ELSEVIER

Journal of Neuroscience Methods 115 (2002) 63–66

JOURNAL OF NEUROSCIENCE METHODS

www.elsevier.com/locate/jneumeth

Monitoring of idebenone treatment in patients with Friedreich's ataxia by high-pressure liquid chromatography with electrochemical detection

Rafael Artuch [a,*], Catrina Colomé [a], Maria Antònia Vilaseca [a], Asun Aracil [b], Mercè Pineda [b]

[a] *Serveis de Bioquímica, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, Universitat de Barcelona, 08950 Esplugues, Barcelona, Spain*
[b] *Serveis de Neurologia, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain*

Received 12 October 2001; received in revised form 17 December 2001; accepted 18 December 2001

Abstract

Idebenone is a quinone analog that is applied in the treatment of several neurological disorders including Friedreich ataxia and mitochondrial encephalomyopathies. Our aim was to develop an easy and sensitive analytical HPLC-procedure for the determination of idebenone in the serum of patients treated with this drug. Serum samples from nine paediatric patients diagnosed with Friedreich ataxia and receiving idebenone treatment were analyzed. Idebenone was separated from serum by reverse high-pressure liquid chromatography and analyzed using an electrochemical detection procedure. No interferences were observed during analysis of patient samples obtained prior to idebenone treatment. Calibration of idebenone concentration indicated a linear range between 500 pmol/l and 5 μmol/l and calculation of within-run and between-run coefficients of variation suggested adequate analytical quality for reliable determination. In agreement with previously reported data, during drug therapy, idebenone serum concentrations (basal conditions, range 0.1−0.49 μmol/l) were greatly elevated 90 min after an oral dose (range 0.66–3.63 μmol/l). Thus, we have developed a simple and rapid method that offers adequate analytical quality for accurate idebenone determination.

Keywords: Idebenone; Ubiquinone; Friedreich ataxia; HPLC; Electrochemical detection; Pediatric patients

1. Introduction

Idebenone ((6-)10-hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone) is a quinone analog which is used in the treatment of several neurological disorders: Friedreich ataxia (Rustin et al., 1999a,b; Schöls et al., 2001), mitochondrial encephalomyopathies (Pisano et al., 1996; Napolitano et al., 2000) and senile dementia (Wakabayashi et al., 1992). Idebenone acts as an antioxidant through the action of its quinone ring and diffuses more rapidly than ubiquinone across biological membranes owing to the modification of the composition and length of its side chain (Gillis et al., 1994). These properties allow idebenone to reach the tissues more easily than ubiquinone and efficiently protect cells from peroxidative damage (Rustin et al., 1999a,b).

Several procedures have been developed to analyze quinone compounds such as ubiquinone (Finckh et al., 1995; Artuch et al., 1998), although there are few reports concerning idebenone determination (Wakabayashi et al., 1992; Pisano et al., 1996; Hu et al., 2000). Our aim was to develop an easy and sensitive analytical HPLC procedure for the determination of idebenone in serum of patients receiving treatment with this drug.

2. Materials and methods

2.1. Patients

Idebenone was analyzed in the serum of nine paediatric patients diagnosed with Friedreich ataxia (five males

* Corresponding author. Tel.: +34-93-280-4000; fax: +34-93-280-3626.
E-mail address: vilaseca@hsjdbcn.org (R. Artuch).

0165-0270/02/$ - see front matter
PII: S0165-0270(01)00533-7

and four females). Ages ranged from 10 to 18 years; median 15 years. Samples were first analyzed before the start of the therapy. Once the treatment was applied (5 mg/kg per day p.o. during 12 months), two samples were analyzed in each patient: The first one just before an oral dose to monitor the minimum serum concentrations, and the second sample 90 min after the oral dose of idebenone to determine the maximum concentration, according to the pharmacokinetic parameters reported by Pisano et al. (1996). Serum samples were obtained by venous blood puncture, centrifuged at $2000 \times g$ (10 min at 4 °C) and stored in darkness at −70 °C until the moment of the analysis.

This study was performed in accordance with the Helsinki Declaration of 1975, as revised in 1996, and was approved by the ethics committee of the Hospital Sant Joan de Déu.

2.2. Reagents

The idebenone calibrator was a generous gift from Takeda Chemical Industries LTD. About 500 pmol/l–5 μmol/l of idebenone dissolved in methanol (Romil-SpS, Cambridge, UK) was used for calibration and linearity studies. They were stored in the same conditions as serum samples. A 5 μmol/l idebenone solution was stable for at least 1 month.

2.3. Sample preparation

Idebenone was extracted from 50 μl of serum. About 25 μl of 4 mol/l HCl (Merck, Darmstadt, Germany) was added to hydrolyze serum samples (vortex 1 min). After incubation at 60 °C for 20 min, 1 ml of *n*-hexane (Romil-SpS) was added to extract idebenone (vortex 1 min). The sample was then centrifuged at $2000 \times g$ (10 min, 4 °C) and 0.8 ml of the hexane layer were recovered, filtered (0.45 μm filters, Teknokroma) and evaporated under nitrogen stream. Finally, the desiccated sample was stored at −70 °C and diluted in 250 μl of mobile phase (80% methanol, 20% water with 20 mmol/l of lithium perchlorate) just before the start of the analysis. Calibrator solutions (50 μl) were added to control serum and treated like the samples. Then, 30 μl of both samples and calibrators were injected onto the chromatograph.

2.4. Chromatographic conditions

Idebenone was separated by reverse HPLC (Serie 200, Perkin–Elmer, Norwalk, CT, USA). Column: Nucleosil C18 (5 μm, 25×0.4 cm, Teknokroma). Mobile phase: 80% methanol, 20% MiliQ-water containing 20 mmol/l of 95% lithium perchlorate (Sigma-Aldrich, Steiheim, Germany). It was prepared daily and filtered with 0.45 μm nylon membrane filters (Teknokroma). Flow: 1.2 ml/min of mobile phase passed through the column at room temperature. Total chromatographic time: 20 min.

Once separated, idebenone was analyzed by electrochemical detection (Coulochem II, ESA, Chelmsford, MA, USA) with one analytical cell (model 5010, ESA). The first electrode was attached at −400 mV to reduce the oxidized idebenone, while the analytical electrode voltage was +200 mV to oxidize the reduced idebenone. Gain 20 nA. Chromatographic data were managed using the Turbochrom Navigator Program (Perkin–Elmer).

3. Results

Under these conditions, idebenone retention time was 7.5 min. Hydrodynamic voltammogram for idebenone showed a maximum rate of oxidation at +200 mV. No increment in idebenone response was observed in increasing the potential of the analytical electrode up to +600 mV.

The calibration curve of idebenone was linear over the range 500 pmol/l–5 μmol/l. Within-run analytical coefficients of variation for idebenone were 7% (0.1 μmol/l), 6.3% (0.5 μmol/l) and 5.7% (1 μmol/l). Between-run coefficient of variation was 8.7% (0.5 μmol/l). No interferences were observed when analyzing patients samples prior to treatment (Fig. 1A). After the start of the therapy, idebenone concentrations ranged between 0.1 and 0.49 μmol/l, median 0.29 μmol/l in basal conditions (just before the oral dose, Fig. 1C). About 90 min after the oral doses, idebenone ranged between 0.66 and 3.63 μmol/l, median 1.31 μmol/l (Fig. 1D).

4. Discussion

Idebenone has been effective in the treatment of several neurological disorders (Rustin et al., 1999a,b; Pisano et al., 1996). Several reports about the determination of quinone compounds have been published. However, the analysis of idebenone concentrations has scarcely been reported. A HPLC with electrochemical detection procedure was reported by Wakabayashi et al. (1992). This procedure is of sufficient quality for idebenone determination, especially for the accurate quantification of the residual concentrations. It uses a platinum catalyst reduction prior to the detection of idebenone. Moreover, the potential of the analytical cell is +700 mV in contrast to the potential applied in our method, which is much lower. Under our conditions, the stability of the baseline was achieved in only 15 min. Moreover, we did not observe the presence of interfering materials. When increasing the potential to +600 mV, several unknown metabolites appeared, complicating the resolution of idebenone. Furthermore, with this

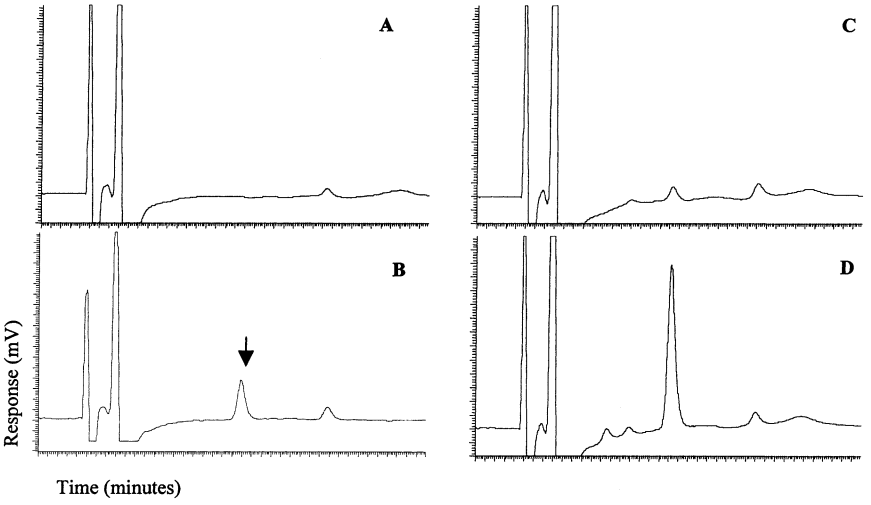

Fig. 1. (A) Chromatogram of a control serum sample. (B) Chromatogram of a control serum sample plus 50 μl of 1 μmol/l calibrator solution. The arrow indicates the idebenone peak. (C) Chromatogram of a patient on treatment in basal conditions (0.28 μmol/l) and (D) 90 min after the oral dose (3.24 μmol/l).

procedure only one electrochemical cell is used for the analysis of idebenone.

Another HPLC with ultraviolet detection procedure was developed by Pisano et al. (1996). In this report, the extraction of plasma idebenone was optimized. We used similar conditions to those reported by these authors. However, we tested ultraviolet detection after chromatographic separation of idebenone. In these conditions, at least 400 μl of serum was required to measure idebenone concentrations reliably, in contrast to the 50 μl used by our HPLC with electrochemical detection procedure. Moreover, the detection of the serum idebenone residual concentrations was difficult, at least in our experience. The detection limits obtained with ultraviolet detection systems are much higher than those obtained by electrochemical detection (Bowers, 1984).

Recently, Hu et al. (2000) reported an HPLC method coupled to mass spectrometric detection for quantification of idebenone. This method did not offer a better detection limit than electrochemical detection. Moreover, this is a sophisticated analytical procedure that is not readily available in most laboratories.

We tried to use an internal standard for idebenone analysis. However, all of the internal standards used (coenzymes Q1, Q2, Q3 and Q4, and butylated hydroxytoluene from Sigma) had a maximum response at higher voltages than idebenone. Such an increment in the voltage affected the resolution of the chromatograms because of the appearance of interfering compounds. The coefficient of variation obtained without internal standardization suggest that the analytical quality is adequate.

There are few reports about idebenone monitoring during treatment of neurological disorders with this drug. The results reported in our group of patients receiving idebenone treatment are in agreement with other reported series (Pisano et al., 1996).

In conclusion, this method is simple and rapid, and it offers adequate analytical quality for idebenone determination.

Acknowledgements

This work was supported by a grant (FIS: 98/0049-01). We are very grateful to Montserrat Gallart for her skilful technical assistance.

References

Artuch R, Moreno J, Quintana M, Puig RM, Vilaseca MA. Serum ubiquinone-10 in pediatric population. Clin Chem 1998;44:2378–9.

Bowers LD. Liquid chromatography. In: Kaplan LA, Pesce AJ, editors. Clinical Chemistry, Theory, Analysis, and Correlation. St Louis: The CV Mosby Company, 1984:100–17.

Finckh B, Kontush A, Commentz J, Hübner C, Burdelski M, Kohlschütter A. Monitoring of ubiquinol-10, ubiquinone-10, carotenoids, and tocopherols in neonatal plasma microsamples using high-performance liquid chromatography with coulometric electrochemical detection. Anal Biochem 1995;232:210–6.

Gillis JC, Benfield P, McTavish D. Idebenone. Drugs and Aging 1994;5:133–52.

Hu P, Jiang J, Xie HH. Determination of idebenone in plasma by HPLC/MS. Acta Pharmacol Sin 2000;21:306–8.

Napolitano A, Salvetti S, Vista M, Lombardi V, Siciliano G, Giraldi C. Long-term treatment with idebenone and riboflavin in a patient with MELAS. Neurol Sci 2000;21:S981–2.

Pisano P, Durand A, Autret E, Desnuelle C, Pinsard N, Serratrice G, et al. Plasma concentrations and pharmacokinetics of idebenone and its metabolites following single and repeated doses in young patients with mitochondrial encephalomyopathy. Eur J Clin Pharmacol 1996;51:167–9.

Rustin P, Munnich A, Rötig A. Quinone analogs prevent enzymes targeted in Friedreich ataxia from iron-induced injury in vitro. Biofactors 1999;9:247–51.

Rustin P, von Kleist-Retzow JC, Chantrel-Groussard K, Sidi D, Munnich A, Rötig A. Effect of idebenone on cardiomyopathy in Friedreich's ataxia: a preliminary study. Lancet 1999;354:477–9.

Schöls L, Vorgerd M, Schillings M, Skipka G, Zange J. Idebenone in patients with Friedreich ataxia. Neurosci Lett 2001;306:169–72.

Wakabayashi H, Nakajima M, Yamato S, Shimada K. Determination of idebenone in rat serum and brain by high-performance liquid chromatography using platinum catalyst reduction and electrochemical detection. J Chromatogr 1992;573:154–7.