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Short Communication

Determination of idebenone in rat serum and brain by high-performance liquid chromatography using platinum catalyst reduction and electrochemical detection

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ABSTRACT

A high-performance liquid chromatographic determination of idebenone, a new cerebral metabolism-improving agent, in rat serum and brain has been developed. After separation of idebenone on a reversed-phase column, idebenone was reduced once in a platinum catalyst reduction column connected on-line, then monitored quantitatively by electrochemical detection. A linear relationship between the peak-height ratio of idebenone to the internal standard and idebenone concentration was observed in the range 0.015–50 ng with a detection limit of 5 pg (signal-to-noise ratio = 5). This method was satisfactorily rapid and sensitive, and was successfully applied to the determination of idebenone in rat serum and brain tissues.

INTRODUCTION

6-(10-Hydroxydecyl)-2,3-dimethoxy-5-methyl-1,4-benzoquinone (idebenone, Fig. 1), a new agent which improves cerebral metabolism and has effects on neurological deficits caused by cerebral vascular disorders, has been used clinically for the treatment of senile dementia.

Several reports concerned with the determination of quinone compounds have appeared [1–3]. The ubiquinones and ubiquinol (reduced form) have been determined using UV and electrochemical detection (ED) [4], and both have also been determined by high-performance liquid chromatography (HPLC) with ED [5,6]. Vitamin K_1 has been determined using fluorometric detection after post-column electrochemical reduction [7]. There are a few methods for the analysis of idebenone, the details of which are unknown.

If idebenone is hydrogenated using a platinum catalyst, the quinone ring can be reduced to form the corresponding hydroquinone compound. This hydroquinone derivative is extremely susceptible to oxidation. By connecting the reduction column to a chromatographic column, a more rapid and effective analytical method can be attained.

In this paper, a rapid and sensitive analytical procedure for the determination of idebenone in rat serum and brain tissue using a reduction column has been developed.

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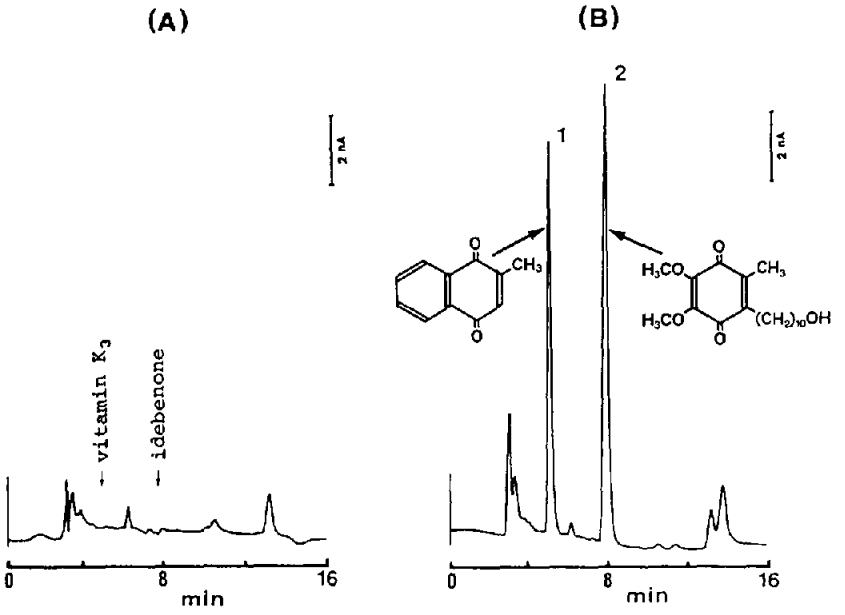

Fig. 1. Typical chromatograms of (A) drug-free serum and (B) the serum taken from idebenone-administered (50 mg/kg) rat. Peaks: 1 = vitamin K_3 (2.0 ng); 2 = idebenone (2.4 ng).

EXPERIMENTAL

Reagents

Crystalline idebenone and its reduced compound were provided by Takeda (Osaka, Japan). All other chemicals and solvent employed were of analytical reagent or HPLC grade, and were used without further purification.

Stock solutions (10 mg per 10 ml) of idebenone and vitamin K_3 were prepared by dissolving each in ethanol (HPLC grade) and diluted to appropriate concentrations prior to use. The stock solutions were shielded from light during storage.

Apparatus and chromatographic conditions

The equipment consisted of an LC-6A HPLC system (Shimadzu, Kyoto, Japan) and a Rheodyne 7125 injection valve (Cotati, CA, USA) with a 100-μl loop. The column was a Shim-pack CLC-ODS (5 μm particle size, 150 mm × 6 mm I.D.) (Shimadzu). The electrochemical detector was an EICOM ECD 100 (Eicom, Kyoto, Japan) with a glassy carbon working electrode and an Ag/AgCl reference electrode. The applied oxidation potential was set at +0.7 V.

The chromatograms were obtained at ambient temperature. As the mobile phase, 85% methanol containing 0.05 *M* sodium perchlorate was used. The flow-rate of the mobile phase was 1.0 ml/min. The data were recorded and evaluated with a Shimadzu C-R3A recorder. A stainless-steel column (10 mm × 4.6 mm I.D.) was uniformly packed with platinum catalyst (5% on alumina, 10 μm, provided by Toa Electronics, Tokyo, Japan) by tapping, and purged with water at a flow-rate of 10 ml/min for 5 min.

Sample preparation of rat serum and brain tissue

Idebenone was suspended in 5% gum arabic solution to make a concentration of 25 mg/ml. The suspension was injected intraperitoneally (50 mg/kg) into male Wistar rats, weighing 250–300 g. The rats were sacrificed by decapitation 1 h after the drug administration. Blood was collected by venipuncture into centrifuge tubes, and centrifuged at 1500 *g* for 10 min at room temperature. The sera thus obtained were stored at −80°C until assayed. Brains were quickly removed, and the tissues of cortex, hippocampus, striatum and cerebellum were separated.

Assay of idebenone in rat serum and brain tissue

To 0.1 ml of serum, 10 ng of vitamin K_3 (10 ng per 100 μl of ethanol) as an internal standard

were added with shaking. To the mixtures, 4 ml of cyclohexane–benzene (1:4, v/v) were added and extracted by vortex-mixing for 2 min. To 50–100 mg of the brain tissues, 0.5 ml of 0.1 *M* perchloric acid and 2 ng (2 ng per 20 μl of ethanol) of vitamin K_3 as an internal standard were added. The tissues were homogenized in a glass homogenizer at 4°C. To the homogenates, 6 ml of cyclohexane–benzene (4:1, v/v) were added with mixing for 2 min. Extracts of serum or brain homogenate were centrifuged at 1500 *g* for 10 min. The organic layers were evaporated to dryness under nitrogen gas. The residue was dissolved in 100 μl of ethanol. The ethanolic solution was then filtered through the Column Guard (Millipore, 0.45 μm), and a 20-μl aliquot of the clear filtrate was injected into the HPLC system.

Recovery test

Aliquots of 1, 10 and 20 ng of each idebenone as an ethanolic solution were added to the normal rat serum or brain. The procedures followed were as described above.

RESULTS AND DISCUSSION

Optimization of HPLC–ED conditions

The maximal response for the determination of idebenone was obtained at an applied potential of +0.7 V. The combination of a 85% methanol, 0.05 *M* sodium perchlorate and the flow-rate of 1.0 ml/min gave the best retention time, peak height and separation factor for idebenone and vitamin K_3. Variations in the flow-rate have a greater influence on the electrochemical response than variations in methanol concentration.

The platinum catalyst was most effective in reducing idebenone at a column length of 10 mm, compared with 30 and 50 mm. This phenomenon is the result of the peak broadening that occurs when idebenone passes through the longer reduction columns. The relationship between reduction ability and idebenone was linear in the range approximately up to 2000 ng of idebenone.

HPLC of idebenone

Idebenone and vitamin K_3 have retention times of 7.8 and 4.9 min, respectively, and both materials were clearly separated [resolution (R_S) = 5.4].

The calibration curve for idebenone was constructed by plotting the peak-height ratio of idebenone against the internal standard. The calibration curve for idebenone was linear over the range 0.015–50.0 ng per 20-μl injection; its concentration in sera was usually in the range 100–300 ng/ml [8]. The detection limit was found to be 5 pg for idebenone (signal-to-noise ratio = 5).

The HPLC method based on ED is more sensitive (100-fold) and selective than the previous UV (275 nm) detection method. The within-day and day-to-day relative standard deviations (R.S.D.s) were 3.5% (n = 10) and 4.1% (n = 10), respectively.

Extraction of idebenone and vitamin K_3 from the serum and brain was examined with several solvents: chloroform, diethyl ether, benzene, cyclohexane and hexane. The most effective extraction solvents were found to be cyclohexane–benzene (1:4, v/v) for serum and cyclohexane–benzene (4:1, v/v) for brain tissue.

Recovery of idebenone spiked to rat serum and brain

The recovery rates for idebenone spiked to serum and whole brain at three different concentrations were more than 96% (R.S.D. 4.9%, n = 10) and 91.0–94.0% with R.S.D.s of 4.2–5.6% (n = 10), respectively (Table I).

TABLE I

RECOVERIES OF IDEBENONE SPIKED TO RAT SERUM AND BRAIN

Idebenone added	Recovery (mean ± S.D., n = 10) (%)	R.S.D. (%)
Rat serum (ng per 0.1 ml)		
1.0	96.4 ± 4.7	4.9
10.0	98.7 ± 3.0	3.0
20.0	98.3 ± 2.9	3.0
Rat brain (ng per 0.1 g)		
1.0	91.1 ± 5.1	5.6
10.0	93.4 ± 3.9	4.2
20.0	94.0 ± 4.1	4.4

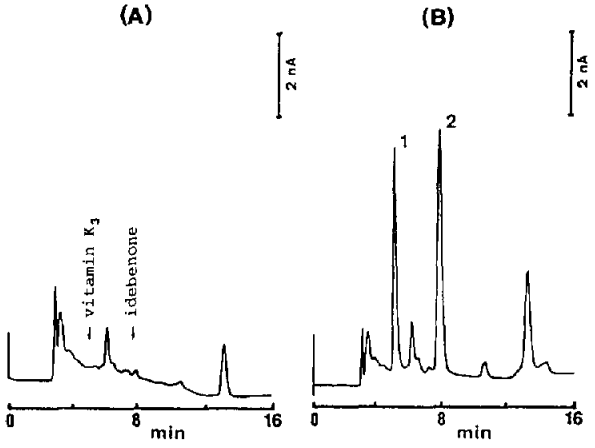

Fig. 2. Chromatograms of (A) drug-free brain and (B) cerebellum taken from an idebenone-administered (50 mg/kg) rat. Peaks: 1 = vitamin K_3 (0.5 ng); 2 = idebenone (0.7 ng).

Determination of idebenone in rat serum and brain tissue after intraperitoneal administration of idebenone

Fig. 1 shows typical chromatograms of drug-free serum and the serum taken from an idebenone-administered rat. Several unknown peaks were observed on the chromatograms of the drug-free serum, but no interference was encountered for the assay of idebenone. The serum idebenone level in rats 1 h after an intraperitoneal injection of idebenone (50 mg/kg) was found to be 252 ng per ml of serum. The chromatograms of drug-free brain and brain tissue taken from the idebenone-administered (50 mg/kg) rat are shown in Fig. 2, and the idebenone contents are shown in Table II. These values correspond well with those obtained from the literature [9].

TABLE II

BRAIN DISTRIBUTIONS AFTER INTRAPERITONEAL ADMINISTRATION (50 mg/kg) OF IDEBENONE IN NORMAL RAT

Brain tissue	Found (mean ± S.D., n = 5) (ng/g of tissue)
Cortex	87.6 ± 9.6
Hippocampus	21.4 ± 9.8
Striatum	54.5 ± 5.3
Cerebellum	64.3 ± 10.9

Reduced form of idebenone

6-(10-Hydroxydecyl)-2,3-dimethoxy-5-methylhydroquinone, a reduced form of idebenone, is one of its metabolites [10]. The reduced hydroquinone is unstable in air and light. When an equal volume of 0.4% ascorbic acid was first added to a volume of rat serum, the recoveries (95.7–98.4%) of the reduced form of idebenone were satisfactory, and satisfactory separation of idebenone and its reduced form (retention time 6 min) was also achieved with the proposed method. At 15 min after the administration, the concentration of idebenone was 110 ± 21 ng/ml; that of the reduced form was 22 ± 8 ng/ml (n = 2).

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