Eur J Clin Pharmacol (1996) 51 : 167–169

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Plasma concentrations and pharmacokinetics of idebenone and its metabolites following single and repeated doses in young patients with mitochondrial encephalomyopathy

Received: 2 January 1995 / Accepted in revised form: 1 April 1996

Abstract *Objective*: The pharmacokinetics and tolerance of idebenone after single or repeated doses have been studied in young patients with mitochondrial encephalomyopathy.
Results: No significant adverse effects were noted. In 3 out of 7 patients idebenone induced overall stimulation and improvement in arousal. Plasma concentrations of idebenone and its main metabolites were determined and the pharmacokinetic parameters of idebenone after single and repeated doses were estimated. During the single dose study, the mean plasma concentrations of idebenone and its main metabolites and mean pharmacokinetic parameters were comparable to published results (C_{max} = 452.2 ng · ml^{-1}, t_{max} = 2.3 h, AUC = 26 μg · ml^{-1} · h, $t_{1/2\beta}$ = 16.5 h). During the repeated doses study, no significant difference was found between mean residual plasma concentrations of idebenone on Day 2 (47 ng · ml^{-1}) and Day 5 (70.6 ng · ml^{-1}), and mean $t_{1/2\beta}$ of idebenone after the single and after repeated dose studies, i.e., there was no evidence of accumulation. Although idebenone did not appear to accumulate during this study, the coadministration of anticonvulsants, often prescribed during mitochondrial encephalomyopathy, can affect its pharmacokinetics.

Key words Idebenone; mitochondrial encephalomyopathy, young patients, pharmacokinetics

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Introduction

Mitochondrial encephalomyopathy can be caused by defects in the mitochondrial respiratory complexes in skeletal muscle and also in the nervous system as well as in a variety of viscera, which led to the concept of a multiple system syndrome.

Until now, only ubidecarenone (Co Q10) has been reported to improve mitochondrial myoencephalopathic patients. Co Q10 therapy requires prolonged administration before any response can be demonstrated [1].

Idebenone (3-hydroxy-6-(10-hydroxydecyl-2-methoxy-5-methyl-1,4-benzoquinone or CV 2619) is a benzoquinone derivative, which stimulates the respiratory and phosphorylating activity in brain mitochondria, and improves disorders of cerebral energy metabolism [2]. It is currently marketed in Japan for patients with cerebrovascular disorders and dementia [3], Ihara et al. [4, 5] have initiated successful therapy with coenzyme Q10 and idebenone in two patients with mitochondrial encephalomyopathy.

Pharmacokinetic studies of idebenone have been done in healthy volunteers [6, 7] and elderly [7] subjects, showing comparable pharmacokinetic profiles in the two populations. The objective of the present work was to study the pharmacokinetics and tolerance of

Table 1 Demographic, clinical characteristics and treatment of patients in the pharmacokinetic study

Patient	Sex	Age (years)	Height (cm)	Weight (kg)	Idebenone single dose (mg)	Idebenone repeated dose (mg·1day)	Concomitant medication
1	F	16	157	48	70	50-50-50	
2	M	18	170	42	60	40-40-40	Co-enzyme Q
3	M	18	160	39	60	40-40-40	
4	F	6	108	25	40	30-20-20	Co-enzyme Q, l-carnitine
5	M	6	128	26	40	30-20-20	Phenobarbital, co-enzyme Q, l-carnitine, ascorbic acid
6	M	5	96	15	20	20-10-20	
7	F	8	117	34	20	40-30-30	Hydrocortisone, acebutolol, sodium valproate

idebenone after single or repeated doses in young patients with mitochondrial encephalomyopathy.

Materials and methods

Seven patients aged 3 to 18 years, hospitalised for follow-up of mitochondrial myoencephalopathy were included in the study. Demographic and clinical characteristics and drug treatment are summarised in Table 1. All but patient one had normal renal and hepatic function. An increase in alkaline phosphatases, transaminases and gamma GT was noted in patient 5 and was probably related to current phenobarbital treatment. At baseline, two patients had increased LDH activity probably related to the muscle disorder. A complete blood count in all patients before the study was normal or showed only borderline alterations. The research protocol was approved by the local Ethics Committee.

Idebenone (Takeda, Japan) was provided in 10 and 30 mg coated tablets. After an overnight fast, subjects were given in an open trial, a single oral dose of idebenone (1.5 mg · kg^{-1}). A standardised meal was ingested 4 h after dosing. Venous blood samples were taken through an indwelling catheter 0.5, 1, 2, 3, 4, 6, 8, 12 h and 1, 1.5, 2, 3 and 4 days after the dose. Subjects were then given idebenone 3-times a day for 5 days during the principal meals (07.00, 12.00 and 20.00 h); the daily administered dose was 3 mg · kg^{-1}. Residual level samples were drawn by direct venous puncture every morning at 08.00 h for 4 days. On the 5th day, after an overnight fast, subjects were given their dose of idebenone and remained fasting for a further 4 hours. Venous blood samples were drawn through an indwelling catheter at 0.5, 1, 2, 3, 4, 6, 8 and 12 h, and 1, 1.5, 2, 3 and 4 days after the dose. Blood samples were centrifuged (2500 rpm for 4 min at 4° C) and plasma was separated and stored at −20° C until analysis. Adverse events and cardiovascular function (blood pressure and heart rate) were recorded.

Idebenone and its two main metabolites, QS4 [6-(3-carboxypropyl)-3-hydroxy-2-methoxy-5-methyl-1,4-benzoquinone] and QS6 [6-(3-carboxypropyl)-2,3-dimethoxy-5-methy-1,4-benzoquinone] in plasma were determined by HPLC. Briefly, the appropriate internal standards of idebenone (QSA9, Takeda) and its metabolites (KS3, Takeda) were added to the plasma samples, which were hydrolysed with 6N hydrochlohydric acid at 70° C for 20 min. The samples were then extracted into diethylether/methylene chloride (7/3 v/v) and back-extracted into 1.5% sodium hydrogencarbonate solution. For the determination of idebenone the organic layer was acidified with one drop of acetic acid and evaporated to dryness. The residue was taken up in the mobile phase (n-hexane/isopropyl alcohol/acetic acid, 1000/45/1, v/v) and injected onto a silica chromatography column (Sil 100, 5 µm, Lichrospher Merck). The flow rate was 1 ml · min^{-1}. For determination of both metabolites, the aqueous layer from back-extraction with sodium hydrogencarbonate was acidified with 6N HCL 0.2 ml and re-extracted into diethylether/methylene chloride. The organic layer was evaporated to dryness: the residue was taken up in the mobile phase (acetonitrile/water/acetic acid, 50/50/0.3, v/v) and injected onto a reversed phase C18 column (Nucleosil 5 µm Interchrom). The oven temperature was 40° C and the flow rate was 1 ml · min^{-1}. UV detection (280 nm) was used for the determination of both indebenone and its metabolites. All concentration values were derived by reference to calibration curves constructed each day using the ratio of the peak height of idebenone or its metabolites and the corresponding internal standard. The methods used were linear over the measured concentration ranges and the coefficient of variation was less than 10% both for idebenone and its metabolites. Intra- and inter-day reproductibility and accuracy were also shown to be satisfactory. The limits of detection were fixed at 5, 10 and 20 ng · ml^{-1} respectively for idebenone, QS4 and QS6.

The estimation of the pharmacokinetic parameters of idebenone after single or repeated oral doses and statistical analysis of these two sets of values were obtained using the Siphar software package (Version 4.0; Simed®). Model adjustment and macroconstant calculation were done using the weighted least squares method with Powell's minimization algorithm. An open two-compartment model with (single dose) or without (repeated doses) lag time was used for the calculations. Statistical analysis used Student's t-test for paired data.

Results and discussion

During the single dose study, the mean plasma concentrations of idebenone and its main metabolites and mean pharmacokinetic parameters of idebenone (Table 2a) were comparable to values previously published (8,9). The maximum concentration (C_{max}) was 452 (160) ng · ml^{-1}, time to reach maximum concentration (t_{max}) was 2.3 (1.9) h, total area under the curve (AUC) was 2.67 (20 µg · ml^{-1} · h), and terminal elimination half-life $t_{1/2\beta}$ was 16.5 (5.5) h.

The mean pharmacokinetic parameters of idebenone after multiple doses are listed in Table 2b. Mean residual plasma concentrations (62.5 (30.3) ng · ml^{-1}) and the C_{max} (321 (159) ng · ml^{-1}) of idebenone were lower than those reported in the literature. Conversely, the mean $t_{1/2\beta}$ (22.3 (16.6) h) was higher than the published result [6]. This might be due to pre-existing impairment of hepatic function, interaction with drugs given with idebenone (valproïc acid) or variation in

Table 2 Individual and mean pharmacokinetic parameters of idebenone during single (Table 2a) and multiple dosing (Table 2b) studies; $t_{1/2\beta}$ terminal elimination half-life; AUC total area under the curve; C_{max} maximum concentration; t_{max} time to reach maximum concentration; C_{min} 1 to 5 residual concentration measured just before the first to fifth doses of idebenone

a)

Patient	$t_{1/2\beta}$ (h)	AUC ($\mu g \cdot ml^{-1} \cdot h$)	C_{max} ($ng \cdot ml^{-1}$)	t_{max} (h)
01	13.9	2.32	596	1
02	25.5	1.90	180	6
03	18.6	1.30	400	1
04	13.4	2.90	595	1
05	8.2	2.56	599	1
06	16.0	2.59	333	4
07	19.9	5.11	462	2
Mean	16.5	2.67	452.2	2.3
SD	5.5	1.20	160	1.9

b)

Patient	$t_{1/2\beta}$ (h)	C_{max} ($ng \cdot ml^{-1}$)	$C_{min}2$ ($ng \cdot ml^{-1}$)	$C_{min}3$ ($ng \cdot ml^{-1}$)	$C_{min}4$ ($ng \cdot ml^{-1}$)	$C_{min}5$ ($ng \cdot ml^{-1}$)
01	13.4	419	72	6	100	10
02	14.1	192	10	50	60	53
03	38.9	190	70	27	34	38
04	18	–	59	65	60	61
05	17.3	–	54	30	31	72
06	5.3	181	64	86	160	78
07	52.3	492	0	49	85	84
Mean	22.3	320.5	47	56.7	75.5	70.6
SD	16.6	158.8	29.5	24.4	44.7	22.7

bioavailability; it has been shown that [3] post-breakfast administration increases plasma idebenone concentrations, but there was no statistically significant accumulation of idebenone. Indeed, no significant difference was found between:

1) Mean residual plasma concentrations of idebenone on Day 2 (47 (29.5) $ng \cdot ml^{-1}$) and Day 5 (70.6 (22.7) $ng \cdot ml^{-1}$)

2) The mean $t_{1/2\beta}$ of idebenone in the single and repeated dose studies. AUCs over a dosing interval at steady state were not compared to AUC values obtained from single dose data, since the dosing schedules were different (1.5 $mg \cdot kg^{-1}$ vs 1 $mg \cdot kg^{-1}$ three times a day for 5 days for simple and repeated dose studies, respectively).

Concentrations of metabolites during single and repeated dose studies were generally too low to provide an adequate description of the metabolite kinetics.

During this short-term pharmacokinetic study, no significant adverse effects were noted. Overall stimulation and improvement in arousal were induced in 3 out of 7 patients. A long-term clinical study should be performed in order to evaluate the efficacy and tolerance of chronic idebenone treatment in young patients with encephalomyopathy.

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