Changes in the Prooxidant and Antioxidant Status of Tissues during Paraneoplastic Processes

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Electron paramagnetic resonance was used to identify paramagnetic centers in the blood and liver of laboratory rats with S-45 sarcoma and mice with Ehrlich carcinoma. Paraneoplastic changes in prooxidant and antioxidant activity of the blood and liver tissue, mitochondrial respiration, and NO metabolism and inhibition of antioxidant processes contribute to impairment of cell membrane structures, erythrocyte hemolysis, and hypoxia. An important role of these processes in the pathogenesis of paraneoplastic anemia is confirmed by the positive effect of antioxidant and membrane-stabilizing therapy.

Key Words: *S-45 sarcoma; Ehrlich carcinoma; paraneoplasia; antioxidant system; nitric oxide*

Surgical, chemotherapeutic, and radiation methods of therapy are extensively used in the treatment of oncological patients. Despite technical success of therapeutic procedures, this disease often causes death of patients. It is probably associated with paraneoplastic changes in intact organs and tissues. They not only accompany and increase the severity of primary tumors, but also serve as the major cause of disability and death [7,8]. The processes observed under conditions of malignant growth have an important role in tumor disease. Study of the mechanisms for paraneoplasia and pathogenetic correction of paraneoplastic changes would allow us to prolong lifetime of oncological patients.

Changes in the prooxidant and antioxidant state of tissues underlay most pathological processes. Here we studied the changes in oxidation-reduction processes during malignant tumor growth, evaluated their role in the pathogenesis of paraneoplastic changes, and developed methods for correction of these abnormalities.

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MATERIALS AND METHODS

Experiments were performed on 60 male rats of various strains (220-250 g). The study was conducted on days 30 and 40 after transplantation of S-45 sarcoma. Sixty male mice (25-30 g) were examined on days 14 and 21 after transplantation of Ehrlich carcinoma. Electron paramagnetic resonance (EPR) spectra of the blood and liver were recorded before and after treatment with the antioxidant and membrane-stabilizing drug Plaferon LB using a RE-1307 radiospectrometer. The test drug in a daily dose of 10 μg/100 g was injected intraperitoneally over 10 days before the start of study [2,4]. The concentration of free nitric oxide (NO) was estimated using sodium diethyldithiocarbamate as a spin trap. The results were analyzed by Student's *t* test.

RESULTS

Study of blood samples showed that the EPR signal of oxidized ceruloplasmin and NO increases, while the Fe^{3+} signal decreases during tumor growth (Tables 1 and 2). Signals of Fe^{2+}, Mn^{2+}, and methemo-

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globin were identified in the EPR spectrum of the blood at the same stage of tumor growth.

The increase in the signal of oxidized ceruloplasmin and Fe^{2+} reflects a decrease in activity of the blood antioxidant system. Ceruloplasmin oxidizes Fe^{2+} to Fe^{3+}, which promotes Fe^{3+} incorporation into apotransferrin. Fe^{2+} ions generating free radicals and activating oxidation processes are removed from the blood. Tumor growth is accompanied by increased formation of free radicals and activation of lipid peroxidation (LPO). We revealed not only an increase in the signal of oxidized ceruloplasmin and Fe^{2+}, but also a decrease in the signal of Fe^{3+} transferrin.

Increased amount of Mn^{2+}-containing complexes preventing activation of antioxidant enzyme superoxide dismutase, and elevation of Fe^{2+} concentration have an important role in stimulation of LPO and damage to membrane structures [3].

NO activates peroxidation processes and causes damage to membrane structures. The signal of NO was observed in EPR spectra of animals with tumors. Increased formation of NO is probably related to the impairment of local blood circulation and microcirculation. Tumor growth is accompanied by tissue hypoxia. Under these conditions the nuclear factor NFkB activates inducible NO synthase, which increases NO formation [6].

LPO-activating agents Fe^{2+}, Mn^{2+}, and NO produce damage to cell membrane structures (*e.g.*, erythrocytes), which results in hemolysis. The EPR signal of methemoglobin was revealed in blood samples from animals with tumors.

Study of EPR spectra in the liver revealed dysfunction of the antioxidant system and changes in mitochondrial respiration of cells. We revealed an increase in the strength and decrease in the half-width of free radical signals. The appearance of high-intensity FeS signal reflected an increase in the ratio of the semiubiquinone form of ubiquinone in the overall free radical signal and transition of NADH dehydrogenase into the reduced from. Probably, electron transport in the NADH-ubiquinone-oxide reductase chain is impaired in hepatocyte mitochondria. It should be emphasized that ubiquinone activates LPO. Therefore, increased contribution of ubiquinone into the overall signal of free radicals should intensify LPO. Mitochondrial respiration is also impaired in the cytochrome P-450 site of microsomes. Changes in electron transport can be associated with stress-produced hypercatecholaminemia, activation of phospholipase A_2, accumulation of fatty acids, and influence of semiubiquinones that promote generation of superoxide radicals [5].

Neoplasm growth was also accompanied by an increase in the signal of ferricytochrome P-450, which reflects the inhibition of detoxification processes. Phospholipid component of microsomal membranes plays a role in function of the monooxy-

TABLE 1. EPR Signals in the Blood from Rats and Mice under Normal Conditions and during Tumor Growth (before and after Plaferon LB Therapy, *M*±*m*)

EPR signals	Normal	C-45 (day 30), Ehrlich (day 14)		C-45 (day 40), Ehrlich (day 21)	
		not treated	Plaferon LB therapy	not treated	Plaferon LB therapy
Methemoglobin		24.5±0.4	17.9±0.6*	22.0±0.5	10.4±0.4*
		4.0±0.3		9.8±0.3	9.0±0.2
Fe^{3+}	33.0±1.3	21.5±0.5	31.5±0.7*	28.3±0.5	28.2±0.4
g=4.2	31.0±1.3	26.2±0.7	29.0±0.8**	25.5±0.7	27.4±0.4**
Ceruloplasmin	20.2±0.8	27.6±0.7	23.2±0.7*	37.6±0.7	25.2±0.7*
	50.8±0.7	52.0±0.5	51.2±0.7	55.2±0.4	50.5±0.5**
Mn^{2+}		16.0±0.3	15.3±0.3	12.8±0.4	10.7±0.4**
g=2.14		10.0±0.3	7.5±0.2*	11.3±0.5	9.1±0.4*
Fe^{2+}		31.4±0.5	19.9±0.4*	29.7±0.7	29.5±0.9
g=2.2		19.0±0.5	8.5±0.4*	20.2±0.5	15.0±0.4*
FeS-NO		15.3±0.3	11.5±0.4*	16.7±0.3	17.8±0.3***
g=2.03		11.2±0.5	5.3±0.3*	14.2±0.4	10.3±0.4*
NO	16.0±0.5	20.8±0.4	17.4±0.7*	47.7±1.0	37.5±0.8*
g=2.02	18.3±0.7	25.1±0.5	20.3±0.4*	32.0±0.8	25.0±0.5*

Note. Here and in Table 2: *g*, splitting factor. *$p<0.001$, **$p<0.01$, and ***$p<0.05$ compared to untreated animals.

TABLE 2. EPR Signals in the Liver of Rats and Mice under Normal Conditions and during Tumor Growth (before and after Plaferon LB Therapy, *M*±*m*)

EPR signals	Normal	C-45 (day 30), Ehrlich (day 14)		C-45 (day 40), Ehrlich (day 21)	
		not treated	Plaferon LB therapy	not treated	Plaferon LB therapy
Intensity of free radicals	25.0±0.9	26.1±0.4	25.0±0.3***	28.8±0.5	24.6±0.4*
	27.0±0.8	39.1±1.2	30.0±0.5*	38.5±1.3	37.0±0.8
Half-width of free radicals	12.0±0.5	7.6±0.4	10.5±0.5**	10.2±0.2	12.7±0.4*
	14.2±0.5	12.5±0.7	14.0±0.5	12.3±0.6	12.0±0.4
P-450	12.1±0.7	25.1±0.4	13.6±0.4*	15.8±0.5	14.3±0.7
g=2.25	10.0±0.6	17.7±0.7	14.3±0.5*	20.0±0.5	19.5±0.6
FeS	25.0±1.2	27.3±0.6	21.5±0.4**	34.9±0.6	27.2±0.4*
g=1.94	41.7±1.3	53.5±0.7	53.3±0.3	71.4±1.7	65.5±1.2*
Mn^{2+}	10.0±0.3	12.8±0.9	9.8±0.4**	14.1±0.6	12.2±0.4**
g=2.14	0.5±0.2	1.2±0.3	1.0±0.2	1.3±0.3	1.2±0.2
FeS-NO		22.1±0.5	12.2±0.8*	19.3±0.5	20.1±0.5
g=2.03		1.1±0.2	1.0±0.2	1.4±0.2	1.5±0.2

genase system, which depends on cytochrome P-450 activity. This component of microsomal membranes determines the course and type of rate-limiting reactions in detoxification processes. Activation of LPO can damage microsomal membranes and change physicochemical properties of the lipid fraction, which leads to dysfunction of the monooxygenase system and suppression of detoxification processes.

EPR signals of Mn^{2+} and Fe^{2+} in the liver tissue serve as a criterion for damage to membrane structures of hepatocytes and erythrocytes. This hypothesis was confirmed during studying erythrocyte resistance. We showed that malignant tumor growth is accompanied by changes in osmotic and chemical resistance of erythrocytes, which plays an important role in the development of paraneoplastic anemia [1].

Plaferon LB therapy was followed by the decrease in EPR signals of Fe^{2+} and Mn^{2+} (promoters of free radicals in the blood), recovery of Fe^{3+} transferrin activity, significant decrease in the concentrations of oxidized ceruloplasmin and methemoglobin, and reduction of NO and FeS-NO signals. The EPR spectrum of the liver tissue was characterized by a decrease in the strength and widening of free radical signals.

Our results show that malignant tumor growth is accompanied by significant changes in the prooxidant and antioxidant status of tissues. These abnormalities play a role in the development of paraneoplastic changes in cell membrane structures (*e.g.*, paraneoplastic dysfunction of erythrocytes). Treatment with Plaferon LB increases activity of the organism, protects membrane structures, and improves mitochondrial respiration in cells.

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