

R. Artuch¹
A. Aracil²
A. Mas³
E. Monrós⁴
M. A. Vilaseca¹
M. Pineda²

Cerebrospinal Fluid Concentrations of Idebenone in Friedreich Ataxia Patients

Abstract

We studied plasma and cerebrospinal fluid (CSF) concentrations of idebenone in five Friedreich ataxia patients on treatment with this antioxidant, and plasma and CSF ubiquinone-10 (Q₁₀) concentrations in 15 controls. CSF idebenone concentrations were below the detection limit in 3 Friedreich ataxia patients and no association could be demonstrated between plasma and CSF idebenone values. Q₁₀ CSF concentrations (median: 2.25 nmol/L) were approximately 300 times lower than those of plasma (median: 0.77 μ mol/L). No correlation was observed between plasma and CSF Q₁₀ concentrations. A significantly positive correlation was observed between CSF total protein values (range 8.1–107.5 mg/dL; median: 29.5) and CSF Q₁₀ concentrations (Spearman test: $r=0.664$; $p=0.01$). Our findings suggest that less idebenone is distributed to the brain than to other tissues, although CSF does not appear to be an appropriate material for treatment monitoring of idebenone and other quinoid compounds.

Key words

Friedreich ataxia · idebenone · ubiquinone-10 · cerebrospinal fluid

Introduction

Idebenone (6-[10-hydroxydecyl]-2,3-dimethoxy-5-methyl-1,4-benzoquinone) is a quinone analogue with antioxidant properties which is used in the treatment of several neurological disorders: Friedreich ataxia (FRDA: OMIM 229300) [17], mitochondrial encephalomyopathies [11,15], and senile dementia [7,14]. Idebenone diffuses more rapidly than ubiquinone-10 (Q₁₀) across biological membranes, owing to the modification of the composition and length of its side chain [17]. Among biological membranes, the blood-brain barrier (BBB) restricts the diffusion of many drugs, although lipid-soluble drugs are probably not subject to any restriction, especially those with a molecular mass under 400–600 Da [13]. According to previously reported data, idebenone should be able to pass through the BBB because i) its effectiveness in the treatment of central nervous system disorders has been demonstrated [1,7,11,15], and ii) it has been detected in rat brain after idebenone administration [10,21]. However, in spite of the growing body of evidence for the effectiveness of idebenone treatment in central nervous system disorders in humans, its transport across the BBB remains obscure and difficult to demonstrate and, to the best of our knowledge, no data regarding this subject have been published. Furthermore, several authors reported no neurological improvement in FRDA patients after idebenone treatment [2,9], although ultrasound cardiac measurements improved.

Affiliation

¹ Biochemistry Department, Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain

² Neurology Department, Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain

³ Pharmacy Department, Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain

⁴ Genetics Department, Hospital Sant Joan de Déu, University of Barcelona, Esplugues, Spain

Correspondence

Rafael Artuch · Biochemistry Department, Hospital Sant Joan de Déu · Passeig Sant Joan de Déu 2 · 08950 Esplugues Barcelona · Spain · E-mail: rartuch@hsjdbcn.org

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Our aims were to determine plasma and CSF concentrations of idebenone in FRDA patients being treated with this antioxidant in order to determine whether it passes through the BBB, and to analyze other CSF quinoid compounds (Q_{10}) which occur naturally in the central nervous system in control populations.

Materials and Methods

Subjects

We studied five genetically confirmed FRDA patients (age range 11–19 years, median 15), with intronic GAA repeat expansions ranging from 600 to 790 (Table 1). All patients had been receiving idebenone treatment (10 mg/kg/day divided into 3 doses) throughout the previous year. Plasma samples from patients were obtained before and after the oral doses of idebenone, while CSF was collected only after the oral doses (Table 1), except in the case of patient 2 (who underwent surgery). We also analyzed plasma and CSF samples from a control population of 15 pediatric patients (age range 9–18 years, median 13) sent to our laboratory, to rule out viral or bacterial meningitis. The exclusion criteria were microbiological diagnosis of infectious diseases, the presence of chronic disorders and/or pharmacological treatment. All FRDA patients subjected to this study were volunteers, and informed consent was obtained. Samples from patients and controls were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2000. The Clinical Ethics Committee of the Hospital Sant Joan de Déu approved the study.

Methods

For idebenone and Q_{10} determination, all plasma and CSF samples were immediately frozen at -80°C until the time of analysis. CSF cell count (leukocytes, erythrocytes) and biochemical parameters (glucose and protein concentrations) were analyzed in the 5 FRDA patients and the 15 control samples in the emergency laboratory, according to standard procedures.

Plasma and CSF idebenone concentrations in the 5 FRDA patients were measured by reverse-phase HPLC (Series 200, Perkin-Elmer, Norwalk, CT, USA) with electrochemical detection (Coulchem II, ESA, Chelmsford, MA, USA) according to a previously reported procedure [1]. Plasma and CSF Q_{10} concentrations from the 15 controls were determined by reverse-phase HPLC with electrochemical detection, according to a previously reported procedure [3]. Four hundred μL of CSF were used for both idebenone and Q_{10} determination. The molecular genetic analysis of FRDA patients was performed as previously reported [1].

Statistical analysis

Data were expressed as median and range. The Spearman test was applied for simple correlation studies in the control group. Statistical studies were performed using the SPSS program, version 11.0.

Results

Plasma and CSF values of the different variables studied in the five FRDA patients are summarized in Table 1. In 3 patients, CSF idebenone concentrations were below the detection limit of the

Table 1 Biochemical results of plasma and CSF samples from the 5 FRDA patients. Idebenone concentrations are expressed as nmol/L

Pat.	GAA repeats	Baseline idebenone		Time after dose (minutes)	Post-dose idebenone	
		Plasma	CSF		Plasma	CSF
1	650	335	–	140	2520	n.d.
2	790	116	n.d.	138	870	8.0
3	680	372	–	120	1290	n.d.
4	600	274	–	80	853	12.5
5	710	77	–	40	140	n.d.

n.d.: non detectable

procedure (2.5 nmol/L). No association could be demonstrated between plasma and CSF idebenone values or between the number of GAA repeats and idebenone values. CSF protein concentrations (range 6.1–27.1 mg/dL; median 13.5) did not correlate with idebenone values. With respect to other quinoid compounds in the 15 controls, plasma Q_{10} concentrations ranged between 0.55–1.01 $\mu\text{mol/L}$ (median: 0.77), while median Q_{10} CSF values were approximately 300 times lower than those for plasma (range 1.18–4.91 nmol/L; median 2.25). No correlation was observed between plasma and CSF Q_{10} concentrations. A significantly positive correlation was observed between CSF total protein values (range 8.1–107.5 mg/dL; median: 29.5) and CSF Q_{10} concentrations (Spearman test: $r = 0.664$; $p = 0.01$).

Discussion

Increased oxidative damage has been proposed as a causative mechanism for several neurological disorders, including FRDA [17]. Idebenone, a lipophilic antioxidant used in the treatment of this disease, has shown a protective effect against free radical damage in rat brain tissue [8]. Furthermore, idebenone has been detected in rat brain after treatment [10,21]. In FRDA patients, the effect of idebenone on neurological symptoms is controversial, and a mild improvement in the international cooperative ataxia rating scale [19] has been reported in a series of young patients [1]. Although all these data suggest that idebenone passes readily through the BBB, no evidence for this hypothesis has been reported in humans. In fact, several authors observed no neurological improvement after idebenone therapy in FRDA patients, although they reported significant reductions of interventricular septal thickness and left ventricular mass [2,9]. These tissue differences may reflect distribution of idebenone to the brain in smaller quantities than to the heart [2]. However, no plasma idebenone concentration monitoring was reported in these sources. According to our previous experience, plasma idebenone concentrations in FRDA patients taking 5 mg/kg/day showed a wide range of values [1]. Furthermore, higher plasma idebenone values were associated with mildly improved neurological functions in our FRDA patients. This dispersion suggested that some patients may respond better than others, probably as a consequence of idebenone availability. Nevertheless, we also observed non-progression of myocardiopathy in our group of patients [1].

supporting the hypothesis that, under idebenone therapy, the heart probably responds better than the CNS in FRDA patients.

According to our present results, only two patients showed CSF concentrations around the detection limit of our procedure and these values were 100fold lower than those observed in plasma. Therefore, no association could be established between plasma and CSF idebenone concentrations. As previously reported [14,15], plasma idebenone values were significantly higher in the sample collected after the oral dose, while this effect was not observed in CSF concentrations. Furthermore, owing to the undetectable CSF idebenone concentrations in three patients, no relationship could be observed between CSF idebenone and the number of GAA repeats or the CSF protein concentration. All these observations might suggest that idebenone does not pass easily through the BBB; however, we should take into account that idebenone and other lipophilic antioxidants (tocopherol, Q_{10}) may be efficiently transported only by proteins [5,16]. In CSF, the concentration of proteins is much lower than that of plasma [18], and these might be the most important determinants of CSF idebenone concentrations.

All these data led us to determine the concentrations of CSF Q_{10} , which is a quinone synthesized in almost all tissues, including the central nervous system. To our knowledge, no data regarding CSF Q_{10} values have been reported. According to our results, CSF Q_{10} values were 300 times lower than those observed in plasma, and no association between Q_{10} values in the two specimens could be demonstrated. The positive correlation observed between CSF Q_{10} and total protein concentrations would support the hypothesis that Q_{10} CSF values depend mainly on protein concentration. Consequently, we might assume that Q_{10} (and other quinoid compounds) pass through the BBB, but CSF is not an appropriate specimen for lipophilic antioxidant monitoring. Therefore, the CSF/plasma ratio of idebenone or Q_{10} would not be representative for the blood-brain passage of these compounds.

Similar observations have previously been reported in several studies involving CSF tocopherol, which was also extremely low compared to plasma concentrations [12]. While some authors demonstrated increased CSF tocopherol values after treatment with this vitamin [20], other authors could not confirm this result [12], so the data has remained controversial. As in the case of idebenone and Q_{10} , CSF does not seem to be an appropriate specimen for monitoring tocopherol treatment, since CSF concentrations probably depend on the lipoprotein concentration rather than on passage through the BBB.

Some heredo-ataxic syndromes are caused by primary Q_{10} deficiency or by isolated tocopherol deficiency. Furthermore, neurological signs and symptoms of both disorders may be partially corrected by oral Q_{10} [4] and tocopherol treatment [6], supporting the hypothesis that lipophilic antioxidants may readily cross the BBB.

In conclusion, in view of the previous findings and of data reported here, it is probable that less idebenone is distributed to the brain than to the heart. However, it is also probable that idebenone crosses the BBB, although CSF is not an appropriate material for idebenone treatment monitoring. This observation would

be valid for other quinoid compounds and lipophilic antioxidants such as Q_{10} .

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