Original Article *J. Clin. Biochem. Nutr.*, **40**, 194–202, May 2007

Protective Effects of Coenzyme Q_{10} on Decreased Oxidative Stress Resistance Induced by Simvastatin

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Received 9 October, 2006; Accepted 25 October, 2006

Summary **The effects of simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), on oxidative stress resistance and the protective effects of coenzyme Q (CoQ) were investigated. When simvastatin was administered orally to mice, the levels of oxidized and reduced CoQ_9 and CoQ_{10} in serum, liver, and heart, decreased significantly when compared to those of control. The levels of thiobarbituric acid reactive substances induced by Fe^{2+}-ascorbate in liver and heart mitochondria also increased significantly with simvastatin. Furthermore, cultured cardiac myocytes treated with simvastatin exhibited less resistance to oxidative stress, decreased time to the cessation of spontaneous beating in response to H_2O_2 addition, and decreased responsiveness to electrical field stimulation. These results suggested that oral administration of simvastatin suppresses the biosynthesis of CoQ, which shares the same biosynthesis pathway as cholesterol up to farnesyl pyrophosphate, thus compromising the physiological function of reduced CoQ, which possesses antioxidant activity. However, these undesirable effects induced by simvastatin were alleviated by coadministering CoQ_{10} with simvastatin to mice. Simvastatin also reduced the activity of NADPH-CoQ reductase, a biological enzyme that converts oxidized CoQ to the corresponding reduced CoQ, while CoQ_{10} administration improved it. These findings may also support the efficacy of coadministering CoQ_{10} with statins.**

Key Words: **coenzyme Q_{10}, ubiquinol-10, HMG-CoA reductase inhibitor, statin, oxidative stress**

Introduction

Hypercholesterolemia is a well-known risk factor for coronary heart disease and arteriosclerosis. 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), the late-limiting enzyme in the biosynthesis of cholesterol through the mevalonate pathway, converts HMG-CoA, a trimer of acetyl CoA molecules generated from fatty acid oxidation, to mevalonic acid, which is then further metabolized to eventually produce cholesterol [*1*]. Among various drugs developed to reduce serum cholesterol levels and subsequent risk for coronary events, HMG-CoA reductase inhibitors (statins), have proven to be extremely useful drugs [*2–5*].

While statins are very clinically effective drugs, adverse reactions such as rhabdomyolysis [*6*, *7*] have been reported. Adverse reactions often affect tissues and cells with high energy metabolism, such as skeletal muscles, myocardium

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and smooth muscles, and to date, ultrastructural changes, including mitochondrial dysfunction [*8*, *9*] and swelling, increased serum creatine kinase, induction of necrosis [*10*] or apoptosis [*11–15*], and abnormalities in calcium homeostasis [*16*] have been reported. However, the contributing factors and protective effects have not been elucidated.

Coenzyme Q (CoQ) is an essential carrier for the mitochondrial electron transport system and plays an important role in energy production. Exogenous CoQ_{10} has been shown to improve energy metabolism and activate energy production by cardiac myocytes. CoQ is distributed not only in mitochondria, but also in other subcellular fractions [*17–19*], and some CoQ always presents as reduced CoQ (H_2CoQ) [*18*]. H_2CoQ is unstable when exposed to air, and can easily be oxidized into oxidized CoQ. In this manner, chemically unstable H_2CoQ is thought to exist in the body to serve as an antioxidant. We have reported previously [*20–22*] that a novel cytosolic NADPH-CoQ reductase is responsible for the reduction of CoQ_{10} to H_2CoQ_{10} in biomembranes. Furthermore, we observed that prolonged supplementation with CoQ_{10} caused a significant increase in the NADPH-CoQ reductase activity, and protected partially hepatotoxicity induced by carbon tetrachloride and H_2O_2. In addition, enhanced levels of H_2CoQ_{10} and NADPH-CoQ reductase activity showed more resistant to oxidative stress than those of normal levels. These results suggested that H_2CoQ_{10} with NADPH-CoQ reductase constituted a fundamental defense system against oxidative stress in cellular membrane. According to some reports, another reductases such as NAD(P)H:quinone oxidoreductase 1 (NQO1, DT-diaphorase) [*23*, *24*], thiredoxin reductase [*25*] and lipoamide dehydrogenase 1 [*26*, *27*] may also involve in the reduction of CoQ to H_2CoQ. The *in vitro* experiments using low-density lipoprotein (LDL) [*28*, *29*], biological membranes [*30*] or lecithin liposome membranes [*31*, *32*] have clarified that H_2CoQ possesses potent antioxidant activity. To date, two mechanisms for the antioxidant activities of H_2CoQ have been clarified. In one mechanism, H_2CoQ directly eliminates lipid peroxyl radicals [*31*, *32*], and in the other, H_2CoQ indirectly acts as an antioxidant by regenerating α-tocopherol from α-tocopheroxyl radicals formed by a reaction between α-tocopherol and lipid peroxyl radicals (α-tocopherol-saving action). It has been clarified that H_2CoQ exhibits potent antioxidant activity through these two mechanisms.

CoQ is supplied exogenously through diet [*33*] or endogenously through biosynthesis [*34*, *35*]. Therefore, supply reduction in either exogenous or endogenous CoQ may affect its physiological functions. Because cholesterol and CoQ share the same biosynthesis pathway until farnesyl pyrophosphate [*34*, *35*], inhibition of HMG-CoA reductase, which is the rate-limiting enzyme in cholesterol biosynthesis, is likely to affect the metabolism and physiological functions of CoQ, including H_2CoQ [*35*]. In fact, many investigators have pointed out that statin administration lowers CoQ in serum (plasma) [*9*, *36–38*] and tissue [*36*], and that statins hinder normal function of the heart [*39*] and skeletal muscles [*40*].

In this study, in order to administer HMG-CoA reductase inhibitors safely and efficiently, we elucidated the change of oxidative stress resistance induced by simvastatin and its protective effects of CoQ_{10} were investigated.

Materials and Methods

Reagents

All reagents were commercially available and were of reagent grade. The solvent for high performance liquid chromatography (HPLC) was purchased from Wako Pure Chemical Industries, Ltd., Osaka. Commercially available reagent grade methanol for HPLC was used after distillation. Authentic CoQ_9 and CoQ_{10} for HPLC were donated by Nisshin Pharma Inc., Tokyo. As simvastatin, 5 mg Lipovas tablets (pharmaceutical drug, Banyu Pharmaceutical Co. Ltd., Tokyo), and as CoQ_{10}, 30 mg LivLon Soft Capsules (dietary and health food, Nisshin Pharma Inc., Tokyo) were used in the experiment for oral administration to mice. Each compound was dissolved in drinking water and homogenized by ultrasonic treatment.

Animals

Ten week-old male specific pathogen-free (SPF) ICR mice were used. To prepare cultured cardiac myocytes, female SPF ICR mice on the fourth day of gestation were used. All mice were purchased from SLC Japan (Shizuoka). Simvastatin and CoQ_{10} were administered orally, and by measuring daily water intake for each mouse for three days before the study, drug solutions were prepared such that each mouse would consume the predetermined dosages. Simvastatin and CoQ_{10} were mixed in drinking water just prior to administration, and drinking water was prepared daily. Based on body weight, the mice were divided into four groups of five mice each [(1) control group (no simvastatin or CoQ_{10}), (2) simvastatin group (1 mg/day of simvastatin), (3) CoQ_{10} group (3 mg/day of CoQ_{10}), and (4) simvastatin + CoQ_{10} group (1 mg/day of simvastatin + 3 mg/day of CoQ_{10})], and the dosage of simvastatin and CoQ_{10} was set as follows: each mouse was weighed daily, and simvastatin and CoQ_{10} were administered for two consecutive weeks. Simvastatin and CoQ_{10} were administered orally to pregnant mice from the fourth to fifteenth day of gestation (fetal ventricular myocardium was excised from pregnant mice on the fifteenth day of gestation). Mice were fed Lab MR Stock (standard feed, SLC Japan). All animal experiments were conducted in accordance with the manual compiled by the Kobe University Animal Ethics Committee.

Cultured cardiac myocyte preparation and observation

The fetal ventricular myocardium was excised from pregnant mice prepared as described above from the fourth to fifteenth day of gestation, and cultured cardiac myocytes were prepared according to the method of Goshima *et al.* [*41*]. Cardiac myocytes were cultured using Eagle MEM containing 10% fetal bovine serum under 5% CO_2 in air at 37°C for 2 days. To avoid antioxidant contamination, including CoQ_{10}, originating from fetal bovine serum, cells were washed using 10 ml of Dulbecco's phosphate-buffered saline twice 1 h before the study, and after replacing culture solution with Eagle MEM free of fetal bovine serum, cells were incubated under 5% CO_2 in air at 37°C for at least one hour. A 1- to 2-mm-diameter cardiac myocyte sheet containing at least 10^4 cells was produced after cultivation for 2 days. The spontaneous beating of cultured cardiac myocyte sheet was measured in an incubator at 36 ± 1°C and under 5% CO_2 in air using a phase contrast microscope. Electrical field stimulation was performed by the method of Nakamura *et al.*, as follows [*42*]: two platinum electrodes, 1 Hz, pulse length of 50 ms, and pulse amplitude of 100 V.

Preparation of mitochondria and cytosolic fraction from mice liver and heart

Intracellular fractions of mice heart and liver were prepared by differential centrifugation as described previously [*18*]. The purity of the cytosol and mitochondrial fractions was determined by measuring their marker enzyme activities.

Measurement of total serum cholesterol level

Total serum cholesterol was measured spectrophotometrically by the enzyme method [*43*].

Measurement of CoQ levels in serum, cytosolic fraction and tissue

Reduced and total CoQ (sum of oxidized and reduced CoQ) levels were measured by HPLC-electrochemical detector in accordance with the method of Okamoto *et al.* [*44*]. CoQ in the cytosolic fraction was expressed as pmol per mg protein.

Measurement of lipid peroxidation induced by Fe^{2+}-ascorbate

Lipid peroxidation was carried out using 50 mM ascorbic acid and 5 mM $FeSO_4$ according to the method of Takei *et al.* [*45*]. Thiobarbituric acid reactive substances (TBARS) were extracted using 3 ml of n-butanol and were measured by the fluorometrical method (Ex: 515 nm, Em: 553 nm). The standard was 1,1,3,3-tetraethoxypropane in the TBARS assay.

Measurement of NADPH-CoQ reductase activity in cytoplasm

NADPH-CoQ activity in cytoplasm of liver and heart was measured according to the method of Takahashi *et al.* [*46*]. This activity was expressed as the amount of CoQ_{10} (pmol) reduced per minute per mg protein.

Protein content and data analysis

Protein content was determined as described by Lowry *et al.* [*47*]. The Student's unpaired *t* test were used for statistical analysis and statistical significance was assigned for any p values less than 0.05.

Results

Changes in reduced and total CoQ levels in mouse serum, heart, liver, and their cytosolic and mitochondorial fractions induced by simvastatin administration (Table 1)

When 1 mg/kg body weight simvastatin was orally administered to mice everyday for two weeks, total serum cholesterol decreased significantly when compared to controls (no simvastatin or CoQ_{10}). Furthermore, coadministration of simvastatin and CoQ_{10} lowered serum cholesterol in a similar manner. This result shows that CoQ_{10} itself does not affect the cholesterol lowering activity of simvastatin.

Simvastatin administration significantly lowered the levels of both serum CoQ_9, the predominant homologue in mice, and CoQ_{10}, as compared to those of control. Furthermore, simvastatin also singnificantly lowered reduced, oxidized and total CoQ_{10} levels, and did not affect the ratio of reduced CoQ to total CoQ.

When 3 mg/kg body weight CoQ_{10} was orally administered to mice everyday for two weeks, irrespective of whether CoQ_{10} was administered alone or with simvastatin, serum levels increased by about 6-fold. These results show that simvastatin administration does not affect the serum CoQ_{10} increasing level by CoQ_{10} administration. CoQ_{10} itself did not affect the levels of serum CoQ_9, the main homologue in mice.

Like the levels of CoQ_9 and CoQ_{10} in serum, the levels of CoQ_9 and CoQ_{10} in the liver and heart were significantly lowered by simvastatin administration. Furthermore, this lowering action was observed in mitochondrial and cytosolic fractions of liver and heart.

Oxidative stress resistance-decreasing effects of simvastatin

Changes in oxidative stress resistance due to simvastatin administration were evaluated using heart and liver mitochondria and cultured cardiac myocytes.

The TBARS levels produced using Fe^{2+}-ascorbate in heart mitochondria at 15 min after the start of the reaction for the simvastatin group was significantly higher when compared to the other groups (Table 2). At two hours after the start of the reaction, the TBARS level (mM) in the control, simvastatin, CoQ_{10} and simvastatin + CoQ_{10} groups was 0.67, 0.81, 0.63 and 0.66, respectively, and CoQ_{10} clearly lowered significantly the TBARS level as compared to that

Table 1. Effect of simvastatin on serum cholesterol and Coenzyme Q contents in Serum, Heart and Liver

	Administration			
	Control	Sim	CoQ_{10}	Sim + CoQ_{10}
Serum				
Cholesterol (mg/dl)	131 ± 25	94 ± 31*	141 ± 15	90 ± 22*
Total CoQ_9 (ng/ml)[1]	71.9 ± 3.1	60.2 ± 8.9*	75.8 ± 3.4	66.1 ± 6.4*
Reduced CoQ_9 (ng/ml)	58.0 ± 2.2	48.0 ± 8.8*	62.9 ± 3.7	54.3 ± 6.8
Reduced/Total (%)[2]	80.4 ± 3.0	79.3 ± 3.9	83.0 ± 1.9	81.2 ± 5.5
Total CoQ_{10} (ng/ml)[1]	24.4 ± 1.3	19.3 ± 1.5**	155.3 ± 48.2**	143.7 ± 56.8**
Reduced CoQ_{10} (ng/ml)	19.2 ± 1.7	15.4 ± 1.5**	128.7 ± 37.3**	115.6 ± 54.2**
Reduced/Total (%)[2]	78.5 ± 2.6	80.1 ± 4.9	83.3 ± 2.9*	78.0 ± 6.1
Liver				
Total CoQ_9 (μg/g)[1]	39.3 ± 5.0	30.5 ± 3.1*	44.6 ± 2.8*	37.4 ± 5.6
Reduced CoQ_9 (μg/g)	24.7 ± 4.9	18.4 ± 2.6*	29.8 ± 2.6*	23.5 ± 5.7
Reduced/Total (%)[2]	62.6 ± 6.0	60.2 ± 4.9	66.8 ± 3.7*	63.2 ± 6.7
Total CoQ_{10} (μg/g)[1]	3.3 ± 0.6	2.5 ± 0.3*	9.1 ± 1.8**	8.8 ± 2.3**
Reduced CoQ_{10} (μg/g)	2.0 ± 0.4	1.4 ± 0.3*	6.1 ± 1.4**	5.6 ± 1.5**
Reduced/Total (%)[2]	59.8 ± 4.1	57.3 ± 4.2	66.0 ± 4.9*	64.0 ± 4.1*
Mitochondria				
Total CoQ_{10}[1] (pmol/mg protein)	13.7 ± 1.3	10.0 ± 1.5**	21.8 ± 2.2**	17.0 ± 1.1*
Cytosol				
Total CoQ_{10}[1] (pmol/mg protein)	2.5 ± 0.2	2.4 ± 0.1*	3.3 ± 0.2**	3.0 ± 0.2**
Heart				
Total CoQ_9 (μg/g)[1]	214.5 ± 36.0	171.8 ± 22.4*	225.4 ± 30.9	186.1 ± 6.3*
Reduced CoQ_9 (μg/g)	91.5 ± 8.7	71.0 ± 10.1*	98.8 ± 9.5	81.9 ± 8.9*
Reduced/Total (%)[2]	43.4 ± 5.8	41.4 ± 2.8	44.4 ± 6.4	45.0 ± 4.0
Total CoQ_{10} (μg/g)[1]	24.8 ± 5.2	21.0 ± 3.2*	26.2 ± 3.1	23.1 ± 3.4
Reduced CoQ_{10} (μg/g)	8.5 ± 1.2	7.0 ± 1.2*	9.9 ± 1.2	8.0 ± 1.4
Reduced/Total (%)[2]	35.0 ± 6.8	33.8 ± 3.3	38.2 ± 4.8	35.2 ± 6.5
Mitochondria				
Total CoQ_{10}[1] (pmol/mg protein)	284.8 ± 18.7	247.8 ± 17.7*	313.9 ± 11.4*	270.9 ± 20.8
Cytosol				
Total CoQ_{10}[1] (pmol/mg protein)	17.4 ± 0.8	13.4 ± 1.1*	16.8 ± 1.5	15.9 ± 1.0

Control: untreated group, Sim: simvastatin-treated group, CoQ_{10}: CoQ_{10}-treated group, Sim + CoQ_{10}: simvastatin and CoQ_{10}-cotreated group. The values given are means ± SD (n = 5). [1] Total CoQ_n is the sum of both reduced and oxidized form of CoQ_n. [2] Reduced CoQ_{10}/Total CoQ_{10} × 100. *p<0.05, **p<0.005 vs control (untreated) group.

of simvastatin group.

Like heart mitochondria, simvastatin administration increased the amount of TBARS with time in liver mitochondria. However, unlike heart mitochondria, suppression of TBARS by CoQ_{10} was not seen for the first hour of the reaction, and significant suppression was confirmed only after two hours (Table 3).

Changes in oxidative stress (H_2O_2) resistance [*42*] were determined using cultured cardiac myocyte sheets prepared from fetal ventricular myocardium obtained from pregnant mice on oral simvastatin administration in terms of spontaneous beating and electrical field stimulation response (Fig. 1). Prior to the experiment, cultured cardiac myocytes were incubated with 10 μM CoQ_{10} (CoQ_{10}-treated group) for 6 hours, 3 μM simvastatin (simvastatin-treated group) for 2 hours, or 10 μM CoQ_{10} for 6 hours and 3 μM simvastatin for 2 hours (simvastatin and CoQ_{10}-cotreated group). Under microscopic observations, in this point, any morphological changes of cells were not observed.

When 50 μM H_2O_2, an active oxygen species, was added to untreated cultured cardiac myocytes, beating stopped after 17 min. Furthermore, immediately after cessation, electric field stimulation was applied to the cells. When the time for the cells to respond to the stimulation was measured, electrical field-stimulated beating continued for 28 min. With cardiac myocytes treated by simvastatin, the

Table 2. Protective effect of Coenzyme Q_{10} on lipid peroxidation of heart mitochondria induced by Fe^{2+}-ascorbate

Group	TBARS (mM)			
	15 min	30 min	1 h	2 h
Control	0.46 ± 0.04[e)]	0.67 ± 0.03	0.61 ± 0.02[d)]	0.67 ± 0.01[f)]
Sim	0.60 ± 0.05[b)]	0.72 ± 0.03	0.71 ± 0.07[a)]	0.81 ± 0.02[c)]
CoQ_{10}	0.46 ± 0.01[e)]	0.57 ± 0.02[b), f)]	0.57 ± 0.03[d)]	0.63 ± 0.04[f)]
Sim + CoQ_{10}	0.41 ± 0.01[f)]	0.61 ± 0.06[d)]	0.60 ± 0.02[d)]	0.66 ± 0.02[f)]

Control: untreated group, Sim: simvastatin-treated group, CoQ_{10}: CoQ_{10}-treated group, Sim + CoQ_{10}: simvastatin and CoQ_{10}-cotreated group. The values given are means ± SD (n = 5). [a)]p<0.05, [b)]p<0.01, and [c)]p<0.001 vs control (untreated) group at each indicated time. [d)]p<0.05, [e)]p<0.01, and [f)]p<0.001 vs simvastatin-treated group at each indicated time.

Table 3. Protective effect of Coenzyme Q_{10} on lipid peroxidation of liver mitochondria induced by Fe^{2+}-ascorbate

Group	TBARS (mM)			
	15 min	30 min	1 h	2 h
Control	0.69 ± 0.02	0.86 ± 0.08	0.80 ± 0.04[d)]	0.85 ± 0.05[c)]
Sim	0.71 ± 0.03	0.89 ± 0.03	0.94 ± 0.06[b)]	0.94 ± 0.02[a)]
CoQ_{10}	0.65 ± 0.03[c)]	0.74 ± 0.05[a), d)]	0.63 ± 0.08[b), e)]	0.66 ± 0.08[b), e)]
Sim + CoQ_{10}	0.72 ± 0.03	0.88 ± 0.03	0.89 ± 0.06[a)]	0.86 ± 0.05[c)]

Control: untreated group, Sim: simvastatin-treated group, CoQ_{10}: CoQ_{10}-treated group, Sim + CoQ_{10}: simvastatin and CoQ_{10}-cotreated group. The values given are means ± SD (n = 5). [a)]p<0.05 and [b)]p<0.01 vs control (untreated) group at each indicated time. [c)]p<0.05, [d)]p<0.01, and [e)]p<0.001 vs simvastatin-treated group at each indicated time.

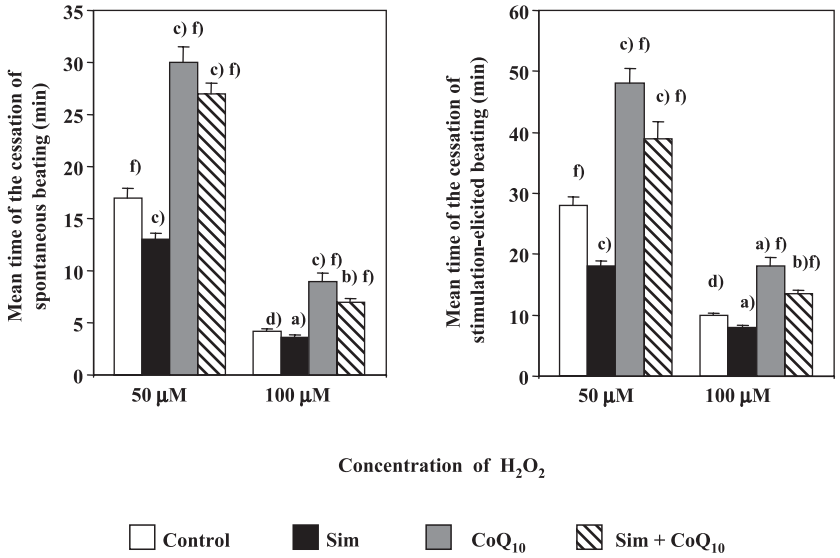

Fig. 1. Protective effect of Coenzyme Q_{10} on beating impairment of cultured cardiac myocytes induced by simvastatin. The value given are means ± SD (n = 5). [a)]p<0.05, [b)]p<0.01, and [c)]p<0.001 vs control (untreated) group. [d)]p<0.05, [e)]p<0.01, and [f)]p<0.001 vs simvastatin-treated group.

length of time to cessation was 13 min, and the electric field stimulation response time was 18 min, thus suggesting that simvastatin lowers oxidative stress resistance. On the other hand, CoQ_{10} increased the oxidative stress resistance of cardiac myocytes, and when compared to those of simvastatin group, spontaneous beating and electric field stimulation response time were significantly longer. The results were the same when 100 μM H_2O_2 was added to cardiac myocytes, but the effects of CoQ_{10} on spontaneous beating and electric field stimulation response time were not as great as when 50 μM H_2O_2 was added.

NADPH-CoQ reductase activity-lowering effect of simvastatin (Fig 2)

NADPH-CoQ reductase is one of the CoQ reductases in the cytoplasm, and we have reported previously that NADPH-CoQ reductase activity changes due to various oxidative stresses [*48*].

As shown in Fig. 2, when compared to the control group, cytoplasmic NADPH-CoQ reductase activity in the liver and heart in the simvastatin group were significantly lower when compared to those of control. CoQ_{10} administration increased NADPH-CoQ reductase activity in the liver and heart, and the NADPH-CoQ reductase activity in the simvastatin and CoQ_{10}-cotreated group was higher than that of the simvastatin group.

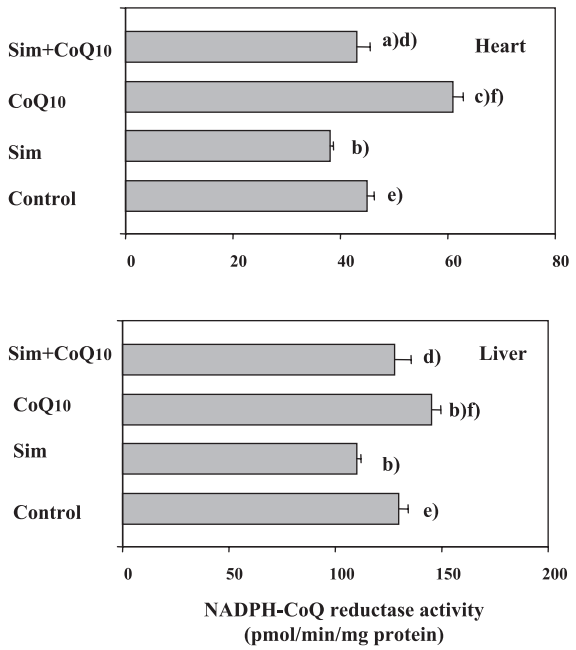

Fig. 2. Effect of simvastatin on cytosolic NADPH-CoQ reductase activities in heart and liver. The value given are means ± SD (n = 5). [a)]$p<0.05$, [b)]$p<0.01$, and [c)]$p<0.001$ vs control (untreated) group. [d)]$p<0.05$, [e)]$p<0.01$, and [f)]$p<0.001$ vs simvastatin-treated group.

Discussion

The nutritional sufficiency of CoQ is affected by both endogenous and exogenous CoQ, and a reduction in one source is thought to markedly affect its physiological function. We reported previously [*49*] that serum CoQ_{10} levels in patients on total parenteral nutrition (TPN) decreased significantly after TPN and never reached zero following TPN. These results suggest that the decrease in serum CoQ_{10} levels after TPN is dependent on dietary (exogenous) CoQ_{10} and that the residual serum CoQ_{10} levels following TPN are dependent on biosynthesized (endogenous) CoQ_{10}. However, the physiological changes induced by insufficient supply of endogenous CoQ_{10} have been unknown.

The CoQ biosynthesis pathway for eukaryotic cells has already been clarified [*34*, *35*]. CoQ shares the same biosynthetic pathway as cholesterol up to farnesyl pyrophosphate, and statins, which inhibit the rate-limiting enzyme of cholesterol biosynthesis, block the upstream section of the CoQ biosynthesis pathway. Therefore, statins affect CoQ biosynthesis greatly. Furthermore, the CoQ level in the body has been reported to decrease after the age of 20 years, and this decrease is marked in tissues with high energy metabolism, such as the heart [*50*]. Decreased CoQ_{10} might be thus a serious problem, as most hypercholesterolemia patients who take statins are elderly.

Decrease in CoQ levels induced by oral administration of HMG-CoA reductase inhibitor

As shown in Table 1, oral administration of simvastatin significantly decreased not only serum cholesterol levels, but also serum and tissue CoQ_9 and CoQ_{10} levels. Simvastatin also decreased both reduced and oxidized forms of CoQ_9 and CoQ_{10}. Many investigators have pointed out that statins lower serum and tissue CoQ levels [*9*, *36–38*], but the effects of decreased CoQ on the body have not been fully elucidated. Folkers *et al.* reported that patients on statin therapy exhibited cardiac dysfunction [*39*]. The most important physiological action of CoQ_{10} is to improve energy metabolism by serving as an essential factor in the mitochondrial electron transfer system, and this action is accepted as the physiological action of CoQ_{10}. Therefore, reduced CoQ levels may affect skeletal muscles and myocardium with active energy metabolism. In fact, muscle pain or weakness with high creatine kinase levels is the major adverse effects of statins [*2*]. In the present study, the levels of CoQ, in cytoplasm where NADPH-CoQ reductase are present, as well as in the mitochondria were measured (Table 1), and the results confirmed that statin administration reduces CoQ levels in mitochondria and cytoplasm among intracellular organelles.

Decreased oxidative stress resistance due to oral administration of HMG-CoA reductase inhibitor

In addition to energy production activation, another important physiological action of CoQ_{10} is as an antioxidant *in vivo*. Therefore, when simvastatin administration lowers CoQ levels in the body, endogenous antioxidative function may be affected to some degree. In general, orally taken oxidized CoQ_{10} is absorbed by epithelial cells in the small intestine and transported via the lymphatic system to the liver, and is reduced by NADPH-CoQ reductase to become H_2CoQ_{10}, which has potent antioxidant action [*20–22*]. In human serum (plasma), CoQ_{10} is mostly present as H_2CoQ_{10}, and even if oxidized CoQ_{10} is administered exogenously, the ratio of reduced CoQ_{10} to total CoQ_{10} remains the same [*51*]. Therefore, H_2CoQ_{10}, which is extremely unstable in air, is thought to exist in the body due to its role as an antioxidant.

In the present study, oral administration of statin decreased the resistance of heart and liver mitochondria to lipid peroxidation induced by Fe^{2+}-ascorbate and transiently increased the production of TBARS (Tables 2 and 3). This increase in TBARS was alleviated by oral administration of CoQ_{10}. Furthermore, oral administration of CoQ_{10} increased also NADPH-CoQ reductase activity, which was decreased by statin administration (Fig. 2). On the other hand, simvastatin also induced cessation of both spontaneous beating and stimulation-elicited beating in cultured cardiac myocytes (Fig. 1). This simvastatin-contractile impairment was prevented partially by pretreatment of cultured cardiac myocytes with 10 μM CoQ_{10}. These results suggest that by coadministering CoQ_{10} and statin, sufficient amounts of H_2CoQ_{10} are supplied to the body and maintained, thus alleviating the decreased oxidative stress resistance caused by statin administration.

Necessity of CoQ_{10} during oral administration of HMG-CoA reductase inhibitor

In 2001, the Ministry of Health, Labour and Welfare in Japan permitted the use of CoQ_{10} as a food additive as long as no claims were made about its pharmacological effectiveness and application. Now, many CoQ_{10}-containing dietary and helath supplements are commercially available in Japan. While people mostly take CoQ_{10} for health maintenance or nutritional supplementation, as the size of the elderly population continues to increase in Japan, it is necessary to consider to the adverse reactions caused by drugs that are frequently prescribed to the elderly. The present study showed that statin administration decreased the body's resistance to oxidative stress, and one of the factors may be decreased CoQ levels. Therefore, we clarifies that when administering statin, it is desirable to elevate CoQ to normal levels, thus maintaining resistance to various oxidative stresses.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 16500429) from the Ministry of Education, Science and Culture, Japan, and Grand-in-Aid for Health Science Research as well as Grand-in-Aid for Cooperative Research (A) from Kobe Gakuin University, Japan. This research was also carried out in Coenzyme Q_{10} Biofunctional Research Center endowed by Shiseido Pharmaceutical Co. Ltd., Tokyo, Japan.

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