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The Malignant Tumor Growth and Reactive Forms of Oxygen and Nitrogen

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ABSTRACT

The purpose of this study was investigation of lipid peroxidation and nitric oxide (NO) concentration in blood and cancer tissue (in center and periphery) during sarcoma C-45 growth with the use of Electronic-paramagnetic-resonance method (EPR). Experiments have been carried out on 30 rats of mixed population (body mass 120-150 g). For detection of free NO concentration in blood, the spin-traps - Na diethyl-dithio-carbamate was used. The received data were analyzed statistically using the Student's t criterion. Have been stated, that in the process of sarcoma C-45 growth in central and particularly in peripheral region of the cancer tissue the NO synthesis is exaggerated that probably results in dilatation of supplying blood vessels in cancer tissue increasing trophicity and activating processes of proliferation. At the late period of cancer growth NO synthesis in central region of cancer decreases that could be explained by conversion of NO into peroxinitrite causing cell destruction. Along with that, lipid peroxidation in cancer tissue and organism is activated, which is manifested by increased intensity of oxidized ceruloplasmin, Met-Hemoglobinemia and increased concentration of Mn^{2+} and Fe^{2+} ions.

KEYWORDS: *sarcoma C-45, blood, cancer tissue, lipid peroxidation, met-Hb, ceruloplasmin, nitric oxide*

It is well known that the active forms of oxygen and nitrogen play an important role in cell injuries via the lipid peroxidation that makes the basis for oxidative destruction of cell membranes.

There are suggestions that nitric oxide is involved in the processes directed against cancer growth. It protects cell membranes from exo- and endogenous injuries, reveals citotoxic and mutagen activity comprising the nitrosoactive deamination, DNA strand disorders and etc.

The end result of nitric oxide dual (positive and negative) effect depends on its concentration, duration of generation, and state of antioxidant system [1].

Noteworthy, the vasodilatatory effect of NO. If we take into consideration the fact, that cancer growth demands increased blood supply which is provided via the angiogenesis [2] and vasodilatation, investigation of NO concentration in cancer tissue is of great value. The topicality of this question supported by other investigators [3].

The cancer tissue contains inducible synthase of NO (iNOS). This enzyme produces NO, which in turn probably supports cancer tissue blood supply and growth.

Coming from the aforesaid, the importance of NO production and its quantitative evaluation at different stages of cancer growth is obvious [4].

The aim of present study was investigation of oxidative processes and NO in blood and cancer tissue (in central and peripheral regions) during the sarcoma C-45 growth.

MATERIAL AND METHODS

Experiments have been carried out on pubertal white rats (weight 120-150 g) on the 30th, 40t^{h} and 50th day after implantation of Sarcoma C-45.

electronic-paramagnetic signals were studied by the Electronic-Paramagnetic-Resonance (EPR) method (Radio spectrometer PE-1307). Materials from blood, and cancer tissue were placed in polyethylene tubes and kept in liquid nitrogen (-180°C). For detection of free NO concentration in blood, the spin-traps - Na diethyl-dithio-carbamate was used.

RESULTS AND DISCUSSION

Investigations have shown that in the central region of the cancer tissue intensive signals of Mn^{2+} and Fe^{2+} ions are revealed indicating destructive processes developed in the cancer tissue. The received data are presented in *Tab.1* and *Tab.2.*

In the peripheral region of the cancer tissue intensity of the above-mentioned ions is reduced (30th day after C-45 implantation) and increases in the dynamic of the cancer growth (40th and 50th day). Obtained data indicates that destructive processes are more intensive in the central region of the cancer (initially). In the periphery cell destruction intensity depends on the period of cancer development. Mn^{2+} and Fe^{2+} ions are quite powerful promoters of free radical oxidative processes, continuously stimulating oxidative processes in cancer tissue. Therefore, super oxidative processes are much more intensive in the central region of the cancer tissue than in the periphery.

On the 30th day after C-45 implantation the intensive signal of NO was detected in the cancer tissue that probably results in dilatation of supplying blood vessels in cancer tissue increasing trophicity and activating processes of proliferation. It is remarkable that NO signal decreases in the central region of the cancer, and increases in the peripheral region of the cancer in the process of cancer growth. Along with that, the intense signal of ribonucleotid reductase is revealed in peripheral region of cancer as well.

Oxygen reactive form promoters, Mn^{2+} and Fe^{2+} ions high concentration after interaction with superoxidradicals increases probability of NO conversion into the peroxinitrite in this portion of the cancer. The mentioned reaction could be considered, as a reason for decreased NO concentration in the central region of the cancer tissue. Peroxinitrite is characterized by high free radical activity, thereby aggravating the destructive processes in the cancer tissue

The sharp increase of NO concentration in peripheral region of the cancer tissue at the late period of the sarcoma growth results in blood vessel dilatation and increased permeability, thereby improves trophicity and provides farther proliferation of the peripheral region of the cancer tissue.

The peroxidative processes are activated in blood of the experimental animals as well. The oxidized ceruloplasmin, Mn^{2+} and Fe^{2+} ions high concentration and Met-Hb signals are detected. The signal of Fe^{3+} transferrin decreases indicating suppression of erythro- and hemopoiesis eventually leading to hypoxia.

The EPR signal g=2,01 characteristic for blood formed elements adrenergic structures inactivation was increased. It is probably the result of the erythrocytes membrane lipoperoxidation and surface receptors inactivation.

Thus, it could be concluded that in the process of sarcoma C-45 growth the lipid peroxidation in cancer tissue and organism is exaggerated, which is manifested by inactivation of antioxidant enzymes, developed met-memoglobinemia and increased concentration of Mn^{2+} and Fe^{2+} ions.

Days after sarcoma C-45 implantation	***Mn^{2+} g=2,14***		***Fe^{2+} g=2,44***		***Ribonucleotidreductase RR***		***NO***	
	Center	Periphery	Center	Periphery	Center	Periphery	Center	Periphery
30 1	31,0±0,3	13,8±0,3	43,9±0,9	14,7±0,1	31,1±0,4	30,1±0,3	137,2±3,0	32,9±0,7
40 2	28,1±0,5 $P_{2,1}<0,001$	17,0±0,5 $P_{2,1}<0,001$	40,4±0,8 $P_{2,1}<0,002$	16,9±0,5 $P_{2,1}<0,005$	27,4±0,4 $P_{1,2}<0,01$	34,2±0,2 $P_{1,2}<0,001$	56,1±1,3 $P_{2,1}<0,001$	57,9±2,1 $P_{2,1}<0,001$
50 3	29,9±0,6 $P_{3,2}<0,005$	24,4±0,9 $P_{3,2}<0,001$	37,3±0,7 $P_{3,2}<0,02$	64,7±6,0 $P_{3,2}<0,001$	28,7±0,1 $P_{3,.2}<0,05$	34,1±0,4 $P_{2,3}>0,05$	22,7±0,5 $P_{3,2}<0,001$	66,9±3,8 $P_{3,2}<0,05$ $P_{3,1}>0,001$

Tab.1 *The EPR specter of cancer tissue in rats.*

Days after sarcoma C-45 implantation	***Inactive receptors*** *g=2,01*	***Met-Hb*** *g=6,0*	***Fe^{3+}*** *transferrin*	***Ceruloplasmin*** *g=2,056*	***Mn^{2+}*** *g=2,14*	***Mo***	***Fe^{2+}*** *g=2,44*	***FeS-NO*** *g=2,03*	***NO***
Norm	0,9± 0,1	_	33,0±2,3	20,0 ±1,2	2,0±0,8	_	_	_	16
30 1	2,8±0,09	24,5± 0,4	28,3±0,8 $P_{0.1.}<0,01$	27,6 ±0,7	16,0±0,4	14,1±0,6	31,4±0,7	11,5±0,2	26
40 2	2,5 ± 0,01	22,0±0,5	21,4±0,8 $P_{0.2.<}0,001$	37,6 ±0,8	12,8±0,3	13,6±0,4	29,7±0,9	11,5±0,6	28
50 3	2,9 ± 0,01 P<0,001	24,0± 0,3 P<0,001	21,4±0,8 $P_{0.3.}<0,002$	35,6±0,5 P<0,001	10,3±0,3	13,0±0,5	28,4±0,2	10,8±0,5	26

Tab.2 *The blood EPR specter of rats in norm and during sarcoma C-45 growth.*

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Содержание реактивных форм кислорода и азота в злокачественных опухолях

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Р Е З Ю М Е

Методом электронно-парамагнитного резонанса (ЭПР) в процессе роста злокачественной опухоли С-45 у лабораторных крыс изучены ЭПР центры и содержание NO в центре, на периферии опухолевой ткани и, также, в крови. В процессе роста опухоли в центральной и, особенно, в периферической части опухолевой ткани усиливается синтез оксида азота, в результате которого, повидимому, происходит дилатация кровеносных сосудов опухоли, улучшается её трофика, усиливается пролиферация. Параллельно росту опухоли в её центре содержание NO уменьшается, что должно быть связано с преобразованием оксида азота в пероксинитрит, вызывающего деструкцию и разрушение клеток и, соответственно, снижение синтеза NO в них. В опухолевой ткани обнаруживается наличие сигнала RR (рубонуклеотидредуктазы), который в центре опухоли при её росте не меняется. На периферии же - отмечается его увеличение. На основании анализа полученных данных можно заключить, что в процессе роста саркомы С-45 увеличивается интенсивность свободнорадикального окисления как в самой опухоли, так и в крови, что проявляется увеличением интенсивности ЭПР сигналов Mn^{2+} и Fe^{2+}, усилением сигнала окисленного церулоплазмина и появлением ЭПР сигнала метгемоглобина.

КЛЮЧЕВЫЕ СЛОВА: *саркома С-45, кровь, опухолевая ткань, пероксидация липидов, метгемоглобин, церулоплазмин, окись азота*