

Early report

Quinone-responsive multiple respiratory-chain dysfunction due to widespread coenzyme Q₁₀ deficiency

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Background The respiratory-chain deficiencies are a broad group of largely untreatable diseases. Among them, coenzyme Q₁₀ (ubiquinone) deficiency constitutes a subclass that deserves early and accurate diagnosis.

Methods We assessed respiratory-chain function in two siblings with severe encephalomyopathy and renal failure. We used high-performance liquid chromatography analyses, combined with radiolabelling experiments, to quantify cellular coenzyme Q₁₀ content. Clinical follow-up and detailed biochemical investigations of respiratory chain activity were carried out over the 3 years of oral quinone administration.

Findings Deficiency of coenzyme Q₁₀-dependent respiratory-chain activities was identified in muscle biopsy, circulating lymphocytes, and cultured skin fibroblasts. Undetectable coenzyme Q₁₀ and results of radiolabelling experiments in cultured fibroblasts supported the diagnosis of widespread coenzyme Q₁₀ deficiency. Stimulation of respiration and fibroblast enzyme activities by exogenous quinones in vitro prompted us to treat the patients with oral ubiquinone (5 mg/kg daily), which resulted in a substantial improvement of their condition over 3 years of therapy.

Interpretation Particular attention should be paid to multiple quinone-responsive respiratory-chain enzyme deficiency because this rare disorder can be successfully treated by oral ubiquinone.

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Introduction

Mitochondrial encephalomyopathies represent a heterogeneous group of genetic disorders caused by various types of respiratory-chain dysfunction. The respiratory chain consists in five major complexes (complexes I–V) and catalyses the transduction of energy from respiratory substrates into a proton-motive force that is used to synthesise ATP. Coenzyme Q₁₀ (ubiquinone) transfers reducing equivalents from various dehydrogenases to complex III (ubiquinone cytochrome *c* reductase) and acts as a transmembrane hydrogen carrier.^{1,2} No cure for mitochondrial encephalomyopathy is currently available and most therapeutic trials have failed to provide biochemical evidence of improved respiratory-chain function.

We report quinone-responsive mitochondrial encephalomyopathy in two siblings with multiple respiratory enzyme deficiency ascribed to a deficiency of coenzyme Q₁₀ biosynthesis in various tissues, and assessed the efficacy of oral ubiquinone therapy in these patients.

Patients and methods

Patients

Patient 1 was a boy who was born after a term pregnancy and normal delivery (birthweight 3600 g, length 54 cm, head circumference 35.5 cm) to healthy parents from western France. His older brother (by 5 years) was healthy, but his two older sisters (patients 2 and 3) were also affected. He did well during the first weeks of life, but nystagmus was noted early, and he gradually developed severe myopia and bilateral visual loss. At 10 years, he was diagnosed with retinitis pigmentosa with optic nerve atrophy of the left eye and cataract of the right eye. Bilateral sensorineural deafness was suspected early (at 3 months) and was further confirmed by detection of impaired auditory evoked potentials at 1 year (50 dB). He could sit at 6 months and walk unaided at 18 months. Nephrotic syndrome was diagnosed at 3 years and resulted in terminal renal failure that required kidney transplantation at 9 years. Progressive ataxia, dystonia with generalised amyotrophy, and increased deep tendon reflexes of lower limbs substantially hindered his ability to walk unaided. At 12 years, he could no longer ride his bicycle or walk unaided and he became progressively wheelchair-bound. He had a long face with large ears, a gaping mouth, and increased salivation, but no specific dysmorphic features were noted. Magnetic resonance imaging showed abnormal signals in the periventricular and cerebellar white matter, with moderate ventricular dilatation (not shown). Hypertrophic normokinetic cardiomyopathy was found at age 11 years. Metabolic investigation showed normal plasma and cerebrospinal fluid lactate and no organic aciduria. Oxidation of ¹⁴C-fatty acids by circulating lymphocytes was normal (not shown).

Patient 2, the oldest sister (by 3 years) of patient 1, was not seen at our hospital but had a similar, although more severe, clinical course. Delayed motor development was noted at 6 months of age. She walked unaided at 2 years, but progressive ataxia and pyramidal involvement gradually developed and she lost the ability to walk at 3 years. Bilateral sensorineural deafness and visual loss were detected at 18 months. A computed tomography scan of the brain showed generalised hypodensity of white matter. She developed nephrotic syndrome with glomerular sclerosis and died aged 8 years after rapid neurological deterioration.

Patient 3, the older sister (by 1 year) of patient 1, had a milder form of the disease. At 1 year of age, she had bilateral sensorineural deafness, nystagmus, and myopia. Despite moderate ataxia, she could walk unaided at 3 years. She developed nephrotic syndrome with glomerular sclerosis and terminal renal failure that required kidney transplantation at 8 years. She is moderately mentally disabled but has never required wheelchair or motor assistance.

Metabolic and biochemical analysis

In patients 1 and 3, we measured lactate, pyruvate, and their molar ratios by enzymatic methods as indexes of the oxidation-reduction status in plasma and cerebrospinal fluid.³ Urinary organic acids were studied by gas chromatography and mass spectrophotometry.⁴

Mitochondria-enriched fractions were prepared from an open biopsy of the deltoid muscle (120 mg).⁵ Circulating lymphocytes were isolated on a Ficoll cushion. Cultured skin fibroblasts and lymphoblastoid cell lines from the two patients and 163 controls were grown under standard conditions.⁶

Spectrophotometric assays of respiratory-chain enzymes, polarographic tests, and mitochondrial membrane potential measurements were carried out on skeletal muscle mitochondria, circulating lymphocytes, or cultured skin fibroblasts.^{5,7} Enzymes involved in coenzyme Q₁₀ biosynthesis were measured in cultured skin-fibroblast homogenates. Mevalonate kinase activity,⁸ farnesyl pyrophosphate synthetase,⁹ squalene synthase,¹⁰ and 4-hydroxybenzoate trans-prenyltransferase¹¹ were measured.

Lipids were extracted with petroleum ether/methanol (ratio 12/18) and analysed by reverse-phase high-performance liquid chromatography on a C18 column (3 µmol/L; 4.6 mm × 10 cm; Rainin Instrument Company Inc, Woburn, MA, USA). Lipids were separated on a binary convex gradient from 90% methanol/water (v/v 9/1) to 100% methanol/2-propanol/hexane (v/v/v 2/1/1) at a flow rate of 1.5 mL/min. In addition to its specific retention time, coenzyme Q₁₀ was identified by its redox properties. We used an ultraviolet light detector at 210 nm and 275 nm to monitor lipids.¹⁰ For analysis of coenzyme Q₁₀ biosynthesis, fibroblasts were incubated with 0.5 mCi RS-[5-³H]mevalonic acid (10 Ci/mmol, Du Pont NEN, Boston, MA, USA) for 2 h. After lipid extraction, the upper solvent phase (p-ether) was separated by high-performance liquid chromatography as described above, and radioactivity was measured with a radioactivity flow detector (Radiomatic Instrument, Tampa, FL, USA). To study the formation of the decaprenyl-pyrophosphate side-chain, polar lipids in the lower solvent phase (methanol) were enzymatically dephosphorylated and analysed by high-performance liquid chromatography with a gradient system from 100% methanol/water (v/v 4/1) to 100%

	Activity (nmol/min/mg protein)			Activity ratio (complex IV vs variable)		
	Patient 1	Patient 3	Controls*	Patient 1	Patient 3	Controls†
Complex II	23	20	25 (12–40)	4.6	4.8	5.3 (0.5)
Complex III	92	92	96 (43–146)	1.2	1.1	1.4 (0.2)
Complex IV	107	96	126 (61–187)	· ·	· ·	· ·
G3PD	22	16	26 (9–54)	4.9	6.0	4.6 (0.6)
Complex II + III	21	22	41 (13–65)	5.1	4.4	3.2 (0.3)
G3PD + complex III	11	12	32 (9–71)	9.7	8.0	4.2 (0.4)

*Median (range). †These values (means [SD]) follow a gaussian distribution.¹³
G3PD=glycerol-3-phosphate dehydrogenase.

Table 1: Respiratory-chain enzyme activity in circulating lymphocytes of patients 1 and 3 and of 163 controls

methanol/2-propanol (v/v 4/1). The radiolabelled intermediates were detected by a radioactivity flow detector and identified according to their retention time.

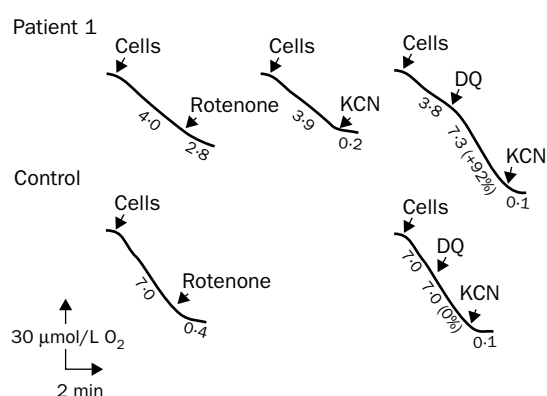
The yeast aminoacid sequence of hexaprenyl pyrophosphate synthetase (COQ1, SwissProt P18900, www.expasy.ch/sprot) was used as template to screen the human dbEST database by use of the BLAST similarity searching program (TBLASTN option; version 1.4.11), and primers for reverse-transcription PCR amplification of total RNA were designed from the derived sequences. Total RNA extracted from either heart biopsies or cultured skin fibroblasts was reverse transcribed with the GeneAmp RNA PCR kit (Applied Biosystems, Norwalk, CT, USA), and complementary DNA (cDNA) was amplified with specific oligonucleotides (30 cycles of 30 s at 95°C, 30 s at 45–55°C, depending on the primer used, and 1 min at 72°C). Amplification products were purified on a 2% low-melting-point agarose gel and sequenced directly with 3.2 pmol amplification primer, 100 ng DNA, and 8 µL sequencing reaction mixture (Dye Terminator Cycle Sequencing Kit, Perkin-Elmer Cetus, Norwalk, CT, USA) on an automatic fluorimetric DNA sequencer (Applied Biosystems).

Results

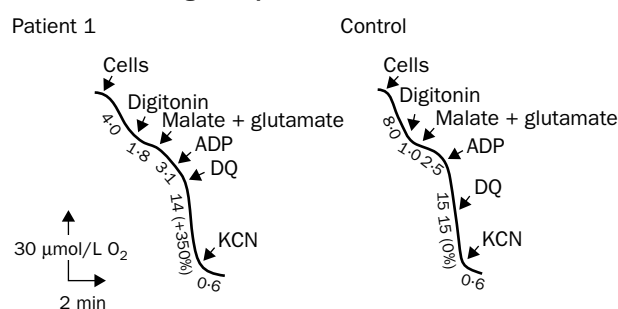
Respiratory enzyme activity in circulating lymphocytes (table 1) and skeletal muscle mitochondria from patients 1 and 3, and cultured skin fibroblasts from patient 1 (not shown), was within the normal range. However, quinone-dependent activities in lymphocytes (complex I and III, complex II and III, glycerol-3-phosphate and complex III) were in the lowest absolute control values, and activity ratios, which are used to detect unbalanced respiratory chain enzyme functions,^{12,13} differed substantially from control (table 1). This difference suggested abnormal coenzyme Q₁₀ pool function in the patients' lymphocyte mitochondria. Fibroblast respiration averaged 60% of the control mean, was poorly sensitive to rotenone (a specific inhibitor of complex I), but fully sensitive to potassium cyanide (a specific inhibitor of cytochrome oxidase; figure 1). Similar results were seen in fibroblasts and circulating lymphocytes from patient 3 (not shown). Ragged red fibres and lipid storage anomalies were not seen in muscle samples from patient 1.

Coenzyme Q₁₀ deficiency was also indicated by the large effect of decylubiquinone (an exogenous coenzyme Q₁₀ analogue) on oxidative activities in fibroblasts from patient 1 (figure 1). Decylubiquinone stimulated oxygen uptake (92% stimulation) and fully restored malate-glutamate oxidation in permeabilised fibroblasts from patient 1 (figure 1). Decylubiquinone also significantly increased the glycerol-3-phosphate cytochrome c reductase activity of fibroblasts in patient 1 (83% stimulation). Idebenone, coenzyme Q₄, and coenzyme Q₆ similarly corrected the deficient enzyme activities in patients' fibroblasts (not shown). These findings

Effect of respiratory-chain inhibitors and DQ on respiration rate of intact fibroblasts



Effect of DQ on oxidation of malate plus glutamate by mitochondria of digitonin-permeabilised fibroblasts



Effect of DQ on G3P cytochrome c reductase of freeze-thaw fibroblasts

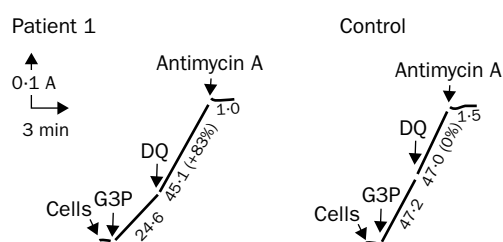


Figure 1: **Effect of an exogenous quinone analogue on respiration, mitochondrial substrate oxidation, and respiratory-chain enzyme activity of cultured skin fibroblasts from patient 1 and controls**

Numbers along the traces are nmol/min/mg protein. Numbers in parentheses are percentages of rates measured before adding 80 $\mu\text{mol/L}$ decylubiquinone (DQ). Quantities used: 3 $\mu\text{mol/L}$ rotenone; 0.3 mmol/L potassium cyanide (KCN); 0.002% digitonin; 10 mmol/L malate; 10 mmol/L glutamate; 0.2 mmol/L ADP; 10 mmol/L glycerol-3-phosphate (G3P); 1 $\mu\text{mol/L}$ antimycin A.

suggest that exogenous quinones readily cross intact cell and mitochondrial membranes. Similar results were obtained in fibroblasts from patient 3 and in circulating lymphocytes from both patients (not shown).

Direct evidence of quinone deficiency was eventually given by measurement of coenzyme Q_{10} in controls' and patients' fibroblasts. No coenzyme Q_{10} was detected in either patient's fibroblasts (controls 117–200 ng/mg protein). To estimate the ability of fibroblasts from controls and from patient 1 to synthesise coenzyme Q_{10} (figure 2), cells were grown in the presence of ^3H -mevalonate. Substantial incorporation of ^3H into cholesterol, squalene, and dolichol was seen in controls and patients (figure 3). A ^3H -coenzyme Q_{10} peak was detected in controls, but we noted no incorporation in fibroblasts from patient 1 (figure 3). Indirect evidence of trans-prenyltransferase

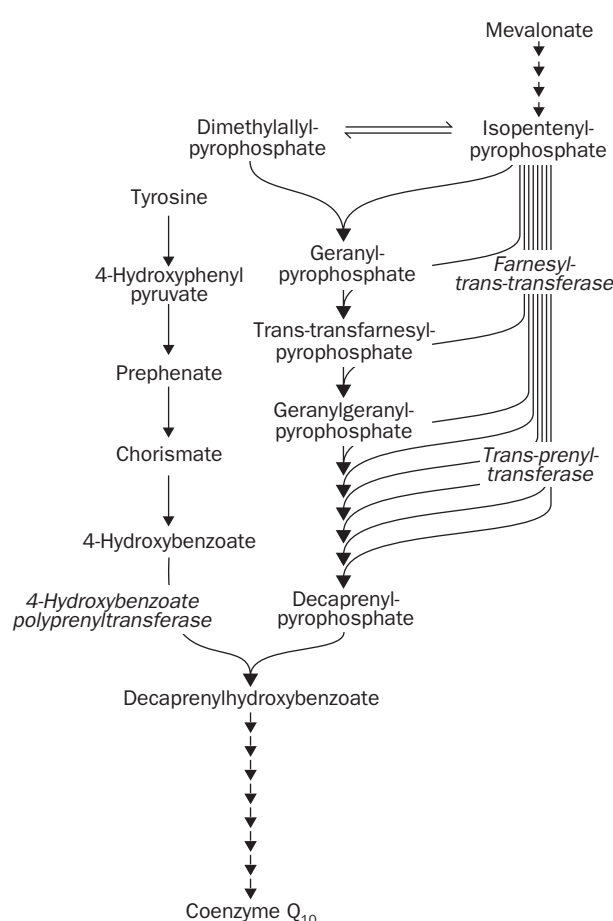


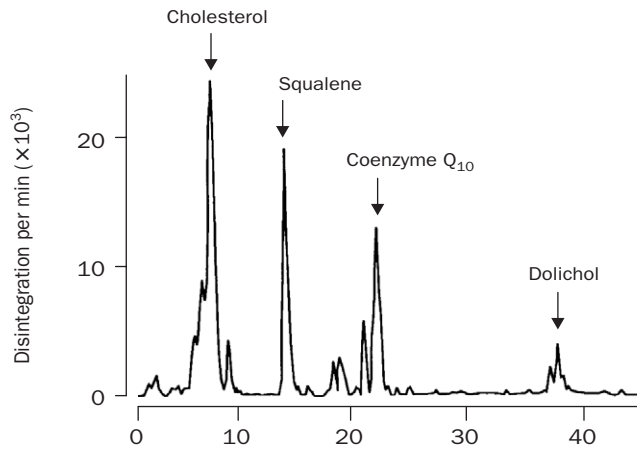
Figure 2: **Coenzyme Q_{10} biosynthesis pathway**

Enzymes are shown in italics.

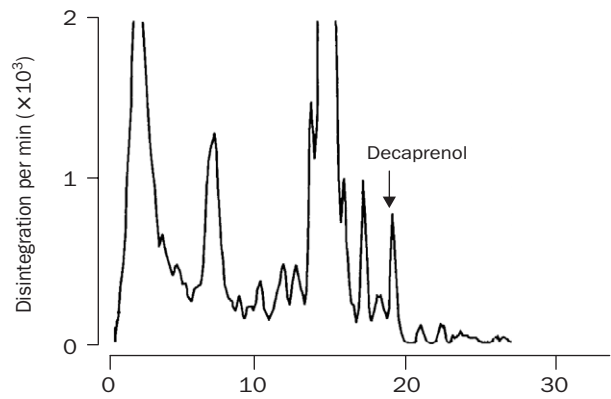
deficiency, which is not directly quantifiable in mammals, was provided by the low level of eluted decaprenol in fibroblasts from patient 1 (figure 3). However, we sequenced the human homologue of yeast trans-prenyltransferase cDNA and failed to detect the disease-causing mutation in the coding sequence in the patients' trans-prenyltransferase gene (not shown), and the very low concentrations of trans-prenyltransferase mRNA precluded quantification of the specific transcripts in control fibroblasts and lymphoblastoid cell lines.

Because of in-vitro correction of the respiratory-chain enzyme deficiency by quinone analogues, patient 1 was given oral idebenone 5mg/kg daily for 2 months (this drug readily crosses the blood-brain barrier). Muscle weakness and ataxia initially improved and in-vitro stimulation by decylubiquinone of glycerol-3-phosphate cytochrome c reductase in lymphocytes (a sensitive measure of coenzyme Q_{10} depletion) fell from 170% to 40% and 28% after 12 days and 74 days, respectively, of idebenone administration (figure 4). After 2 months, however, clinical symptoms worsened and stimulation of glycerol-3-phosphate cytochrome c reductase by decylubiquinone increased to 98%, whereas plasma idebenone (2 $\mu\text{mol/L}$) remained unchanged. For this reason, patient 1 was given oral ubiquinol (90 mg daily) instead of idebenone, and his condition improved. Although he had been wheelchair-bound, after 2 months of therapy the boy could stand, walk unaided, and ride his bicycle for more than 3 km. His bodyweight, muscle bulk, head control, and precise movements also improved. Drooling and cataract

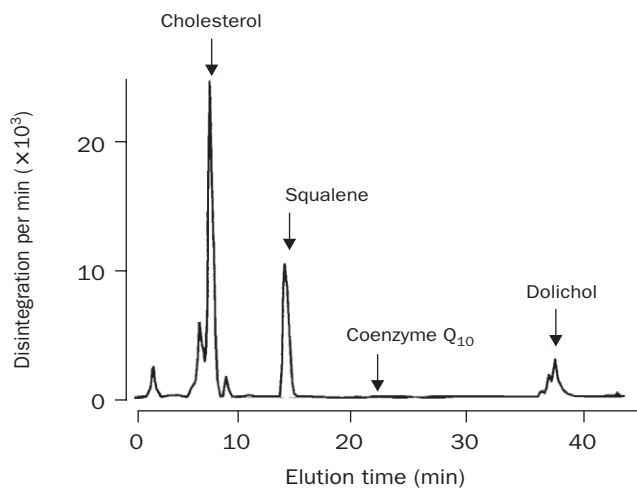
Control, p-ether extracts



Control, methanol extracts



Patient 1, p-ether extracts



Patient 1, methanol extracts

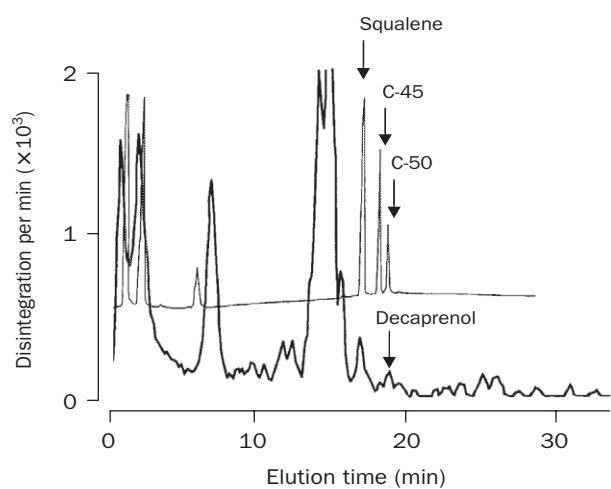


Figure 3: Synthesis of coenzyme Q₁₀ and decaprenyl compounds by cultured skin fibroblasts from patient 1 and controls
 Elution profiles of p-ether and methanol phase extracts from control and patient 1 skin fibroblasts incubated with ³H-labelled mevalonate. The dotted line corresponds to the elution profile of standard compounds.

completely resolved. His ability to smile and follow with the eyes completely recovered (table 2). Simultaneously, in-vitro stimulation by decylubiquinone of glycerol-3-phosphate cytochrome *c* reductase activity in lymphocytes

fell from 130% to 47% and 0% after 3 months and 10 months, respectively (figure 4).

Patient 3 was also given oral ubiquinone (90 mg daily). Her skills and general condition greatly improved: she could pronounce an increasing number of words, rapidly gained the ability to write her name and catch a ball, and her interaction with her environment improved.

	Before treatment	After 6 months of treatment
Stand unaided	Difficult	Yes
Walk unaided	Difficult, few metres	Yes, hundreds of metres
Ability to run	No	About 10 m
Ride tricycle	No	Yes, several hours a day
Throw ball in a basket	No	Yes
Ability to climb stairs unaided	No	Yes
Ability to get up from chair without hands	Difficult	Yes
Falls per week	Many	1-2
Stand up after falling	No	Yes
Dress alone	No	Yes, except buttons
Put on shoes	No	Yes, except laces
Autonomous morning toilet	No	Better and better
Ability to colour pictures	Little	Yes
Ability to cut meat	No	Yes
Drizzling	Yes	No
Anal-sphincter control	No	Yes
Smile	Very few	Yes, happy behaviour
Personality	Prostrated	Outgoing

Table 2: Functional and cognitive improvement in patients given ubiquinone

Discussion

Unlike previous cases, in whom quinone deficiency was confined to neuromuscular tissues,¹⁴⁻¹⁶ our patients had widespread multitissue quinone depletion. This finding provided the unique opportunity to investigate the mechanism of coenzyme Q₁₀ depletion in what we believe to be a novel but treatable inborn error of metabolism.

The incorporation of ³H-mevalonate into cholesterol and dolichol but not into coenzyme Q₁₀ suggests that a specific step of endogenous coenzyme Q₁₀ synthesis is impaired. The very low concentrations of labelled decaprenyl-diphosphate in patients' cell extracts were consistent with a deficiency of trans-prenyltransferase, the enzyme that elongates the prenyl side-chain of the quinone (figure 2). However, no mutation in trans-prenyltransferase cDNA could be identified,

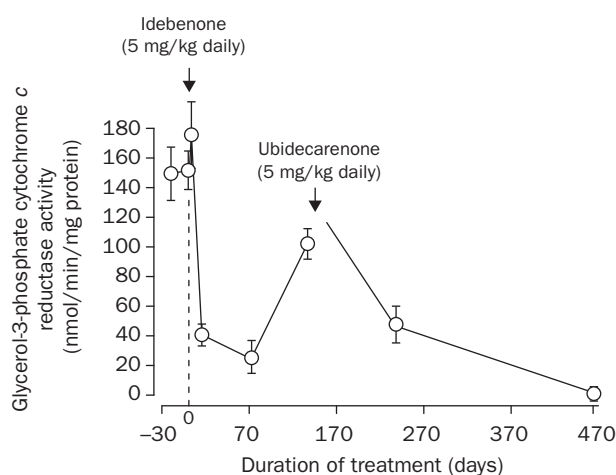


Figure 4: Effect of oral quinone treatment on respiratory-chain activity in circulating lymphocytes from patient 1

The stimulation of glycerol-3-phosphate cytochrome c reductase activity by decylubiquinone (80 $\mu\text{mol/L}$) is shown. The progressive decrease in stimulation is accounted for by the gradual repletion of the mitochondrial quinone pool after oral treatment. Values are means (SD) of three activity measurements.

suggesting that the disease-causing mutation lies either elsewhere in the trans-prenyltransferase gene or in another gene involved in the regulation of trans-prenyltransferase activity. Of note, the blockade of ubiquinone synthesis in the yeast mutant *coq7* may result from either a primary deficiency of the accumulating substrate-transforming enzyme (CoQ7p) or the putative impairment of an essential step in the activation of the enzyme.¹⁷

Assessment of coenzyme Q_{10} -deficient fibroblasts showed that oxidation of some substrates (pyruvate, glycerol-3-phosphate, or dihydro-orotate) is selectively sensitive to coenzyme Q_{10} depletion. The corresponding enzyme activities are therefore the most sensitive markers for coenzyme Q_{10} deficiency in human beings. Our study also shows that quinone analogues (decylubiquinone, idebenone, coenzyme Q_{4} , coenzyme Q_{6}) can cross the plasma and mitochondrial membranes as they restore normal enzyme activities, thus making the diagnosis of quinone-responsive respiratory-chain deficiency feasible in vitro.

Since the first description of hypermetabolic mitochondrial myopathy by Luft and colleagues,¹⁸ several hundred patients with respiratory-chain deficiency have been studied, but responsiveness to ubidecarenone has seldom been observed. Indeed, only a few cases of unambiguous coenzyme Q_{10} depletion have been described,¹⁴⁻¹⁶ in which patients had mitochondrial encephalomyopathy with central-nervous-system and skeletal-muscle abnormalities, epileptic seizures, cognitive impairment,¹⁴ general weakness, and exercise intolerance.¹⁴⁻¹⁶ Recurrent myoglobinuria triggered by fever and convulsions,¹⁴ or after moderate exercise, has been also reported.¹⁵ In these cases, coenzyme Q_{10} deficiency was seen in skeletal muscle but not in circulating lymphocytes or in skin fibroblasts, precluding thorough investigation of impaired coenzyme Q_{10} synthesis. The exact frequency of coenzyme Q_{10} deficiency, which accounts for 1% of all respiratory-chain deficiencies, is difficult to establish because of the difficulty in diagnosing this disorder.

Our patients have allowed us to investigate the consequences of quinone deficiency on respiratory-chain function and to identify a novel disorder of coenzyme Q_{10} synthesis.

Further investigations should help decide the respective roles of antioxidant effect and quinone dependence in the clinical improvement of the patients given quinones as therapy. Whatever the mechanism, it is important that quinone-dependent multiple respiratory-chain enzyme deficiency be recognised, because this form of mitochondrial dysfunction seems to respond well to oral quinone administration.

Contributors

Agnès Rötig determined and studied the human trans-prenyltransferase cDNA sequence in the patients. Eeva-Liisa Appelkvist, Gustav Dallner, and Lars Ernster analysed the coenzyme Q_{10} content of patients' fibroblasts and coenzyme Q_{10} biosynthesis. Dominique Chretien did enzymological investigations of the respiratory chain in lymphocytes and muscle mitochondria. Noman Kadhom cultured the skin fibroblasts. Patrick Ederly and Arnold Munnich were in charge of the patients' clinical management and quinone treatment. Marc Lebeideau referred the patients. Vanna Geromelo and Pierre Rustin did enzymological studies on cultured skin fibroblasts. All investigators helped to write the paper. Lars Ernster died during the preparation of the paper.

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