

Note

Coenzyme Q10 Decreases TNF- α and IL-2 Secretion by Human Peripheral Blood Mononuclear Cells

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Summary The beneficial effect of coenzyme Q10 (CoQ10) on human health occurs through various mechanisms including the possibility of immunomodulation. Therefore, the purpose of study was to examine the in vitro effect of CoQ10 on cytokine production and superoxide anion generation by human peripheral blood mononuclear cells (PBMC). 2×10^6 /mL PBMC obtained from 19 volunteers were incubated for 24 h without or with 0.6, 1.25, 2.5 and 5.0 μM of CoQ10. The production of the following cytokines were examined: IL-1 β , IL-1ra, IL-6, IL-10, IL-2 and IFN γ . Superoxide anion production was examined by incubation of 4×10^6 /mL cells with CoQ10 and 2×10^{-3} mM phorbol myristate acetate (PMA) for 60 min. The production of the proinflammatory cytokines IL-1 β , IL-6 and IFN γ and that of the anti-inflammatory cytokines IL-1ra and IL-10 by PBMC was not affected by CoQ10, whereas TNF α secretion was significantly decreased when the cells were incubated with 0.6 and 1.25 μM of CoQ10. On the other hand, increasing doses of CoQ10 caused mild, but statistically significant inhibition of IL-2 secretion. The generation of superoxide anion by PBMC did not differ significantly between cells incubated with or without CoQ10 at concentrations between 0.3 and 5.0 μM . The results suggest that CoQ10 exerts a certain effect on cytokine production by PBMC related to its capacity to modulate human immune function.

Key Words coenzyme Q10, cytokines, peripheral blood mononuclear cells, superoxide anion

Coenzyme Q10 (CoQ10) is an essential component in the respiratory chain at the mitochondrial level, and therefore plays an important role in human life. There is a considerable amount of data suggesting that CoQ10 exerts a beneficial effect on a broad spectrum of pathological conditions, such as preventing atherosclerosis by attenuation of LDL oxidation and endothelial lesions (1, 2), improving the functional status and quality of life in patients with end-stage heart failure (3), and even enhancing sperm motility with a consequent effect on male infertility (4). In addition, evidence indicates that dietary supplementation of CoQ10 along with other antioxidants exerts an anti-aging effect in both humans (5) and mice (6, 7). It is notable that CoQ10 has been shown to possess a certain anti-cancer effect (8). Premkumar et al. (9) have reported a decrease in both carcinoembryonic and carbohydrate-CA-15 antigens in breast cancer patients treated with tamoxifen supplemented with a combination of CoQ10, riboflavin and niacin. It has been proposed that the ben-

eficial role of CoQ10 is related both to its antioxidant activity and its ability to modulate the function of the immune system by acting at the mitochondrial level (10). Based on this concept and following observations that the level of CoQ10 has been found to be decreased in pathological conditions accompanied by intensive generation of free radicals, researchers advise in these cases therapy supplementation with CoQ10 (11–13). A few models, such as phagocytic rate, circulating antibodies, and viral and parasitic infections have been applied to establish the relationship between CoQ10 and the immune system. Regarding the close relationship between cytokine production and proper function of the immune system, we hypothesized that CoQ10 may modulate cytokine production by human peripheral blood mononuclear cells (PBMC). However, a comprehensive review of the literature (PubMed) failed to elicit reports on the effect of CoQ10 on cytokine production by PBMC. In addition, there is uncertainty in the literature as to the antioxidant role of CoQ10. The present work was conducted to examine cytokine production and superoxide anion generation by PBMC following incubation with CoQ10.

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Table 1. Effect of CoQ10 on the production of pro-inflammatory cytokines by human PBMC.

CoQ10	IL-1 β (ng/mL)	<i>p</i> value	IL-6 (ng/mL)	<i>p</i> value	TNF- α (pg/mL)	<i>p</i> value	IFN γ (ng/mL)	<i>p</i> value
0	4.47 \pm 0.59		109 \pm 11		222 \pm 48		27.7 \pm 1.8	
0.6 μ M	4.33 \pm 0.53	NS	118 \pm 12	NS	185 \pm 41*	0.0042	27.0 \pm 2.1	NS
1.25 μ M	4.21 \pm 0.61	NS	120 \pm 12	NS	182 \pm 41*	0.0097	27.2 \pm 1.8	NS
2.5 μ M	4.75 \pm 0.58	NS	121 \pm 12	NS	228 \pm 55	NS	26.8 \pm 2.0	NS
5.0 μ M	4.40 \pm 0.59	NS	117 \pm 12	NS	206 \pm 47	NS	26.3 \pm 2.1	NS

PBMC were incubated without (control) and with various concentrations of CoQ10 as detailed in "Materials and Methods." The results are expressed as the mean \pm SE ($n=19$) for each experimental point. The paired *t*-test has been applied for statistical evaluation. $p<0.05$ was considered as statistically significant. NS, not significant.

Materials and Methods

Subjects. Nineteen healthy adult volunteers were enrolled in the study after its approval by the Hospital Human Studies Committee, and informed consent was obtained from these participants.

CoQ10 preparation. A stock solution of 1 mM of CoQ10 (Sigma, Israel) was prepared in absolute ethanol and stored at -20°C . Further dilutions of CoQ10 were made in RPMI-1640 medium (Biological Industries, Beit Haemek, Israel) containing 1% penicillin, streptomycin and nystatin, and supplemented with 10% fetal calf serum (designated as complete medium, CM). Control cultures were incubated either with complete medium or with ethanol at a final concentration of 0.5%, corresponding to that with the drug added.

Cell preparation and culture conditions. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by Histopaque-1077 (Sigma) gradient centrifugation. The cells were washed twice in phosphate buffered saline (PBS) and suspended in CM.

Effect of CoQ10 on cytokine production. To investigate the effect of CoQ10 on cytokine production, 2×10^6 PBMC suspended in 1 mL CM were incubated with or without CoQ10 at the following concentrations: 0.6, 1.25, 2.5 and 5.0 μ M. For TNF- α , IL-1 β , IL-1ra, IL-6 and IL-10 assessment, the cultures were incubated for 24 h with 10 ng/mL lipopolysaccharide (LPS, *Escherichia coli*, Sigma). For IL-2 and IFN γ testing cultures were incubated for 48 h with 1 μ g/mL phorbol myristate acetate (PMA, Sigma) and ionomycin (Sigma) 0.5 μ g/mL. At the end of the incubation period the culture media were collected, cells were removed by centrifugation at 1,500 rpm for 10 min and supernatants were collected and kept at -70°C until assayed.

Cytokine content in the supernatants. The concentration of cytokines in the supernatants was tested using ELISA kits (Biosource International, Camarillo, CA, USA) specific for human cytokines as detailed in the guideline provided by the manufacturer. The detection levels of these kits were: 15 pg/mL for IL-6, and 30 pg/mL for TNF- α , IL-1 β , IL-1ra, IL-2, IL-10 and IFN γ .

Superoxide anion generation. Superoxide anion generation by PBMC was detected by the method of Johnston et al. (14). Briefly, 4×10^6 cells suspended in 1 mL of PBS supplemented with 1 gr% glucose, 0.5 mM

CaCl $_2$ and 80 nM cytochrome C (from horse heart, Sigma) were incubated at 37°C for 60 min in the absence or presence of various concentrations of CoQ10 prepared as described above and without or with 2×10^{-3} mM PMA. At the end of the incubation period, the cells were sedimented by centrifugation at 1,500 $\times g$, the supernatants were collected and the absorbance was determined at 550 nm. The amount of superoxide anion produced was directly proportional to the amount of reduced cytochrome C calculated by subtracting the OD values obtained with PMA from those without PMA. The specificity of the reaction was confirmed by an almost complete inhibition of cytochrome C reduction following addition of superoxide dismutase to the incubation mixture.

Statistics. Data was analyzed using analysis of variance (ANOVA) with repeated measures for each cytokine, and paired *t*-tests to compare the difference between the various concentrations of CoQ10 and cells incubated without the drug. Probability values of $p<0.05$ were considered as significant. The results are expressed as mean \pm SE of at least 15 individuals.

Results

Notably, 0.5% of ethanol added to the cultures did not exert any effect on the production of any of the cytokines tested.

Effect of CoQ10 on pro- and anti-inflammatory cytokine generation

The production of the proinflammatory cytokines IL-1 β , IL-6 and IFN γ by PBMC was not affected following incubation with the four doses of CoQ10 tested ($F_{4;76}=1.53$; $p=0.2$, $F_{4;72}=0.9$; $p=0.468$ and $F_{4;64}=0.95$; $p=0.44$, respectively, Table 1). The secretion of TNF- α was significantly decreased when PBMC were incubated with CoQ10 ($F_{4;76}=3.87$; $p=0.0065$). Incubation of cells with 0.6 and 1.25 μ M of CoQ10 lowered the release of TNF- α by 16.7 and 17.75%, respectively ($p=0.0042$ and $p=0.0097$, respectively). However, the production of this cytokine was not affected when higher concentrations of the drug were added.

The production of the anti-inflammatory cytokines IL-1ra and IL-10 by human PBMC was not affected by the four doses of CoQ10 tested ($F_{4;72}=2.6$; $p=0.59$ and $F_{4;72}=1.15$; $p=0.34$, respectively, Table 2).

Table 2. Effect of CoQ10 on the production of anti-inflammatory cytokines and superoxide anion by human PBMC.

CoQ10	IL-1ra (ng/mL)	<i>p</i> value	IL-10 (ng/mL)	<i>p</i> value	$^{\cdot-}\text{O}_2$ (nmol/ 10^6 cells)	<i>p</i> value
0	7.81±0.73		3.86±0.33		7.2±0.6	
0.6 μM	7.16±0.90	NS	3.61±0.26	0.218	7.2±0.9	0.42
1.25 μM	6.92±0.92	NS	3.40±0.24	0.065	6.5±1.0	0.49
2.5 μM	7.10±1.03	NS	3.66±0.41	0.288	7.0±0.5	0.56
5.0 μM	6.9±0.97	NS	3.34±0.35	0.078	6.0±0.7	0.076

PBMC were incubated without (control) and with various concentrations of CoQ10 as detailed in "Materials and Methods." The results are expressed as the mean±SE for each experimental point ($n=19$ for cytokines and $n=10$ for superoxide anion production). The paired *t*-test has been applied for statistical evaluation. $p<0.05$ was considered as statistically significant. NS, not significant.

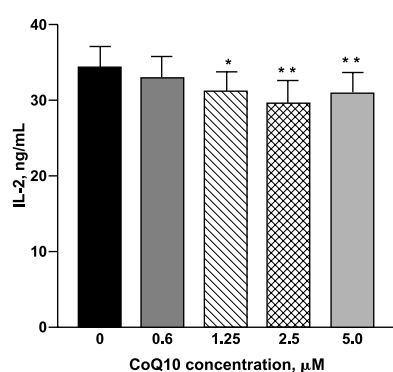


Fig. 1. Effect of CoQ10 on IL-2 production by peripheral blood mononuclear cells of healthy subjects. The results are expressed as the mean±SE. The paired *t*-test has been applied for statistical evaluation. $p<0.05$ was considered as statistically significant. Asterisks represent statistically significant differences from cells incubated without the substance. $p<0.01$; $p<0.001$.

Increased doses of CoQ10 caused mild, but statistically significant, inhibition of IL-2 secretion ($F_{4;60}=6.08$; $p=0.00035$, Fig. 1). At concentrations of 1.25, 2.5, and 5.0 μM of CoQ10, the production of IL-2 was reduced by 9.1% ($p=0.003$), 13.4% ($p=0.0003$), and 9.4% ($p=0.001$), respectively. A lower concentration of CoQ10 (0.6 μM) did not exert any significant effect on IL-2 secretion.

Superoxide anion generation

The generation of superoxide anion by PBMC did not differ significantly between cells incubated for 1 h without or with CoQ10 at concentrations between 0.6 and 5.0 μM (Table 2).

Discussion

The results of this study show that the production of the proinflammatory cytokines IL-1 β , IL-6 and IFN γ by PBMC was not affected following incubation with the four doses of CoQ10 used. The secretion TNF- α was significantly decreased when PBMC were incubated with 0.6 and 1.25 μM of CoQ10. However, the production of this cytokine was not affected when higher concentrations of CoQ10 were added. Our findings differ from those reported by Fuller et al. (15), who found that

CoQ10 suppressed the increased production of inflammatory mediators including IL-6 by human dermal fibroblasts, an effect enhanced by the addition of carotenoids. On the other hand, our results are in agreement with those reported by Schmeizer et al. (16) who have demonstrated a significant decrease of the proinflammatory cytokine response of LPS induced cells from a human monocytic line, following pre-incubation with the reduced form of CoQ10, ubiquinol-10. It is notable that other lipid-soluble vitamins and compounds such as vit. E and lycopene exert immunomodulatory effects as we have shown previously (17, 18). Studies have shown that CoQ10 exerts a synergistic effect when added to anti-cancer drugs. Thus, in untreated patients with breast cancer in whom IL-1 β , IL-6, TNF- α and endothelial growth factor were elevated, tamoxifen, administered for 1 y, caused a significant reduction in these cytokines. However, when administration of tamoxifen to one group of patients was combined with riboflavin and niacin, and in another group with CoQ10, this effect was achieved after 45 and 90 d, respectively (19). The decrease in TNF- α production by PBMC following incubation with CoQ10 observed in the present work is in accordance with the findings reported by Cammer (20), who has achieved a restoration of the number of cultured oligodendrocytes damaged by treatment with TNF- α by addition of CoQ10.

It is a general belief that CoQ10 plays a key role in the production of sub-cellular energy and since it acts as an antioxidant, it prevents lipid peroxidation and scavenges superoxide anions (21). Kon et al. (22) have reported that CoQ10 administered to 18 male athletes reduced exercise-induced muscular injury caused by oxidative stress. Similar results have been reported in rats with perfusion injuries (23). It has been suggested that the capacity of CoQ10 to dismutate the free radical superoxide anions can play a role in the anti-aging process (24). It is notable that in our work the secretion of IL-2 was decreased following incubation of PBMC with 1.25–5.0 μM of CoQ10. However, a higher dose (10 μM) of CoQ10 did not affect the secretion of either TNF- α or IL-2. It is possible that this phenomenon may be explained by the anti-oxidant effect of CoQ10, as has been reported by Daghini et al. (25), who have shown

that antioxidant vitamins express a biphasic effect in vitro on oxidative stress and angiogenesis in human umbilical vascular endothelial cells. On the other hand, there are reports showing that the activity of CoQ10 as an antioxidant remains controversial, at least under physiological conditions (26, 27). Thus, Sohal et al. (28) have shown that, although supplementation of CoQ10 to mice increased its endogenous content, the rate of superoxide anion generation determined in various tissues did not differ significantly from that in controls. Researchers from the same group provided evidence that augmentation of submitochondrial particles derived from skeletal muscle, liver and kidney of mice kept on a diet containing 123 mg/kg of CoQ10 did not affect the rate of superoxide anion generation (29). Although these reports are in accordance with the lack of any antioxidant effect of CoQ10 observed in the present work, we wish to emphasize that our experiments have been performed in vitro, a fact that may be a limitation.

It is conceivable that the beneficial effect of CoQ10 in humans is mediated at least in part through inhibition of TNF- α production, as well as by other activities such as increasing the capability of phagocytic cells to engulf targets, elevation of circulating antibody levels, prevention of apoptosis and even its possible anti-cancer effect (8, 10, 30), mechanisms that may explain the fundamental role of CoQ10 in the function of the immune system.

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