EFFECT OF UBIQUINONE ON PHOSPHOLIPID TURNOVER AFTER RADIATION INJURY

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The ubiquinones are biologically active substances used in medicine for the treatment of diseases associated with a disturbance of energy states [11]. Ubiquinones are known to be members of the class of natural regulators of lipid metabolism of the isoprenoid type [13]. Administration of ubiquinone-9 to irradiated animals has a radioprotective effect, expressed as some lengthening of the survival time of the irradiated animals [2]. Correlation probably exists between the protective action of ubiquinone and its ability to partially abolish the marked activation of cholesterol formation in the liver caused by irradiation of animals in a lethal dose [3]. The writers showed previously that injection of ubiquinone causes changes in the phospholipid composition of liver mitochondria and microsomes of intact and irradiated rats [5]. It can be tentatively suggested that modification of the phospholipid composition of the liver cell membranes is due to changes in the phospholipid turnover rates after administration of ubiquinone. The aim of this investigation was to study turnover (synthesis and breakdown) of phospholipids in the liver and also in radiosensitive organs (intestine and spleen) of normal and γ-irradiated animals treated with ubiquinone-9.

EXPERIMENTAL METHOD

Male Wistar rats weighing 150-180 g were irradiated on a "Gupos" apparatus in a dose of 8 Gy. After irradiation the control and irradiated animals were deprived of food for 48 h and given only water. Ubiquinone in sunflower oil was injected subcutaneously three times in the course of 48 h. The total dose of ubiquinone was 200 mg/kg and the total volume of oil injected was 1.5 ml per rat. The last injection of ubiquinone was given 3 h before decapitation of the animals. [^{3}H]-L-serine was injected intraperitoneally in a dose of 30 μCi per rat 1 h before decapitation. In the experiments to study phospholipid breakdown, [2-^{14}C]-glycerol was injected 24 or 48 h before decapitation in a dose of 50 μCi per animal. Lipids were extracted from the tissues, chromatography carried out, and concentration and radioactivities of phospholipids determined as described in [4]. The test objects were the liver, small intestine, and spleen of the rats.

EXPERIMENTAL RESULTS

Injection of ubiquinone-9 into normal and irradiated rats caused changes in the phospholipid composition of the animal's organs and in the intensity of uptake of the labeled precursor into individual components of polar lipids (Table 1). Injection of ubiquinone caused an increase in the concentrations of phosphatidylserine (PS) and phosphatidylcholine (PC) in the liver; in the case of PC the effect was preserved during irradiation. The concentration of phosphatidylethanolamine (PEA) in the liver, which was increased as a result of irradiation of the animals, returned to normal after injection of ubiquinone. In the intestine, on the other hand, ubiquinone caused a decrease in the PEA concentration and caused the PS level to fall to trace amounts in both normal and irradiated animals. In the spleen of irradiated animals, and also of rats receiving ubiquinone, the concentration of sphingomyelin (SM) increased; in response to the combined action of the two factors, however, the increase was not significant. Injection of an oily solution of ubiquinone-9 into the animals thus induced organ-specific changes in the phospholipid concentrations in the tissues. The possibility cannot be ruled out that partial modification of the phospholipid composition could be ascribed to the action of the oil. It has been found, for example, that injection of oil leads to an

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TABLE 1. Effect γ-Irradiation on Phospholipid Content and Incorporation of [^{3}H]Serine into Phospholipids in Rat Organs (M ± m)

Organ	Experimental conditions	SM		PEA		PC		PS	
		A	B	A	B	A	B	A	B
Liver	control	35±5	2,0±0,03	280±45	2,46±0,05	658±38	15,58±0,48	21±4	2,85±0,27
	irradiation	45±3	2,0±0,09	422±22*	3,72±0,06*	746±9	23.613±1,0	24±2	2,88±0,09
	ubiquinone	34±10	1,57±0,2	218±6	1,69±0,25	909±71*	14,75±0,25	43±6*	1,58±0,42*
	irradiation + ubiquinone	31±5	1,39±0,12	310±14	3,28±0,02	877±12*	13,24±0,5	28±3	1,64±0,13*
Intestine	control	30±1	0,89±0,23	137±28	2,25±0,5	171±5	2,65±0,11	43	2,01
	irradiation	23±12	0,64±0,02	105±1	2,73±0.12	168±2	1,46±0,1*	46	11,89*
	ubiquinone	27±9	0,97±0,4	85±11*	1,52±0,24	137±14	2,55±0,22	0	0
	irradiation + ubiquinone	36±16	1,01±0,02	107±15	1,41±0,27	228±22	3,4±0,16	0	0
Spleen	control	23±2	0,24±0,02	114±17	0,36±0,01	242±4	0,84±0,06	58±1	1,34±0,02
	irradiation	36±5*	0,35±0,008	138±19	0,51±0,01*	238±18	1,32±0,01*	56±3	1,32±0,01
	ubiquinone	38±7*	0,42±0,027*	122±19	0,56±0,02	224±20	1,09±0,1	63±4	1,49±0,2
	irradiation + ubiquinone	30±6	0,38±020	137±23	0,87±0,01*	267±8	1.53±0.34*	60±2	1 34±0,015

Legend. A) Concentration of phospholipid (in μg P_i/g wet weight of tissue). B) Total radioactivity of phospholipids (in cpm/g tissue); *) Changes significant at the P = 0.05 level.

TABLE 2. Ratio of Specific Radioactivities of PEA/PS and PC/PEA in Rat Organs after Irradiation and Injection of Ubiquinone

Organ	Experimental conditions	PEA / PS	PC / PEA
Liver	control	0,07 —	2,6 —
	irradiation	0,07 (100)	3,7 (142)
	ubiquinone	0,18 (300)	1,78 (68)
	irradiation + ubiquinone	0,19 (316)	1,36 (52)
Intestine	control	0,32 —	1,06 —
	irradiation	1,0 (312)	0,33 (31)
	ubiquinone	— —	1,05 (100)
	irradiation+ ubiquinone	— —	1,23 (118)
Spleen	control	0,12 —	1,2 —
	irradiation	0,14 (114)	1,5 (125)
	ubiquinone	0,17 (140)	1,0 (83)
	irradiation + ubiquinone	0,30 (250)	1,07 (89)

Legend. Numbers in parentheses indicate percent of control.

increase in the total phospholipid content in the rat liver [7]. Phospholipid synthesis in the organs of the rats was investigated by the use of [^{3}H]serine as radioactive precursor. It has been shown [8] that biosynthesis of PS in rat liver homogenates takes place by a Ca^{++}-dependent base exchange reaction, not requiring sources of energy. In animal tissue this is evidently the main pathway of PS synthesis. Since serine was the source of radioactivity in the experiments conducted as described above, it can be postulated that radioactive PEA could be formed as a result of decarboxylation of PS, and labeled PC as a result of methylation of labeled PEA molecules. We know that SM also is synthesized with the aid of L-serine through its reaction with palmitoyl-CoA, with the formation of sphingosine [9]. Injection of ubiquinone causes a tendency for synthesis of all phospholipids investigated to be inhibited in the liver, and this was found most clearly in the case of PS, for which the intensity of incorporation of the label was depressed both under normal conditions and after γ-irradiation (Table 1). During the study of phospholipid turnover by the use of [2-^{14}C]glycerol as labeled precursor [4], no significant changes were found in PC and PEA synthesis in the liver after irradiation and injection of ubiquinone. This is evidence that the principal pathway of

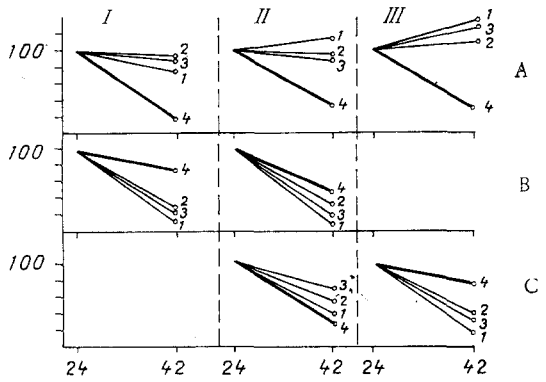

Fig. 1. Effect of ubiquinone on phospholipid breakdown in organs of intact and γ-irradiated rats. Abscissa, time after injection of [2-^{14}C]glycerol (in h); ordinate, specific radioactivity of phospholipids (in percent). I) SM, II) PEA, III) PC. A) Liver, B) small intestine, C) spleen. 1) Irradiation, 2) ubiquinone, 3) irradiation + ubiquinone, 4) control.

biosynthesis of the basic phospholipids through phosphorylethanolamine and phosphorylcholine remains unchanged, whereas synthesis by the path of L-serine utilization, quantitatively unimportant compared with the main pathway, is modified under the influence of irradiation and ubiquinone loading.

Analysis of changes in radioactivity of the phospholipids in radiosensitive organs (intestine and spleen) is difficult, because phospholipids from the liver may reach these organs through exchange with plasma lipoproteins. Loading with ubiquinone does not lead to changes in the intensity of incorporation of [^{3}H]serine into intestinal phospholipids, but the effect of ubiquinone is expressed as disappearance of PS. The intensity of synthesis of SM in the spleen was appreciably increased and some activation of incorporation of the precursor into PEA and PC also observed. To analyze the intensity of phospholipid synthesis in rat organs it is useful to examine the ratio between specific radioactivities of PEA/PS, which gives an approximate estimate of the rates of conversion of PEA from PS as a result of decarboxylation, and also to examine the ratio between specific radioactivities PC/PEA, which characterizes the intensity of formation of PC from PEA by methylation (Table 2). The results are evidence that ubiquinone loading considerably activates decarboxylation of PS and inhibits the conversion of PEA into PC in the rat liver and spleen. Under these circumstances this effect is preserved in the organs of irradiated animals also. Ubiquinone loading normalizes conversion of PEA into PC when sharply depressed as a result of irradiation. It can be concluded that ubiquinone loading modifies the effect of radiation on phospholipid metabolism in these organs studied in rats, and partially restores the normal level of their conversion.

Analysis of the data showed that accumulation or exhaustion of individual phospholipids in rat organs as the result of γ-irradiation and ubiquinone loading does not always correspond in direction to the change in intensity of synthesis of these lipids. For example, the most effective action of ubiquinone on phospholipid turnover in the liver is inhibition of synthesis of PS together with an increase in its concentration, which may evidently be linked with inhibition of PS breakdown in the liver. To study the intensity of elimination of labeled phospholipid molecules from rat organs [2-^{14}C]glycerol was used: It was injected into rats 24 and 42 h before the measurements. For the sake of clarity, values of specific radioactivities are normalized relative to the control (Fig. 1). Clear inhibition of phospholipid excretion both after irradiation and after ubiquinone loading were observed in the liver. In the rat intestine, on the other hand, all phospholipids were more actively excreted as a result of irradiation and ubiquinone loading, and in the spleen activation of PC breakdown was observed, with no significant change in PEA elimination. In the liver and radiosensitive tissues opposite changes in the rate of elimination of phospholipids were thus observed under the influence of γ-irradiation and ubiquinone loading. Inhibition of phospholipid elimination from the liver may perhaps be connected with changes in phospholipase activity. We know that injection of the antioxidant α-tocopheryl acetate causes liver mitochondrial phospho-

lipase activity to fall [1]. Delay of excretion of phospholipids from the liver of irradiated animals may also perhaps be explained by inhibition of phospholipase activity, but the available evidence is contradictory. For example, increased phospholipase activity has been demonstrated in the erythrocytes of rabbits irradiated in a dose of 1000 R [6], whereas on the other hand inhibition of lecithinase activity has been found in rat serum during the first two days after irradiation in a dose of 360 R [10]. Delayed excretion of phospholipids from the liver 48 h after irradiation is evidently a sign of repair of the cell membranes, and in this sense ubiquinone has a positive effect. Acceleration of phospholipid excretion from the intestine of irradiated animals may perhaps reflect the more rapid breakdown of radiosensitive intestinal cells, for the phospholipase activity of the intestinal mucosa falls during the first day after irradiation [12].

Injection of ubiquinone-9 thus has a varied and organ-specific action on phospholipid turnover and content in the organs of normal and γ-irradiated animals, which is manifested as some degree of normalization of the intensity of synthesis in the organs of irradiated rats and delay of phospholipid breakdown in the animals' liver. This situation may perhaps play a definite role in the protective effect of ubiquinone on irradiated animals.

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