Biochemistry (Moscow), Vol. 69, No. 5, 2004, pp. 520-526. Translated from Biokhimiya, Vol. 69, No. 5, 2004, pp. 639-646.

Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM03-174, March 28, 2004.

Protection of Rat Myocardium by Coenzyme Q during Oxidative Stress Induced by Hydrogen Peroxide

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Received July 11, 2003
Revision received December 11, 2003

Abstract—Ubiquinone Q_{10} (coenzyme Q) is an important component of the mitochondrial electron transport chain and an antioxidant. The purpose of this work was to find out whether an increase in the level of coenzyme Q in the heart changes its maximal working capacity and resistance to oxidative stress. Male Wistar rats were treated with coenzyme Q (10 mg/kg body weight per day) for six weeks, and this increased its content in the myocardium by 63%. The myocardial content of malonic dialdehyde and activities of key antioxidant enzymes were unchanged, except nearly 2.5-fold decrease in the activity of superoxide dismutase. The maximal working capacity of the isolated isovolumic heart did not change, but under conditions of oxidative stress induced by 45-min infusion of hydrogen peroxide (70 μM) into coronary vessels the contractile function of these hearts decreased significantly more slowly. This was associated with less pronounced lesions in the ultrastructure of cardiomyocytes and lesser disorders in the oxidative metabolism of mitochondria that suggested increased antioxidant protection of the myocardium.

Key words: isolated heart, isoproterenol, antioxidant protection, oxidative metabolism, mitochondria

Ubiquinone Q_{10} (coenzyme Q) is an important component of the mitochondrial electron transport chain, and during its functioning it is subjected to successive one-electron reductions with generation of semiquinone ($^{\cdot}QH$) and a completely reduced form, ubiquinol (QH_2):

$$Q + e^- + H^+ \leftrightarrow {}^{\cdot}QH + e^- + H^+ \leftrightarrow QH_2.$$

Similarly to the natural phenol α-tocopherol, reduced phenolic forms of coenzyme Q (QH_2) are antioxidants, and the *in vivo* high antioxidant effect of ubiquinone Q_{10} is determined by its direct interaction with lipid radicals of $LO_2^{\cdot}$ or $LO^{\cdot}$ type with intermediate production of the ubisemiquinone radical ($^{\cdot}QH$) according to reactions described in [1-4]:

$$LO_2^{\cdot}\ (LO^{\cdot}) + QH_2 \rightarrow LOOH\ (LOH) + {}^{\cdot}QH,$$

$$LO_2^{\cdot}\ (LO^{\cdot}) + {}^{\cdot}QH \rightarrow LOOH\ (LOH) + Q.$$

The ubiquinol-dependent bioregeneration of free phenoxyl radicals of α-tocopherol (α-$TO^{\cdot}$) produced during interaction of vitamin E (α-TOH) with lipid radicals occurs by a similar mechanism [1-4]:

$$LO_2^{\cdot}\ (LO^{\cdot}) + \alpha\text{-TOH} \rightarrow LOOH\ (LOH) + \alpha\text{-}TO^{\cdot},$$

$$\alpha\text{-}TO^{\cdot} + QH_2 \rightarrow \alpha\text{-TOH} + {}^{\cdot}QH,$$

$$\alpha\text{-}TO^{\cdot} + {}^{\cdot}QH \rightarrow \alpha\text{-TOH} + Q.$$

Thus, the high antioxidant and tocopherol-sparing effects of the reduced form of ubiquinone Q_{10} are determined by reduction of two lipid or two tocopheroxyl radicals by a single molecule of ubiquinone Q_{10}. This process is biologically reasonable, because during oxidative reactions not the essential component (vitamin E) is spent but another natural liposoluble antioxidant ubiquinone Q_{10}, and the demand for the latter can be satisfied by biosynthesis and also by rapid regeneration in the mitochondrial electron transport chain.

The direct antioxidant effect of coenzyme Q in mitochondria has been shown experimentally: the decrease in its reduction on addition of antimycin A was accompanied by increased oxidation of membrane lipids [5]. On the contrary, in the presence of Krebs cycle sub-

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strates the intensity of free radical reactions was sharply suppressed [5, 6]. Note, that by mature age the coenzyme Q concentration in organs is decreased, especially in the heart muscle [7, 8]. Because coenzyme Q can inhibit peroxidation in membranes and protect DNA and proteins against the damage caused by free radicals [9, 10], it is used in treatment of cardiovascular system diseases associated with development of oxidative stress [11-13]. However, many aspects of the preventive effect of coenzyme Q in undamaged heart are not clear. In particular, it is not known whether this effect is associated with increase in the maximal aerobic power of the heart indicated by nearly twofold increase in the rate of NADH oxidation with increase in the coenzyme Q content in submitochondrial particles [14]. Therefore, in the present work we studied the effects of exogenous coenzyme Q on its concentration in rat blood and myocardium, on the maximal working capacity of the heart, and also on activities of key antioxidant enzymes in cardiomyocytes and the myocardium resistance to reactive oxygen species.

MATERIALS AND METHODS

Experimental animals. Male Wistar rats with body weight of 275 ± 10 g were given daily for six weeks through an esophageal probe a solubilized form of ubiquinone Q_{10} (10 mg/kg body weight) (Kudesan preparation, Akva-MDT, Moscow, Russia); the control animals were treated with the same amount of the solubilizing solution. In addition to ubiquinone, the Kudesan preparation contained a small amount of α-tocopherol (1.5 mg/kg) and cremophor as a detergent.

Biochemical studies. The heart tissue was minced and homogenized in distilled water at the weight/volume ratio of 1 : 4 with a Polytron (Teflon/glass) homogenizer (Hijiriseiko, Japan). The homogenate was centrifuged at 2000 rpm for 5 min, and the supernatant fluid was used to extract coenzyme Q_{10} by the method described in [15] with some modifications. To 100 ml of the supernatant fluid of the myocardium homogenate or blood serum 220 μl of ethanol and 550 μl of hexane were added, the mixture was carefully shaken for 10 min, centrifuged at 3000 rpm for 3 min, and 500 μl of the upper layer of hexane was taken. The residue was supplemented with 550 μl of hexane, and the extraction procedure was repeated. The combined hexane extracts were evaporated to dryness, the lipid residue was dissolved in 100 μl of ethanol, and the oxidized form of ubiquinone was reduced by addition of 10 μl of 5% sodium tetrahydroborate in ethanol. The reduced extract (11 μl) was analyzed by HPLC with electrochemical detection on a Coulochem II device (Environmental Sciences Associate, Inc., USA), and the data were treated according to a computer program of the same firm.

Secondary products of free radical lipid oxidation (mainly malonic dialdehyde) were determined in the myocardium homogenate by reaction with 2-thiobarbituric acid [16]. Activities of antioxidant enzymes were determined in the supernatant fluids resulting by centrifugation of the myocardium homogenates at 800*g* for 10 min. The activity of catalase was determined at 240 nm by the rate of utilization of hydrogen peroxide with a Hitachi-557 recording spectrophotometer (Japan) [17]. The activity of Se-containing glutathione peroxidase was determined at 340 nm in a coupled glutathione reductase system by the rate of NADPH oxidation, with *tert*-butyl hydroperoxide as a substrate, using an FP-901 chemical analyzer (Labsystems Oy, Finland) in the regime of kinetic operation [18]. The activity of Cu,Zn-superoxide dismutase (SOD) was determined by inhibition of reduction of nitroblue tetrazolium with the superoxide radical O_2^- generated in the xanthine–xanthine oxidase system, by kinetics of formazan production measured with a Hitachi-557 recording spectrophotometer at 560 nm [19]. The amount of catalase or glutathione peroxidase required for oxidation of 1 μmol H_2O_2 or GSH per 1 min, respectively, was taken as the unit of the enzyme activity under conditions of determination. The amount of SOD required to 50% inhibit the reduction of nitroblue tetrazolium to formazan was taken as the unit of the enzyme activity under conditions of the experiment. The protein content in the specimens was determined by the Lowry method.

Study on the contractile function of myocardium. Rats were anesthetized with urethane (2 g/kg body weight), the isolated hearts were perfused at 37°C through the aorta using a peristaltic pump with modified Krebs–Henseleit solution saturated with carbogen (95% O_2 and 5% CO_2) and containing substrates providing for the maximal working capacity of the heart [20]: glucose (11 mM), pyruvate (5 mM), and insulin (10 units/liter). The initial rate of the flow was adjusted to keep the perfusion pressure in the aorta at the average level of 70 mm Hg. To measure the pressure, a catheter equipped with a latex balloon was introduced into the left ventricle cavity. The balloon was filled with a fluid volume sufficient for maintaining the end diastolic pressure in the ventricle at the level of 10-15 mm Hg. The pressure changes in the balloon were recorded with a Gould Statham P23Db electromanometer and a Gould 2400S recorder (USA). The contractile function intensity (CFI) of the isovolumic heart was characterized by the product of the developed pressure and heart rate. This parameter is known to change proportionally to oxygen consumption by the cardiac muscle. The scheme of the experiment included determination of the maximal CFI in the course of gradual increase in the rate of perfusion, i.e., on additional activation of the contractile apparatus by Ca^{2+} entering through ion channels activated by distension [21]. Under these conditions, the maximal working capacity of the

heart occurred on addition to the perfusate of isoproterenol (0.1 μM); afterwards the perfusate was supplemented with 70 μM hydrogen peroxide to induce free radical oxidation, and the antioxidant protection system of the myocardium was assessed under conditions of the same perfusion rate and continued introduction of isoproterenol.

Study of myocardium ultrastructure. Small pieces of the myocardium from side wall of the left ventricle were placed into ice-cold isotonic KCl to prevent contracture-associated changes, and then these specimens were prefixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, fixed with 1% osmic acid in the same buffer, dehydrated in alcohol solutions of increasing concentration, and were embedded in araldite. Ultrathin sections 70-80 nm in thickness were prepared using an OM-3 microtome (Reichert, Austria), stained with uranyl acetate and lead citrate, and viewed with a JEM-100-CX electron microscope (Japan) at the accelerating voltage of 80 kV.

Study of oxidative metabolism of myocardium. Mitochondria were isolated from some of the hearts and treated as described in [22]. In the mitochondria, the rate of oxygen consumption was determined in state 3 of the respiratory chain (in the presence of both oxidation substrates and ADP in the incubation medium) and in its state 4 (in the presence of oxidation substrates but without ADP in the incubation medium). The rate of oxygen consumption was determined with a Clark-type electrode at 25°C using a YSI 53 polarograph (Yellow Spring Instruments, Inc., USA). The rate of production by mitochondria of reactive oxygen species, such as superoxide radicals $O_2^{\cdot-}$, was determined by the EPR signal of a Tiron (4,5-dihydroxy-1,3-benzenedisulfonic acid, disodium salt monohydrate) spin trap. EPR spectra were recorded with an E-109E spectrometer (Varian, USA) at room temperature.

Reagents. Reagents from Sigma, Aldrich, ICN, Cambridge Isotope Labs (USA) and Serva (Germany) were used.

Data processing. The results were processed using *t*-test and ANOVA test the Origin 6.1 program (Microcal Software, Inc., USA). The results are presented as mean value ± mean error (M ± m).

RESULTS AND DISCUSSION

Changes in parameters of the myocardial antioxidant systems. Treatment of rats of ubiquinone Q_{10} during six weeks increased 14-fold its blood concentration compared to the control group (the results will be presented in another paper), whereas the level of ubiquinone Q_{10} in the myocardium increased by 63% (Table 1). Six weeks after the treatment with ubiquinone Q_{10} had been abolished, its contents in the blood plasma and myocardium became virtually normal (the results will be presented in another paper). In earlier works introduction of exogenous ubiquinone Q_{10} increased its contents only in the blood plasma and liver [23-25].

Table 1. Effect of introduction to rats of solubilized form of ubiquinone Q_{10} for six weeks on parameters of free radical reactions in the myocardium

Parameter	Control (n = 10)	Ubiquinone Q_{10} (n = 10)
Content of ubiquinone Q_{10}, mg/g wet tissue	5.7 ± 0.5	9.3 ± 0.5*
Content of malonic dialdehyde, nmol per mg protein	2.2 ± 0.14	2.3 ± 0.1
Activity of superoxide dismutase, units/mg protein	73.9 ± 7.1	30.3 ± 1.1*
Activity of catalase, units/mg protein	15.9 ± 2.0	13.9 ± 2.2
Activity of glutathione peroxidase, units/g protein	675 ± 42	560 ± 45

* $p < 0.05$ with respect to the control.

After the treatment with ubiquinone Q_{10} for six weeks, the content in the myocardium of malonic dialdehyde, a product of free radical lipid oxidation, was unchanged, and no significant changes were recorded in activities of the main antioxidant enzymes catalase and glutathione peroxidase (Table 1). Surprisingly, the activity of superoxide dismutase was nearly 2.5-fold decreased in the animals treated with ubiquinone Q_{10} for six weeks compared to the corresponding control (Table 1).

Changes in the contractile function of the heart. The introduction to rats of ubiquinone Q_{10} for six weeks failed to significantly influence the increase in the body weight as compared to the control animals, and dry weight of the heart in both groups of animals also varied insignificantly (in the range of 0.45-0.49%). Parameters of the contractile function of the heart in the control animals and the baseline parameters in the animals treated with ubiquinone Q_{10} were also virtually the same: the systolic pressure was 135 ± 5 mm Hg, heart rate was 4.7 ± 0.2 Hz, perfusion pressure (at the perfusion rate of 14 ml/min) was 69 ± 2 mm Hg. Increase in the perfusion rate through the coronary vessels from 14 to 26 ml/min in the control group increased the developed pressure by 55% and the heart rate by 40%. As a result, the value of contractile function intensity increased by 76% and nearly twofold after the introduction of isoproterenol (Fig. 1). In the animals treated with ubiquinone Q_{10}, the CFI value increased similarly, along with increase in the heart rate and resulting pressure (by 37 and 51%, respectively). Nevertheless, the increase in the end diastolic pressure (its value under conditions of the experiment characterized elasticity of the myocardium) in this group was lower.

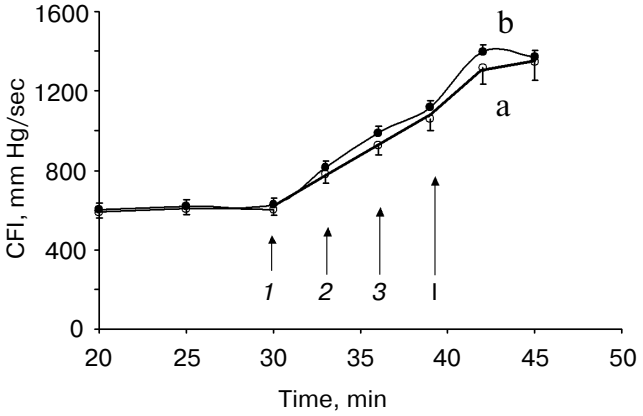

Fig. 1. Effect of elevated functional load on the contractile function intensity (CFI) (the product of developed pressure and heart rate, mm Hg/sec) of the isovolumic rat heart: a) control (n = 8); b) hearts of rats treated with ubiquinone Q_{10} (n = 7). After the stabilization period, the rate of coronary perfusion was increased stepwise from 14 to 26 ml/min (arrows *1-3*), and on this background 0.1 μM isoproterenol (I) was added.

The end diastolic pressure began to significantly decrease even during the initial perfusion of the heart at the constant rate (Fig. 2), and this decrease became more pronounced with a further increase in the perfusion rate and under the influence of isoproterenol.

Ubiquinone Q_{10} is an important link in the mitochondrial system of electron transport; therefore, it was supposed that introduction of exogenous ubiquinone Q_{10} should increase the aerobic power of the myocardium. However, in our experiments introduction of ubiquinone Q_{10} had virtually no effect on the maximal working capacity of the isolated heart. In studies on athletes the long-term taking of ubiquinone Q_{10} more than twofold increased its plasma level, but no significant changes were found in the oxygen consumption, anaerobic and respiratory threshold, heart rate, and arterial pressure during and after the stepwise elevated exercise [26]. Nevertheless, the decrease in the end diastolic pressure under elevated the load in the rats treated with ubiquinone Q_{10} suggested better distensibility of the myocardium that seems to be decisive for the maximal working capacity of the heart, which is executing the pump function.

Oxidative stress. On addition of 70-μM hydrogen peroxide into the perfusate, the contractile function of the isolated heart assessed by the contractile function intensity gradually decreased in both groups. The decrease in the contractile function intensity was mainly caused by the corresponding decrease in the developed pressure, whereas the heart rate in both groups retained the level recorded after the introduction of isoproterenol. In the animals treated with ubiquinone Q_{10} the decrease in the developed pressure (Fig. 3) and correspondingly in the contractile function intensity was markedly slower. A significant difference between the groups was evident even after 5 min and continued over the subsequent observation period.

Pronounced lesions in the ultrastructure of cardiomyocytes were found by electron microscopy in the myocardium specimens from the control group, which were taken after the perfusion with H_2O_2 was finished.

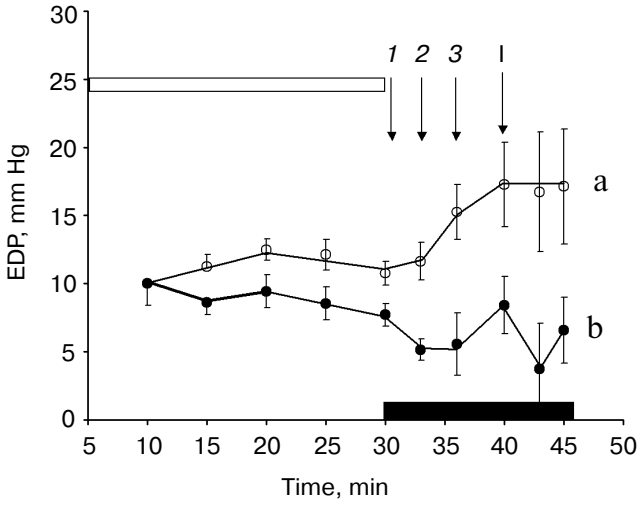

Fig. 2. Effect of elevated functional load on the end diastolic pressure (EDP) of the isovolumic rat heart (designations of the curves the same as in Fig. 1). After the stabilization period (white band), the rate of coronary perfusion (ml/min) was increased stepwise (arrows *1-3*), and on this background 0.1-μM isoproterenol (I) was added. In the rats treated with ubiquinone Q_{10}, the end diastolic pressure was significantly different from that in the control rats ($p < 0.05$) within the period shown by the black band.

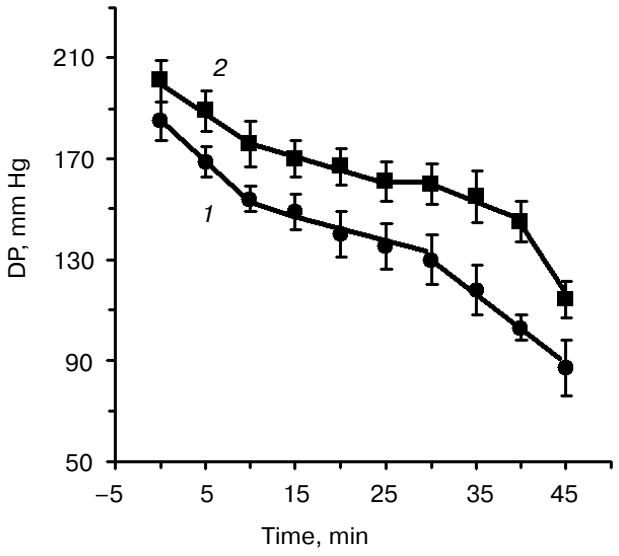

Fig. 3. Changes in the developed pressure (DP) under the influence of H_2O_2 (70 μM) constantly added into the perfusate (time 0) with an infusion pump. The values are presented as M ± m; *1*) control group; *2*) group treated with ubiquinone Q_{10}.

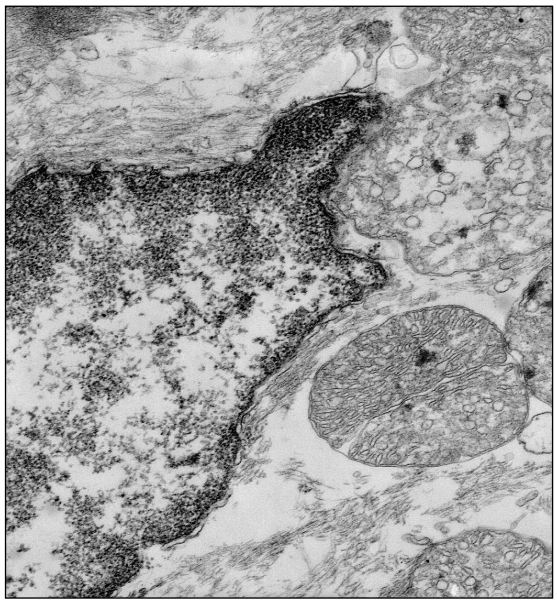

Fig. 4. Region of a cardiomyocyte of the rat treated with the solvent after introduction of H_2O_2. Edema of the sarcoplasm, accumulation of chromatin in the nuclear periphery, swelling and enlargement of mitochondria, and appearance in them of dense inclusions are seen. Magnification ×40,000.

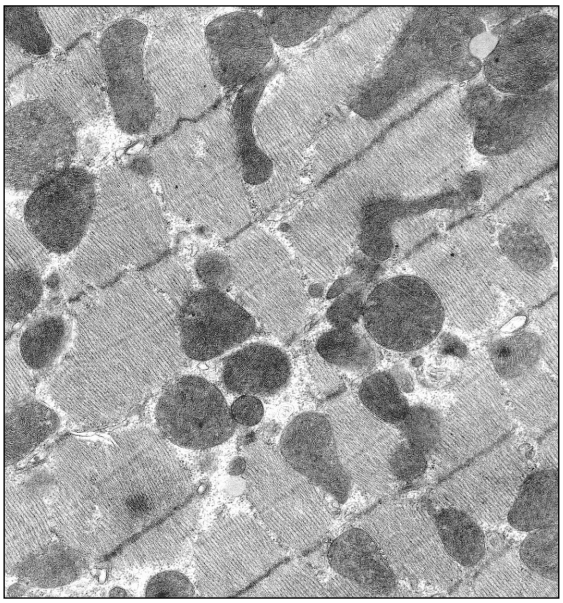

Fig. 5. Region of a cardiomyocyte of a rat treated with ubiquinone Q_{10} after introduction of H_2O_2 (70 μM). There is a regular alternation of myofibrillar disks; some of Z-disks are twisted; mitochondria display a dense matrix; in some mitochondria, cristae are homogenized and individual lipid inclusions are seen. Magnification ×22,000.

About 70% of the cardiomyocytes were disconnected and displayed pronounced abnormalities, such as large vacuoles and expressed swollen mitochondria with dense (possibly calcium-containing) inclusions (Fig. 4). In the nuclei of these cells, the margination of chromatin was observed (Fig. 4); moreover, edema of the sarcoplasm and overcontraction or lysis of myofibrils was also often observed. Only about one third of the cardiomyocytes appeared normal ultrastructure, but they also displayed enlarged profiles of T-tubules, twisted Z-disks, and thinned and partially lysed myofibrils. These changes corresponded to those observed after the introduction of 300 μM H_2O_2 [27].

Surprisingly, most of the cardiomyocytes from the myocardium of rats treated with ubiquinone Q_{10} virtually retained the intact structure after oxidative stress (Fig. 5). Main organelles, such as myofibrils and mitochondria, appeared normal, and the mitochondria had a dense matrix with poorly distinguishable closely packed cristae. Sometimes there were observed mitochondria with homogenized cristae, twisted Z-disks, and a small number of lipid inclusions. In the perinuclear zone, ribosomes and polyribosomes were accumulated, and individual lysosomes appeared.

The effect of oxidative stress on the myocardium was also assessed six weeks after the treatment with ubiquinone Q_{10} had been terminated, and the contractile function intensity was similarly suppressed in both groups. Morphological changes were more pronounced than in the myocardium isolated from rats after the treatment with ubiquinone Q_{10} for six weeks: about half of the cardiomyocytes displayed edema of the sarcoplasm and mitochondria, and lysis of myofibrils and lipid infiltration of the sarcoplasm were also observed. In the other half of the cardiomyocytes, the structure of myofibrils was intact and mitochondria were slightly enlarged. Thus, termination of the treatment with ubiquinone Q_{10} was associated with decrease in the antioxidant protection of the myocardium.

Oxidative metabolism. In mitochondria isolated from the hearts of the rats subjected to oxidative stress the respiration rate and value of the respiratory control (the ratio of oxygen consumption rates in states 3 and 4) were very low and nearly the same in the control and experimental groups (Table 2). In the presence of antimycin A the rate of succinate-dependent generation of superoxide radicals in mitochondria isolated from the hearts of the rats treated with ubiquinone Q_{10} was about fourfold lower than in mitochondria isolated from the hearts of the control rats.

Thus, the long-term treatment with ubiquinone Q_{10} significantly increased resistance of the isolated heart to oxidative stress caused by the addition of H_2O_2. This was manifested by a retarded decrease in the contractile function of the heart, by less pronounced lesions of the cardiomyocyte ultrastructure, and by lesser disorders in the respiration rate of mitochondria. Enrichment of mito-

Table 2. Respiratory control and generation of superoxide radicals in mitochondria isolated from the hearts of the rats treated with ubiquinone Q_{10} for six weeks

Group	Respiration rate, nmol O_2/min per mg protein		Respiratory control	Rate of O_2^- generation, nmol/min per mg protein
	state 3	state 4		
Control (4)	10.08 ± 1.02	4.60 ± 0.81	2.19 ± 0.11	1.71 ± 0.63
Ubiquinone Q_{10} (5)	12.05 ± 2.94	6.01 ± 1.19	1.96 ± 0.17	0.38 ± 0.14*

* $p < 0.05$ compared to control.

chondrial membranes with antioxidants is known to efficiently protect them against oxidative stress [28]. In particular, addition of ubiquinone Q_{10} to the rats' diet for six weeks was favorable for restoration of the heart functional parameters after oxidative stress caused by frequent stimulation [29], ischemia-reperfusion [30], or isoproterenol. This was associated with the less pronounced structural lesions and decreased rate of production of superoxide radicals in the isolated mitochondria [30].

The results of the present work are mainly consistent with these observations. Increase in content of the natural antioxidant coenzyme Q in the myocardium seems to promote an increase in the resistance of cell membranes to oxidation; however, we have concurrently observed a twofold decrease in the activity of one of the key antioxidant enzymes, myocardial superoxide dismutase. Superoxide dismutase is considered to be one of the main components of the antioxidant system of the cell [31], and decrease in its level increases the vulnerability of myocardium to superoxide anion radicals generated during oxidation of doxorubicin, whereas addition of superoxide dismutase to the perfusate prevents its toxic effect [32]. On the other hand, incubation of hepatocytes with ubiquinone Q_{10} fully prevented the doxorubicin-caused decrease in respiration and potential [10]. Note that a significant decrease in the activity of superoxide dismutase, which is one of key antioxidant enzymes in the rats treated with ubiquinone Q_{10}, was not accompanied by increase in contents of free radical oxidation products (in particular, MDA) in the myocardium or by decrease in its H_2O_2-dependent resistance. This is consistent with data on the protective effect of catalase and not of SOD in the case of H_2O_2 addition to the perfusate [27], and in our experiments, it seems to be associated with a very active utilization of hydroxyl radicals produced during homolysis of H_2O_2 according to the following reactions:

$$HO^{\cdot} + QH_2 \rightarrow H_2O + {}^{\cdot}QH,$$

$$HO^{\cdot} + {}^{\cdot}QH \rightarrow H_2O + Q,$$

while the efficient utilization of initial superoxide anion radicals failed to significantly affect the total antioxidant potential of the tissue. The significant decrease in the SOD activity in the myocardium of animals in response to long-term treatment with ubiquinone Q_{10} has been recorded for the first time, and this phenomenon is likely to be associated with changes in the balance between low- and high-molecular-weight antioxidants in the cell.

Thus, the findings of the present work clearly show a high efficiency of ubiquinone Q_{10} in protection of the myocardium against oxidative stress, and this is promising for the use of coenzyme Q preparations in complex therapy of cardiovascular diseases, including atherosclerosis, ischemic heart disease, and severe cardiac failure.

This work was supported by the Russian Foundation for Basic Research (project Nos. 02-04-50049, 02-04-499951, and 02-04-59062).

REFERENCES

1. Lankin, V. Z., Tikhaze, A. K., and Belenkov, Yu. N. (2000) *Kardiologiya*, **40**, 48-61.
2. Zenkov, N. K., Lankin, V. Z., and Men'shchikova, E. B. (2001) *Oxidative Stress. Biochemical and Pathophysiological Aspects* [in Russian], MAIK Nauka/Interperiodika, Moscow.
3. Zenkov, N. K., Kandalintseva, N. V., Lankin, V. Z., Men'shchikova, E. B., and Prosenko, A. E. (2003) *Phenolic Bioantioxidants* [in Russian], Siberian Division, Russian Academy of Medical Sciences, Novosibirsk.
4. Lankin, V. Z. (2003) in *Free Radicals, Nitric Oxide, and Inflammation: Molecular, Biochemical, and Clinical Aspects* (Tomasi, A., et al., eds.) Vol. 344, IOS Press, Amsterdam, pp. 8-23.
5. Landi, L., Cabrini, L., Sechi, A. M., and Pasquali, P. (1984) *Biochem. J.*, **222**, 463-466.
6. Mellors, A., and Tappel, A. L. (1966) *J. Biol. Chem.*, **241**, 4353-4356.
7. Beyer, R. E., Morales-Corral, P. G., Ramp, B. J., Kreitman, K. R., Falzon, M. J., Rhee, S. Y., Kuhn, T. W., Stein, M., Rosenwasser, M. J., and Cartwright, K. J. (1984) *Arch. Biochem. Biophys.*, **234**, 323-329.
8. Sugiyama, S., Takasawa, M., Hayakawa, M., and Ozawa, T. (1993) *Biochem. Mol. Biol. Int.*, **30**, 937-944.
9. Ernster, L., and Dallner, G. (1995) *Biochim. Biophys. Acta*, **1271**, 195-204.

10. Lenaz, G., Fato, R., Castelluccio, C., Cavazzoni, M., Estornell, E., Huertas, J. F., Pallotti, F., Parenti Castelli, G., and Rauchova, H. (1994) *Mol. Aspects Med.*, **15 (**Suppl.), S39-S46.
11. Langsjoen, P. H., and Langsjoen, A. M. (1999) *Biofactors*, **9**, 273-284.
12. Tran, M. T., Mitchell, T. M., Kennedy, D. T., and Giles, J. T. (2001) *Pharmacotherapy*, **21**, 797-806.
13. Kapelko, V. I. (2003) *Russian Medical Journal*, **21**, 1185-1189.
14. Lenaz, G., Parenti Castelli, G., Fato, R., D'Aurelio, M., Bovina, C., Formiggini, G., Marchetti, M., Estornell, E., and Rauchova, H. (1997) *Mol. Aspects Med.*, **18 (**Suppl.), S25-S31.
15. Lass, A., Forster, M. J., and Sohal, R. S. (1999) *Free Rad. Biol. Med.*, **26**, 1375-1382.
16. Lankin, V. Z., and Mikheeva, L. P. (1975) in *Bioantioxidants* [in Russian], Nauka, Moscow, pp. 151-156.
17. Aebi, H. E. (1983) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.) 3rd Ed., Vol. 3, Verlag Chemie, pp. 273-286.
18. Lankin, V. Z., and Gurevich, S. M. (1976) *Dokl. Akad. Nauk SSSR*, **226**, 705-708.
19. Beauchamp, C., and Fridovich, J. (1971) *Analyt. Biochem.*, **44**, 276-287.
20. Kapelko, V. I., Kupriyanov, V. V., Novikova, N. A., Lakomkin, V. L., Zueva, M. Y., Steinschneider, A. Y., and Saks, V. A. (1988) *J. Mol. Cell. Cardiol.*, **20**, 465-479.
21. Kitazake, M., and Marban, E. (1989) *J. Physiol. (Lond.)*, **414**, 455-472.
22. Korkina, O. V., Khatkevich, A. N., Kapelko, V. I., and Ruuge, E. K. (2001) *Kardiologiya*, **41**, 53-56.
23. Reahal, S., and Wrigglesworth, J. (1992) *Drug Metab. Dispos.*, **20**, 423-427.
24. Turunen, M., Appelkvist, E. L., Sindelar, P., and Dallner, G. (1999) *J. Nutr.*, **129**, 2113-2118.
25. Lonnrot, K., Tolvanen, J. P., Porsti, I., Ahola, T., Hervonen, A., and Alho, H. (1999) *Life Sci.*, **64**, 315-323.
26. Weston, S. B., Zhou, S., Weatherby, R. P., and Robson, S. J. (1997) *Int. J. Sport Nutr.*, **7**, 197-206.
27. Miki, S., Ashraf, M., Salka, S., and Sperelakis, N. (1988) *J. Mol. Cell. Cardiol.*, **20**, 1009-1024.
28. Huertas, J. R., Martinez-Velasco, E., Ibanez, S., Lopez-Frias, M., Ochoa, J. J., Quiles, J., Castelli, G., Parenti, Mataix, J., and Lenaz, G. (1999) *Biofactors*, **9**, 337-343.
29. Rosenfeldt, F. L., Pepe, S., Ou, R., Mariani, J. A., Rowland, M. A., Nagley, P., and Linnane, A. W. (1999) *Biofactors*, **9**, 291-299.
30. Lakomkin, V. L., Korkina, O. V., Tsyplenkova, V. G., Timoshin, A. A., Ruuge, E. K., and Kapelko, V. I. (2002) *Kardiologiya*, **42**, 51-55.
31. Sarvazyan, N. A., Askari, A., and Huang, W. H. (1995) *Life Sci.*, **57**, 1003-1010.
32. Chan, E. M., Thomas, M. J., Bandy, B., and Tibbits, G. F. (1996) *Can. J. Physiol. Pharmacol.*, **74**, 904-910.