Results of Idebenone Treatment in Patients with Ocular Mitochondrial Myopathy

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Abstract

The effectiveness of idebenone treatment has been evaluated, by means of an open uncontrolled study, in 5 patients affected with ocular mitochondrial myopathy (OMM) with ophthalmoplegia and mitochondrial DNA deletions. The rationale for this study relies on: i) there is not an effective treatment for OMM; ii) in mitochondrial diseases oxygen free radicals can be produced in excess in affected tissues, owing to decreased mitochondrial respiratory chain function; iii) idebenone acts as protecting factor against membrane lipid peroxidation. All patients were ambulatory, one of them was also affected by insulin-dependent diabetes and another one suffered from attitudinal tremor on left arm. Patients were orally treated with idebenone at doses of 90 mg/day for 6 consecutive months. The treatment dropped out for 15 days in a patient. Before and after idebenone treatment the following parameters were evaluated: i) anaerobic lactate threshold during submaximal incremental exercise; ii) visual (VEPs) and brainstem auditory (BAEPs) evoked potentials (in 3 cases).

No clear modifications of exercise lactate increment was observed. VEPs and BAEPs were abnormal in basal conditions. While BAEPs worsened in one case, VEPs showed clear improvement in two cases, suggesting that idebenone medium-term treatment can interfere with central nervous system function parameters in OMM.

Key words: mitochondrial myopathies, therapy, exercise test, evoked potentials.

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Ocular mitochondrial myopathies (OMM) are mitochondrial disorders characterized by the presence, in the affected tissues, of deletions of mitochondrial DNA (mtDNA) [2, 16]. The disease mainly affects ocular and skeletal muscles, but also can show progression of symptoms with age indicating that tissues other than skeletal muscle can be affected, for instance the central nervous system (CNS) [13]. A part from tissue distribution of mtDNA mutation, other factors can influence clinical expression of the disease in terms of altered synthesis of the respiratory chain enzyme complex subunits or tRNA molecules: extension of the mtDNA deletions along the mtDNA circular molecule, heteroplasmy with coexistence in different proportions of wild-type and deleted mtDNA and complementation between them.

Several therapeutic approaches have been attempted in OMM, with the aim to bypass the electron transport chain block or stimulate respiratory chain activity in wild-type mitochondria. Since there is not an effective treatment of the disease, we report in this paper the results concerning the effectiveness of the idebenone treatment in some OMM patients.

Idebenone is a synthetic derivative from benzoquinone, already used in the therapy of human neurodegenerative disorders for its activity as protecting factor against membrane lipid peroxidation induced by oxygen free radicals [19]. The rationale for a therapeutic use of idebenone in OMM lies on the hypothesis that oxygen free radicals can be produced in excess in affected tissues, as a consequence of decreased mitochondrial chain function [11].

Materials and Methods

The study has been performed on 5 subjects (Table I); 4 patients, 2 males and 2 females, were affected with Chronic Progressive External Ophthalmoplegia (CPEO) and single large scale deletion of mtDNA and the other one, a 47 yr-old woman, with CPEO and multiple mtDNA deletions. The patients had previously been selected according to the following criteria:

i) results of muscle biopsy taken from the deltoid or the quadriceps, in particular the presence of ragged red fibers

Table I. Muscle biopsy results and exercise lactate values before (b) and after (a) idebenone treatment.

Patients (age/sex)	RRF*	COX-$	COX activity^	LT	LT lactate
(1)	3%	4%	82%	b 40%	b 4.55 (360)
26/M				a 40%	a 3.01 (201)
(2)	0%	0%	97%	b 50%	b 3.10 (267)
24/M				a 50%	a 2.82 (243)
(3)	4%	8%	55%	b 50%	b 4.58 (361)
45/F				a 50%	a 2.71 (213)
(4)	9%	6%	37%	b 50%	b 6.60 (528)
58/F				a 50%	a 2.65 (191)
(5)	7%	6%	69%	b 40%	b 4.04 (318)
47/F				a 50%	a 3.05 (291)

* % of ragged red fibers out of total number of fibers.
$ % of negative type 1 fibers.
^ measured on muscle isolated mitochondria and expressed as % of the average of normal controls (n = 12).
LT: lactate threshold power output (% of the pnPOmax).
LT lactate: blood lactate levels corresponding to the LT expressed in nmol/L and as normalized value (in parentheses).

(RRF) and cytochrome c oxidase negative (COX-) fibers, enzymatic activity alterations of respiratory chain complexes and mtDNA deletions. Methods used have been described elsewhere [17];

ii) mild severity of the skeletal myopathy;

iii) absence of cardiac and respiratory involvement.

All patients were ambulatory, one of them being also affected by insulin-dependent diabetes (case 2) and another one suffering from postural tremor on the left arm (case 1).

The study protocol was submitted for the approval to the University Institutional Board for Human Investigation and an informed consent was obtained from all the subjects.

The patients were orally treated with idebenone (Daruma, Lederle) at doses of 90 mg/day for 6 consecutive months. Routine blood examination was bimonthly performed to exclude collateral side effects of the treatment.

In basal conditions and after the treatment exercise lactate kinetics and CNS evoked potentials were evaluated as follows:

1) Exercise lactate kinetics

To assess exercise lactate kinetics of the patients, a submaximal test was performed starting from a low power output level and by progressively incrementing the load of the performance, according to a protocol already used in mitochondrial myopathy evaluation [18]. In such circumstances, the exercise is initially produced in aerobic conditions and then, as the exercise level reaches the anaerobic threshold, in anaerobic conditions.

After having determined the predicted normal maximal power output (pnPOmax) of the patient, corresponding to the maximal consumption of oxygen calculated on the basis of the anthropometric characteristics of the subject, the exercise was conducted at an increasing power output starting from 10% of the pnPOmax and reaching the exhaustion point, the incremental load being 10% of the pnPOmax each time. The patients performed a series of 3-minute exercise bouts, with intervening 2-minute rest intervals. Blood lactate was determined in venous samples from an antecubital vein in basal conditions and during each interstep interval.

Anaerobic threshold was calculated as lactate anaerobic threshold (LT), the exercise output level (% of the pnPOmax) at which the increment of lactate assumes an exponential trend [1], by applying logarithmic transform to lactate data.

Lactate values both absolute and normalized to the basal ones were compared to 5 age-matched control subjects.

One-way analysis of variance (Kruskal-Wallis test) was performed to compare lactate data between controls and patients before and after idebenone treatment. Wilcoxon test was utilised to compare, in the patients, lactate values corresponding to LT before and after the treatment.

2) Evoked potentials

In 3 cases, changes in CNS following idebenone intaking were assessed using Brainstem Auditory (BAEPs) and pattern Visual (VEPs) Evoked Potentials, performed either before and after 6 months of therapy.

BAEPs were recorded with the active electrode (clip) placed on the earlobe of the stimulated ear (A1 and A2) and the reference one in the Cz position (10-20 International System) using a silver/silver-chloride surface electrode 9 mm in dia, fixed with collodion. The ground electrode was taped on forehead. Acoustic stimuli consisted of alternating polarity clicks, lasting 0.1 msec, and with an intensity 60 dB above the acoustic threshold previously established, at a stimulus rate of 10 Hz. A continuous masking noise, 40 dB less than the stimulus intensity, being delivered to the controlateral ear. 2000 trials were averaged using a 1 Hz - 3 kHz filter bandpass. Responses were recorded twice to ensure relia-

bility. In the analysis of BAEPs waveforms, the absolute latency of wave I and the I-III, I-V, and III-V interpeak latencies were measured, as well as the IV-V complex/wave I amplitude ratio [12].

VEPs were obtained using as stimulus a black-and-white checkerboard pattern, at contrast level of 50% and at mean luminance of 70 cd/m2, counterphase modulated at 1 Hz (2 reversal/sec) [14]. The spatial frequency was 3 c/deg and all recordings were made with full-field monocular stimulation. The whole stimulating field subtended 14 x 12 deg of the visual field and subjects were seated 132 cm far from the monitor. Responses were recorded using the same silver/silver-chloride cup electrodes, 9 mm in dia, emploied for BAEPs recording, fixed over the scalp with collodion and filled with saline jelly: the active one 5 cm above the inion on the midline (Oz'), the reference one in Fpz' (= middle point between Fpz and Fz of 10-20 International System). The ground electrode was placed on the forearm.

For BAEPs and VEPs control values see reference 13.

Results

No patients but one, case 3, complained of side effects: the woman referred nausea and gastralgia, the reason why the treatment was dropped out for 15 days.

1) Exercise lactate kinetics

No significant difference was observed in baseline absolute lactate values between controls and patients before or after treatment.

Progressive increment of exercise lactate was greater in patients before treatment than in controls. Post-treatment exercise lactate levels were lower, but not significantly, with respect to pre-treatment condition (Fig. 1). While in controls, as expected, LT was not detected until 60% of the pnPOmax, in patients it occurred at 40 or 50% of the pnPOmax both before and after the treatment (Table I). No significant difference was present in normalized lactate values, corresponding to LT, between pre- and post-treatment condition. Lactate values corresponding to 60% of the pnPOmax showed reduction after the treatment: in fact the lactate increment at this level was significant higher, in comparison to controls, only before idebenone treatment ($p < 0.05$) and no more after (Fig. 1).

2) Evoked potentials

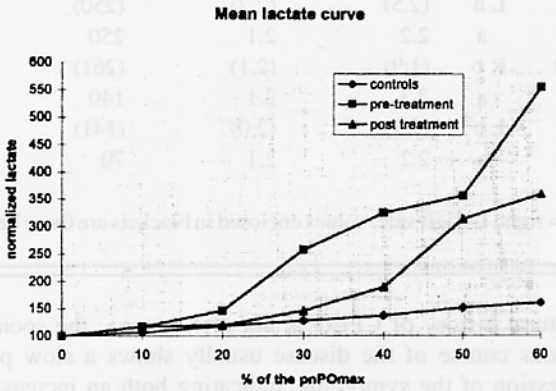
VEPs and BAEPs were abnormal in all the 3 tested cases in basal conditions. After the treatment, BAEPs showed in one case (case 1) loss of III and V waves, previously clearly evident; in the remaining 2 minimal worsening (right III-V interpeak prolongation in case 2 and bilateral IV-V wave amplitude reduction in case 3). Taking into account VEP recording, evidence of improvement was detected in cases 1 and 3; no clear changes for the last one (Table II).

Discussion

Idebenone is a therapeutic agent considered to be effective both in protecting cellular membranes from oxidative stress and enhancing efficacy of mitochondrial respiratory chain. The pharmacologic characteristics of idebenone suggest that it could be useful in the treatment of primary mitochondrial diseases such as OMM.

Figure 1. Mean normalized values of lactate during exercise. The comparison at 60% of the pnPOmax was significant ($p < 0.05$) between pre-treatment patients and controls.

	controls	*pre-treatment*	*post-treatment*
0	*100*	*100*	*100*
10	*106*	*117*	*117*
20	*118*	*146*	*119*
30	*128*	*257*	*146*
40	*137*	*324*	*189*
50	*151*	*356*	*313*
60	*160*	*555*	*359*



OMM are characterized by primary mitochondrial myopathy in skeletal muscle and often in other tissues, such as the CNS. MtDNA deletions are frequently the causative molecular events, as single large scale mutation in sporadic cases or as multiple deletions in dominant autosomal inherited ophthalmoplegia.

Biochemical consequences of the mtDNA mutation are responsible for an impairment of the aerobic cell metabolism. Such consequences of mtDNA mutations have been extensively investigated both at muscle histological and biochemical levels. The position of the deletion along the circular mtDNA molecule and, over all, its size can be determinant in the occurrence of a respiratory chain defect [8]. The size of the mtDNA deletion seems to play a fundamental role, considering that the deletion often involves tRNA genes, thus affecting the translation of mitochondria-encoded enzyme subunits [4].

Several therapeutic strategies have been adopted in OMM to compensate respiratory chain insufficiency to furnish ATP in the affected tissues. Contradictory results are reported in the literature [6]. In the absence of therapeutic approaches able to modify mtDNA content in the involved tissues, strategies that could limit the cellular relevance of the molecular defect could be useful in modifying the natural course of the disease. In fact, even if the

Table II. BAEPs and VEPs findings, obtained before (b) and after (a) idebenone intaking in 3 patients.

Case		BAEPs			VEPs				
		I-III (ms)	III-V (ms)	IV-V / I (%)	N70 amplitude (μV)	N70 latency (ms)	P100 amplitude (μV)	P100 latency (ms)	RCTs (ms)
(1)	Rb	(2.6)	(1.6)	(87)	(N.O.)	(N.O.)	(N.O.)	(N.O.)	(N.O.)
	a	2.5	N.O.	N.O.	1.23	120	1.68	146	98
	L b	(2.7)	(1.8)	(100)	(N.O.)	(N.O.)	(N.O.)	(N.O.)	(N.O.)
	a	2.6	N.O.	N.O.	0.9	114	0.72	130	N.O.
(2)	R b	(2.7)	(1.8)	(210)	(0.1)	(108)	(1.40)	(132)	(57)
	a	2.3	2.3	270	2.85	104	3.72	134	60
	L b	(2.5)	(2.2)	(250)	(0.4)	(108)	(1.10)	(154)	(51)
	a	2.2	2.1	250	1.62	116	1.50	150	72
(3)	R b	(1.9)	(2.1)	(261)	(0.8)	(120)	(1.32)	(164)	(88)
	a	2.3	2.1	140	2.73	98	4.77	128	58
	L b	(2.1)	(2.0)	(141)	(1.08)	(114)	(4.7)	(150)	(70)
	a	2.2	2.1	70	1.0	100	4.98	134	68

R = right, L = left side; values enclosed in brackets are those before treatment; RCTs: Retino-Cortical time; N.O. = responses not obtained.

natural history of CPEO is not well known, the spontaneous course of the disease usually shows a slow progression of the symptoms, indicating both an increasing damage to skeletal muscle and the possible involvement of other tissues. The latter aspect is sometimes observed in mitochondrial pathology and can explain the shifting from one syndrome to another as is the case of Kearns-Sayre syndrome reported as natural evolution, with increasing age, of Pearson's disease [7]. Events linked to the ageing process, such as: 1) modifications of the mtDNA deletion pattern, 2) the accumulation of spontaneous mutations or 3) oxygen free radicals production could theoretically be involved in this progression [5].

The putative pharmacologic properties of the idebenone derive from its chemical structure, analogous to physiological ubiquinone, to which it competes for electron redox exchange. In fact an imbalance in the electron transport chain between ubisemiquinone and cytochrome b566 with respect to cytochrome aa3, can occur in mitochondrial diseases mainly due to cytochrome c oxidase deficiency [10]. This imbalance can drive oxygen free radical formation and consequently production of hydroperoxides and the deleterious lipid peroxides. These events can in turn be responsible for increased susceptibility of mtDNA to further mutation [15] and enhance the effects of the natural aging process on mtDNA.

In literature there are only sporadic reports on the use of idebenone in mitochondrial encephalomyopathies: in one case a subject affected with MELAS syndrome was successfully treated with this drug [3].

In this study, performed on 5 patients affected with OMM, the effects of 6 month idebenone therapy were evaluated by determining the exercise LT, as expression of aerobic metabolic efficiency during contraction, and some aspects of CNS function as described by BAEPs and VEPs.

As far as lactate increase during incremental submaximal exercise is concerned, no significant modification was observed after the treatment as deducted by the calculation of LT. However it has to be mentioned that lower lactate values were detected after the treatment, especially at the end of the exercise protocol used.

Concerning the results obtained by means of evoked potentials it has to be pointed out that, while in one case BAEPs showed worsening, clear improvement in 2 cases, over the three evaluated, was observed by means of VEPs.

In interpreting these results several points have to be considered. First of all the pharmacokinetics of the idebenone which indicates that the drug can reach high concentration in the cerebral tissues, owing to its lipid solubility [20]. On the contrary, at our knowledge, there are no data which indicate its concentration in the skeletal muscle tissue. A low distribution into the skeletal muscle could justify the small effects observed in exercise LT, considering that in the experimental animal tissues idebenone is able to reduce lactate increment in some pathological conditions [9].

The controversial effects of idebenone treatment on evoked potentials could be related to the different severity of the disease among the subjects tested. In fact the patient in which BAEPs worsened was that one with clearest evidence of CNS involvement. In this sense it would be interesting to compare idebenone effects to evoked potentials in the larger group of patients with mitochondrial myopathy, published in an our previous paper [13]. On the other hand, VEPs results indicate a clear improvement on visual performance, which is frequently involved in mitochondrial diseases.

Obviously depending on the too much exiguous number of patients examined in this study, the results obtained are far to be considered definitive. Nevertheless, both the improvement found in VEPs testing, even if modest, and the different behaviour showed by VEPs and BAEPs, seem worth being verified on further studies and more reliable conditions, first of all a more extended group of patients.

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