GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Content of Trace Elements in the Lymph and Blood during Pyrogenal-Induced Fever and Complete Freund's Adjuvant-Induced Low-Grade Fever

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 148, No. 12, pp. 611-614, December, 2009
Original article submitted August 25, 2009

Experiments on rats showed that complete Freund's adjuvant-induced low-grade fever and pyrogenal-induced fever are accompanied by an increase in the content of Mn, Cu, Zn, and Se in the thoracic duct lymph. Our results indicate that the lymphatic system supplies trace elements to the circulation, which compensates for a deficiency in trace elements and maintains their systemic circulation. The decrease in the content of some trace elements in the blood during fever is probably associated with their excessive consumption (*e.g.*, to maintain the antioxidant defense system). During experimental low-grade fever and pyrogenal-induced fever, the lymphatic system serves as the reservoir for trace elements and plays a role in the regulation of trace element balance in biological fluids.

Key Words: *low-grade fever; fever; microelements; lymph; blood*

Trace elements (TE) play an important role in the maintenance of cellular and membrane homeostasis and regulate the intracellular functions. The lymphatic system has a modulatory effect on metabolic transformations of TE under various pathological conditions. Variations in the content of TE affect functional activity of lymphatic vessel lymphangions, which closes the circuit of autoregulation [3]. Moreover, the metabolism of TE forms the basis for other metabolic transformations [1,4,5,8]. On the one hand, the concentration and activity of antioxidant compounds (*e.g.*, antioxidant enzymes) in blood plasma and lymph depend on the content of TE. On the other hand, TE can directly increase prooxidant activity in biological fluids and tissues, thus aggravating the injury [3]. Our studies showed that experimental fever and low-grade fever are accompanied by an increase in the amount of antioxidant enzymes in the central lymph and blood.

Here we studied the dynamics of TE (Cu, Mn, Zn, Se, and Fe) in the thoracic duct (TD) lymph and venous blood plasma during pyrogenal-induced fever and experimental low-grade fever.

MATERIALS AND METHODS

Experiments were performed on 96 albino rats. Low-grade fever was induced by injection of complete Freund's adjuvant (CFA, single dose 0.15 ml; Difco laboratories) with 0.1% killed and dried *Mycobacterium butyricum* into the pads of both hindlimbs. This treatment was conducted in accordance to the requirements for a prolonged temperature response. Fever was induced by an intramuscular injection of pyrogenal in a

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0007-4888/09/1486-0852

daily dose of 100 μg/kg for 3 and 10 days. Control rats (3 groups) received an equivalent volume of the apyrogenic solution under similar conditions. The animals were anesthetized with sodium ethaminal in a dose of 50 mg/kg. The lymph was obtained by puncture of the TD ostium (close to the opening into the venous angle). The blood was taken from the lower vena cava. The animals were euthanized by a narcotic drug in the lethal dose. The contents of Mn, Cu, Zn, Se, and Fe were measured in biological fluids by means of mass spectrometry. The results were analyzed by parametric Student's *t* test.

RESULTS

A 3-fold injection of pyrogenal was accompanied by an increase in the contents of Mn, Cu, and Se in the lymph of TD (by 23.2, 33.0, and 34.1%, respectively, compared to the control). The content of Fe was reduced by 60%, while the concentration of Zn remained unchanged under these conditions. As differentiated from the lymph, the content of Mn, Cu, Zn, Se, and Fe in blood samples was decreased in all periods of the study (by 41.7, 44.3, 18.3, 25.7, and 35.5%, respectively). The directionality of changes in the contents of Mn, Se, and Fe in the central lymph on day 11 after 10-fold treatment with LPS was similar to that observed during the previous period. The concentrations of Mn and Se increased by 44.4 and 29.4%, respectively. Fe content was reduced by 37.5%. However, the amount of Zn was elevated by 35.8%. Cu concentration in the lymph and blood plasma decreased by 17.9 and 21.2%, respectively. The directionality of changes in the contents of Zn, Fe, and Cu (decrease) in the blood of animals was similar after 10-fold and 3-fold treatment with pyrogenal. The concentration of Mn remained unchanged, while the content of Se increased by 27.7% (Table 1).

Variations in the amount of TE under conditions of experimental low-grade fever differed from those observed during pyrogenal-induced fever. For example, the content of Fe remained unchanged in biological fluids. Moreover, no changes were found in the concentrations of Cu and Zn in blood samples. The contents of Mn, Cu, Zn, and Se in the TD lymph increased by 38.0, 18.5, 43.3, and 29.5%, respectively. The concentration of Mn in blood plasma was reduced by 31.2%, while the content of Se increased by 31.4%.

Mn is rapidly eliminated from the circulation into tissues (primarily into the liver). This element serves as an essential coenzyme of redox reactions in cell mitochondria. Mn enters the composition of a key enzyme, which protects the cell from reactive oxygen species (Mn-containing superoxide dismutase, Mn-SOD). It should be emphasized that there are 2 forms of SOD in mammals. The 1st form contains Mn, while the 2nd form includes Cu and Zn. These forms of SOD are characterized by different location in the cell. Mn-SOD is located in mitochondria, while Cu,Zn-SOD is present in the cytosol [14]. Endogenous pyrogen interleukin-1 (IL-1) serves as a stimulator of Mn-SOD synthesis. Mn^{2+} ions cause spontaneous production of IL-1 [4]. At the low content of Mn, Mn-SOD production is suppressed due to substrate deficiency and low stimulatory effect of IL-1. Hence, the increase in lymph Mn content can increase the antioxidant potential during activation of lipid peroxidation (LPO) under pathological conditions. Increased Mn concentration in the lymph probably has a detoxifying effect, since Mn neutralizes products of protein catabolism. This TE acts synergistically with Mg during activation of glutamine synthase [2].

Iron ions have a strong effect on LPO. Increasing the concentration of iron in blood plasma is accompanied by strong activation of LPO in various tissues, including the heart, liver, spleen, and plasma. Fe^{2+} ions are present in all systems for generation of reactive oxygen species from O_2 (mitochondrial, microsomal, and xanthine oxidase systems). They are essential for the production of OH^- [2]. Iron plays an important role in activation of LPO during degradation of lipid peroxides with the formation of reactive alkoxyl radical responsible for chain branching. Moreover, elevated content of Fe in the medium reduces significantly glutathione peroxidase (GPx) activity. GPx is one of the major antioxidant enzymes. It should be emphasized that Fe enters the composition of catalase. The decrease in Fe concentration in the central lymph and blood plasma during fever is probably related to transferring deficiency, changes in protein synthesis, and translocation of free Fe to the lymphoreticular tissue (the site of activation of antibody production).

Cu accumulation in the TD lymph is of particular significance. This TE initially binds to metalloprotein in the liver. Then, Cu is incorporated into ceruloplasmin and Cu-containing enzymes. Cu can play a role of the prooxidant (increase in the production of O_2^- and OH^-) or antioxidant, which depends on the concentration of this TE [7,9]. However, activation of LPO in mitochondrial and liver microsomes of rats was reported under conditions of reduced activities of SOD, catalase, and GPx under conditions of copper deficiency is followed by a significant [6]. In addition to Zn, Cu serves as an essential component of SOD, has antioxidant activity, and protects the cell from damage.

Zn ions are not involved in oxidation-reduction processes, but stabilize the sulfhydryl groups and protect them from oxidation by Cu and Fe ions [11]. Zn stimulates conversion of essential fatty acids to prostaglandins, prevents inactivation of NO by LPO

TABLE 1. Content of TE in TD Lymph and Blood Plasma of Rats with CFA-Induced Low-Grade Fever and Pyrogenal-Induced Fever (μmol/liter)

Parameter		Control-1	CFA administration	Control-2	Threefold treatment with pyrogenal	Control-3	Tenfold treatment with pyrogenal
Lymph	Cu	22.05±1.68	27.12±1.17***	21.69±3.71	28.85±2.16***	19.08±1.29	15.67±1.19***
		n=7	*n*=8	*n*=9	*n*=10	*n*=8	*n*=7
	Mn	0.071±0.007	0.098±0.11**	0.069±0.010	0.085±0.009***	0.063±0.008	0.091±0.007*
		n=7	*n*=8	*n*=9	*n*=10	*n*=8	*n*=7
	Zn	19.08±1.15	27.34±1.89*	19.55±1.05	21.78±1.97	18.89±1.81	25.66±2.03***
		n=7	*n*=8	*n*=9	*n*=10	*n*=8	*n*=7
	Se	6.61±0.71	8.56±0.83**	6.94±0.59	9.31±0.89***	6.33±0.61	8.19±0.75**
		n=7	*n*=8	*n*=9	*n*=10	*n*=8	*n*=7
	Fe	17.19±1.67	15.45±1.23	18.85±2.11	7.54±1.02*	17.67±1.88	11.05±1.54*
		n=7	*n*=8	*n*=9	*n*=10	*n*=8	*n*=7
Blood plasma	Cu	31.56±2.78	28.78±2.09	33.60±2.18	18.73±1.04*	30.12±1.97	24.61±2.56**
		n=7	*n*=7	*n*=7	*n*=8	*n*=7	*n*=11
	Mn	0.115±0.014	0.079±0.010*	0.108±0.012	0.063±0.016*	0.116±0.011	0.104±0.013
		n=7	*n*=7	*n*=7	*n*=8	*n*=7	*n*=11
	Zn	27.88±0.67	29.19±1.18	27.59±0.83	22.56±0.90**	25.79±1.03	20.21±0.89**
		n=7	*n*=7	*n*=7	*n*=8	*n*=7	*n*=11
	Se	12.93±0.64	17.00±0.85*	13.78±0.54	10.24±0.43***	14.53±0.73	18.55±0.95*
		n=7	*n*=7	*n*=7	*n*=8	*n*=7	*n*=11
	Fe	25.81±2.07	23.61±1.87	26.76±1.96	17.26±2.18**	24.93±2.15	15.31±1.82*
		n=7	*n*=7	*n*=7	*n*=8	*n*=7	*n*=11

Note. *n*, number of animals. *$p<0.001$, **$p<0.01$, and ***$p<0.05$.

products (indirect vasodilatory effect), and induces the expression of metalloproteins and immunophillins [10]. Immunophillins are a component of the general mechanism of cell protection from stress. Moreover, Zn stimulates the release of TNF-α. This endogenous pyrogen is released from depots under the influence of glucocorticoids [13]. It should be emphasized that the concentration of glucocorticoids increases during fever. Zn deficiency is characterized by reduction of blood steroids and, therefore, regulatory dysfunction of these hormones [12]. The increase in Zn concentration in the lymph under conditions of low-grade fever and long-term treatment with pyrogenal is probably related to activation of enzymes for nucleic acid metabolism and protein metabolism. The immunostimulatory effect of Zn is of particular importance. This action is realized at the level of intracellular messenger systems. Zn induces the production and potentiates the effect of some cytokines that stimulate natural killer cells [4,12]. The increase in Zn concentration during 10-day fever probably has the membrane-stabilizing effect, which is typical of this TE. The observed changes are of particular importance upon prolonged exposure to the pathogenic factor. Zn deficiency is characterized by activation of membrane LPO, which results in membrane labilization and development of nonspecific membrane "fluidity".

Se has a strong antioxidant effect, which is related to the presence of this TE in antioxidant enzymes (*e.g.*, GPx). Rat GPx is the major enzyme that catalyzes reduction of H_2O_2 and organic peroxides into water and hydroxy derivative, respectively, by glutathione. In the composition of GPx, Se suppresses the synthesis of inflammatory mediators (leukotrienes). This effect is mediated by the following two mechanisms: (1) inhibition of 5-lipoxygenase due to metabolic transformation of activators (fatty acid peroxides) and increase in the amount of inhibitors (hydroxy acids); and (2) competition for the substrate (5-OOH arachidonate). Se serves as an indirect inhibitor of leukotrienes, which

reduces the inflammatory response. Moreover, this TE promotes production of endogenous antioxidants of the protein or lipid nature [7].

Our results suggest that the increase in the concentration of Mn, Cu, Zn, and Se in the TD lymph during CFA-induced low-grade fever and fever is associated with their transport into the lymph flowing from the liver and intestine. The lymphatic system supplies TE to the circulation, which compensates for the deficiency in these compounds and maintains systemic circulation. The decrease in the content of TE in blood plasma during fever is probably associated with their excessive consumption (*e.g.*, for maintenance of the antioxidant defense system). During experimental low-grade fever and pyrogenal-induced fever, the lymphatic system serves as the reservoir for TE and plays a role in the regulation of their balance in biological fluids of the organism.

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