EFFECT OF UBIQUINONE-9 ON LIPIDS OF RAT LIVER NUCLEI AND CHROMATIN DURING CHRONIC IRRADIATION

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Interest in the effect of chronic γ-irradiation on lipids of nuclei and chromatin is due to the structural and functional role of lipids not only in the nuclear membrane, but also in nonmembranous nuclear structures, including chromatin [15] and the nuclear matrix [13]. Lipids affect activity of nuclear DNA- and RNA-polymerases [11, 12], and they undergo changes in the cell cycle [2], during tumor transformation [10, 14], and under the influence of ionizing radiation in lethal and sublethal doses [4, 7].

Ubiquinone-9 is not only a natural antioxidant, occurring in rats, but it also has a specific effect on lipid metabolism, lowers the cholesterol level, and alters the rate of synthesis and breakdown of phospholipids in rat tissues [6]. Ubiquinone exerts its effect at the whole body level and also at the cellular level when added to the incubation medium [9]. Ubiquinones have been shown to have some degree of radioprotective action and to normalize the lipid composition of the liver microsomes and mitochondria, when modified by the action of lethal doses of radiation [6].

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing initially 60-70 g. Three groups of animals, each containing 30 rats were used: 1) control, 2) irradiation, and 3) irradiation + ubiquinone-9. The animals were irradiated in a chamber with a ^{137}Cs radiation source at a dose rate of 0.129 Gy/day for 155 days, until the total dose of 20 Gy had been given. The irradiated animals received ubiquinone-9 mixed with cottage cheese in a dose of 8 mg/kg body weight. Animals of groups 1 and 2 received cottage cheese alone. When the dose of irradiation reached 20 Gy the animals were killed by decapitation. Methods of isolation of nuclei and chromatin, of determining protein and DNA, of thin-layer chromatography of lipids, and of quantitative estimation of lipids were all described previously [5]. The concentrations of cholesterol and of free fatty acids (FFA) were determined [16], and to construct a calibration curve, arachidonic acid was used. Phenylmethylsulfonyl fluoride was added to the solutions during isolation of nuclei and chromatin in a concentration of 0.5 mM. The DNA protein ratio in the chromatin was 1:1.9.

EXPERIMENTAL RESULTS

The content of total phospholipids in the homogenate, nuclei, and chromatin, and the content of individual phospholipids in the liver homogenate were unchanged by irradiation (Table 1). Alers and co-workers [1] likewise found no changes in the phospholipid content in the liver of rats after chronic irradiation in a dose rate of 0.6 Gy/day and in a total dose of 15 Gy, either immediately after irradiation or later.

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TABLE 1. Content of Phospholipids (in μg phospholipid/mg protein) in Rat Liver Homogenate after Chronic Irradiation in a Dose of 20 Gy and Peroral Ubiquinone-9 (M ± m, n = 3)

Variant	SF	PCh + PS	PEA	CL
Control	4,23±0,57	46,04±2,01	20,21±2,45	3,12±0,32
Irradiation	4,38±0,17	43,85±3,58	22,52±1,97	3,17±0,41
Irradiation + ubiquinone-9	4,72±0,19	41,64±9,36	22,08±2,15	3,20±0,47

TABLE 2. Content of Individual Phospholipids (in μg phospholipids/mg protein) in Rat Liver Nuclei and Chromatin after Chronic Irradiation in a Dose of 20 Gy and Peroral Ubiquinone-9 (M ± m, n = 3)

Variant	SF		PCh + PS		PEA		CL	
	nuclei	chromatin	nuclei	chromatin	nuclei	chromatin	nuclei	chromatin
Control	4,25±0,84	4,78±0,53	17,13±0,60*	21,13±1,38	5,05±0,95	5,64±0,33	2,02±0,07	5,32±0,86*
Irradiation	4,25±0,35	4,91±1,22	29,68±0,22*	22,02±2,72	13,83±2,13	7,53±0,93	2,49±0,76	1,51±0,07*
Irradiation + ubiquinone-9	4,68±1,47	3,11±0,14	20,00±2,13*	20,62±3,24	7,45±0,42	6,92±2,12	3,51±0,96	1,18±0,07*

Legend. Here and in Table 3 *$p < 0.05$ indicates significant differences.

TABLE 3. Content of Free Fatty Acids and Cholesterol (in μg lipids/mg protein) in Rat Liver Homogenate, Nuclei, and Chromatin after Chronic Irradiation in a Dose of 20 Gy and Peroral Ubiquinone-9 (M ± m, n = 3)

Variant	Homogenate		Nuclei		Chromatin	
	FFA	cholesterol	FFA	cholesterol	FFA	cholesterol
Control	46,7±4,0	7,0±0,36	60,1±9,8	13,4±1,13	59,2±10,3	10,6±2,2
Irradiation	33,3±3,81	7,5±0,43	47,9±13,7	19,6±1,9*	45,7±14,4	13,4±0,44*
Irradiation + uniquinone-9	39,0±5,7	6,2±0,25	57,1±4,9	8,4±0,23*	66,1±15,9	5,9±0,32*

An increase in the quantity of phospholipids in the total fraction of phosphatidylcholine with phosphatidylserine (PCh + PS) and in the content of phosphatidylethanolamine (PEA) was found in the liver nuclei of the irradiated rats (Table 2). Ubiquinone-9 had a normalizing effect on the content of these phospholipids. The content of sphingomyelin (Sph) and of cardiolipin (CL) was unchanged after irradiation. During the investigation of nuclear lipids it was impossible to calculate changes in the content of the phospholipids taking place in the nuclear membrane, and the contribution of possible changes in the lipid content in nonmembranous nuclear structures.

A considerable decrease in the CL content was found in the liver chromatin of the irradiated rats (Table 2). Previously, after acute irradiation of rats in a dose of 10 Gy we observed changes in the content of PCh and PEA and a decrease in the CL content in the chromatin [4]. The ratio of the specific radioactivity of the chromatin CL to the specific radioactivity of the nuclear CL increased after irradiation, evidence of its high metabolic activity [3]. The decrease in the CL content in the liver chromatin of rats after chronic irradiation evidently reflects a change in its metabolism.

The content of free fatty acids (FFA) in the liver homogenate, nuclei, and chromatin of the irradiated rats had a tendency to fall (Table 3). Peroral administration of ubiquinone-9 had a normalizing effect.

The cholesterol content in the rat liver homogenate showed virtually no change after irradiation (Table 3). A very small decrease in the cholesterol content of the liver 1 week after the end of exposure to irradiation has been described [1]. We observed an increase in the cholestol content in the nuclei after irradiation, and there was a tendency for the cholesterol content in the chromatin to increase. Previously, after acute γ-irradiation of rats in a dose of 10 Gy we observed a change in the cholesterol content in the early period after irradiation [4]. Because of the limited information on the role of cholesterol in intranuclear structures, it is very difficult to interpret the changes observed in the content of cholesterol in the nucleus and chromatin after chronic irradiation of animals. Nikitin and co-workers [8] found a significant increase in the cholesterol content in the liver nuclei of aging rats. The changes we found in the cholesterol content in the nuclei after chronic irradiation of the animals had a similar tendency. Administration of ubiquinone-9 significantly reduced the cholesterol content in the nuclei and chromatin of the irradiated rats (Table 3).

Thus in rats subjected to chronic irradiation with a dose rate at which any possible shortening of the lifespan is due mainly to radiation-induced carcinogenesis, changes were found in the lipid composition of the cell nuclei and chromatin of the liver, a radioresistant organ, and ubiquinone-9 was found to have a normalizing effect on the lipid content, a fact which could prove useful in the prevention of possible late sequence of irradiation.

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