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OXIDATIVE METABOLISM OF RAT BLOOD IN THE COURSE OF ALVEOLAR HEPATIC CARCINOMA PC–1 GROWTH

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Aim: **To evaluate oxidative metabolism of rat blood in the course of alveolar hepatic cancer growth *in vivo*. *Methods*: The oxidation imbalance was assessed by the rise in the values of the integral index of oxidation stress. The structural and functional state of erythrocyte membranes was investigated by spin electron paramagnetic resonance spectroscopy. *Results*: The growth of alveolar carcinoma was found to be associated with intensification of lipid peroxidation processes with increased blood content of conjugated dienes, malonic dialdehyde against the background of decreased concentration of endogenous antioxidants tocopherol and retinol. Destabilization of the structural state of erythrocyte membranes of rat tumor hosts at the development of oxidation stress was studied, which was characterized by nonspecific structural changes of membrane sorption centres, reduction in specific capacity in the protein–lipid contact area and its increase in the phospholipid bilayer, rise in the degree of order and polarity. *Conclusion*: Alveolar carcinoma growth in rats resulted in intensification of free radical lipid peroxidation processes with a shift of the prooxidant–antioxidant balance to the left and development of oxidation stress.**
Key words: **oxidative metabolism, alveolar carcinoma, rat, homeostasis, membrane.**

In the normal state when body systems function in the conditions of physiological optimum, there is a prooxidant–antioxidant balance which is an essential mechanism of oxidative homeostasis. The balance is of labile nature, represents a resultant of opposing processes and is characterized by a variable mode of functioning within the limits compatible with viability and preservation of homeostasis.

In the stress setting caused by malignant tumor growth, catabolic processes are intensified, the amount of oxidized or partially oxidized active forms of oxygen, including radical products, increases, the oxidant–antioxidant balance shifts to the left towards domination and activation of lipid peroxidation (LP). The disorders in the functioning of LP–antioxidants system are manifested by intra– and intersystemic imbalance of the LP level and antioxidant content which may be defined by the following three types of reactions: compensation; overstress (compensating and not compensating the LP increase); exhaustion. It is just the development of the "oxidant peroxidant stress" that is likely to play a particular role among the factors of productive tumor intoxication owing to its practical significance and reactivity. Besides, lipoperoxides produce a co–cancerigenic effect, i. e. significantly enhance the action of veritable carcinogens [1–3]. Free radical LP reactions proceed most intensively in phospholipid structures and especially in the membrane lipid bilayer. Lipid oxidation in cellular membranes results in their increased permeability, microviscosity, reduced fluidity and inhibited enzymatic activity. The rate of lipoperoxidation reaction is determined by the extent of fatty acids unsaturation and depends on the structural organization of the lipid phase of biological membranes (molecular mobility of lipids, strength of lipid–lipid and protein–lipid interactions). At the same time, covalently bound associations of membrane proteins are formed in the membrane hydrophobic interior. The disorders in the structure and properties of interrelated membrane components affect the general metabolic state of the cell, its homeostasis and life time [4–6].

The purpose of this study was to evaluate oxidative metabolism of rat blood in the course of alveolar hepatic cancer growth.

MATERIALS AND METHODS

The experiments were performed on 70 white random–bred male rats 150–200 g of weight, reared at the nursery of the State Institution "N. N. Alexandrov Research Institute of Oncology and Medical Radiology" (Minsk, Belarus). All animal procedures were carried out under the rules of local Ethics Committee. The experimental animals were divided into 4 groups: group 1 — intact rats (n = 10), groups 2–4 — rats with inoculated alveolar hepatic carcinoma PC–1 (n = 20 in each group). The inoculation was performed by subcutaneous injection of 0.5 ml of PC–1 20% cellular suspension in Hanks' solution, the final concentration of the cells not exceeding $2 \cdot 10^6$. All experiments were carried out under neuroleptanalgesia (Droperidol and Phentanyl, 2 : 1, 0.3 ml per 100 g of animal mass i.m.) on days 8, 16, 30 after PC–1 inoculation.

The intensity of blood oxidative metabolism in the experimental animals was assessed by the level of LP and antioxidant system (AOS) processes. The primary (conjugated dienes (CD)) and secondary (malonic dialdehyde (MDA)) LP products, the content of non–enzymatic endogenous antioxidants (tocopherol (E) and retinol (A)) were determined in blood serum. The CD

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Abbreviations used: 5–, 12–DSA — doxylstearic acid; A — retinol; AOS — antioxidant system; CD — conjugated dienes; E — tocopherol; EPR — electron paramagnetic resonance; K — oxidation stress rate; LP — lipid peroxidation; MDA — malonic dialdehyde.

content was evaluated by spectrophotometry in hexane extract from the difference in optical density between the experimental and control samples at 233 nm [6]. MDA was evaluated by spectrofluorometry from the colour reaction with thiobarbituric acid [7]. The levels of E and A antioxidants were determined by spectrofluorometry [8]. To evaluate the imbalance in the LP–AOS system, the integral index — oxidation stress rate K — was used [9]. The K rate was calculated employing the obtained values of LP and AOS from the formula:

where i–values are obtained from rats with inoculated PC–1 and n – values — from intact animals. With equilibrium in the LP–AOS system being maintained, the integral rate K≈1. With the LP processes being enhanced, the K value increases. The use of the integral index makes it possible to concurrently assess the LP process level, AOS efficiency and determine the extent of imbalance in the LP–AOS system.

The structural and functional state of erythrocyte membranes was investigated by spin electron paramagnetic resonance (EPR) spectroscopy. The experimental animals' blood was sampled after their decapitation. The erythrocytes were derived from the blood taken with heparin solution (1 : 10) by 4–fold centrifugation in sodium chloride isotonic solution containing 10 mmol/l of Tris–HCl (pH 7.2) for 5 min at 1,500 rpm. 50 μl of the erythrocyte suspension (cell concentration 1.5 million cells in 1 ml of the solution) were mixed with ethanol solution of the spin probe (Fig. 1), the final concentration of 12–doxylstearic acid (DSA) probe I (5,10) in the sample being 10^{-8} mM, of 5–DSA probe I (12,3) — 10^{-6} mM (Sigma, USA). The concentration of ethanol did not exceed 1% (v/v). EPR spectra were registered by PC–100X spectrometer under the following conditions: SHF power 10 mW, modulation amplitude 0.7 mT, magnetic field scan rate 12 mT/min, field scan centre 339 mT, sample temperature in the resonator 37 °C.

The following biophysical parameters were calculated by EPR spectra (Fig. 2) to define the local environment (microviscosity) of the spin probes in spin–labelled erythrocyte membranes: membrane specific capacity in protein–lipid contact area (C_1) and phospholipid bilayer (C

(4.2% and 5.6% respectively, $p > 0.05$). The oxidation stress rate rose 1.5–fold in animals with inoculated PC–1 compared to that in the intact group ($p < 0.05$). In the subsequent period of observation, intensification of lipoperoxidation processes in the course of PC–1 tumor growth (day 16) in the experimental animals correlated with increased levels of primary (CD by 33%) and secondary (MDA by 24%) LP products which is indicative of the toxic phase of tumor intoxication ($p < 0.05$). A decrease in the content of endogenous antioxidants (tocopherol 1.4–fold and retinol 1.2–fold) was observed in the study group of rats with PC–1 vs. the control ($p < 0.05$). The oxidation imbalance in the LP–AOS system functioning increased 2.8–fold compared to that in the intact group. The rise of the integral index of oxidation stress may be regarded as a stage of a relative compensatory overstress in the functioning of antioxidant body defense against free radical intermediates.

In the terminal stage of the neoplastic process in the rat tumor hosts (day 30), CD and MDA concentrations in blood were growing exponentially (up to 185.7% and 143.1% respectively) compared to the control values ($p < 0.05$). Increased accumulation of the products of incomplete metabolism and metabolism of the tumor itself in all body sectors (intercellular, tissue, organic) in the terminal stage of rat alveolar hepatic carcinoma growth is related to the reduction in the content of antioxidants — tocopherol to 65.3% and retinol to 72.2% compared to the values in the intact group ($p < 0.05$). The oxidation stress rate in rat tumor hosts exceeded the norm 5.6–fold. In the terminal stage of alveolar carcinoma growth a pronounced oxidant imbalance is likely to occur with a shift to the left towards predominance of free radical lipoperoxidation processes and with decompensated exhaustion in the antioxidant defence functioning.

The analysis of the dynamic properties of some areas of erythrocyte membrane by means of selectively bound spin probes demonstrated that the development of neoplastic disease in the experimental animals was accompanied by re–distribution of specific capacities of membrane binding centres. In the initial period of tumor growth (day 8) in the rats, a decrease in the binding ability of erythrocyte membrane was established in protein–lipid contact area for nitroxyl radicals I (5,10) and I (12,3) by 19.5% and 13.1% respectively compared to the control values. In the phospholipid bilayer area, the binding ability of the membranes of erythrocyte population cells increased 1.2–fold in the experimental animals compared to intact ones. The growth of alveolar carcinoma in the rats was associated with a fall of membrane specific capacity in the protein–lipid interaction area 1.5–1.6–fold and a rise of the capacity in the phospholipid bilayer area by 33–59.4% on the 30th day after inoculation.

The phospholipid bilayer is the basic structural unit of biological membranes, possessing concurrently fluidity and microviscosity. The study ascertained elevation of the degree of order of spin–labelled erythrocyte membranes by 13.8–27.5% in rat tumor hosts in the terminal stage of the disease vs. the parameter value in the control group ($p < 0.05$).

The development and growth of malignant tumor is related to activation of the processes of free radical LP initiating the processes of phospholipolysis. A moderate rise in the level of lysophospholipids in the cellular membrane promotes an increase in the activity of hexose monophosphate shunt enzymes, membrane transport ATPases; considerable accumulation of lysophospholipids makes it possible for lipid bilayer to convert into monolayer with disruption of permeability for Na^+, K^+ ions, formation of hydrophilic channels and enzyme solubilization.

The disturbance of the structural organization of the phospholipid bilayer in the course of tumor growth in rats is associated with an increase in the time of correlation of rotational diffusion of I (5,10) and I (12,3) nitroxyl radicals 1.4–1.5–fold on the 30th day after PC–1 inoculation. The correlation time is known to be determined by solvate environment of nitroxyl radicals and to depend on reorganizations of phosphate groups in the rotation and polarity area.

The comparative analysis of the data demonstrated that the development of the malignant disease in the experimental animals was accompanied by a statistically significant rise in the values of polarity parameters $2A_{||}$ and $2A_{\perp}$ (by 117.7–123.3% and 103–107.5% respectively) compared to those in the control group. The increase in the polarity in the depth of the lipid layer of erythrocyte membranes in the course of tumor growth may occur in consequence of elevated membrane permeability and accumulation of a great number of polar molecules of water because of enhancing LP processes and opening polar groups of protein molecules.

On the whole, the study showed that alveolar carcinoma growth in rats resulted in intensification of free radical LP processes with a shift of the prooxidant–antioxidant balance to the left and development of oxidation stress. Increased formation and accumulation of primary and secondary LP products causes irreversible exhaustion in antioxidant defense system functioning and a statistically significant oxidation imbalance in rat tumor hosts.

The development of oxidation stress brings about a disorder in the structural and functional state of erythrocyte plasmatic membranes which is manifested by non–specific structural changes of sorption centres with reduction in specific capacity in the protein–lipid interaction area and its increase in the phospholipid bilayer. The rise in microviscosity and the fall in fluidity of cellular membranes in the course of tumor growth entail elevation of the degree of order and polarity of the phospholipid bilayer, increased correlation time which is associated with degradation of viscous elastic properties and deformability, changes in the form and size of erythrocytes, enhancement of aggregating ability.

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ПОКАЗАТЕЛИ ОКИСЛИТЕЛЬНОГО МЕТАБОЛИЗМА КРОВИ КРЫС В ПРОЦЕССЕ РОСТА АЛЬВЕОЛЯРНОЙ КАРЦИНОМЫ РС–1 ПЕЧЕНИ

Цель: **исследовать окислительный метаболизм крови крыс в процессе роста альвеолярного рака печени.** ***Методы*****: окислительный дисбаланс оценивали по повышению интегрального показателя оксидантного стресса. Структурное и функциональное состояние мембран эритроцитов крыс исследовали методом электронного парамагнитного резонанса.** ***Результаты*****: установлено, что рост альвеолярной карциномы сопровождается активацией процессов перекисного окисления липидов с повышением содержания в крови диеновых конъюгатов, малонового диальдегида на фоне снижения концентрации эндогенных антиоксидантов токоферола и ретинола. При развитии оксидантного стресса отмечена дестабилизация структурного состояния мембран эритроцитов крыс — носителей опухолей, характеризующаяся неспецифическими перестройками центров сорбции мембраны, уменьшением удельной емкости в области белок-липидных контактов и ее увеличением в фосфолипидном бислое, повышением упорядоченности и полярности.** ***Выводы*****: рост альвеолярной карциномы у крыс приводит к активации процессов перекисного окисления липидов со сдвигом равновесия прооксиданты—антиоксиданты влево и развитию оксидантного стресса.**

Ключевые слова**: окислительный метаболизм, альвеолярный рак, крыса, гомеостаз, мембрана.**