BIOPHYSICS AND BIOCHEMISTRY

Effects of Dalargin on Free Radical Processes in the Blood of Rats Exposed to Moderate Hypothermia

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Moderate hypothermia stimulates LPO processes in rat plasma and erythrocytes and simultaneously increases antioxidant activity in the plasma and SOD activity erythrocyte. Dalargin prevents stimulation of LPO in the blood in hypothermia by reducing the generation of active oxygen species and maintaining the level of low-molecular antioxidants.

Key Words: *lipid peroxidation; antioxidant activity; hypothermia; dalargin; rat blood*

Global and local hypothermia is used in medical practice mainly for reducing oxygen demands of tissues and elimination of ischemic and hypoxic phenomena. Exposure to hypothermia alleviates the consequences of stroke, infarction, and ischemia–reperfusion [14]. On the other hand, exposure of non-narcotized animals to hypothermia is fraught with stimulation of free radical processes (FRP) in tissues. Free radical homeostasis of cells and tissues is supported by coordination of enzymatic and nonenzymatic systems involved in generation of reactive oxygen species (ROS), on the one hand, and systems for their elimination, on the other. Hypothermia can shift the balance towards excessive generation of free radicals and lead to antioxidant deficiency, which, in turn, leads to modification of the biological membranes chemical composition, ultrastructural organization, permeability, and of activities of membrane enzymes [15]. Hence, the search for substances correcting the FRP in tissues under conditions of hypothermia is an important problem.

Endogenous regulatory peptides and their synthetic analogs play an important role in inhibition of stress-induced FRP. One of these synthetic analogs is dalargin, a D-Ala^2, Leu^5, Arg^6-enkephalin opioid hexapeptide. Dalargin intraperitoneally injected to rats reduces the intensity of LPO processes in various tissues under conditions of stress [6,7], including hypothermia [4]. However, the mechanisms of antioxidant effects of dalargin remain not quite clear.

We studied possible mechanisms of correcting effect of dalargin on FRP in the blood of rats exposed to moderate hypothermia.

MATERIALS AND METHODS

Experiments were carried out on outbred male albino rats (n=46; 180-200 g). Hypothermia was induced by cooling the animals in Plexiglas boxes with circulating cold (10°C) water jackets. Body temperature was measured rectally. Body temperature was reduced from 38 to 30°C over 20 min; immediately after that the animals were decapitated. Pharmacopeial dalargin (Microgen) was injected in a single intraperitoneal dose of 100 μg/kg (131 nmol/kg) 30 min before exposure to cold or decapitation. Controls were injected with the same volume of saline. Blood after decapitation was collected in tubes with heparin (25 units/ml) or 1.8%

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sodium citrate and centrifuged at 1500 rpm for 5 min. Plasma was collected and erythrocytes were washed 3 times in 0.9% NaCl prepared on 10 mM Tris-HCl buffer (pH 7.4).

Conjugated dienes were measured in whole blood [3], MDA in the plasma and erythrocytes [1]. The content of reduced glutathione in erythrocytes was measured by Ellman's method [2]. Activities of SOD and catalase in hemolysates were measured [5]. Hemoglobin was measured using commercial reagents (Allwex Diagnosticum). Antioxidant activity of plasma components was evaluated by the kinetics of oxidation of 2,6-dichlorophenolindophenol reduced form by oxygen [10]. The content of uric acid in the plasma was measured by the enzymatic method using Allwex Diagnosticum kit.

The data were statistically processed by Student's *t* test using Statistica software.

RESULTS

The concentration of uric acid in the plasma increases by 66.2% in moderate hypothermia (Table 1). Uric acid is forming with generation of superoxide radical during purine nucleotide metabolism (with the formation of hypoxanthine and xanthine) under the effect of xanthinoxidase [11]. Hypoxanthine accumulates during body temperature reduction presumably because of more rapid catabolism of purine nucleotides as a result of stimulation of contractile and noncontractile thermogenesis under the effect of catecholamines.

Previous study [12] on mouse caudal arteries also showed that moderate cold exposure (28°C) stimulated, in contrast to warm exposure (37°C), ROS generation in smooth muscle cell mitochondria. ROS forming during this exposure promote vasoconstriction, but can also be released into circulation. These data indicate that the production of ROS in tissues increases significantly at the initial stages of hypothermia (30°C). Is this process paralleled by stimulation of oxidative modification of the most important biomolecules of the blood? In order to answer this question, we studied the intensity of LPO processes in the blood. Analysis of LPO products showed that the content of conjugated dienes in the blood increases by 19%, of plasma and erythrocyte MDA levels by 24 and 29.3%, respectively, in hypothermia in comparison with the control. Hence, ROS forming in moderate hypothermia promote oxidative destruction of plasma and erythrocyte membrane lipids.

Antioxidant activity of plasma hydrophilic elements is significantly (25%) higher in hypothermia than in the control (Table 2). High antioxidant activity in hypothermia seems to be due to accumulation in the blood of such antioxidants as ascorbic acid, uric acid, bilirubin, and transferrin- and ceruloplasmin-

TABLE 1. Blood Levels of Uric Acid and LPO Products in Rats Exposed to Moderate Hypothermia and Injected with Dalargin (*M*±*m*; *n*=8)

Group	Plasma level of uric acid, μmol/liter	Conjugated dienes, E_{232}/ml	Plasma MDA, μmol/liter	Erythrocyte MDA, μmol/liter
Control	242.8±15.1	1.41±0.09	1.92±0.06	52.3±2.4
Dalargin+control	260.6±20.4	1.54±0.05	1.46±0.07	56.7±1.3
Hypothermia	403.6±20.8**	1.69±0.05*	2.39±0.16*	67.6±0.8***
Dalargin+hypothermia	325.6±29.0**+	1.37±0.07	1.24±0.06***++	56.2±0.6+

Note. *p<0.05, **p<0.01, ***p<0.001 compared to the control. +p<0.01, ++p<0.001 compared to hypothermia.

TABLE 2. Antioxidant Activities of Plasma Hydrophilic Components, Erythrocyte Glutathione Content, and SOD and Catalase Activities in Rats Exposed to Moderate Hypothermia and Injected with Dalargin (*M*±*m*; *n*=8)

Group	Antioxidant activities of hydrophilic components, %	Glutathione, μmol/liter	SOD activity, arb. units/mg Hb	Catalase activity, μmol H_2O_2/mg Hb/min
Control	59.2±1.4	2.43±0.10	7.69±0.32	41.3±1.03
Dalargin+control	65.7±1.0**	4.38±0.16***	7.51±0.29	40.6±1.13
Hypothermia	74.1±0.6***	2.01±0.05*	9.48±0.41x	38.7±1.53
Dalargin+hypothermia	63.5±0.9	2.43±0.05+	9.58±0.43x	38.8±1.44

Note. *p<0.05, **p<0.01, ***p<0.001, xp<0.02 compared to the control. +p<0.001 compared to dalargin+control.

type proteins binding alternating valence metals and preventing their participation in Haber–Weiss reaction.

It is known that erythrocytes act as specific acceptors ("scavengers") of free radicals which help to control oxidative stress due to high level of reduced glutathione. The content of reduced glutathione in erythrocytes decreased by 17.3% in hypothermia in comparison with the control (Table 2). Drop of reduced glutathione level in erythrocytes under conditions of oxidative stress promotes iron release from hemoglobin [13]. Free iron ions stimulate LPO processes and erythrocyte hemolysis. Stimulation of intravascular hemolysis of erythrocytes was detected in moderate hypothermia [4].

Analysis of the enzymatic component of the antioxidant defense showed that erythrocyte SOD activity increases by 23% in hypothermia (Table 2). In contrast to SOD, catalase activity does not change in hypothermia. The increase in plasma antioxidant activity and erythrocyte SOD activity in moderate hypothermia are presumably compensatory shifts aimed at reduction of FRP in the blood.

Hence, all these data indicate that moderate hypothermal exposure is associated with the development of oxidative stress in the blood. This is paralleled by activation of various components of the blood antioxidant system.

In order to correct the FRP processes in the blood, the animals were injected with opioid peptide dalargin before cooling. Injection of dalargin to control animals led to a negligible (7.3%) increase of plasma level of uric acid (Table 1). In hypothermia, accumulation of uric acid in the plasma of animals injected with dalargin was less pronounced than in animals exposed to hypothermia and not receiving the peptide. The decrease of uric acid formation under the effect of dalargin was presumably a result of peptide inhibition of xanthine oxidase [7], leading to less intense formation of $O_2^{\bullet-}$ and H_2O_2. Dalargin is characterized by venodilating effect and reduces the arterial tone [9]. Presumably, dalargin stimulates opioid receptors in the arterial endothelium, this leading to stimulation of NO synthesis, causing vasodilatation, and reducing peripheral vascular resistance [8]. Presumably, hypothermal exposure after dalargin injection is not associated with the above-mentioned rapid production of ROS in smooth muscle cells' mitochondria and their release into circulation.

Dalargin did not appreciably modify the content of conjugated dienes in blood lipids and MDA in erythrocytes of control normothermal animals (Table 1). However, dalargin prevented accumulation of conjugated dienes under conditions of moderate hypothermia. In addition, dalargin modified essentially plasma MDA content (Table 1). In controls, dalargin reduced plasma MDA content by 26% 30 min after injection. Injection of dalargin before hypothermia (30°C) led to reduction of plasma MDA level in comparison with the control (by 38.2%) and in comparison with 30°C hypothermia without peptide injection (by 50%). Moreover, dalargin prevented elevation of MDA level in erythrocytes in hypothermia (Table 1).

In controls, dalargin slightly though significantly elevated antioxidant activity of hydrophilic antioxidants (Table 1). In moderate hypothermia, the peptide prevented elevation of antioxidant activity of these compounds. In controls, dalargin elevated (by 80%) glutathione content in erythrocytes (Table 2). In hypothermia, dalargin completely blocked the decrease in glutathione level in erythrocytes. Measurement of activities of erythrocyte antioxidant enzymes revealed no effect of dalargin on SOD and catalase activities in the control and hypothermia (Table 2).

Hence, our results indicate that short-term moderate hypothermal exposure stimulates LPO processes in the blood. Preinjection of dalargin in general prevented stimulation of LPO processes in the blood under conditions of hypothermia at the expense of inhibition of ROS generation and maintenance of high activity of antioxidant defense components.

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