Effects of Coenzyme Q10 on Rat Liver Cells under Conditions of Metabolic Stress

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Under conditions of metabolic stress induced in Wistar rats by 5-day starvation with subsequent refeeding, supplementation with coenzyme Q10 in doses of 10 and 100 μg/kg of body weight resulted in significant increase in liver weight after the experiment. Percent ratio of liver cell populations was changed, which was detected by flow cytometry. In addition, specific effects of low dose of coenzyme Q10 (10 mg/kg body weight) on hepatocytes was observed, which manifested in increased number of mitoses and percentage of S-phase cells, enhanced expression of D1 and Rb-protein expression, and reduced percent of apoptotic hepatocytes. Adaptive effects of coenzyme Q10 are associated with enhanced expression of Hsp25, Hsp70, and Hsp90 in hepatocytes during metabolic stress.

Key Words: *coenzyme Q10; hepatocytes; stress; nutrition*

Coenzyme Q10 (CoQ10) transfers electrons in mitochondrial respiratory chain, participates in elimination of reactive oxygen metabolites, decelerates the development of apoptosis induced by oxidative stress. CoQ10 can be found in mitochondria, cytosol, and transport vesicles. Supplementation of rat food with CoQ10 increases ATP content, blocks oxidative stress, and reduces DNA damage in cells [8,11]. CoQ10 restores functional activity of immune system in old rats [4]. In cell culture, CoQ10 prevents mitochondrial disturbances induced by hydrogen peroxide due to inhibition of the destabilizing effect of Bax protein on mitochondrial membranes [7].

The objective of this work was to investigate CoQ10 effects on expression of heat shock proteins (Hsp) and cell cycle regulators and on proliferation of liver cell during metabolic stress induced by starvation with subsequent full-scale restoration of feeding.

MATERIALS AND METHODS

Experiments were carried out on 10-week-old Wistar rats obtained from vivarium of Institute of Nutrition, Russian Academy of Medical Sciences. The animals were randomized into groups (n=5; Table 1), control groups received standard food. Experimental groups daily received CoQ10 in doses of 10 or 100 mg/kg of body weight *per os* in 50 μg of vegetable oil.

The animals were decapitated, organs were immediately removed, weighed, and placed into complete medium RPMI-1640 with 10% FCS (fetal calf serum). Hepatic cells were separated as described previously [1]. Experiments were performed in 3 repetitions, the results were presented as $M\pm m$. The data were processed by ANOVA. The groups were compared pairwise using Student's t test. Newman–Keuls multiple comparisons were used for nonparametric analysis of several groups. Between-group differences were statistically significant at $p\leq 0.05$.

RESULTS

During starvation, CoQ10 supplementation in a dose of 100 mg/kg promotes maintenance of the body weight, and in doses 10 and 100 mg/kg CoQ10 significantly increases body weight after end of starvation period (Table 2). The trend to liver weight increase was observed after administration of 10 and 100 mg/

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TABLE 1. Animal Groups

Group	Experiment conditions	Time of experiment, days									
		1	2	3	4	5	6	7	8	9	10
1	Control group	Vivarium ration					–				
2	Starvation, 5 days+water *ad libitum*	Starvation					–				
3	The same	Starvation+CoQ10, 10 mg					–				
4	The same	Starvation+CoQ10, 100 mg					–				
5	Starvation, 5 days+100% ration, 5 days	Starvation					Vivarium ration				
6	The same	Starvation+CoQ10, 10 mg					Vivarium ration				
7	The same	Starvation+CoQ10, 100 mg					Vivarium ration				
8	Control group	Vivarium ration									

Note. "–" decapitation.

kg CoQ10. CoQ10 significantly increases liver weight at the end of starvation.

Flow cytometry showed that CoQ10 affects the ratio of cell populations in the liver, which was seen from characteristics of histogram peak (Fig. 1). CoQ10 in a dose of 10 mg/kg produced a more pronounced effect on hepatocyte proliferation, it increased the percent of mitoses and S-phase cells, but little affected over liver cells (Table 3, Fig. 2). Taking into account the role of CoQ10 as a regulator of mitochondrial respiratory chain [7], the observed effects can be explained by substantial differences between hepatocytes and other liver cells by the number of mitochondria. These findings attest to regulatory effects of CoQ10 on the expression of cyclin D1 protein and Rb-protein coordinating hepatocyte passage through the cell cycle phases. During refeeding CoQ10 produced an adaptive effect and increased the percent of mitoses in hepatocytes. CoQ10 supplementation during starvation 2-fold increased lymphocyte count in the liver, which was determined by migration of new cells into the liver, rather than by proliferation of resident clones, since parameters of cell cycle remain within normal values (Table 3). This is a consequence of adaptation maintaining immune response in the liver.

The adaptive effects of CoQ10 are realized through molecular shaperones, Hsp; expression of Hsp increases during starvation and refeeding after stress (Table 4). CoQ10 in a dose of 10 mg/kg more efficiently induces Hsp expression during starvation, which positively correlates with observed effects of CoQ10 on hepatocyte proliferation. During refeeding, Hsp90 expression increased twofold. The decrease in Hsp90 and Hsp70 levels in the liver is known to inhibit he-

TABLE 2. Effects of CoQ10 on Rat Body Weight and Liver Weight ($M \pm m$)

Parameter	Control	Starvation, 5 days			Starvation, 5 days+100% ration, 5 days		
		starvation	Q10, 10 mg/kg	Q10, 100 mg/kg	starvation	Q10, 10 mg/kg	Q10, 100 mg/kg
Body weight before the experiment	181±12	167±10	183±11	183±12	190±11	192±15	186±13
Body weight after the experiment	215±13	148±4	160±2	170±3*	171±10	226±4*	222±9*
Changes in body weight, %	+19.6±2.0	-13.5±2.0	-12.8±1.0	-7.5±0.7*	+9.0±0.8	+18.2±2.0**	+18.9±4.0**
Liver weight after the experiment	9.0±1.1	4.85±0.70	5.29±0.60	5.44±0.70	7.7±0.6*	9.61±0.50*	9.18±0.60*

Note. Here and in Tables 3 and 4: *$p \leq 0.05$, **$p \leq 0.001$ compared to the control.

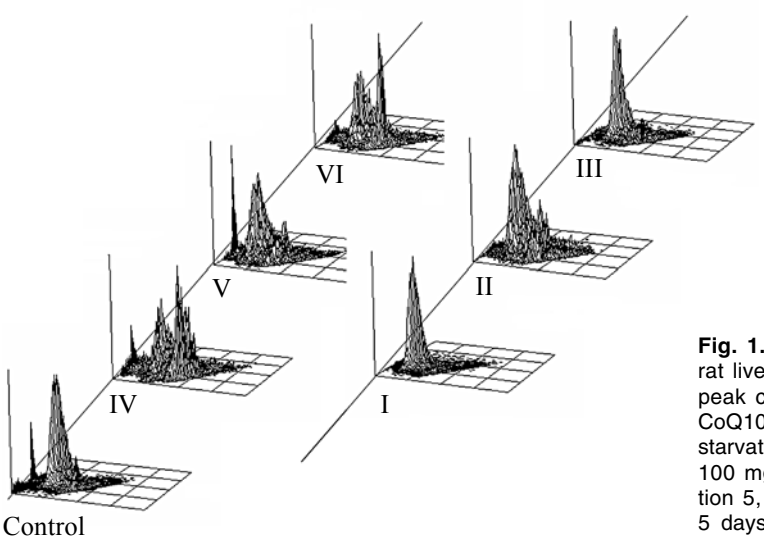

Fig. 1. Effects of CoQ10 on changes in cell population ratio in rat liver. 3D presentation of hepatic cell populations. The middle peak corresponds to hepatocytes responding by proliferation to CoQ10 supplementation during starvation. I: starvation 5 days; II: starvation, 5 days+CoQ10, 10 mg; III: starvation, 5 days+CoQ10, 100 mg; IV: starvation, 5 days+100% ration, 5 days; V: starvation 5, days+100% ration 5 days+CoQ10, 10 mg; VI: starvation, 5 days+100% ration, 5 days+CoQ10, 100 mg.

patocyte proliferation and increase apoptosis [5,6]; we observed significant inhibition of hepatocyte apoptosis under the influence of CoQ10 during starvation, as well as proliferation intensification (Table 3). Activity of Hsp27, Hsp70, and Hsp90 is associated with nuclear transcriptional factor NF-κB regulating apop-

TABLE 3. Effects of CoQ10 on Proliferation of Rat Liver Cells during Stress (*M*±*m*)

Parameter	Control	Starvation, 5 days			Starvation, 5 days+100% ration, 5 days		
		starvation	Q10, 10 mg/kg	Q10, 100 mg/kg	starvation	Q10, 10 mg/kg	Q10, 100 mg/kg
Hepatocytes							
G1-phase, %	78.7±6.2	73.2±5.8	62.7±5.1	74.4±6.8	74.5±3.1	81.8±7.5	79.7±6.7
S-phase, %	13.4±3.8	16.5±6.9	28.9±9.7	15.3±5.5	10.3±2.6	12.4±4.1	13.3±4.6
G2/M-phase, %	7.4±3.9	9.8±4.8	8.1±2.7	10.2±6.7	8.5±2.2	7.7±1.2	7.8±2.1
D1 cyclin, %	14±2.6	20±3.9	33±5.5	20±4.5	16.3±3.8	15±2.4	15±5.2
p-Rb-protein, %	4.9±0.8	5.9±0.8	8.4±1.2	6.1±0.9	4.5±0.9	4.9±0.7	4.9±0.7
Mitosis, %	5.6±0.9	5.9±0.7	7.0±1.1	5.6±0.7	5.6±0.7	6.1±0.7	6.2±0.8
Apoptosis, %	4.9±0.4	6.9±2.5	4.8±0.7	4.7±0.8	5.0±0.7	5.2±0.4	5.3±0.5
DNA, mean	92±12	118±10	137±11	106±12	102±7	108±7	105±7
Lymphocytes							
Lymphocytes	10.5±1.5	5.0±2.6	9.4±4.7**	12.0±4.7**	9.8±1.2	11.5±2.1	14.5±4.8
G1-phase, %	55.5±6.4	44.3±6.4	49.5±7.1	53.7±11.0	63.4±4.1	72.2±6.6	70.9±6.2
S-phase, %	17.7±3.4	22.3±2.5	18.9±2.6	21.0±5.1	13.9±1.6	13.6±2.8	14.8±2.9
G2/M-phase, %	26.9±4.6	33.7±5.0	31.8±5.4	25.4±6.8	26.7±3.9	14.5±3.7	14.5±3.5
D1 cyclin, %	17.2±3.1	30.0±5.3	22.5±3.8	24.0±4.8	16.8±2.2	16.0±2.5	20.3±3.4
p-Rb protein, %	4.3±0.7	7.2±0.9	7.0±0.9	7.1±0.8	4.2±1.0	4.8±0.6	5.0±0.8
Mitosis, %	4.1±0.5	3.8±0.6	4.5±0.4	5.3±0.4	4.1±0.5	3.7±0.6	3.6±0.5
Apoptosis, %	3.8±0.4	5.4±0.9	4.1±0.7	3.9±0.7	5.9±1.3	4.1±0.6	4.0±0.5
DNA, mean	102±11	129±13	128±9	128±11	126±8	112±7	119±9

Note. mean: fluorescence intensity.

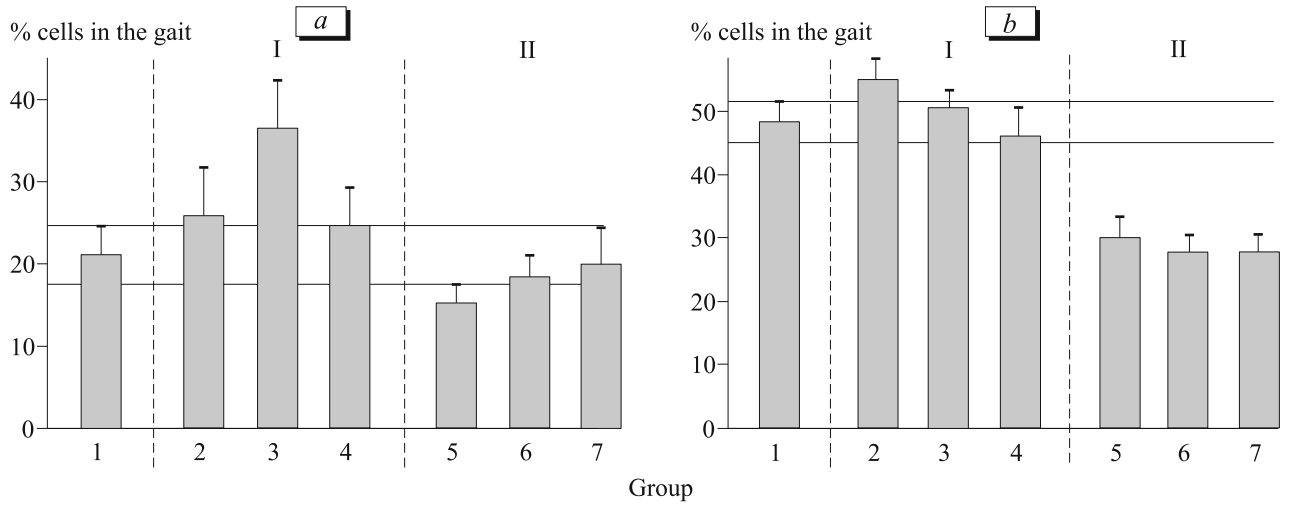

Fig. 2. CoQ10 regulates cell proliferation in rat liver during metabolic stress. *a*) hepatocytes, percentage of dividing cells; *b*) lymphocytes, percentage of dividing cells.

TABLE 4. Effects of CoQ10 on Hsp in Rat Liver during Stress (*M*±*m*)

Parameter, %	Control	Starvation, 5 days			Starvation, 5 days+100% ration, 5 days		
		starvation	Q10, 10 mg/kg	Q10, 100 mg/kg	starvation	Q10, 10 mg/kg	Q10, 100 mg/kg
Hsp25	10±2	24±3	36±6	28±5	31±5	45±5	51±4
Hsp70	18±3	23±3	38±6	33±3	42±4	56±6	70±2
Hsp90	2.1±0.2	2.9±0.3	4.6±0.4*	4.1±0.4*	4.6±0.3	5.0±0.7	8.1±0.7

totic signals, cell responses to nutrients, and ATP level [2,10]. Hsp90 regulates expression of certain proteins promoting degradation of NF-κB-inducible kinase [3]. Hsp70 stabilizes intermediate complex NF-κB/IκBa/IκB, which delays its proteasome degradation and prolongs NF-κB-dependent signals [12].

Thus, adaptive effects of CoQ10 during metabolic stress in rats induced by changes in nutrition were demonstrated. The mechanism of CoQ10 action is associated with activation of Hsp expression, regulation of hepatocyte proliferation via changes in the expression of D1 cyclin and Rb-protein, as well as with inhibition of hepatocyte apoptosis.

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