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Coenzyme Q_{10} responsive ataxia: 2-year-treatment follow-up.

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Abstract

We assessed the clinical outcome after coenzyme Q_{10} (CoQ_{10}) therapy in patients presenting ataxia associated with CoQ_{10} deficiency and in cases with ataxia and no apparent CoQ_{10} deficiency.

We performed an open-label prospective study in 14 patients classified into two groups according to CoQ_{10} values in muscle (deficient or not). Patients were evaluated clinically (ICARS scale, MRI and video-tape registration) at baseline and every 6 months during a period of 2 years after CoQ_{10} treatment (30 mg/Kg/day).

Patients with CoQ_{10} deficiency showed a statistically significant reduction of ICARS scores (Wilcoxon test: $p = 0.018$) after 2 years of CoQ_{10} treatment as compared to baseline conditions. In patients without CoQ_{10} deficiency, no statistically significant differences were observed in total ICARS scores after therapy, although one patient from this group showed a remarkable clinical amelioration.

Biochemical diagnosis of CoQ_{10} deficiency was a useful tool for the selection of patients who are good candidates for treatment, since all of them responded to therapy. However, the remarkable clinical response in one case without CoQ_{10} deficiency highlights the importance of treatment trials for identification of patients with CoQ_{10}-responsive ataxia.

Key words: coenzyme Q10 deficiency**;** mitochondrial disorders; ataxia; cerebellum; pediatric patients.

Introduction

Primary CoQ_{10} deficiency has been described as an autosomal recessive disease with heterogeneous phenotypes: among them, cerebellar ataxia is the most frequent one.[14] Mutations in 5 different nuclear genes involved in CoQ_{10} biosynthesis have been described: *COQ2 (*MIM #609825),[19] *PDSS1 (*MIM #607429),[20] *PDSS2 (*MIM #610564)[21], *CABC1* (MIM #606980) genes[22,23] and *COQ9.*[24] Mutations in *CABC1* have been associated with the ataxic form of CoQ_{10} deficiency syndromes.

The response to replacement therapy in the ataxic form of CoQ_{10} deficiency is variable. Some patients with ataxia had good clinical outcome after CoQ_{10} supplementation,[12-15] whereas others with genetically confirmed CoQ_{10} deficiency showed variable response.[23]

Our goal was to assess clinical outcome after CoQ_{10} therapy in 7 patients with ataxia and CoQ_{10} deficiency in muscle and/or fibroblasts and in 7 patients with ataxia but without CoQ_{10} deficiency.

Methods

Patients

We have analyzed CoQ_{10} content in 289 muscle and 165 cultured skin fibroblasts. We performed an open-label prospective study. Fourteen eligible patients were those with congenital cerebellar ataxia of unknown origin as main symptom (9 females and 5 males; age range, 2-38 years; mean, 11 years). Friedreich ataxia, ataxia associated with vitamin E deficiency, ataxia-telangiectasia, ataxia with oculomotor apraxia, late-onset forms of ataxia (spinocerebellar ataxias types 1 and 2), ataxia associated with mutations in mitochondrial DNA and other known inherited forms of ataxia were excluded in basis of clinical, biochemical and molecular genetic grounds. The main clinical features are listed in Table 1. We classified these 14 patients into two groups based on CoQ_{10} content in muscle or fibroblasts and on mitochondrial respiratory chain enzyme activities, following previously reported criteria:[25] Group 1: 7 patients with CoQ_{10} deficiency in muscle (range 1.31 – 2.60 nmol/citrate synthase Units [mean, 1.99; standard error of the mean = 0.27]; compared with our reference values (muscle: 2.7 – 8.5 [mean: 5.4]). These seven patients presented a clear reduction in CoQ_{10}-dependent mitochondrial respiratory chain enzymes, as previously reported.[25] In all patients, CoQ10 deficiency was further confirmed in fibroblasts (data not shown). Group 2: 7 patients without CoQ_{10} deficiency in muscle (range 2.7 – 6.7 nmol/citrate synthase Units [mean = 4.12: standard error of the mean = 1.29]). All patients presented CoQ_{10} values in fibroblasts related to citrate synthase in the normal range (data not shown).

Muscle CoQ10 reference values were established in 37 paediatric patients, as previously reported [25]. For fibroblasts, skin biopsies were taken from 65 patients of similar ages (2-16 years, average 9.5) for differential diagnosis of inborn errors of metabolism:

exclusion criteria were diagnosis of mitochondrial energy metabolism disorder and other genetic-metabolic conditions involving mitochondria.

The study protocol was approved by the ethics committee of the Hospital Sant Joan de Déu. Informed consent was obtained from parents and adult patients.

Biochemical and molecular procedures

Screening for inborn errors of metabolism was applied in all patients, including analysis of plasma amino acids, sialotransferrin, biotinidase activity and urine organic acids with normal results. Celiac disease was also routinely rule out.

Mitochondrial respiratory chain enzymes were measured by spectrophotometric procedures.[26] Muscle and fibroblast CoQ_{10} concentrations were determined by reverse-phase high-pressure liquid chromatography (HPLC, Waters, MA, USA) with electrochemical detection (Coulochem II, ESA, MA, USA) as previously reported.[26]

We performed genetic analysis of four genes involved in the CoQ_{10} biosynthetic pathway (*COQ2, PDSS1*, *PDSS2* and *CABC1)* in 6 patients belonging to group 1 (cases 1, 2, 3, 4, 5, and 6) by direct sequencing of each exon and their exon-intron boundaries, in an ABI Prism 3130xl autoanalyser (Applied Biosystems, Foster City, CA).

Treatment protocol

The patients were evaluated clinically and biochemically at baseline and every 6 months after CoQ_{10} administration (dodecarenone) for a period of 2 years. CoQ_{10} dosage was chosen according to previously reported regimens[27] and was the same for all patients (30 mg/Kg/day orally 3 times per day). Patients reported in the present article were treated with oral CoQ_{10} by compassionate use. No other pharmacological treatments, such as carnitine or vitamins and antioxidants were simultaneously applied.

Clinical evaluation: Neurological evaluations were always performed by the same investigator. The neurological status of our 14 patients was assessed using the

International Cooperative Ataxia Rating Scale (ICARS),[28] where higher scores indicate a more severe disease: posture and gait (0-34 points), kinetic functions (0-52 points), dysarthria-speech (0-8 points) and oculomotor movement disorders (0-6 points). We compared ICARS scores at baseline and at 6, 12 and 24 months after the initiation of therapy. We considered the patients improved when we observed a significant reduction in posture and kinetic function scores. Video recordings of the patients were taken at baseline and every six months after the introduction of CoQ_{10} therapy, and cranial MRI and electroneurophysiological studies were obtained at the beginning and at the end of the study.

Biochemical monitoring: To control CoQ_{10} treatment compliance, EDTA-blood samples were taken at baseline and every 6 months after the start of therapy. All samples were collected in the fasting state (and 10-12 hours after the last oral CoQ_{10} doses) and plasma samples were stored at –80ºC prior to CoQ_{10} analysis.

Statistical analysis

Mann-Whitney U and Student-t tests were applied to compare ICARS scores, plasma CoQ_{10} levels and the age between groups 1 and 2, and the Wilcoxon test was used to compare paired ICARS scores data before the start of the therapy and 6, 12 and 24 months after therapy. Statistical significance was considered as $p<0.05$. Calculations were performed with the SPSS 17.0 program.

Results

All patients enrolled in this study completed the period established of CoQ10 treatment except for patients 6 and 13, who are still being followed-up (18 months period, Fig. 1), since they were enrolled later on. No putative side effects were observed during the duration of the therapy in any case (dermatologic, gastrointestinal, haematologic, hepatic, neurologic and ophthalmic effects).

Mutational screening of the *COQ2*, *PDSS1*, *PDSS2* and *CABC1* genes only revealed a single base change in the *COQ8* gene in patient 2, who was a heterozygous carrier of the c.1825C>G (p.L609V) mutation in exon 15.

Total ICARS, posture and gait and kinetic function scores, and the ages of group 1 and 2 patients, are reported in Table 2 (range, mean and standard error of the mean values). No statistically significant differences were observed when we compared total ICARS, posture and gait, kinetic function scores and age between the two groups at baseline (Mann-Whitney U and Student-t tests).

Clinical data and results of ICARS scores of patients at the beginning and after CoQ_{10} treatment are illustrated in Table 1 and Figure 2. All group 1 cases showed an improvement in total ICARS scores after 24 months of therapy (Table 1, Figure 2). These patients showed a statistically significant reduction of ICARS (in total values Wilcoxon test: **p = 0.018),** posture and gait **(p= 0.017)** and kinetic function scores **(p= 0.042)** when they were compared at baseline and after 2 years of CoQ_{10} treatment. In baseline conditions, oculomotor disorders were limited to nystagmus in 2 out of 7 patients and abnormal ocular pursuit in 1 patient. The ICARS scale evaluation in group 2 after CoQ_{10} therapy showed that case 14 clearly improved, 3 patients showed slight improvement, and 3 worsened. There were no statistically significant differences either

in total ICARS (p = 0.343) or in kinetic function and posture and gait scores between patients at baseline and after 24 months of CoQ_{10} therapy (Table 1, Figure 2). Considering patients individually, cases 5, 6 and 14 showed the best clinical response. The videotapes show patient 6 at baseline and at the end of the investigation: there was a clear improvement in kinetic functions (video1, supplemental data) and in gait and posture (video 2, supplemental data). Case 2, the patient with a nucleotide change in *CABC1* gene, did not show a clear response to the treatment. Conversely, case 14, with no CoQ_{10} deficiency, showed a remarkable reduction in ICARS score (from 16 to 7 points).

Regarding CoQ_{10} monitoring, plasma values at baseline ranged from 0.33 to 1 µmol/L (reference values: 0.41 - 1.15 µmol/L) and increased after supplementation in all patients, confirming that all of them had good treatment compliance. No significant differences were observed in mean plasma CoQ_{10} concentrations after the start of therapy between group 1 (range: 3.1 – 13.6; average: 8.8 µmol/L) and group 2 (range: 4.8 – 13.3; average: 8.3 µmol/L).

Neurophysiological studies did not show neuropathic or myopathic patterns in any patient prior to the start of therapy. In group 1, MRI evidenced cerebellar atrophy in all patients, 4 of whom (cases 2, 3, 5 and 7) had severe atrophy. In case 2, the very severe cerebellar atrophy observed at the start of the therapy did not changes noticeably after 1 year on CoQ_{10} (Figure 3). Case 6, who had a remarkable clinical response to therapy, showed only moderate cerebellar atrophy with vermian involvement (Figure 3). In group 2, only 2 out of 7 patients showed cerebellar atrophy whereas in the other 5 (cases 8, 11, 12, 13 and 14), the MRI was normal or revealed only slight atrophy. Case 14 had a normal MRI.

Discussion

The ataxic form is the most common of the CoQ_{10} deficiency syndromes.[29] To our knowledge, no studies have reported long-term follow-ups of these patients after CoQ_{10} supplementation.

Concerning genetic studies, only case 2 harboured a heterozygous nucleotide change in *CABC1 gene*, but we failed to detect any other change in patients. Therefore, in all likelihood, other genes are associated with the ataxic form of CoQ_{10} deficiency syndromes, but they remain to be identified.

According to several authors, the response to treatment in CoQ_{10} deficiency patients with ataxia is usually good, but variable.[13,14,23] As no agreement on CoQ_{10} dosage exists at present, we established a 30 mg/kg.day regimen following previously reported experiments.[27] This dose proved to be effective, since patients presented a positive clinical outcome (Figure 2).

In all our patients from group 1, neurological outcome based on ICARS scale after CoQ_{10} therapy was good, especially for posture and gait scores (all patients decreased the score in this subtest). Although statistically significant improvement was observed for kinetic function, 5 out of 7 patients improved in this subtest and 2 remained stable. Interestingly, the only case with a proven mutation in *CABC1*, did not respond any better than other patients with no mutations in the genes we screened for. The severe cerebellar involvement in this case may explain this moderate response. This is not too surprising, as there have been other patients with ataxia and CoQ_{10} deficiency who showed no clinical improvement after treatment.[23]

The response to treatment of group 2 was more variable, ranging from good in one case to none or worsening. As recently described, the CoQ_{10} amount in patients carrying mutations at *CABC1* gene may be only mildly decreased in muscle, and normal in fibroblasts.[22,23] Therefore, it seems prudent to sequence this gene in patients with ataxia

of unknown origin and with good clinical outcome after CoQ_{10} supplementation. Moreover, it seems necessary to improve the biochemical tools to detect patients with CoQ10 deficiency for therapeutical trials.

Some variables may influence the ICARS score evolution after CoQ_{10} or other therapies, such as the age of patients at the start of the therapy, the severity of the disease (evaluated as total ICARS score), treatment compliance or physiotherapy.[30, xx] Our results, showed no statistically significant differences in ICARS scores and patient age between group 1 and 2 at the start of therapy, suggesting that these variables did not explain the different clinical outcomes. Neither did treatment compliance differ between the two groups, according to plasma CoQ_{10} values. Concerning physiotherapy, only 3 patients from group 2 were under physiotherapy concomitantly to CoQ_{10} therapy. This fact might explain, as previously suggested [XX], the improvement observed in cases 10 and 14 belonging to group 2.

Cerebellar atrophy has been related with the ataxic forms of CoQ_{10} deficiency,[12-15] although no MRI studies in large series are available for these patients. According to our results, MRI alterations in group 1 were considerably more severe than in group 2. Interestingly, case 6, who showed only a mild vermian atrophy, had the best clinical response among all patients (Video tape). The normal MRI in case 14 may correlate with his very good clinical response.

In conclusion, in the absence of a reliable molecular diagnosis of CoQ_{10} deficiency, biochemical diagnosis was useful in selecting patients for CoQ_{10} supplementation, since all of them responded well to treatment. Furthermore, the remarkable clinical response in one patient with no CoQ_{10} deficiency in fibroblasts or muscle (and the previous observation that some patients may present mutations in *CABC1* gene without CoQ_{10}

deficiency) highlights the importance of treatment trials for the identification of patients with CoQ_{10}-responsive ataxia.

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Author Roles

1. Reserch Project: A. Conception, B. Organization, C. Execution

2. Statistical Analysis: A. Design, B. Execution, C. Review and Critique

3. Manuscript: A. Writing of the first draft, B. Review and Critique

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Video Tape 1. Posture and gait evaluation of case 6 at the start of CoQ_{10} therapy (first part of the video) and after 1 year of CoQ therapy. Gait in tandem position clearly improved

Video Tape 2. kinetic function evaluation of case 6 at the start of CoQ_{10} therapy (first part of the video) and after 1 year of CoQ therapy. Fine motor skills improved and it was noteworthy that the patient recovered the facial expression after therapy.

Figure 1: Patients flow diagram.

Figure 2: Total ICARS, posture and kinetic function scores in groups 1 and 2. Point A: baseline conditions and B: 2 years after CoQ10 treatment. Data are expressed as mean (columns) and standard error of the mean (bars).

Figure 3. MRI results in cases 2 (upper), 6 (middle) and 14 (lower panels) in baseline conditions and after CoQ_{10} therapy (except for case 14). Case 2 presented severe cerebellar atrophy, which did not improve after treatment. In case 6, the cerebellum was slightly affected and did not change after treatment, while case 14 did not show MRI alterations.

Table 1. Clinical data in group 1 (CoQ_{10}-deficient patients) and 2 (no CoQ_{10}-deficient patients) before and after the start of the therapy.

Table 2. Age at the start of therapy and ICARS scores in baseline conditions and after 24 months of CoQ_{10} treatment in patients from groups 1 and 2. Results are expressed as range, mean and standard error of the mean.