Antioxidant and Immunomodulating Effects of Ceruloplasmin in Experimental Influenza Infection

N. K. Berdinskikh, Z. D. Savtsova, O. L. Sanina, S. G. Antonenko, A. A. Orlovskii, M. Yu. Zaritskaya, and O. Yu. Yudina

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Exogenous ceruloplasmin is shown to increase the resistance of mice to influenza virus, reduce the immunodepressive effect of the virus, and improve the biochemical parameters in the acute period of experimental infection. A possible positive effect of ceruloplasmin on delayed complications of influenza is discussed.

Key Words: *ceruloplasmin; experimental influenza infection; immunity; lipid peroxidation; cyclic nucleotides*

Pneumonias of viral etiology developing in complicated influenza infection in humans are often difficult to treat and liable to run a protracted chronic course [10,11]. During influenza an inflammatory process in the lungs takes a chronic course mainly in cases of insufficiency of specific cellular immunity [9]. Agents characterized by immunomodulating and antiinflammatory properties are recommended for restoring the immune system activity suppressed by influenza infection and for preventing chronic inflammatory process in the lungs. Ceruloplasmin (CP), a copper-containing enzyme (E.C. 1.16.3.1), is one of the "acute phase" proteins and a component of the system of the organism's natural defense against various hazardous exposures [8]. The antiinflammatory effect of exogenous CP has been demonstrated [4], as well as its antioxidant and immunomodulating properties [1]. This research was undertaken to find out whether exogenous CP exhibits protective, antioxidant, and immunomodulating activities during an acute influenza infection.

Department of Biochemistry, Laboratory of Immunomodulation, R. E. Kavetskii Institute of Experimental Pathology, Oncology, and Radiobiology, Academy of Sciences of Ukraine, Kiev.. (Presented by T. T. Berezov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out with 300 CC57W male mice aged 2.5 months from the Experimental Breeding Center of the R. E. Kavetskii Institute of Experimental Pathology, Oncology, and Radiobiology, Academy of Sciences of Ukraine. The animals were divided into 3 groups: group 1, intact controls; group 2, animals infected with influenza virus; group 3, animals administered CP during influenza infection. Influenza virus A/PR8/34 was administered intranasally in a dose of 100 EID_{50}/0.2 ml. CP (a commercial preparation manufactured by the Kiev Enterprise for the Manufacture of Bacterial Agents) was injected intraperitoneally in a dose of 1.5 mg/kg 4 times at 2-day intervals starting from day 2 postinfection. The protective effect of ceruloplasmin was assessed from animal mortality during one month after viral infection. Biochemical and immunological parameters were measured on day 14 of infection. The antioxidative effect of CP was evaluated from the content of malonic dialdehyde [3], phospholipids [12], and cholesterol [15]. Serum oxidative activity was assessed after Ravin [14] and protein after Lowry [13]. The system of cyclic nucleotides was evaluated from the content of cAMP, cGMP, and their

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TABLE 1. Effect of CP on Biochemical Parameters of CC57W Mice Infected with Influenza Virus A/PR8/34

Parameter	Group		
	1	2	3
		Lungs	
Malonic dialdehyde, mmol/mg protein	2.2±0.2	5.8±0.8*	3.9±0.7
cAMP, pmol/g	200.0±2.3	172.0±1.6*	215.0±1.3*,**
cGMP, pmol/g	77.0±0.8	58.0±1.2*	69.0±1.1*,**
cAMP/cGMP	2.6	3.0	3.2
Phospholipids, μg P/mg protein	6.5±0.6	4.6±1.1	6.2±0.8
Cholesterol, μg/mg protein	20.1±0.7	39.4±1.5*	19.4±2.0**
		Blood	
Malonic dialdehyde, mmol/mg protein	2.8±0.3	2.2±0.2	2.0±0.2
Oxidase activity, mg%	10.2±3.0	70.4±7.0*	60.3±2.0*
cAMP, pmol/ml	41.0±0.9	22.0±0.7*	42.0±1.1**
cGMP, pmol/ml	22.8±1.1	17.7±0.8*	20.3±0.6**
cAMP/cGMP	1.8	1.2	2.1
		Liver	
Phospholipids, μg P/mg protein	9.5±0.5	6.4±1.8	6.6±1.0
Cholesterol, μg/mg protein	19.6±0.3	24.6±3.0	20.6±0.9
		Spleen	
cAMP, pmol/g	145.0±2.1	90.0±0.5*	256.0±1.6*,**
cGMP, pmol/g	133.0±1.5	53.5±1.1*	126.0±1.3*,**
cAMP/cGMP	1.1	1.7	2.0

Note. Here and in Table 2: asterisks show $p<0.05$; one asterisk *vs.* group 1, two asterisks *vs.* group 2.

ratio using the radioisotope method with standard Amersham kits. For assessment of the immunomodulating effect of CP the following parameters were assessed: the level of serum antihemagglutinins in the standard agglutination inhibition test; *in vitro* cytotoxicity of splenic lymphocytes towards syngeneic target cells infected with influenza virus A/PR8/34 [6] and towards A_5 target cells sensitive to natural killers [7]; the ability of splenic lymphocytes to induce the "graft versus host" (GVH) reaction in (CC57W×C57Bl)F_1 hybrids [5].

RESULTS

Injection of CP increased the resistance of mice to influenza infection. In group 2, 25% of mice died of influenza pneumonia on days 5-14 postinfection, whereas in group 3, 100% of animals survived for 1 month. Influenza was associated with intensification of lipid peroxidation in the lungs, a drastic (7-fold) increase of oxidative activity of the blood, a marked reduction of the cAMP and cGMP content in the lungs, blood, and spleen, and a changed ratio of cyclic nucleotides (Table 1). The content of phospholipids in the lungs and liver had a tendency to drop, while the cholesterol level rose (particularly in the lungs). In other words, changes of the biochemical parameters in mice infected with influenza virus indicated the development of an imbalance of the oxidative processes primarily in the lungs, a target organ for influenza virus, as well as in other organs and tissues. After injection of CP the content of malonic dialdehyde in the lungs showed a clear-cut tendency to drop, and the phospholipid content to increase. The cholesterol level in the pulmonary tissue reliably decreased.

TABLE 2. Effect of CP on Immunological Parameters of CC57W Mice Infected with Influenza Virus A/PR8/34

Parameter	Group		
	1	2	3
Serum antihemagglutinin titers	–	1:64	1:128
Cytotoxicity of splenic natural killers, %	14.3±2.6	10.9±1.5	16.2±1.3**
Specific cytotoxic activity of splenic lymphocytes, %	–	21.9±1.5	40.9±2.3**
Index of GVH reaction	18.9±2.2	10.9±1.3*	19.6±1.4**

Note. Dash: not tested.

Oxidase activity of the blood was somewhat reduced. Administration of CP during the acute period of infection led to an appreciable increase of the content of cAMP and cGMP in all the tissues examined, the increase of cAMP being particularly pronounced. The cAMP/cGMP ratio was higher in all tissues of group 3 mice than in groups 1 and 2. According to the sum of all biochemical parameters, group 3 mice were much closer to intact animals than to untreated mice with influenza pneumonia.

Table 2 shows that injection of CP virtually did not affect the titers of serum antihemagglutinins, but at the same time it increased the activity of natural killers, the intensity of specific cytolysis, and the ability of splenic lymphocytes to induce the GVH reaction. Increased cytolysis values indicate a positive immunomodulating effect of CP on cell-mediated antiinfluenza immunity. Intensification of the GVH reaction may be both a specific effect of CP related to the immunomodulating properties of the agent proper [15] and a result of the diminished secondary virus-induced immunosuppression due to a more benign course of influenza in group 3 mice. The findings suggest that administration of CP in the acute period of influenza infection raises the resistance of mice to influenza virus, reduces the immunodepressive effect of the virus, and improves the biochemical parameters of animals. The sum of CP effects may be indicative of a positive effect of CP on the delayed complications of influenza infection.

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