

Effect of α -tocopherol on redox-status alterations and paraneoplastic disorders of erythrocytes

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ABSTRACT

Have been investigated erythrocytes' membrane resistance (ER), deformability (ED) and electronic-paramagnetic signals of blood and liver tissue of white rats (weight range 220-250 g.) during Walker carcinoma growth before and after treatment with α -tocopherol with the use of methods: spectrophotometry, filtration-computed photometry and electronic-paramagnetic resonance (EPR). Investigations have shown paraneoplastic alterations of redox-potential of blood and liver tissue, disorders of mitochondrial respiration and activity of antioxidant processes, exaggerated lipid peroxidation (POL), reduced ER and ED, altered processes of detoxication. Important role of redox-potential disturbances in paraneoplastic disorders of erythrocytes membrane is confirmed by positive influence of antioxidant and membrane-stabilizing therapy. After treatment with α -tocopherol the above-mentioned disturbances vividly were reduced.

KEY WORDS: cancer, paraneoplasia, erythrocyte, deformability, resistance, alpha-tocopherol

Nowadays, treatment of oncological patients involves surgical, chemical and radiotherapies. However, surgical intervention even carried out technically perfectly and other manipulations in most cases are hopeless and patients' life span is still limited and prognosis is poor. Presumably, the reason is ignorance of those paraneoplastic disorders of "intact" organs and tissues that always accompany malignant tumor growth. They are first "messengers" of grave and cruel disease, because paraneoplastic disorders are manifested long before the malignant growth reveals itself. Paraneoplastic processes aggravate course of prime disease and often play the leading role in the lethal outcome [1.2.3.4.5]. Thus, investigation of disorders of "intact" organs (organs that are not directly affected or involved in the malignant growth) and their pathogenetic correction, alongside with existing methods of treatment, considerably would increase the lifespan of oncological patients.

Malignant growth involves entirely the organism, affects organs and tissues directly or indirectly. Among "victims" are erythrocytes. They are very labile, fragile and no wonder that immediately reflect any deviations occurred in organism. Erythrocytes functional state in turn determines intensity of microcirculation and both are responsible for generalized hypoxia accompanying malignant tumor growth. As far as changes in redox-potential of cells and tissues underlie almost all pathological processes, in an attempt to state mechanisms of erythrocytes' membrane paraneoplastic disorders we decided to investigate processes of oxidation and reduction in case of malignant tumor growth, establish their role in pathogenesis of paraneoplastic disorders and elaborate appropriate methods of their correction.

Proceeding from the aforesaid, the purpose of our research was to study erythrocytes' membrane resistance and deformability, and electronic-paramagnetic signals of blood and liver tissue of laboratory rats in dynamics of experimental malignant tumor growth.

MATERIAL AND METHODS

Experiments have been carried out on pubertal white rats (body weight 220-250 g) on 5th and 12th day after implantation of Walker carcinoma.

Osmotic resistance of erythrocytes was studied according to the kinetics of their lysis by means of highly sensitive photoelectrocolorimetric differential method supplied by

automatic setting, which is characterized by high-resolution power and high precision. It provides computed registration of hemolysis kinetics, processing of results and submission of final results in both integral and differential shapes [6]. The curves of hemolysis were obtained in the moment of photometry by adding of distilled water into the cuvette at the constant speed (0,45 ml/min). The amount of cells: 1000 cells per 1 mcl was achieved according to the optical density at 37°C. Constantly twisting mixer in the cuvette prevented agglutination of erythrocytes. We have deliberately given up agglutination, as we wanted to observe single erythrocyte lysis kinetics in dynamics.

Osmotic resistance was estimated according to the parameter T - time, passed from the instillation of hemolysing mixture (distilled water) up to the development of erythrocyte lysis with maximal rate.

Erythrocytes' deformability was defined by means of computed filtration-photometry method, which completely excludes subjectivity in measuring the time of erythrocytes spreading and passage through the filter paper (Filtrac - 388). 1 ml blood and 20 units of Heparin were centrifuged during 5 minute at 3000 rpm. 0,02 ml from the erythrocytes' suspension was instilled on the filter. The erythrocytes' deformability is inversely proportional to the time needed for passage of cells through the filter. The time of erythrocytes passage was registered and processed by computer

Electronic-paramagnetic signals were studied by the electronic-paramagnetic resonance (EPR) method. Materials from the blood and liver were placed in polyethylene tubes and kept in liquid nitrogen (-196°C).

Intraperitoneal injections of α -tocopherol (1,2 ml/100 g a day, beginning from the next day after Walker carcinoma implantation) were used as membrane stabilizing and antioxidant agent.

Experimental animals were divided into three major groups: Norm (20 healthy rats), control (40 untreated rats) and experimental (40 treated with α -tocopherol rats). Obtained data were analyzed by Student's t criterion.

RESULTS AND DISCUSSION

The results of investigation have shown that in the process of malignant tumor growth intensity of EPR signals of

oxidized ceruloplasmin and nitric oxide (NO) in blood increase, and the signal of Fe³⁺-transferrin, in opposite, reduces (Tab.1). At the same terms of tumor growth in EPR spectrum of blood signals of Fe²⁺, Mn²⁺ and methemoglobin (Met-Hb) appear.

Increased activity of signals of oxidized ceruloplasmin and Fe²⁺ is the good evidence of decreased activity of antioxidant system in blood. It is well known, that ceruloplasmin, oxidizing Fe²⁺ ions into Fe³⁺, promotes inclusion of Fe³⁺ in apotransferrin thereby removing Fe²⁺ ions – the generators of free radicals and activators of peroxidative processes, from blood. Thus, process of free radical formation is supported amplifying the lipid peroxidation (POL). Simultaneously, with increase in signals of oxidized ceruloplasmin and Fe²⁺ ions, decreased signal of Fe³⁺-transferrin is revealed, that is negatively reflected on erythropoiesis.

The increase in signal of Mn²⁺ containing complexes, being inhibitor of activation of antioxidant enzyme – superoxidismutase, and Fe²⁺ ions play the leading role in the process of POL resulting disorder of membrane structures.

On the other hand, disorder of membrane structures is supported by another promoter of processes of peroxidation – NO, fixed in EPR spectrum of blood of experimental animals.

Exaggerated formation of NO, probably, is caused by disordered local hemocirculation and microcirculation. On the background of hypoxia, developed during malignant tumor growth, nuclear NFκB activates iNOS that in turn results in production of NO [7.8.9].

Production of POL promoters - Fe²⁺, Mn²⁺ and NO contributes to destruction of cell membrane structures including erythrocytes that makes favorable condition for hemolysis

Process of hemolysis is confirmed by presence of signal of MetHb in EPR spectrum of blood that has not been detected in norm.

EPR spectrum of liver (Tab.2) confirms worsening of organism's antioxidant system functioning and alterations in activity of mitochondrial respiration of cells: increases intensity of free radicals on the background of reduction of their half width. High intensity of FeS signal points on increased portion of ubiquinone in summary of signals of free radicals and conversion of NAD.H-dehydrogenase in reduced form. Thus, electron transport in hepatocytes' mitochondria is disordered at NAD.H-ubiquinone-oxidoreductase locus. Considering that ubiquinone is the generator of free radicals, increase of its portion in summary of signals of free radicals increases POL respectively. Mitochondrial respiration is disordered in microsomes at cytochrome P-450 locus as well.

The above-mentioned alterations of electron transport could be explained by hyper-catecholaminemia due to stress reaction, activation of phospholipase A₂, accumulation of fatty acids and action of ubisemiquinones, which in turn support generation of free radicals [10].

During malignant tumor growth increased intensity of ferricytochrome P-450 signal indicates decreased processes of detoxication. It could be explained as follows. During bio-transformation of organic substances

the phospholipid component of microsomal membranes plays significant role in functioning of monooxygenase system, which in turn depends on cytochrome P-450. The phospholipid component of microsomal membranes determines course and character of reaction limiting the rate of detoxication processes (passage of substrate in membrane, binding with cytochrome P-450 etc). Activation of POL, revealed at malignant tumor growth, is able to cause disorder of microsomal membranes and alterations of physical and chemical properties of its lipid fraction that is the reason of disordered functioning of monooxygenase system and altered processes of detoxication respectively.

Increased EPR signals of Mn²⁺ and Fe²⁺, detected in liver tissue confirm the process of disorder of cell membrane structures. Appearance of signals of nitrosil complexes of non-hem iron is the good evidence of hypoxia that is confirmed by presence of signals of MetHb.

After treatment with α-tocopherol, intensity of EPR signals of free iron Fe²⁺ and Mn²⁺ - promoters of free radicals were reduced. Activity of Fe³⁺-transferrin was restored, concentration of oxidized ceruloplasmin and MetHb, EPR signals of NO and FeS-NO were significantly reduced. In the EPR spectrum of liver tissue after treatment intensity of free radicals was decreased on the background of increased half width of it.

Disorder of membrane structures is confirmed by the results of investigation of erythrocytes' resistance and deformability. As it has shown (Tab.3), during malignant tumor growth ER and ED are significantly reduced. Erythrocytes' membrane disturbances could be explained by decreased antioxidant protection of organism and activated lipid peroxidation leading to destructive processes.

Decreased resistance and deformability of erythrocytes increases susceptibility to lysis. Probably, the mentioned paraneoplastic disorder of erythrocytes is the major cause of anemia that in most cases accompany malignant tumor growth.

Administration of α-tocopherol in common positively effects on redox-potential of cells and tissues and consequently - ER and ED. After treatment intensity of EPR signals of Fe²⁺ и Mn²⁺ - promoters of free radicals, were reduced, activity of Fe³⁺-transferrin was restored, concentration of oxidized ceruloplasmin, MetHb and signals of NO and FeS-NO were significantly decreased. In the EPR spectrum of liver tissue, intensity of free radicals was decreased on the background of increased half-width of its.

Thus, it could be concluded that the malignant tumor growth is accompanied by alterations in redox-potential of organism that probably play important role in mechanisms of paraneoplastic disorders of membrane structures of cells and erythrocytes in particular. α-tocopherol positively affects on antioxidant system of organism, increases its activity, improves electron transport at NAD.H-ubiquinone-oxidoreductase locus and mitochondrial respiration of cells, decreases process of POL, supports preservation of membrane structures, stabilizes erythrocytes' membrane, improves ER and ED thereby reduces anemia and hypoxic state of organism.

EPR signals	Norm	5 th day after walker carcinoma implantation		12 th day after walker carcinoma implantation	
		Control - untreated rats	Treated with α -tocopherol rats	Control - untreated rats	Treated with α -tocopherol rats
Fe ³⁺ G=4,2	33,0±1,3	22,0±0,5***	27,0±0,6*	20,0±0,6***	32,7±0,6*
Ceruloplasmin G=2,056	20,2±0,8	24,5±0,3***	21,7±0,5*	32,4±0,6***	27,3±0,4*
Mn ²⁺ G=2,14	2,0±0,4	12,7±0,5***	12,0±0,5	14,0±0,7***	10,7±0,3*
Met-Hb G=6,0	---	4,2±0,3	---	16,3±1,0	10,9±0,3*
Fe ²⁺ G=2,2	---	21,5±0,7	18,1±0,5**	24,3±0,6	21,2±0,5*
FeS-NO G=2,03	---	11,0±0,6	11,3±0,4	12,0±0,6	11,0±0,5

*p<0,001, **p<0,01 compared to control and ***p<0,001 compared to norm.

Tab.1 Intensity of EPR signals of blood in norm, during Walker carcinoma growth before and after treatment with α -tocopherol.

EPR signals	Norm	5 th day after walker carcinoma implantation		12 th day after walker carcinoma implantation	
		Control - untreated rats	Treated with α -tocopherol rats	Control - untreated rats	Treated with α -tocopherol rats
I-intensity G=2,00	25,0±0,9	41,1±1,1***	31,2±0,9*	40,8±0,7***	38,0±0,8**
Δ H Half-width	12,0±0,5	9,0±0,4***	13,8±0,6*	10,2±0,2***	12,1±0,3*
FeS G=1,94	25,0±1,2	30,0±1,2	28,5±0,6	37,0±1,1***	30,7±1,0*
Mn ²⁺ G=2,14	10,0±0,3	16,5±0,5***	14,0±0,3*	14,4±0,3***	10,4±0,6*
FeS-NO G=2,03	---	10,0±0,5	5,3±0,5**	11,0±0,6	6,5±0,3*
Fe ³⁺ transferrin G=4,2	33,0±0,7	28,3±0,8	29,8±0,7	24,4±0,8***	29,7±0,6*B
P-450 G=2,25	12,1±0,7	25,1±0,3***	19,7±0,5*	25,6±0,4***	24,3±0,6

*p<0,001, **p<0,01 compared to control and ***p<0,001 compared to norm.

Tab.2 Intensity of EPR signals of liver in norm, during Walker carcinoma growth before and after treatment with α -tocopherol.

NORM	5 th day after walker carcinoma implantation		12 th day after walker carcinoma implantation	
	ER	ED	ER	ED
ER - 260,0±4,3 ED - 2,86±0,12				
Control – untreated rats	208,5 ±2,8***	4,62±0,09***	193,2±3,4***	5,06±0,11***
Treated with α-tocopherol rats	220,2 ±2,2**	3,63±0,11*	254,7±2,6*	2,90±0,09*

*p<0,001, **p<0,01 compared to control and ***p<0,001 compared to norm.

Tab.3 Osmotic resistance (ER) and deformability of erythrocytes (ED – time of erythrocytes passage through the filter) in norm, during Walker carcinoma growth before and after treatment with α-tocopherol.

REFERENCES:

1. Steen A.E., Steen K.H., Bieber T. - Multiple paraneoplasia in the absence of a detectable tumour. // Acta Derm Venereol. 2001 May;81(2):150
2. Waldenstrom J., Paraneoplasia - Tumor products as signals. //Acta Clin Belg. 1986; 41(5):301
3. Erdmann H. - Paraneoplastic syndromes // Onkologie. 1984 Feb;7 Suppl 1:46-54. German
4. Rosenblum M.K., - Paraneoplasia and autoimmune injury of the nervous system: the anti-Hu syndrome. // Brain Pathol. 1993 Jul; 3(3):199-212. Review
5. Panizzon R.G., Speich R., Dazzi H. - Atypical manifestations of pityriasis lichenoides chronica: development into paraneoplasia and non-Hodgkin lymphomas of the skin. // Dermatology. 1992;184(1):65-9
6. Зедгинидзе И.Ш., Хулузаури О.В., Яковлев И.Л. - Новый дифференциальный способ для изучения распределения эритроцитов по резистентности и автоматическое устройство для осуществления способа // Киев «Вища школа», 1983, с.383
7. Reuter U., Chiarugi A. - Nuclear factor-kappa-B as a molecular target for migraine therapy. // Ann. Neurol. 2002. 51: 507-516
8. Kolb H., Kolb-Bachofen V. - Nitric oxide in autoimmune disease: cytotoxic or regulatory mediator? // Immunology today, Viewpoint, 1998
9. Ryan K., Ernst M., et al. - Role of NF-kappa-B in p53-mediated programmed cell death. // Nature 2000. 404: 892-897
10. Bayer R., et al. - The function of coenzyme Q in free radical production and as an antioxidant: a review // Chem. Ser., 1987, vol. 27, N1, p. 145-153

Влияние α-токоферола на изменения редокс-потенциала и паранеопластические нарушения мембран эритроцитов

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РЕЗЮМЕ

Методом электронно-парамагнитного резонанса (ЭПР) изучены биофизические показатели крови и печени лабораторных крыс в процессе роста карциномы Уокера до и после лечения α-токоферолом. Установлено, что в динамике роста опухоли снижается антиоксидантная защита организма - повышается концентрация окисленного церулоплазмينا, в тканях инактивируется митохондриальная супероксиддисмутаза, появляются интенсивные сигналы промоторов свободных радикалов Mn²⁺, Mo⁵⁺, Fe²⁺, в печени активируется цитохром P-450, усиливается перекисное окисление липидов (ПОЛ), способствующее нарушению мембранных структур, развитию гемолиза и, следовательно, гипоксии. Снижается резистентность и деформабельность эритроцитов. После патогенетического лечения вышеуказанные нарушения значительно уменьшаются, что подтверждает ведущую роль изменения редокс-статуса организма в патогенезе паранеопластических нарушений.

Ключевые слова: карцинома Уокера, редокс-статус организма, альфа-токоферол, резистентность/деформабельность эритроцитов, паранеоплазия