate diet. Surgeries were performed under sterile conditions. None rats had purulent wounds. Control animals were intraperitoneally injected with 0.2 ml physiological saline

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15 min before the experiment. Group 1 rats received 0.2 ml richlocaine in a dose of 5 mg/kg. Group 2 animals received 0.2 ml richlocaine and 0.2 ml energostim in doses of 5 and 115 mg/kg, respectively.

Keratinocytes were isolated as described elsewhere [4]. Activities of NADH oxidase, cytochrome *c* reductase [2,8], and lysosomal enzymes β-galactosidase, β-glucosidase, and RNase [1,11], contents of NAD [4] and cathepsin D, activities of antioxidant enzymes superoxide dismutase (SOD), catalase [5], and glutathione peroxidase (rate of NADP reduction) [13], and malonic dialdehyde (MDA) concentration were measured in SF homogenates [9]. The results were analyzed by Student's *t* test using STAT Soft software. The differences were significant at $p<0.05$.

RESULTS

Richlocaine alone and in combination with energostim increased survival of SF, prevented endotoxemia, and increased the content of hydroxyproline. These changes probably contribute to a considerable stimulation of microcirculation and reparative processes in SF under conditions of reduced blood flow [6].

We studied the effect of test preparations on tissue hypoxia that serves as a major cause of endotoxemia. In group 1 rats richlocaine in a dose of 5 mg/kg insignificantly increased ATP content in the necrotic zone. However, ATP content in the intact zone increased by 45%. In these animals the concentrations of NAD and cytochrome *c* in both areas practically did not differ from the control. Redox potential of the energy supply system remained low. In group 2 rats combination therapy with richlocaine and antihypoxant energostim normalized redox potential and NAD/NADH ratio, increased cytochrome *c* content in mitochondria, and partially recovered the capacity of the electron transport chain. Probably, the effects of richlocaine are realized via glycolytic synthesis of ATP. Richlocaine affects Na^+ and K^+ transport and, therefore, modulates ATPase activity in ionic pumps [3]. These enzymes are involved in the glycolytic pathway of ATP synthesis. Combination therapy with richlocaine and energostim activated ATP synthesis via oxidative phosphorylation. ATP content in the intact zone did not differ from normal (Fig. 1).

In group 2 rats activity of cytochrome *c* reductase increased, while the content of cytochrome *c* in the blood decreased to the initial level (Fig. 1). NAD content and NAD/NADH ratio returned to normal in the intact zone of SF. These indexes sharply increased in the necrotic zone, but remained below the control (Fig. 2). Combination therapy normalized NADH oxidase activity in the intact zone. However, enzyme activity in the necrotic zone did not differ in rats of groups 1 and

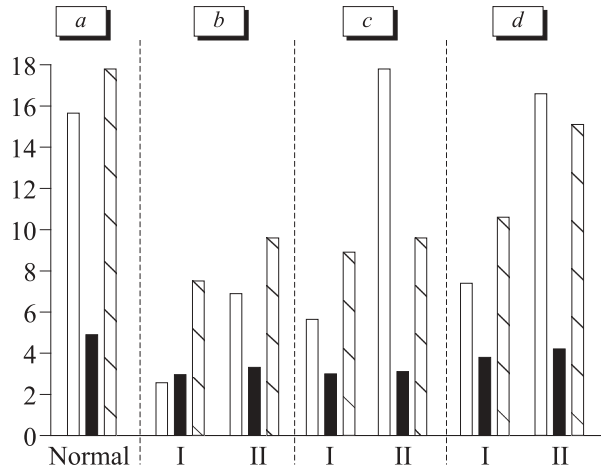

Fig. 1. Effect of treatment with richlocaine alone and in combination with energostim on the contents of ATP (light bars), NAD (dark bars), and cytochrome *c* (shaded bars) in the skin flap (SF, I) and blood (II). Normal (*a*), SF (*b*), SF and richlocaine (*c*), and SF and richlocaine+energostim (*d*).

2 receiving monotherapy and combination therapy with richlocaine, respectively (Fig. 3). Richlocaine monotherapy had no effect on redox potential imbalance, which serves as a major genetic cause of vascular endothelium dysfunction. Richlocaine decreased lactate content [6] and increased glycolytic activity in SF. However, only energostim increased pyruvate concentration and stimulated aerobic glycolysis.

The mitochondrial respiratory chain is involved in electron transport to oxygen and serves as a major source of reactive oxygen species (ROS) and free radicals. Activities of SOD and its cytosolic fraction (Table 1) that eliminate the superoxide anion in the necrotic zone insignificantly increased (by 18.8%) on day 3 after treatment with richlocaine. Activity of glutathione peroxidase neutralizing H_2O_2 in mitochondria increased only in the intact zone. After richlo-

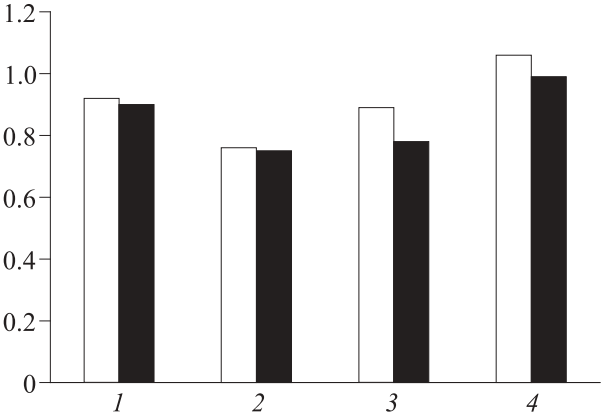

Fig. 2. Effect of treatment with richlocaine alone and in combination with energostim on the redox potential in SF (light bars) and blood (dark bars). Normal (*1*), SF (*2*), SF and richlocaine (*3*), and SF and richlocaine+energostim (*4*).

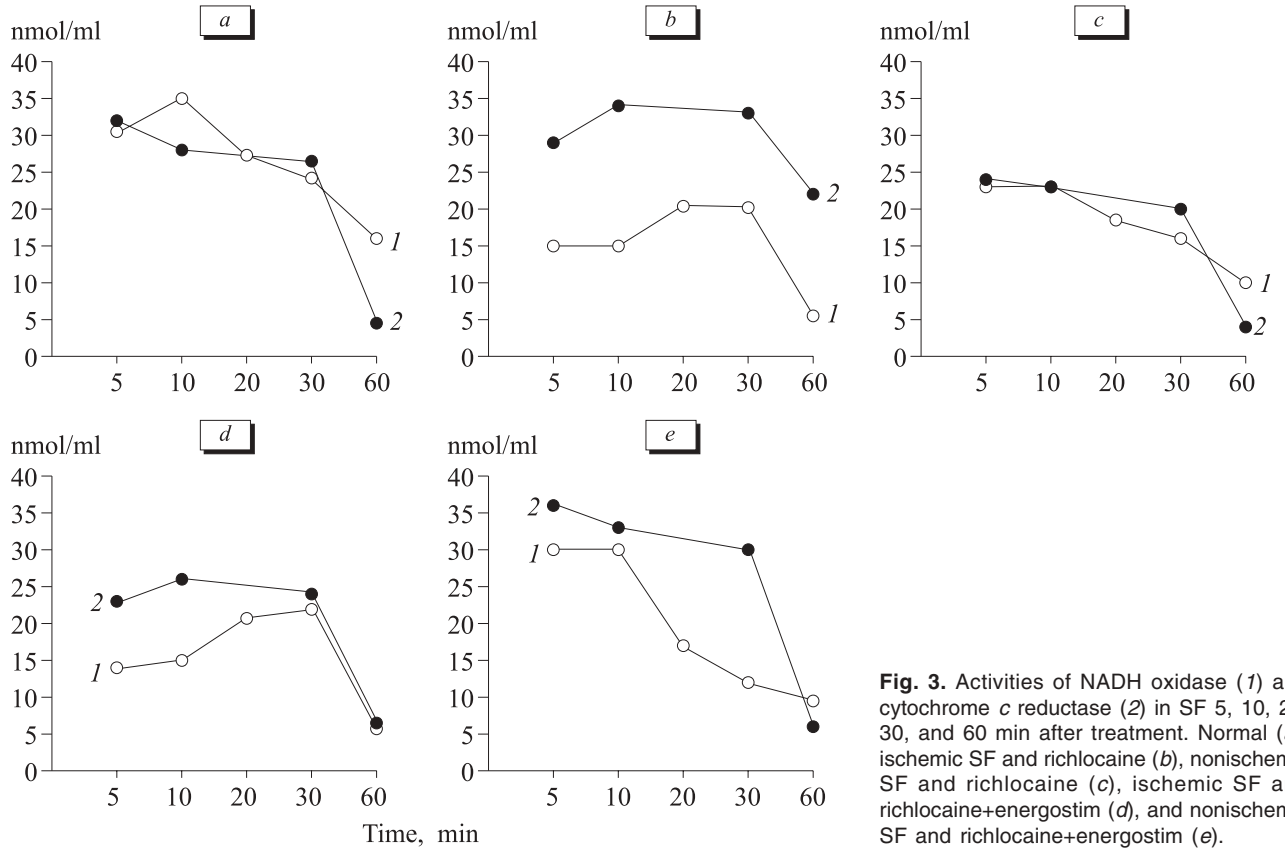

Fig. 3. Activities of NADH oxidase (*1*) and cytochrome *c* reductase (*2*) in SF 5, 10, 20, 30, and 60 min after treatment. Normal (*a*), ischemic SF and richlocaine (*b*), nonischemic SF and richlocaine (*c*), ischemic SF and richlocaine+energostim (*d*), and nonischemic SF and richlocaine+energostim (*e*).

caine treatment the glutathione peroxidase/SOD ratio decreased in the necrotic zone, but surpassed the control. It should be emphasized that this in the intact zone ratio was below normal. Cytosolic SOD activity practically did not differ from that in SF of animals not receiving the preparation. In rats of the richlocaine group activity of the nonmitochondrial enzyme catalase decreased compared to normal and ischemic SF (Table 1). The data suggest that richlocaine activates mitochondrial enzymes of antioxidant protection more signifi-

TABLE 1. Effect of Treatment with Richlocaine Alone and in Combination with Energostim on the System of Antioxidant Protection in Ischemic SF (*M*±*m*)

Parameter	Intact animals (normal)	Control group	Group 1	Group 2
SOD, U/mg protein/min	0.39±0.02	0.28±0.03**	0.48±0.05**	0.49±0.20**
		0.30±0.03	1.0±0.1^{ooo}	0.42±0.03
Cu-Zn SOD, U/mg protein/min	0.21±0.05	0.14±0.03***	0.13±0.02***	0.32±0.04***$^{++oo}$
		0.13±0.03	1.0±0.1^{++}	0.22±0.04^{+++o}
Glutathione peroxidase, nmol NADP/mg protein	2.3±0.4	1.5±0.3**	1.6±0.2^{++}	2.6±0.2^{++oo}
		1.2±0.3**	2.0±0.2**	2.2±0.3^{++}
Glutathione peroxidase/SOD	6.8±0.6	5.35±0.35***	3.3±0.4**	5.4±0.3***^{oo}
		4.0±0.6***	5.0±0.1^{+}	5.5±0.3***$^{+++}$
Catalase, nmol H_2O_2/mg protein/min	77±9	125±19**	59±8***$^{++}$	117±11**^{o}
		59±3***$^{++}$	111±10^{ooo}	67±9^{oo}
MDA, μmol/mg protein	0.95±0.05	1.17±0.08***	1.3±0.1***	0.89±0.04^{+++ooo}
		1.03±0.07	1.0±0.1	0.90±0.03^{+++ooo}

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to intact animals; ^{+}p<0.001, ^{++}p<0.01, and ^{+++}p<0.05 compared to the control; ^{o}p<0.001, ^{oo}p<0.01, and ^{ooo}p<0.05 compared to group 1.

TABLE 2. Effect of Treatment with Richlocaine Alone and in Combination with Energostim on Activity of Lysosomal Enzymes in Ischemic SF (*M*±*m*)

Parameter	Intact animals (normal)	Control group	Group 1	Group 2
Cathepsin D, μg tyrosine/mg protein/min	2.9±0.3	6.1±0.9*	4.2±0.4**$^{++}$	7.8±0.8*$^{+++oo}$
		3.0±0.4	3.2±0.2*	2.2±0.2***$^{+++ooo}$
RNase, μg/mg protein/min	1.0±0.2	2.2±0.3*	1.2±0.3*	2.5±0.3*^{oo}
		0.51±0.11*	1.5±0.1***$^{++}$	1.2±0.2^{++}
β-Galactosidase, mmol p-nitrophenol/mg protein/h	0.82±0.12	1.1±0.2	1.1±0.1***	0.89±0.05^{+++}
		0.84±0.09	0.8±0.2	0.79±0.08
β-Glucosidase, mmol p-nitrophenol/mg protein/h	0.36±0.06	0.9±0.1	1.45±0.15*$^{+}$	0.25±0.05***$^{+o}$
		0.32±0.08	1.80±0.12*$^{+}$	0.34±0.06^{o}

Note. *p<0.001, **p<0.01, and ***p<0.05 compared to intact animals; ^{+}p<0.001, ^{++}p<0.01, and ^{+++}p<0.05 compared to the necrotic zone; ^{o}p<0.001, ^{oo}p<0.01, and ^{ooo}p<0.05 compared to group 1.

cantly than cytosolic enzymes. Combination therapy with richlocaine and energostim normalized redox potential in the energy supply system, improved antioxidant protection, and promoted recovery of the balance between various components in the antioxidant system. After therapy we did not observe enhanced MDA production reflecting hyperactivation of lipid peroxidation. Therefore, test preparations did not violate functional activity of detoxifying systems in keratinocytes.

After combination therapy with richlocaine and energostim activity of the lysosomal enzyme cathepsin D utilizing protein structures increased in the necrotic zone, but remained below the control (Table 2). Enzyme activity in the intact zone returned to normal on day 3. Richlocaine markedly increased activities of RNase and β-galactosidase only in the intact zone. However, β-glucosidase activity increased in both areas. Therefore, richlocaine protected lysosomes from dysfunction in the adaptive synthesis of lysosomal enzyme in considerable amounts. This effect was most pronounced after combination therapy with richlocaine and energostim. Lysosomes were capable of proteolyzing disintegrated structures, which has a positive prognostic importance for survival of SF [4,6,10].

Our results indicate that a pronounced keratinoprotective effect of combination therapy with energostim and richlocaine is based on modulation of the energy supply system, antioxidant protection, and protective function of lysosomes, which increases survival of the skin [6] and relieves endotoxemia.

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