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Participation of xanthinoxidase system in development of oxidative stress under irradiation

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It has been established, that ionizing radiation of 1.0Gy dose causes the activation of prooxidant enzyme xanthinoxidase in spleen lymphocytes that is accompanied by enhanced formation of superoxidase anion-radicals and testifies to the participation of the xanthinoxidase system in radiation induced cell death. The increase in oxidative modification of proteins in splenocytes in three hours after total X-irradiation of rats was shown.

Key words:

Introduction. The development of oxidative stress in cells may be considered a key factor at the programmed death induction caused by the action of radiation. In the process of normal life an animal organism keeps up to a certain ratio between the activity of antioxidant system components, which provide necessary stationary concentration of free radicals, and reactions producing these metabolites. It is known that ionizing radiation leads to the essential disorder of antiradical protection due to the shift balance in prooxidant-antioxidant system towards the excessive generation of active oxygen metabolites (AOM) at simultaneous exhaustion of endogenous enzyme systems and low molecular antioxidants pool (oxidative stress). In these conditions the activation of critical processes for the cell homeostasis maintenance, namely, peroxide lipid oxidation, oxidative modification of proteins and nucleic acids, which is a result of rapid AOM level increase due to their direct formation at water radiolysis and an increase in metabolic generation. Regardless of the evident interrelation between oxidative stress and programmed cell death, apoptosis, still not only the mechanisms, which condition this metabolic situation, remain not ascertain, but also the role of certain radicals in the process of cell self-destruction. Moreover, the question whether oxidative stress is the consequence or the inductor of functional changes, which accompany the processes of programmed cell death, is currently under active examination.

It is known that one of the sources of AOM, superoxidative anion-radicals in particular, is the xanthinoxidase reaction. O_2 surplus, directly and through the formation of high-reactive OH^- and singlet oxygen radicals, assists in initiating and branching of chain free-radi-

cal reactions in cells. Though the importance of xanthinoxidase/xanthindehydrogenase system is high, the data regarding its participation in the destructive effect of the ionizing radiation are not satisfactory enough. Taking into account the role of xanthinoxidase in producing free radicals, it is reasonable to evaluate the contribution of this enzyme system into the development of radiation induced death of lymphoid cells.

Materials and Methods. The experiments were performed on nonlinear male rats, weight 150-170g. One-time total irradiation of the animals was performed using X-ray unit RUM-17 (РУМ-17) in the dose of 1.0 Gy at the following conditions: filters 0.5 mm (*Cu*) and 1 mm (*Al*) skin focusing distance 50 cm, 200 kV, 5 mA, dose power 0.17 Gy/min. The animals were decapitated in three hours after the action of radiation. Spleen lymphoid cells were removed according to the commonly accepted method [2]. The xanthinoxidase activity was determined through the uric acid accumulation [3]. The intercellular level of hypoxanthine and xanthine was determined by including isotopic label C^{14}-adenosine (specific radioactivity 43 mCu/mmole) to the pool of investigated substances [4]. The determination of generation speed of superoxidative anion-radicals was performed by the method [5]. The degree of protein oxidative modification was estimated according to 2,4-dinitrophenylhydrosons accumulation in the cells [6]. The statistics of the obtained results was performed using Microsoft Excel, by the Statistic Analysis method and Student's criteria.

Results and discussion. Xanthinoxidase is a commonly known prooxidant enzyme, represented in cells by two forms, which differ in their acceptor properties, namely xanthin dehydrogenase (DX), for which the final electron acceptor is NAD^+, and by xanthine oxidase (OX), when the function of electron acceptor is performed by molecular oxygen. The realization of dehydrogenase or oxidase activity depends on medium conditions and is determined by the enzyme conformation, the important role in which belongs to oxidized and reductant thiol-groups ratio as well as to the existence of polypeptide fragment (mol. weight 20 kDa) in one of the enzyme subunits [7,8]. A current opinion is that under the ionizing radiation, the DX transforms through several intermediate states into the OX, the functioning of which is connected to monoelectron oxygen reduction with superoxide anion-radicals formation.

The data presented in Table 1 show that the irradiation leads to the activation of enzyme oxidase form. In three hours after the irradiation of the animal (1.0 Gy), statistical increase (1.2 times comparing to the control samples) in the OX activity was observed.

Table 1. Xanthinoxidase activity and total radioactivity of hypoxanthine and xanthine fractions in rat spleen lymphoid cells; M±m, n=5

	Xanthinoxidase activity, nmoles of uric acid/10^8 cells per minute	Total radioactivity, cpm/10^7 cells	
		hypoxanthine	xanthine
Control	60±5	970±84	820±78
1.0 Gy	71±8*	1389±130*	1312±102*

** - the difference with the control is reliable ($p<0.05$)*

Since OX is one of the final enzymes of purine metabolism, its activity in mammals is tightly connected to the intensity of purine transformation. As it is known, purine metabolism disorder induced by radiation is accompanied by the increase in adenylic nucleotides degradation.

In our opinion, a possible reason of the OX activity increase, along with the partial enzyme proteolysis and oxidation of reactive sulphohydril groups [9], may be the accumulation of purine degradation products in the cells, such as hypoxanthine and xanthine, which are specific substrates of the enzyme. Indeed, during the research, the increase in general radioactivity of hypoxanthine and xanthine fractions of spleen cells was found to be 40 and 60 % respectively, which indicates the after-irradiation disorder of purine metabolism, the activation of catabolic branch of adenylic nucleotides conversion, in particular.

The data obtained are confirmed by the results of electronic paramagnetic resonance (Table 2), according to which in three hours after total irradiation of rats (1 Gy) the increase in O_2 speed generation by 1.3 times is observed in splenocytes, which, taking into account a wide activity spectrum of superoxide radicals, especially their capability to initiate free radical chain reactions, determines the formation of pathobiochemical mechanisms of promotion and realization of radiation injuries of critical cell structures. Thus, the increase in intracellular free radicals concentration causes the activation of A2 phospholipase, which, in its turn, leads to the lipid content disorder, inducing non-specific permeability of mitochondrial membranes and proteolytic enzymes penetration into the cytoplasm. In the work [10] the liberation of a mitochondral protease from intermembranous space is shown, which by catalyzing removal of the polypeptide fragment from xanthinoxidase, assists irreversible conversion of dehydrogenaseng activity into oxidizing one. Similar statement is partly founded [11], where the significant increase in xanthinoxidase activity, at the addition to incubation on

Table 2. Generation speed of superoxidizing anion-radicals and degree of protein oxidizing modification in rat splenocytes; M±m, n=5

	nmoles O2/107 cells per minute	Protein oxidizing modification degree, nmoles 2,4-dinitrophenylhy drosons/107 cells
Control	0.5±0.02	4.2±0.2
1.0 Gy	0.65±0.04*	5.0±0.5*

** - the difference with the control is reliable (p<0.05)*

the medium fractions, enriched by nuclei and mitochondria, is demonstrated.

In recent years much attention has been paid to a role of active oxygen metabolites in the processes of protein metabolism, both at normal and different pathological conditions. The increased AOM generating during the oxidative stress development causes not only the activation of lipid peroxide oxidation, disorder of nucleic acids structure, but also the oxidative destruction of protein molecules, which leads to the conformation changes, and therefore, functioning of both soluble and membrane-binding enzymic complexes, receptors, ion channels and is accompanied by deep changes in the cell metabolism [12, 13]. The increase in intracellular content of oxidized proteins may be considered as early criterion of tissue injuries by free radicals and in some cases it amounts to 50-70 % of all the cell proteins.

Therefore, at the next stage of our work we estimated a degree of oxidizing modification of protein molecules during the development of irradiation induced interphase death of the spleen lymphocytes.

The results of our experiments showed that in the splenocytes of rats irradiated by 1.0 Gy dose, there is a statistically reliable increase in phenyl hydrosone content, which amounts to 122% comparing to the control. The data obtained conform with the results of [14], in which the authors found the intensification of protein oxidizing destruction in the cells of radiosensitive organs as a response to ionizing irradiation (Table 2).

Intracellular level of oxidized proteins depends on the AOM generating rate, therefore, on factors that provoke their formation, and on the other hand, is determined by endogenous antioxidants activity, thus being connected to the buffer capacity of prooxidant-antioxidant system. It is known, that AOM increase in the cells, along with other effects, is accompanied by the destruction of main antioxidant (AO) enzymes, suggesting that the increase in the protein oxidizing modification is partially conditioned by the accumulation of AO enzymes, inactivated in the process of catalysis. Along with the modification of proteins by oxygen metabolites there is non-oxidizing way of carbonil derivatives formation with the modification of proteins by aldehydes. P.C. Burcham *et al.* [15] demonstrated that the incubation of proteins with different aldehydes, including MDA, stimulates modification of protein molecules in concentration dependent way. So as the ionizing irradiation leads to the accumulation of lipid peroxidation products, it is possible to suppose that oxidizing modification increase in splenocytes of radiated rats, shown by us, is partially determined by the non-oxidizing mechanism of carbonil derivatives formation.

Therefore, the irreversible changes take place in the spleen lymphocytes at X-ray irradiation in the control system of oxidant-antioxidant cell homeostasis, which result in stimulation of prooxidazing enzyme xanthinoxidase and are accompanied by increase in the generation speed of superoxide radicals and the level of protein oxidizing modification.

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Участие ксантиноксидазной системы в развитии оксидативного стресса при действии лучевого фактора

Резюме

Установлено, что действие ионизирующего излучения в дозе 1,0 Гр вызывает активацию прооксидантного фермента ксантиноксидазы в лимфоцитах селезенки, что сопровождается повышением скорости образования супероксидных анион-радикалов и свидетельствует об участии ксантиноксидазной системы в индуцированой радиацией гибели клеток. Показано увеличение степени окислительной модификации белков в спленоцитах через 3 ч после тотального облучения крыс.

Ключевые слова: ионизирующее излучение, ксантиноксидаза, анион-радикалы, оксидативный стресс, окислительная модификация белков.

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