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The effects of coenzyme Q_{10} treatment on maternally inherited diabetes mellitus and deafness, and mitochondrial DNA 3243 (A to G) mutation

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Summary The characteristic clinical features of diabetes mellitus with mitochondrial DNA (mtDNA) 3243(A-G) mutation are progressive insulin secretory defect, neurosensory deafness and maternal inheritance, referred to as maternally inherited diabetes mellitus and deafness (MIDD). A treatment for MIDD to improve insulin secretory defects and reduce deafness has not been established. The effects of coenzyme Q_{10} (CoQ_{10}) treatment on insulin secretory response, hearing capacity and clinical symptoms of MIDD were investigated. 28 MIDD patients (CoQ_{10}-DM), 7 mutant subjects with impaired glucose tolerance (IGT), and 15 mutant subjects with normal glucose tolerance (NGT) were treated daily with oral administration of 150 mg of CoQ_{10} for 3 years. Insulin secretory response, blood lactate after exercise, hearing capacity and other laboratory examinations were investigated every year. In the same way we evaluated 16 MIDD patients (control-DM), 5 mutant IGT and 5 mutant NGT subjects in yearly examinations. The insulin secretory response assessed by glucagon-induced C-peptide secretion and 24 h urinary C-peptide excretion after 3 years in the CoQ_{10}-DM group was significantly higher than that in the control-DM group. CoQ_{10} therapy prevented progressive hearing loss and improved blood lactate after exercise in the MIDD patients. CoQ_{10} treatment did not affect the diabetic complications or other clinical symptoms of MIDD patients. CoQ_{10} treatment did not affect the insulin secretory capacity of the mutant IGT and NGT subjects. There were no side effects during therapy. This is the first report demonstrating the therapeutic usefulness of CoQ_{10} on MIDD. [Diabetologia (1998) 41: 584–588]

Keywords Deafness, mitochondrial DNA mutation, insulin, C-peptide, lactate.

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Abbreviations: CoQ_{10}, Coenzyme Q_{10}; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OHA, oral hypoglycaemic agents; NDR, without diabetic retinopathy; SDR, diabetic simple retinopathy; PPDR, diabetic preproliferative retinopathy; PDR, diabetic proliferative retinopathy; normo, normoalbuminuria; micro, microalbuminuria; macro, macroalbuminuria; mt DN, mitochondrial DNA; MIDD, maternally inherited diabetes and deafness; ANOVA, analysis of variance; CPR, C-peptide immunoreactivity.

In recent years, mitochondrial DNA mutations have been identified as one cause of diabetes mellitus[1–4]. The most commonly identified mtDNA mutation linked to diabetes is the single base mutation (A-G) at position 3243 within the $tRNA^{LEU(UUR)}$ gene [3, 4]. The clinical features of diabetes mellitus with the 3243 bp mutation are maternal inheritance, progressive insulin secretory defect and a high association with neurosensory deafness. The 3243 bp mutation was identified in about 1 % of Japanese NIDDM patients [3]. Therapeutic trials with CoQ_{10} have been reported to provide clinical benefits in patients with mitochondrial encephalomyopathy and mitochondrial gene mutations[5–14]. Recent reports have also demonstrated clinical benefits on some neurological symptoms of MIDD patients[15, 16]. However, no

Table 1. Clinical characteristics of the CoQ_{10}-treated or untreated diabetic patients, IGT and NGT subjects with mitochondrial mutation of $tRNA^{LEU(UUR)}$ (3243)

Glucose tolerance	DM	IGT	NGT	DM	IGT	NGT
CoQ_{10} treatment	+	+	+	–	–	–
Numbers of subjects	28	7	15	16	5	5
Gender (M/F)	13/15	3/4	7/8	8/8	2/3	2/3
Mutant mtDNA (%)	10.7 ± 13.0	8.7 ± 8.8	9.2 ± 9.2	11.4 ± 12.5	10.2 ± 8.7	8.1 ± 7.2
Age at trial entry	43.5 ± 12.3	38.5 ± 12.2	35.4 ± 9.8	44.4 ± 9.8	36.1 ± 8.5	35.6 ± 7.7
Age at onset of hearing loss	36.1 ± 9.4	35.4 ± 7.3	32.3 ± 5.8	37.4 ± 7.8	33.2 ± 4.4	32.5 ± 3.9
Age at onset of diabetes	38.7 ± 10.2	–	–	39.6 ± 8.7	–	–
Treatment (Diet/OHA/Insulin)	6/8/14	–	–	4/4/8	–	–
Neurosensory deafness (%)	89	43	20	81	40	20
Retinopathy (NDR/SDR/PPDR/PDR)	10/11/2/5	–	–	5/7/1/3	–	–
Nephropathy (Norm/Micro/Macro)	7/11/10	–	–	5/6/5	–	–

Abbreviations used are IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OHA, oral hypoglycemic agents; NDR, without diabetic retinopathy; SDR, diabetic simple retinopathy; PPDR, diabetic preproliferative retinopathy; PDR, diabetic proliferative retinopathy; normo, normoalbuminuria; micro, microalbuminuria; macro, macroalbuminuria

treatment for MIDD to prevent insulin secretory defects and hearing loss has been established.

In this paper, we report the results of a long-term open trial of CoQ_{10} in MIDD patients, mutant IGT and NGT subjects. We found CoQ_{10} treatment prevented progressive insulin secretory defects, exercise intolerance and hearing loss in the MIDD patients. The molecular mechanism by which CoQ_{10} prevents the progression of insulin secretory defects and hearing impairment is discussed.

Patients and methods

Protocol. The study protocol was approved by the Tohoku University Institutional Review Board. Informed consent was obtained from each subject. We divided 44 MIDD patients, 12 IGT subjects and 20 NGT subjects with the 3243 bp mutation into two groups; 28 MIDD patients, 7 IGT subjects (CoQ_{10}-IGT) and 15 NGT subjects (CoQ_{10}-NGT) were treated daily with oral administration of 150 mg of CoQ_{10} (Neuquinon, Tanabe Pharmaceuticals, Osaka Japan) for 3 years. Insulin secretory response, blood lactate after exercise, hearing capacity and other laboratory examinations were investigated every year. The remaining 16 MIDD patients, 5 IGT (control-IGT) and 5 NGT subjects (control-NGT) matched for age and sex, were not treated but were evaluated in the same way in yearly examinations. The clinical characteristics of the two groups are shown in Table 1.

DNA study. Total DNA was isolated from blood and skeletal muscle. A 427 bp fragment encompassing the $tRNA^{LEU(UUR)}$ mutation site located at nucleotide 3,243 was amplified by modified PCR using [α-^{32}P]-dATP (1.7 $Ci \cdot mmol^{-1}$) with forward primer 5′-AAGGTTCGTTTGTTCAACGA (3,029–3,048) and reverse primer 5′AGCGAAGGGTTGTAGTAGCC (3,437–3,456) [17]. The radioactive fragment was digested with Apa1 for 1 h at 37 °C and then followed by electrophoresis on a 5% non-denaturing polyacrylamide gel. Bands were visualized by autoradiography. The percentage of cleaved fragments was determined by densitometric analysis.

Clinical study. The insulin secretory capacity of pancreatic beta cells was evaluated with plasma C-peptide immunoreactivity (CPR) 6 min after 1 mg of glucagon administration and urinary CPR excretion for 24 h. At diagnosis, all patients fulfilled the WHO criteria for diabetes mellitus [18]. Plasma glucose was assayed using the glucose oxidase method. Plasma insulin and CPR were assayed using a radioimmunoassay. Exercise tolerance was assessed by the blood lactate concentration after 5 W ergometer exercise for 15 min. Neurosensory hearing loss was diagnosed by expert otopharyngists. Hearing loss was assessed by pure-tone audiometry. The pure tone average was calculated as the average of 0.5, 1, 2 and 4 kHz as described by Yamasoba [19]. The serum levels of CoQ_{10} were determined by high-performance liquid chromatography [20].

Statistical analysis. All measurements are presented as mean ± SD. Statistical difference was assessed by a repeated measure analysis of variance (ANOVA). Wilcoxon's test was used for paired comparison, and the Mann-Whitney U-test for unpaired comparison. Statistical significance was accepted at the 95% confidence level ($p < 0.05$).

Results

The mean serum CoQ_{10} levels of the MIDD patients before treatment were not significantly reduced as compared with the control subjects (0.84 ± 0.25 μg/ml, vs 1.17 ± 0.29). The levels increased significantly after 3 months of therapy, but did not show any further increase at 1, 2 and 3 years (2 months of therapy, 2.76 ± 1.44, $p > 001$; 1 year, 3.17 ± 2.09, $p < 0.005$; 2 year, 3.05 ± 2.48, $p < 0.005$; 3 year, 3.16 ± 1.99, $p < 0.005$).

Plasma CPR was progressively reduced following glucagon in the control-DM group (before, 2.04 ± 0.72 ng/ml; after 3 years, 1.48 ± 0.48 ng/ml, $p < 0.02$), as shown in Figure 1. Plasma CPR was significantly higher following glucagon in the CoQ_{10}-DM group than that in the control-DM group after 2 years (2.53 ± 0.24 vs 1.78 ± 0.73, $p < 0.001$) and 3 years (1.48 ± 0.48 vs 2.70 ± 0.38, $p < 0.0001$]. CoQ_{10} treatment significantly improved the plasma CPR following glucagon in the MIDD patients (before, 2.53 ± 0.24; after 3 years, 2.70 ± 0.38, $p < 0.02$).

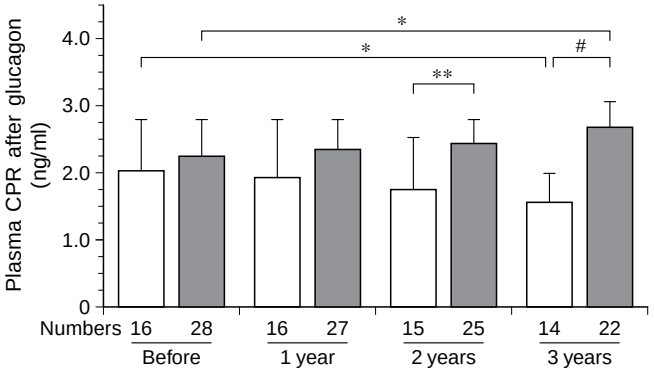

Fig. 1. The effects of long-term CoQ_{10} treatment on plasma C-peptide concentration 6 min after glucagon injection in MIDD patients. Open bars are shown as the control-DM group, closed bars are the CoQ_{10}-DM group. All measurements are presented as mean ± SD. $^{*}p < 0.02$, $^{**}p < 0.001$, $^{\#}p < 0.0001$

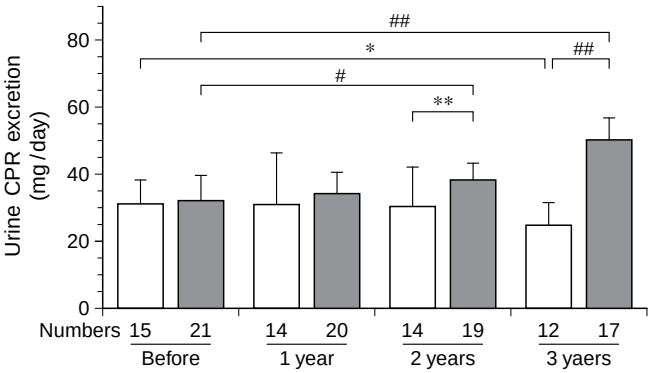

Fig. 2. The effects of long-term CoQ10 treatment on 24 h urinary C-peptide excretion in MIDD patients. Open bars are shown as the control-DM group, closed bars are the CoQ_{10}-DM group. Results are mean ± S.D. $^{*}p < 0.02$, $^{**}p < 0.01$, $\#p < 0.002$, $\#\#p < 0.001$

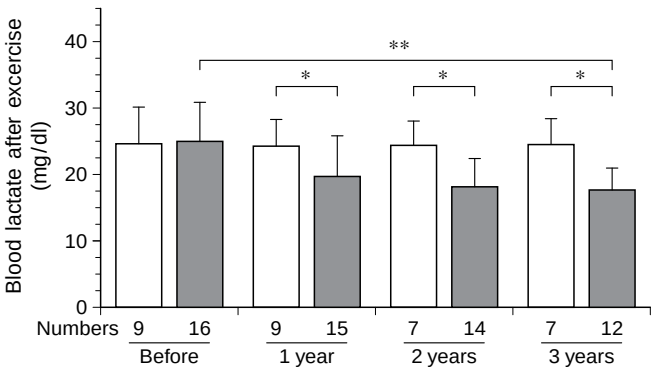

Fig. 3. The effects of long-term CoQ_{10} treatment on plasma lactate after exercise in the MIDD patients. Open bars are shown as the control-DM group, closed bars are the CoQ_{10}-DM group. Results are mean ± S.D. $^{*}p < 0.05$, $^{**}p < 0.01$

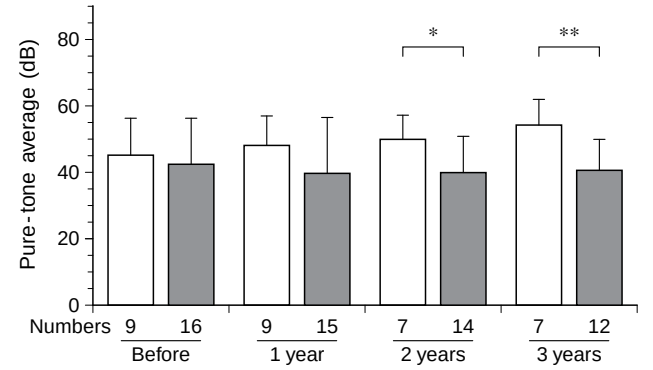

Fig. 4. The effects of long-term CoQ_{10} treatment on pure-tone average in MIDD patients. Open bars are shown as the control-DM group, closed bars are the CoQ_{10}-DM group. Results are mean ± S.D. $^{*}p < 0.05$, $^{**}p < 0.02$

The 24-h urinary excretion of CPR gradually deteriorated in the control-DM group (before, 31.7 ± 7.0 mg/day; after 3 years, 25.2 ± 6.1, $p < 0.02$), as shown in Figure 2. The urinary CPR excretion for 24 h in the CoQ_{10}-DM group was significantly higher than that in the control-DM group after 2 years (38.6 ± 3.4 vs 30.3 ± 11.9, $p < 0.01$) and 3 years (49.3 ± 7.4 vs 25.2 ± 6.1, $p < 0.0001$). CoQ_{10} treatment significantly improved the urinary CPR excretion in the MIDD patients (before, 31.1 ± 9.3; after 2 years, 36.6 ± 3.4, $p < 0.002$; after 3 years, 49.3 ± 7.4, $p < 0.001$).

The blood lactate concentration after exercise gradually decreased in the CoQ_{10}-DM group (before, 24.2 ± 7.12 mg/dl; after 3 years, 16.5 ± 3.61, $p < 0.01$), but was not changed in the control-DM group, as shown in Figure 3. The blood lactate concentration after exercise in the CoQ_{10}-DM group was significantly lower than that in the control-DM group after 1 year (19.0 ± 4.82 vs 23.6 ± 3.76, $p < 0.05$), 2 years (18.7 ± 5.0 vs 24.5 ± 3.27, $p < 0.05$) and 3 years (16.5 ± 3.61 vs 24.4 ± 3.63, $p < 0.001$).

Bilateral neurosensory deafness was demonstrated in 38 (86%) of 44 mutant diabetic patients. Hearing capacity gradually deteriorated in the control-DM group (Fig. 4). The progression rate of hearing loss ranged from 2.2 to 3.1 dB/year in the control-DM group. CoQ_{10} treatment significantly prevented the hearing loss in the MIDD patients after 2 years treatment (40.1 ± 10.2 dB vs 50.2 ± 6.9, $p < 0.05$) and after 3 years treatment (40.8 ± 9.7 vs 53.3 ± 7.9, $p < 0.02$).

After 3 years, one of 5 untreated IGT subjects but none of 7 CoQ_{10}-treated IGT subjects were diagnosed as having diabetes by oral glucose tolerance test. In contrast, one of 5 untreated NGT subjects and one of 15 treated NGT subjects were also diagnosed as IGT. The insulin secretory capacity assessed by the plasma CPR after glucagon and urinary CPR excretion, blood lactate after exercise and hearing capacity were not significantly reduced in the control-IGT group and the control-NGT group after 3 years.

CoQ_{10} treatment for 3 years did not affect the plasma CPR after glucagon, urinary CPR excretion, blood lactate after exercise and hearing capacity in the mutant IGT and NGT subjects (data not shown).

The examined 8 CoQ_{10}-MIDD, 4 control-MIDD, 4 CoQ_{10}-IGT, 3 control-IGT, 5 CoQ_{10}-NGT and 2 control-NGT had normal insulin sensitivity assessed by minimal model analysis. After 3 year trials, the CoQ_{10} treatment did not affect insulin sensitivity in either CoQ_{10}- or control-groups (data not shown). CoQ_{10} treatment did not affect the states of diabetic retinopathy, nephropathy, neuropathy or other diabetic chronic complications. Apart from the improvement of insulin secretory defects, there were no other significant differences in insulin requirement, metabolic control or other clinical parameters between the CoQ_{10}-DM group and the control-DM group. In addition, there were no detectable side effects from CoQ_{10}.

Discussion

In our open trial study, we confirmed that long-term CoQ_{10} treatment prevents progressive insulin secretory defect, exercise intolerance and hearing loss in MIDD patients. However, short-term CoQ_{10} treatment for 1–3 months did not affect the insulin secretory capacity or clinical symptoms in MIDD patients (data not shown). We also investigated the effects of long-term CoQ_{10} treatment on the insulin secretory capacity and hearing capacity in the 12 mutant IGT and 20 mutant NGT subjects. There was only a tendency for the treatment to improve the insulin secretory capacity in the mutant IGT subjects, and we failed to demonstrate an improvement in the glucose tolerance or hearing capacity.

CoQ_{10} is well known to act as an electron carrier of the respiratory chain in mitochondria and has been shown to improve the mutation-associated dysfunction of the respiratory chain in mitochondria. CoQ_{10} has been tested with success in some patients with mitochondrial encephalopathy due to mitochondrial gene mutations [5–14]. A multi-centre open trial of CoQ_{10} treatment showed that sixteen of forty-four patients with mitochondrial myopathies showed at least a 25% decrease in post-exercise lactate levels [12]. However, in the following double-blind trial of three months, the effectiveness of CoQ_{10} treatment for mitochondrial myopathies could not be demonstrated. Thus, the effectiveness of CoQ10 therapy on mitochondrial encephalopathy has not been confirmed. There are a few reports concerning the therapeutic effects on the clinical symptoms of MIDD patients. Suzuki et al. reported that CoQ_{10} administration was effective in improving the symptoms in the legs and the residual urine volume in the bladder of a MIDD patient [15]. They also reported that the insulin-induced oedema observed in MIDD patients responded to CoQ_{10} treatment [16]. Silvestre-Aillaud et al. treated a MIDD patient with a combination of CoQ_{10} and L-carnitine for 6 months, but could not confirm a significant effect either on insulin secretion or on insulin sensitivity [21]. Some reports described CoQ_{10} as deficient in some patients with mitochondrial encephalomyopathy and that its replacement improved the clinical symptoms [22]. However, there was no CoQ_{10} deficiency in the MIDD patient. Long-term CoQ_{10} replacement increased the serum CoQ_{10} concentrations in the MIDD patients.

In recent years, CoQ_{10} has attracted increasing attention with regard to its function in the reduced form (ubiquinol-10) as an antioxidant. Ubiquinol-10 efficiently protects membrane phospholipids and serum low-density lipoprotein from lipid peroxidation and also mitochondrial membrane proteins and DNA from oxidative damage induced by free radicals [23]. Ubiquinol-10 is as effective in preventing oxidative damage to lipids as alpha-tocopherol, and is considered to be the best lipid-soluble antioxidant in humans. Ubiquinol-10 is known to be regenerated from CoQ_{10} by electron transport carriers present in various biomembranes and by some enzymes. Plasma membrane DT-diaphorase has recently been proven to maintain CoQ_{10} in its reduced antioxidant state as ubiquinol-10 and to protect membrane components from free radical damage such as lipid peroxidation [24].

Mitochondrial gene mutations are known to enhance the release of reactive oxygen species from mitochondria and induce a dysfunction in the mitochondrial respiratory chain [25], which might result in the progression of insulin secretory defects and neurosensory deafness. We speculate that the effects of CoQ_{10} on insulin secretion, blood lactate after exercise and hearing capacity in MIDD were due to its anti-oxidation activities. A large-scale double blind cross-over study should be performed to confirm the clinical usefulness of CoQ_{10} on MIDD.

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