Effects of Coenzyme Q_{10} on LDL Oxidation *In vitro*

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Abstract- Coenzyme Q_{10}, a lipid soluble benzoquinone, has excellent antioxidant and membrane stabilizing properties in the cardiac tissue. The present study investigated the effects of coenzyme Q_{10} on *in vitro* LDL oxidation quantitavely. The formation of conjugated dienes, malondialdehyde (MDA) and electrophoretic mobility were monitored as markers of the oxidation of LDL, respectively. Coenzyme Q_{10} showed a decrease the formation of conjugated dienes and MDA, respectively, against oxidation *in vitro*. Coenzyme Q_{10} also reduced electrophoretic mobility of the oxidized human LDL. Thus, results showed that the coenzyme Q_{10} exhibits strong antioxidant activity in $CuSO_4$-mediated oxidation of LDL ($P<0.05$) *in vitro*. The inhibitory effects of the coenzyme Q_{10} on LDL oxidation were dose-dependent at concentrations ranging from 20 nM to 300 nM. Moreover, the effects of coenzyme Q_{10} on LDL oxidation were compared with vitamin E and vitamin C. This study showed that coenzyme Q_{10} is a potent antioxidant to protect LDL against oxidation *in vitro* and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.
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Keywords: Coenzyme Q_{10}; LDL oxidation; Vitamin C; Vitamin E

Introduction

Cardiovascular disease is one of the most causes of mortality in the world. Although an increased concentration of plasma low density lipoprotein (LDL) is believed to be a major risk factor in atherosclerosis, the mechanisms of the LDL oxidation are not completely understood. There is considerable evidence support a role for oxidatively modified LDL in the pathogenesis of atherosclerosis (1,2). The uptake of oxidized LDL (Ox-LDL) by macrophage results in the formation of foam cells and cellular cholesterol accumulated in vascular endothelial cells, and promotes the development of the characteristic fatty streaks found in atherosclerotic lesion (1-5).
Finally, Ox-LDL has been reported to compromise endothelial integrity, a salient feature of atherosclerosis. Antioxidants such as beta-carotene and vitamin E have been shown to decrease formation of atherosclerotic lesions in animal models and epidemiological data suggest that an inverse relationship exist between the intake of antioxidant vitamins and the risk of coronary artery disease (6). According the oxidation hypothesis, antioxidants protect LDL against oxidative stress and delay the formation of modified LDL (7,8). The lipophilic antioxidant vitamin E, beta-carotene, ubiquinol, and lycopen are present in LDL. Vitamin E is thought to be major non-enzymatic antioxidant present in the lipid structures of cells and lipoproteins (9).Vitamin E is a major antioxidant present in the lipoproteins (9).

Coenzyme Q_{10} is a natural human ubiquinone, and it has fundamental role in mitochondrial energy (ATP) production in the respiratory chain (10-12). It plays a role in extra-mitochondrial redox activity in the cell membrane. Coenzyme Q_{10} is also antioxidant, scavenging free radicals and inhibiting lipid peroxidation (13-15). The antioxidant effect of coenzyme Q_{10} is greater than vitamin E (6). Coenzyme Q_{10} is also known to enhance the availability of other antioxidants such as vitamin C, vitamin E and beta-caroten (16).

Since the effects of coenzyme Q_{10} on LDL oxidation have not previously been reported, the objectives of the present study were to assess the effects of plasma pre-incubation with vitamin E, vitamin C and various

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concentration of coenzyme Q_{10} on the modification of LDL induced by $CuSO_4$ *in vitro* by monitoring the formation of conjugated dienes, the formation of MDA and measurement rate electrophoretic mobility (REM).

Materials and Methods

Materials

Coenzyme Q_{10}, disodium ethylene diamine tetra acetate (Na_2EDTA) were purchased from Sigma Chemical Co. Potassium bromide (KBr), sodium chloride (NaCl), disodium hydrogen phosphate ($_{Na2HPO4}$), vitamin E, vitamin C and oil red O were purchased from Wako Pure Chemical Industries, Ltd (Japan).

Blood sampling and *in vitro* addition coenzyme Q_{10}, vitamin E and vitamin C to plasma

Blood samples were taken from ten men. The protocols for the blood sampling were approved by the University of Kanazawa Ethics Committee, and all the informed constants were taken from all the men. Fasting blood samples after an overnight fasting was taken EDTA containing tubes (1.6 mg EDTA/ml blood). To obtain plasma, the centrifugation (3000 rpm for 10 min at 4°C) was started within 1 min following venous puncture to avoid autooxidation of the sample. To minimize oxidation *in vitro*, sodium azide (0.06% wt/vol) was added to plasma immediately after collection. For *in vitro* enrichment of LDL, freshly prepared human plasma was incubated at 37°C for 3 hours in the presence of added vitamin E (100 M), vitamin C (200 M) and various concentration of coenzyme Q_{10} (20, 100, 200 and 300 nM). Coenzyme Q_{10} and vitamin E dissolved in ethanol and vitamin C dissolved in phosphate-buffered saline (PBS) (17). The final concentration of ethanol in the plasma was 5% (vol/vol). Control plasma and plasma contained vitamin C received an equal amount of ethanol and were incubated in the same manner.

Isolation of LDL

The LDL density of 1.019-1.063 was isolated from fresh plasma by single vertical discontinuous density gradient ultracentrifugation (5,18).

Oxidation of LDL
Continuous monitoring of formation of conjugated dienes in LDL

After isolation of total LDL, the protein content of LDL was measured (19). LDL was adjusted to 150 µg/ml of LDL protein with 10 mM PBS, pH7.4. The oxidative modification of LDL was initiated by addition of freshly prepared 10 µM $CuSO_4$ solution at 37°C in a water bath for 5h. The kinetics of LDL oxidation was monitored every 10 minutes by measuring its absorbance at 234 nm. The lag phase was calculated from the oxidation profile of each LDL preparation by drawing a tangent to the slope of the propagation phase and extrapolation into intercept the initial-absorbance axis. The lag phase represented the length of the antioxidant-protected phase during LDL oxidation by coenzyme Q_{10} *in vitro*. The lag time was measured as the time period until the conjugated dienes began to increase (20).

The formation of conjugated dienes was calculated as conjugated dienes equivalent content (nmol/mg-protein) at 5h. The conjugated dienes concentration was calculated by using the extinction coefficient for diene conjugates at 234 nm ($29500 \text{ M}^{-1} \cdot \text{cm}^{-1}$).

Assay of the formation of malondialdehyde (MDA)

Lipid peroxidation end product was determined as malondialdehyde (MDA) according to modified of the method of Buege and Aust. After initiating of oxidation process with $CuSO_4$, the sample mixtures were incubated at 37°C for 5h in a water bath and the reaction terminated by adding EDTA (2 mM). MDA formation was measured in a spectrophotometer at 532 nm. The results were recorded as MDA equivalent content (nmol/mg LDL-protein) (21).

Determination of electrophoretic mobility of LDL

The electrophoretic mobility of n-LDL and Ox-LDL was detemined by agarose gel electrophoresis (1% agarose and 0.1 M barbital buffer) under the condition of 200 V, 80 mA in 0.05 M barbital buffer, then the gel was fixed in ethanol, acetic acid and water 6:1:3 for 15min, oven dried (80°C for 1h), and then stained with oil-red for 16h. After staining, the gel was washed by bleaching solution (ethanol and water 5:3), for until the background adjacent to protein or lipoprotein bands was cleared (22).

Statistical analysis

The data were presented as mean±SD of three experiments performed in duplicate. The variables used to describe the difference between the oxidation curves were lag time, conjugated dienes, MDA and REM. These parameters were obtained using the Mann-Whitney test (using SPSS 13.0 statistical software) for independent data and the differences were considered significant when $P<0.05$.

Results

The effects of vitamin E, vitamin C and dose-gradient concentration of coenzyme Q_{10} on the kinetics of $CuSO_4$-induced LDL oxidation are showed in Figure 1. It clearly shows that $CuSO_4$ dramatically increased oxidation of LDL. The formations of conjugated dienes were decreased by vitamin E, vitamin C and coenzyme Q_{10}. The levels of the conjugated dienes at 5h and lag time of all experiment groups are showed in Figures 2, 3. $CuSO_4$ increased the level of the conjugated dienes in LDL about three-fold and was significantly different from control LDL (414.7±12.5 nmol/mg LDL-protein vs. 153.4±5.941 nmol/mg LDL-protein, $P<0.05$). Vitamin E and coenzyme Q_{10} (300 nM) inhibited the final levels of conjugated dienes in LDL oxidation (325±25.34 and 315±26.65 nmol/mg LDL-protein,

$P<0.05$). Coenzyme Q_{10} showed a dose-dependent inhibition in increase of conjugated dienes at 5h. 20, 100 and 200 nM concentration of coenzyme Q_{10} and vitamin C decreased the level of the conjugated dienes at 5h but were not significantly different from $CuSO_4$-induced group. Vitamin E and coenzyme Q_{10} (300 nM) significantly increased lag time (142.4±8.9 and 160.5±10.2 min vs. 63.9±5.5 min, $P<0.001$ and $P<0.01$), prolongation of the lag time by coenzyme Q_{10} was dose-dependent. The lag time was significantly increased in the LDL separated from pre-incubated plasma with 100 nM, 200 nM and vitamin C. Continuous registration of absorbance of the LDL sample at 234 nm showed that coenzyme Q_{10} (300 nM) exerted the strongest effects, prolonging lag time to more than 2.5-fold of the $CuSO_4$-induced LDL oxidation.
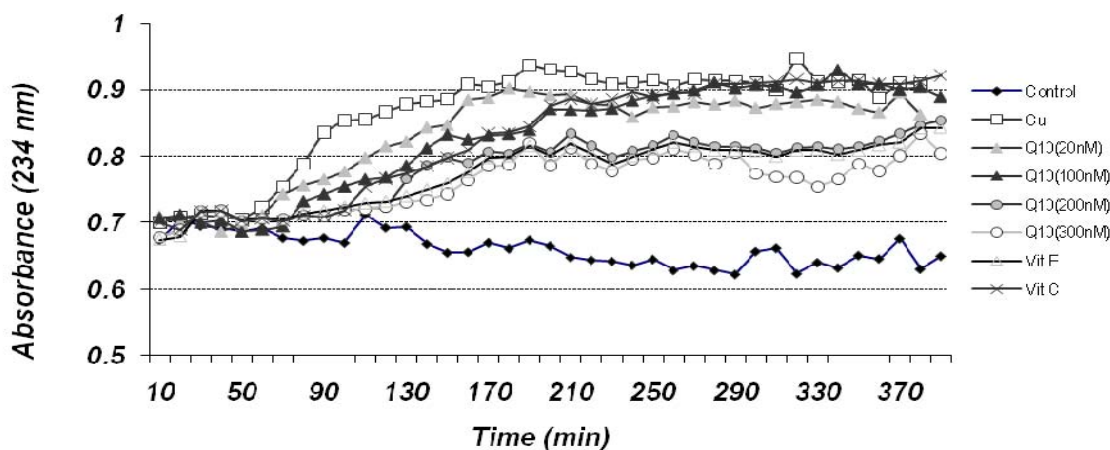

Figure 1. The effects of vitamin E, vitamin C and various concentration of coenzyme Q_{10} on the kinetics of $CuSO_4$-induced LDL oxidation in l0 mM PBS, pH 7.4 at 37°C for 5h. (Control) n-LDL; (Cu) Ox-LDL; (Q_{10} (20nM)) coenzyme Q_{10} (20 nM); (Q_{10} (100nM)) coenzyme Q_{10} (100 nM); (Q_{10} (200nM)) coenzyme Q_{10} (200 nM); (Q_{10} (300nM)) coenzyme Q_{10} (300 nM); (Vit E) vitamin E; (Vit C) vitamin C. Each point represents the means of five experiments.
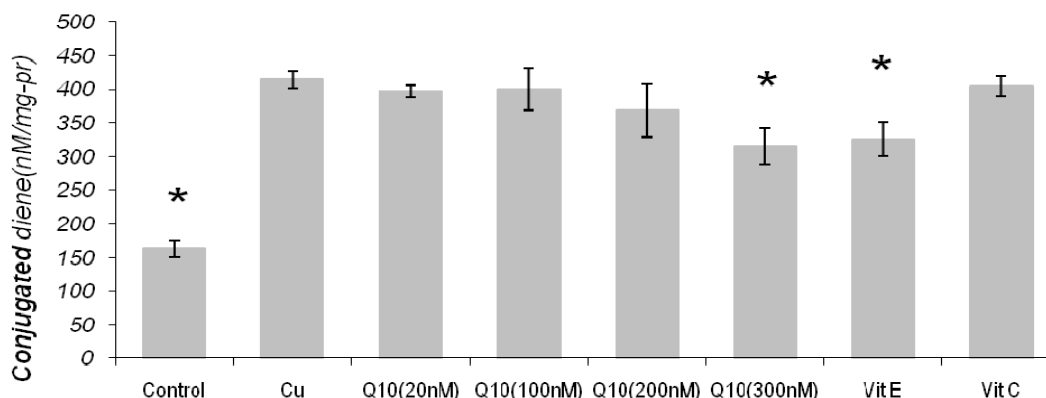

Figure 2. The effects of vitamin E, vitamin C and various concentration of coenzyme Q_{10} on the formation of conjugated dienes of $CuSO_4$-induced LDL oxidation. Abbreviations as in figure 1. Each Point represents the means of five experiments.
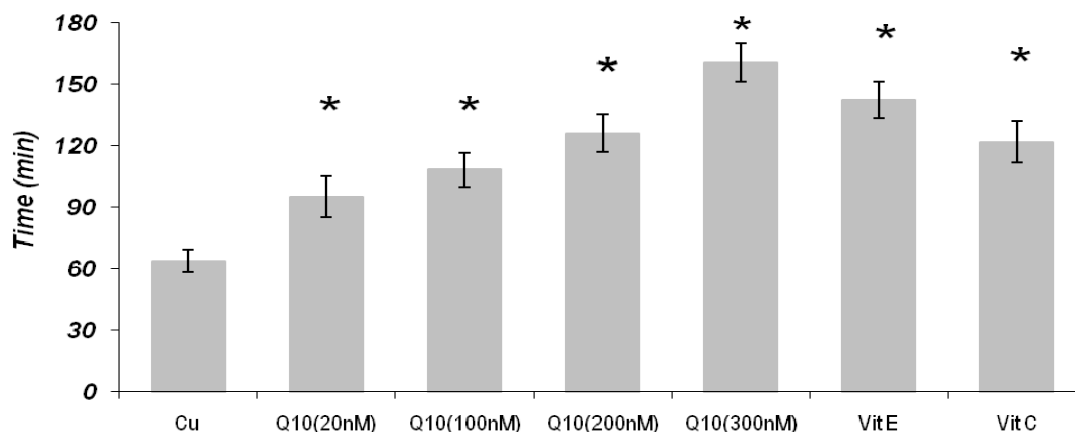
*=significant compared to ox-LDL

Figure 3. Antioxidant effects on lag time of CuSO$_4$-induced LDL oxidation with various concentration of coenzyme Q$_{10}$, vitamin E and vitamin C. Abbreviations as in figure 1 and figure 2.

Concomitantly, the addition of CuSO$_4$ in LDL determined and expressed by measurement of MDA equivalent content. The levels of the MDA at 5h of all experiment groups are showed in Figures 4. Addition of CuSO$_4$ in LDL increased MDA formation about ten-fold and this was significantly increased over control LDL. Vitamin E 100 M significantly inhibited MDA formation about 63.38%. Coenzyme Q$_{10}$ exhibited a dose-dependent inhibition of MDA formation. Coenzyme Q$_{10}$300 nM significantly inhibited MDA formation by about 63.52% respectively. 20, 100 and 200 nM coenzyme Q$_{10}$ and vitamin C decreased the MDA formation by about 36.21%, 38.1%, 42.24% and 55.1% respectively, but were not significantly different from CuSO$_4$-induced group. The MDA formation was significantly decreased in the LDL separated from pre-incubated plasma with coenzyme Q$_{10}$ (200 nM) and vitamin C.

Effect of coenzyme Q$_{10}$ on electrophoretic mobility of LDL: The effects of vitamin E, vitamin C and various concentration of coenzyme Q$_{10}$ on the electrophoretic mobility of CuSO$_4$-induced LDL oxidation are shown in Figures 5. After LDL oxidation by CuSO$_4$, the Ox-LDL moved faster than non-oxidized LDL on agarose gel electrophoresis. Vitamin E, vitamin C and coenzyme Q$_{10}$ (200 and 300 nM) significantly decreased the electrophoretic mobility of LDL oxidized by CuSO$_4$. 20 and 100 nM coenzyme Q$_{10}$ decreased the electrophoretic mobility of LDL, but was not significant.
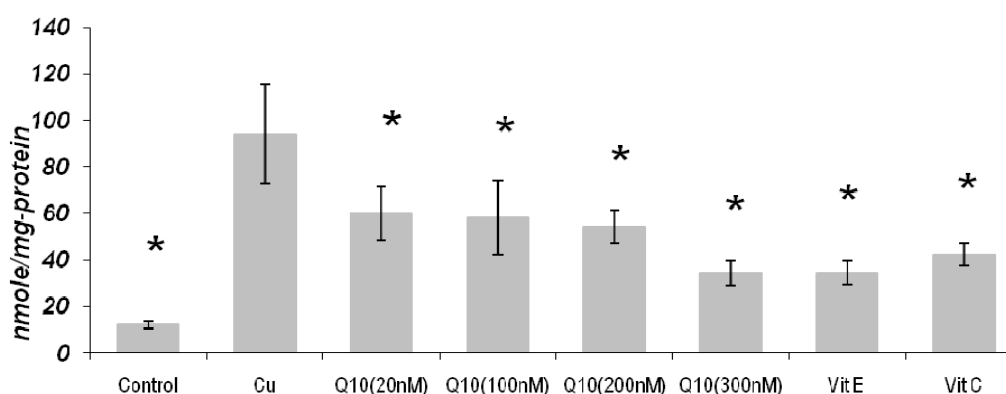

Figure 4. The effects of vitamin E, vitamin C and various concentration of coenzyme Q$_{10}$ on the formation of MDA of CuSO$_4$-induced LDL oxidation. Abbreviations as in figure 1 and figure 2.
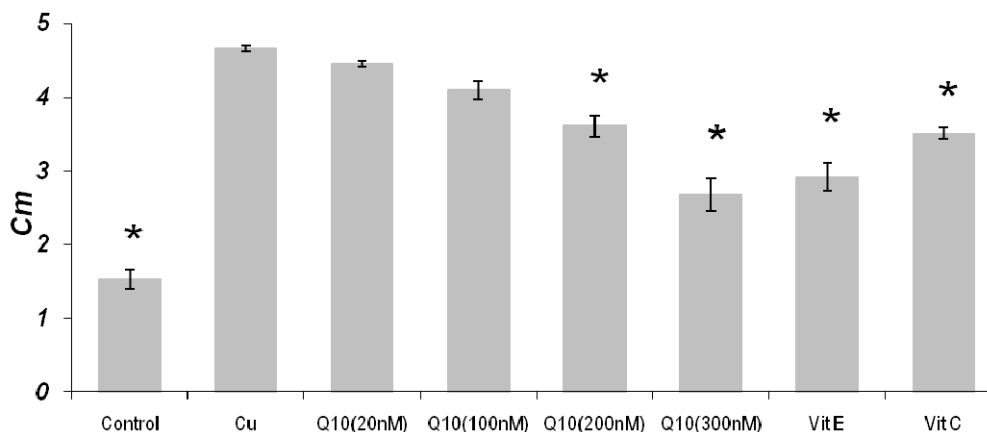
Figure 5. The effects of vitamin E, vitamin C and various concentration of coenzyme Q_{10} on rate the electrophoretic mobility of CuSO₄-induced LDL oxidation. Abbreviations as in figure 1 and figure 2. Each Point represents the means of five experiments.

Discussion

Coenzyme Q_{10}, a lipid soluble benzoquinone, has excellent antioxidant and membrane stabilizing properties in the cardiac tissue (10,13). Coenzyme Q_{10} has beneficial effects in the following areas: 1. Acts as a novel antioxidant. 2. Enhances stamina, endurance and energy levels. 3 Helps to reduce body weight. 4. Normalizes blood pressure. 5. Attenuates immune function. 6. Protects against cardiovascular dysfunction. 7. Increases effectiveness of various chemotherapeutic agents and anti-malarial drugs" (6,16,23).

The oxidative modification of LDL (ox-LDL) is the major factor that stimulates the development of atherosclerosis (3). The oxidative modification of LDL induced by copper ion is related to free radical reaction. LDL oxidation may require the generation of free radicals such as superoxide anion and hydroxyl radicals by the Fenton reaction. After the oxidation by copper ion, polyunsaturated fatty acid of LDL was oxidized, this resulted in an elevation of lipid peroxides and depletion of vitamin E and others antioxidants in ox-LDL (1-3).

The results clearly showed that various concentration of coenzyme Q_{10} have a dose-dependent antioxidant activity by inhibiting the formation of conjugated dienes and MDA, increase lag time and retard electrophoretic mobity of LDL *in vitro*. The results showed that coenzyme Q_{10} especially at 200 and 300 nM concentration similar to vitamin E as a positive control is a potent antioxidant and protect LDL in plasma against oxidation.

In this study, the effect of vitamin C on LDL oxidation showed less advantage than vitamin E and coenzyme Q_{10}, because vitamin C is a water soluble vitamin and the most of vitamin C is separated in the steps of LDL isolation, especially in dialysis. It has been showed in many cases that the protective effect of Coenzyme Q_{10} is associated with its antioxidant properties (24,25). Administration of coenzyme Q_{10} in type I diabetic rats decreased lipid peroxidation and increased superoxide dismutase, catalase, and glutathione levels in rat heart and protected it against oxidative damage (26). The studies showed that coenzyme Q_{10} acts as an oxygen radical scavenger whenever oxygen free radicals are generated, decreases lipid peroxidation in paint industry workers. Also another researcher Baghchi (1997) suggested possible explanations the mechanisms of coenzyme Q_{10} antioxidant: 1. It may act independently as chain-breaking antioxidants, providing hydrogen atoms to reduce peroxyl and/or alkoxyl radicals. 2. A redox interaction may exist between coenzyme Q_{10} and another lipid soluble antioxidant such as vitamin E, in its one-electron oxidized form, vitamin E phenoxyl radical" (27-30). In conclusion, the results of the present study clearly showed that coenzyme Q_{10} is found to posses a good antioxidant activity and various concentrations of coenzyme Q_{10} have a dose-dependent antioxidant activity against LDL oxidation by inhibiting the formation of conjugated dynes and MDA, increasing lag time, and decreasing REM. In conclusion, coenzyme Q_{10} is a potent antioxidant and may be a good alternative to reduce the risk of atherosclerosis and coronary heart disease and other free radical associated health problems.

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