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Biophysical and Biochemical Parameters of Organism During Experimental Malignant Tumor Growth

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Abstract

Have been studied biophysical parameters of blood, liver, spleen and cancer tissue by the method of Electronic-Paramagnetic-Resonance (EPR), activity of antioxidant enzyme catalase by the method of H. Aebi and ceruloplasmin concentration in the blood serum by the method of N. Babenko in white rats with Sarcoma C-45. Investigations revealed, that during Sarcoma C-45 growth antioxidant properties of blood are reduced, mitochondrial superoxiddismutase of tissues is inactivated, production of Mn^{2+}, NO, xantinoxidase, cytochrom P-450 and peroxidation of lipids (POL) are activated, membrane structures' destruction, hemolysis and hypoxia are developed. After treatment with Plaferon LB, mentioned disturbances are vividly improved. It is stated, that administration of Plaferon LB decreases POL, restores antioxidant ability of organism, protects membrane structures, reduces hypoxia. Plaferon LB reveals features of antioxidant and NO modulator, due to which could be explained marked improvement of above-mentioned disturbances.

Key words: *cancer, lipid peroxidation, EPR centers, blood, liver, spleen, catalase, ceruloplasmin, plaferon*

Introduction

Development of pathological process in most cases is closely related to cell injury. Indeed, a large number of chemical, physical, and infectious agents cause irreversible injury by damage to cell membranes.

One important mechanism of membrane damage is injury induced by free radicals, particularly by activated oxygen species [1.2]. Although, free radicals are now thought to be involved in many physiologic processes and are vitally important [3], accumulation of excess amount of free radicals reveals destructive affects on biomembrane, lipid, sulfhydril bonds of proteins, and nucleotides of DNA [4.5]. They may cause DNA damage, enzyme inactivation especially sulfhydril, peroxidation of lipids within cellular and organellar membranes and cause damage of endoplasmic reticulum, mitochondria, and other microsomal components [6.7].

In healthy state organism maintains normal concentration of free radicals. There are several systems, so-called antioxidants (superoxide dismutase, catalase, ceruloplasmin, glutathione peroxidase etc., that contribute to termination or inactivation of free radical reactions.

Because the malignant tumor growth is characterized by exaggerated proliferative processes, increased formation of free radicals and development of various paraneoplastic alterations leading to lethal outcome, investigation of protective systems of organism, biophysical and biochemical parameters of an "intact" organs and tissues (which are not directly involved or affected by the cancer) is very important. Besides,

correction of revealed paraneoplastic disorders would be of great value as well.

Materials and Methods

Experiments have been carried out on pubertal white rats (weight 120-150g) before and after treatment with Plaferon LB on the 21st, 30th and 40th day after implantation of Sarcoma C-45.

Electronic-Paramagnetic signals were studied by the Electronic -Paramagnetic-Resonance (EPR) method (Radiospectrometer PE-1307). Materials from blood, liver, spleen and cancer tissue were placed in polyethylene tubes and kept in liquid nitrogen (-180°C).

Catalase activity was measured by the spectrophotometric technique [8.9]. The method is based on the ability of hydrogen peroxide to form a stable stained complex with molybdenum salts. The reaction is triggered on by addition of 0,1 ml of the serum to 2 ml of 0,03% hydrogen peroxide solution , or 0,1 ml of water is introduced into the blank test. The reaction is stopped in 10 min by addition of 1 ml of 4% ammonium molybdate. The intensity of staining is measured spectrophotometrically at a wavelength of 410 nm against the control test, with 2,0 ml of water instead of hydrogen peroxide.

Ceruloplasmin concentration in the blood serum was determined by the intensity of oxidation of paraphennildiamine hydrochloric acid. The reaction is triggered on by addition of 1ml 0,9% NaCl to 0,2 ml serum, then 0,8 ml from the mixture is added by the 2ml acetat buffer and is kept in thermostate ander 37° C during 1 hour. After incubation, solution is added by 1 ml hydroxilamine and 10 ml 3% NaCl . Coloration of received solution is detected by the Spectrometer "Spectromom -202" at a wavelength of 530 nm [10].

Plaferon injections (10mcg /100g, once a day) were administered during 10 day before experiment.

Plaferon -LB is a drug of protein-peptide nature, which includes biological active substances of endogenous origin [11.12]. Plaferon is received from an amniotic membrane of human placenta and contains some physiologically active substances (interferon, endorphins, enkephalines, cytokines), therefore, Plaferon has different pharmacological effects: antiviral, immunomodulating, antihypoxic, antioxidant, antitoxic [13].

The obtained data was analyzed statistically.

Results

The results of present study revealed decreased antioxidant properties of blood *(Tab.1*) on the 21st day after implantation of Sarcoma C-45 which is manifested by increased intensity of EPR signals of oxidized ceruloplasmin (g=2,056) by 50% in comparison with norm.

At this time EPR signal of Fe^{3+} transferrin (g=4,2) is not changed. On the background of decreased antioxidant protection, lipid peroxidation is activated contributing to destruction of membrane structures and erythrocytes in particular, leading to hemolysis and production of methemoglobin . This last is confirmed by appearance of Met-Hb intensive signal (g=6,0) in the EPR spectrum. Furthermore, presence of signals of Mn^{2+} (g=2,14) and Mo^{5+} containing complexes in blood is the good evidence of membrane structures' destruction. Signal of Mn^{2+} complexes in turn indicates inactivation of mitochondrial superoxiddismutase (SOD), while signal of Mo^{5+} complexes reflects exaggerated production of xantinoxidase and developed ischemia.

In the presence of activated POL, as a result of membrane structures' destruction, inactivation of adrenoreceptors (g=2,01) is obvious. Besides, high concentration of Mn^{2+} ions presented in blood of experimental animals, results in disconnection of adrenoreceptors from system of adenylatcyclase. At last, Mn^{2+} ions are promoters of process of peroxidation. It must be mentioned, that EPR spectrum of blood reveals intensive signal of FeS-NO complexes (g=2,03), indicating activation of NO synthesis.

Inactivation of adrenoreceptors and oppression of protective and compensatory processes of organism causes subsequent aggravation of pathological process.

Manifestation of ferritin bound Fe^{2+} ions which is not detected in norm, indicates destruction of tissue cells.

On the 21st day after tumor implantation EPR spectrum of liver *(Tab.2.)* demonstrates disorder of hepatocytes' mitochondrial respiration at NAD.H: ubiquinon oxidoreductase locus manifested by the increase intensity of signal of free radicals (g=2,0) and decreased half width of it (ΔH).

Sharply increased intensity of signal of Mn^{2+} containing complexes reveals membrane structures' destruction in liver and inactivation of mitochondrial superoxiddismutase. Signal of Mo^{5+} containing xantinoxidase is increased as well, indicating ischemia of hepatocytes.

	INACTIVE RECEPTORS g=2,01	Met-Hb g=6,0	Fe^{3+} TRANSFERR g=4,2	CERULO PLASMIN g=2,056	Mn^{2+} g=2,14	Mo^{5+}	Fe^{2+}	FeS-NO g=2,03
Norm o	0,9±0,1	___	33,0±2,3	20,0±1,2	2,0±0,8	___	___	___
21st day after C-45 implantation x	1,9±0,07	20,0±0,3	32,7±0,3 $P_{0-x}>0,1$	30,6±0,4	12,3±0,5	13,1±0,4	22,7±0,7	16,8±0,4
30th day after C-45 implantation 1	2,8±0,009	24,5±0,4	28,3±0,8	27,6±0,7	16,0±0,4	14,1±0,6	31,4±0,7	15,3±0,2
30th day after C-45 implantation +Plaferon 2	2,1±0,08	17,9±0,7	28,2±0,7 $P_{1-2}>0,1$	23,2±0,8 $P_{0-2}>0,05$	15,3±0,3 $P_{1-2}>0,1$	13,3±0,4 $P_{1-2}>0,1$	19,9±0,5	16,7±0,5
40th day after C-45 implantation 3	2,5±0,06	22,0±0,5	21,4±0,8	37,6±0,8	12,8±0,3	13,6±0,4	29,7±0,9	11,5±0,6
40th day after C-45 implantation +Plaferon 4	2,0±0,09	10,4±0,5	31,5±0,7 $P_{0-1}<0,1$	25,2±0,4	10,7±0,5	10,9±0,3	23,2±0,5	17,8±0,4

Tab.1 *EPR spectrum of blood in rats during Sarcoma C-45 growth before and after treatment with Plaferon.*

Intensified signal of FeS-NO complexes (g=2,03) reflects activation of NO production. In liver, signal of Met-Hb is detected as well.

In the EPR spectrum of spleen *(Tab.3.),* on the 21st day after tumor implantation, signals of Ribonucleotid reductase (RR) and Fe^{3+} transferrin are not changed, while in tumor tissue signal of RR is intensified , which is characteristic for activated proliferative processes.

On the 30th day after tumor implantation, signal of oxidized ceruloplasmin of blood (g=2,056) is slightly increased in comparison with data obtained on the 21st day. Signal of Fe^{3+} transferrin decreases, which might be the result of decreased peroxidative activity of ceruloplasmin diminishing antioxidant ability of apotransferrin and leading to malfunction of erythro- and hemopoiesis.

At the same time, intensity of signal of Mn^{2+} containing complexes increases much more, indicating tissue destruction and disorder of mitochondrial electron transport. In the presence of activated POL, signals, characteristic for adrenoreceptors inactivation are increased as well.

In hepatocytes, on the background of disordered mitochondrial electron transport at NAD.H: ubiquinon oxidoreductase locus, deficit of substrate of mitochondrial respiration takes place, which is revealed by the presence of decreased signal of free radicals in comparison with data of 21st day.

Decreased signal of Mn^{2+} ions could be determined by the efflux of above-mentioned ions in the blood. Signal of FeS-NO (g=2,03) increases extremely, indicating exaggerated production of NO.

Decreased intensity of Fe^{3+} signal probably is the compensatory reaction of organism. On the background of decreased signal of Fe^{3+} transferrin in blood, signal of cytochrom P-450 increases, which is characteristic for

	NORM	21st DAY AFTER C-45 IMPLANT.	30th DAY AFTER C-45 IMPLANT.	30th DAY AFTER C-45 IMPLANT. +PLAFERON	40th DAY AFTER C-45 IMPLANT.	40th DAY AFTER C-45 IMPLANT. +PLAFERON
	0	X	1	2	3	4
I intensity g=2,00	25,0±0,9	35,1±0,8	26,1±0,7	25,0±0,3 $P_{1-2}>0,1$	22,8±0,8	24,6±0,5
ΔH half-width	12,0±0,5	7,2±0,4	7,6±0,3	10,5±0,2	10,2±0,1	12,7±0, $P_{0-4}>0,01$
Met-Hb g=6,0	___	28,1±0,5	21,9±0,8	___	15,7±0,5	2,8±0,5
Fe^{3+}transferrin g=4,2	33,0±2,3	___	28,3±0,8	28,2±0,7 $P_{1-2}>0,1$	21,4±0,8	31,5±0,7
Cytochrom P-450 g=2,25	12,0±0,8	17,7±0,3	25,1±0,4	13,6±0,5 $P_{0-2}>0,1$	15,8±0,3	14,3±0,8 $P_{0-4}>0,05$
FeS g=1,94	25,0±1,2	26,9±0, $P_{0-X}>0,1$	27,3±0,6 $P_{0-1}>0,1$	27,2±0,5 $P_{0-2}>0,1$	21,5±0,6 $P_{0-3}>0,05$	34,9±0,7
Mn^{2+} g=2,14	10,0±1,3	18,0±0,3	12,8±0,7 $P_{0-1}>0,1$	9,8±0,5 $P_{0-2}>0,1$	14,1±0,4	12,2±0, $P_{0-3}<0,05$
Mo^{5+}	8,1±0,3	14,7±0,4	14,5±0,2	9,1±0, $P_{0-2}>0,1$	13,2±0,5	10,5±0,4
FeS-NO g=2,03	10,0±1,4	14,8±0,5	22,1±0,6	32,2±1,0	19,3±0,6	24,1±0,8

Tab.2 *EPR spectrum of liver in rats during Sarcoma C-45 growth before and after treatment with Plaferon*

intoxication and inactivation of processes of detoxication.

On the background of hypoxia, activated POL and inactivated SOD, proliferative processes of organism decrease, which is revealed by the decreased signal of RR in spleen.

On the 40th day after tumor implantation, EPR spectrum of blood reveals further decrease of antioxidant ability of organism (oxidized ceruloplasmin is increased and Fe^{3+} transferrin is decreased). POL is exaggerated, which is revealed by the presence of disordered chain of mitochondrial respiration at NAD.H: ubiquinon oxidoreductase locus, developed ischemia and increased production of generators of oxygen free radicals - ubisemiquinons and xanthinoxidase in liver.

It should be mentioned, that excess amount of NO, revealed by the intensive signal of FeS-NO (g=2,03), in the presence of superoxidradicals and inactivated SOD, converts into peroxinitrit, which is known as high active free radical, thereby makes favorable conditions for further activation of POL. Developed condition in turn decreases RR signal in spleen, indicating decreased proliferative processes.

Biochemical investigation of blood, on the 21st, 30th and 40th day after tumor implantation, in comparison with norm, revealed sharply reduced activity of antioxidant enzyme catalase by 40,5%, 56,2% and 60,3% correspondingly.

Common activity of ceruloplasmin is decreased by 49,6%, 37,6% and 55,1% ($p< 0,001$) correspondingly.

Oxidized ceruloplasmin concentration is increased by 53,0%, 38,0% and 88,1% correspondingly. Ratio of oxidized ceruloplasmin to entire the ceruloplasmin concentration is increased, which points on elevation of quota of inactive ceruloplasmin, reduction of antioxidant ability of organism and activation of lipid peroxidation.

	RIBONUCLEOTID REDUCTASE RR		Fe^{3+} TRANSFERRIN g=4,2 IN SPLEEN
	SPLEEN	CANCER TISSUE	
Norm 0	30,0±1,2	___	33,5±1,5
30th day after C-45 implantation 1	21,2±1,0	27,4±0,4	20,4±1,4
30th day after C-45 implantation+Plaferon 2	25,2±0,7	18,5±0,2	23,5±0,6 $P_{1-2}>0,05$
40th day after C-45 implantation 3	17,7±0,6	32,7±1,0	21,2±1,0
40th day after C-45 implantation +Plaferon 4	21,9±1,1	18,7±0,7	32,1±0,7 $P_{0-4}>0,1$
21st day after C-45 implantation 5	33,1±0,9 $P_{0-5}>0,1$	31,1±0,4	32,8±0,8 $P_{0-5}>0,1$

Tab.3. *EPR signals of spleen and cancer tissue (Sarcoma C-45) in rats before and after treatment with Plaferon.*

According to data, obtained by the experiment with treated animals has been stated, that after treatment with Plaferon-LB, signal of oxidized ceruloplasmin in blood decreases, signal of Fe^{3+} transferrin increases, which corresponds to restored antioxidant ability of blood and erythropoiesis.

Restored antioxidant ability of blood in turn decreases POL, which is manifested by decreased amount of Met-Hb and reduced signal of cytochrom C oxidase.

It is remarkable, that despite treatment, EPR signals characteristic for inactive forms of adrenoreceptors are still increased. It is probably the result of exaggerated production of catecholamines, desensitization of adrenoreceptors and disconnection of adrenoreceptors from system of adenylatcyclase after influence of Mn^{2+} ions presented in blood in excess amount.

On the background of Plaferon-LB, mitochondrial respiratory chain at NAD.H: ubiquinon oxidoreductase locus is restored, which is manifested by the normalization of free radical signals.

Increased signal of NAD.H: dehydrogenase (g=1,94) on the 40th day after tumor implantation probably is the result of Plaferon-LB, which is capable somehow increase mitochondrial membrane permeability.

Signal of Met-Hb is decreased in both - blood and hepatocytes' EPR spectrum reflecting decreased hemolysis.

In case of Plaferon background, signals of Mn^{2+} and Mo^{5+} in the spectrum of blood and liver are lower than in case of untreated animals, indicating decreased production of xanthinoxidase and restored activity of SOD.

Noteworthy, that after treatment with Plaferon, in the EPR spectrum of liver and blood, signal of FeS-NO (g=2,03) is revealed indicating NO modulator ability of Plaferon.

In the spleen tissue, signal of RR and proliferative processes are activated, while in cancer cells mentioned signal and processes are sharply reduced.

In treated animals there is a tendency of catalase activation, however it is still decreased in comparison with norm. On the 30th day after tumor implantation common activity of ceruloplasmin in comparison with untreated animals (control) is increased by 63,2%. Moreover, it reaches the normal level .

On the 30th and 40th day of Sarcoma C-45 growth, Plaferon decreases concentration of oxidized ceruloplasmin by 15,9% and 33,0% correspondingly in comparison with control.

The ratio of oxidized ceruloplasmin to common rate of ceruloplasmin is decreased as well, that points on restored activity of enzyme.

Conclusions

1. During Sarcoma C-45 growth in the organism of experimental rats antioxidant properties of blood are decreased and mitochondrial SOD is inactivated;
2. Production of oxigen active form generators – Mn^{2+}, NO, Xanthinoxidase, Cytochrom P-450 and POL are activated;
3. Protective and compensatory reactions of organism are reduced;
4. Membrane structures and electron transport of mitochondrial respiratory chain are disordered;
5. Hemolysis and hypoxia are developed;
6. After treatment with Plaferon-LB antioxidant protection of blood and activity of hepatocytes' mitochondrial SOD are improved;
7. Intensity of production of oxygen free radical generators - Ubisemiquinon, Xanthinoxidase, oxidized cytochrom P-450 and POL are decreased;
8. Destruction of membrane structures, especially erythrocytes and mitochondrial membranes are decreased contributing to reduction of Hypoxia;
9. Intensity of RR signal in cancer tissue is decreased indicating delayed growth of cancer tissue.

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Биофизические и биохимические параметры организма в динамике экспериментального злокачественного опухолевого роста

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Р Е З Ю М Е

В динамике роста саркомы С-45 изучены биофизические параметры крови, печени, селезенки и опухолевой ткани методом электронно-парамагнитного резонанса (ЭПР), активность антиоксидантного фермента каталазы методом Н. Aebi и концентрация церулоплазмина в сыворотке крови методом Н. Бабенко. Установлено, что при злокачественном опухолевом росте снижаются антиоксидантные свойства крови, в тканях инактивируется митохондриальная супероксиддисмутаза, продукция Mn^{2+}, NO, активируется цитохром Р-450 и ксантиноксидазы, усиливается перекисное окисление липидов (ПОЛ). Происходит деструкция мембранных структур, развивается гемолиз и гипоксия. Плаферон выявляет антиоксидантное и NO-модуляторное свойство. После лечения значительно улучшается антиоксидантная защита организма, снижается ПОЛ, уменьшаются нарушения мембранных структур и уровень гипоксии.

Ключевые слова: *рак, перекисное окисление липидов, ЭПР центры, кровь, печень, селезенка, церулоплазмин, каталаза, плаферон*