Br J Clin Pharmacol 1996; **42**: 707–711

Involvement of CYP2D6 but not CYP2C19 in nicergoline metabolism in humans

Y. BÖTTIGER[1], P. DOSTERT[2], M. STROLIN BENEDETTI*, M. BANI[2], F. FIORENTINI[2], M. CASATI[2], I. POGGESTI[2], C. ALM[1], G. ALVAN[1] & L. BERTILSSON[1]

[1]Department of Medical Laboratory Sciences and Technology, Division of Clinical Pharmacology at the Karolinska Institute, Huddinge Hospital, Sweden and [2]Pharmacia-Upjohn, Pharmacokinetics and Metabolism Department, Milan, Italy

1 Nicergoline, an ergot derivative previously used as a vasodilator, has gained a new indication in treating the symptoms of senile dementia.

2 Nicergoline is rapidly hydrolysed to an alcohol derivative, 1-methyl-10-α-methoxy-9,10-dihydrolysergol (MMDL), which is further *N*-demethylated to form 10-α-methoxy-9,10-dihydrolysergol (MDL). A few individuals display aberrant metabolism of this drug, as shown by their diminished capacity to form the MDL metabolite. The aim of this study was to determine whether defective nicergoline metabolism is associated with the debrisoquine and/or the S-mephenytoin hydroxylation polymorphisms.

3 After a single, oral 30 mg dose of nicergoline, the plasma concentrations of its two metabolites were studied in 15 subjects, divided into three groups with respect to their debrisoquine and S-mephenytoin hydroxylation phenotypes.

4 The pharmacokinetic parameters of MMDL and MDL were similar in the ten subjects who were extensive metabolisers of debrisoquine (five of whom were poor metabolisers of S-mephenytoin) (mean MMDL C_{max} 59 nmol l^{-1} and AUC (0, *t*h) 144 nmol l^{-1}h, mean MDL C_{max} 183 nmol l^{-1} and AUC 2627 nmol l^{-1}h) but were markedly different from the five subjects who were poor metabolisers of debrisoquine (mean MMDL C_{max} 356 nmol l^{-1} and AUC 10512 nmol l^{-1}h, MDL concentrations below limit of quantitation).

5 We conclude that the formation of MDL from MMDL in the metabolism of nicergoline is catalysed to a major extent by CYP2D6 and that the observed interindividual variation in the metabolic pattern of the drug is related to the debrisoquine hydroxylation polymorphism.

Keywords nicergoline CYP2D6 debrisoquine

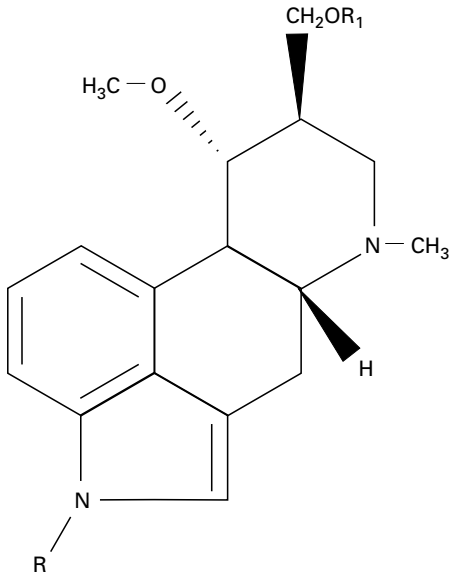
Introduction

Nicergoline (10-methoxy-1,6-dimethylergoline-8β-methanol-bromo-3-pyridinecarboxylate ester), an ergot derivative, has been shown to ameliorate the symptoms of senile mental impairment in several double-blind studies compared with placebo or reference compounds [1–3].

After oral administration nicergoline is rapidly metabolised by hydrolysis of its ester group to form the alcohol derivative MMDL (1-methyl-10-α-methoxy-9,10-dihydrolysergol) which, in turn, is *N*-demethylated to MDL (10-α-methoxy-9,10-dihydrolysergol) (Figure 1). Plasma concentrations of nicergoline in humans are undetectable by conventional methods. Therefore pharmacokinetic studies with nicergoline have been based on the plasma concentrations of the two metabolites. It has been shown that in healthy volunteers, plasma concentrations of MDL are generally much higher than those of MMDL. However, about 5% of subjects achieve far higher concentrations of MMDL than of MDL [4–6], which suggests that the enzyme

* This work was presented as a poster at the 9th International Conference on Cytochrome P450, University of Zurich, July 23–27, 1995.

Correspondence: Dr Ylva Böttiger, Department of Clinical Pharmacology, Huddinge Hospital, S-141 86 Huddinge, Sweden.
*Present address: Zambon Group, Research and Development Division, Milan, Italy.



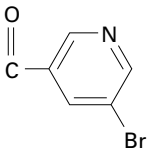
R	R_1	Nomenclature
CH_3		Nicergoline
		hydrolysis
CH_3	H	MMDL
		N-demethylation
H	H	MDL

Figure 1 The structure of nicergoline and its metabolites.

responsible for the *N*-demethylation of MMDL is impaired in these individuals. It is well known that the two hepatic cytochrome P450 enzymes CYP2D6 (the debrisoquine/sparteine hydroxylase) and CYP2C19 (S-mephenytoin hydroxylase) exhibit genetic polymorphisms with an incidence of poor metabolisers among Caucasians of about 7 and 3%, respectively [7, 8]. The aim of this study was to examine the metabolism of nicergoline in poor metabolisers (PM) of debrisoquine and S-mephenytoin to determine whether CYP2D6, CYP2C19, or both, are involved in the metabolism of nicergoline.

Methods

Fifteen subjects were recruited from a population of over 1000 healthy volunteers previously phenotyped with respect to the debrisoquine and S-mephenytoin hydroxylation polymorphisms, as described previously [8]. Five (three men, two women) of the subjects were poor metabolisers (PM) of mephenytoin and extensive metabolisers (EM) of debrisoquine (mean age $\pm$s.d., 30 ± 8 years; mean weight, 73 ± 16 kg), five (four men, one woman) were PM of debrisoquine and EM of mephenytoin (age, 32 ± 4 years; weight 77 ± 11 kg) and five (two men, three women) were EM of both probe drugs (age, 33 ± 8 years; weight, 63 ± 11 kg). All subjects were healthy as defined by medical history, physical examination, electrocardiogram and routine laboratory analysis. One of the subjects had taken 1 g paracetamol on one occasion 4 days before the study. Otherwise, none of the subjects was taking any concomitant medication (except oral contraceptives) for 2 weeks prior to or during the study. None of the subjects consumed any alcoholic beverages for 3 days before and during the study, and none used tobacco.

The study was approved by the Ethics Committee of Huddinge Hospital and by the Medical Product Agency of Sweden. All subjects gave written informed consent.

After an overnight fast, subjects received a single, oral dose of nicergoline (Sermion® film coated 30 mg tablets) at about 08.00 h. Standardized meals were served through the first study day with the first at 2 h post dose. Blood samples (10 ml) were collected via a venous catheter at 0, 0.5, 1, 2, 4, 8, 12, 24, 32, 48 and 72 h after the dose. Plasma was separated after centrifugation (1200 **g** at 4° C for 10 min) and stored in plastic tubes at $-20°$ C pending assay.

Blood pressure and heart rate in resting position were measured at 0, 2, 4, 12, and 24 h after the dose. Subjects were asked whether they had experienced any adverse events or discomfort at 24, 48 and 72 h after dosing and at a post-study examination.

MDL and MMDL concentrations in plasma were determined using a validated, reversed phase h.p.l.c. method with u.v. detection. To 1 ml of human plasma, 0.5 ml of 50 nmol l^{-1} tris (hydroxymethyl)amino methane pH 8.5 was added. Extraction was then carried out twice using 2.5 ml of diethyl ether:n-octanol (8:2, v/v) and centrifugation at 1200 *g* for 5 min. The combined organic phases were re-extracted with 0.26 ml of 5 nmol l^{-1} phosphoric acid. The aqueous phase was washed twice with 1 ml of n-hexane and 0.2 ml of the aqueous phase was injected into the h.p.l.c. system. The analytical separation was performed using a Hypersil ODS column (250×3 mm i.d., particle size 3 μm, Shandon, Cheshire, UK) equipped with a stainless steel precolumn filter (Waters, Milford, MA, USA) with 2 μm frit. The separation of the analytes was carried out at a flow rate of 1 ml min^{-1}, using a 8 mmol l^{-1} H_3PO_4 (adjusted to pH 3 with triethylamine) -acetonitrile mixture (50:50, v/v) as mobile phase. The u.v. detector was set at 225 nm.

The linearity of the h.p.l.c. assay was evaluated from five separate calibration curves obtained on different

days over the concentration range 7–900 nmol l^{-1}. The data were analysed by linear regression (weighting factor 1/y) by plotting the peak area versus concentration of MDL and MMDL added. Correlation coefficients (r) for the regression were always higher than 0.99 for both compounds. The inter-day precision evaluated from quality control samples assayed during the study and expressed as coefficient of variation, ranged from 4.8 to 10.1% for MDL and from 5.3 to 8.0% for MMDL ($n=6$). The mean estimate ($n=6$) of the inter-day accuracy, expressed as recovery, ranged from 95.7 to 101.8% for MDL and from 97.0 to 100.4% for MMDL. The limit of quantitation for MDL and MMDL was 7 nmol l^{-1}.

Plasma pharmacokinetic parameters were calculated by standard non-compartmental methods. The area under the plasma concentration-time curve was calculated to the last detectable concentration, AUC (0, th) using the linear trapezoidal rule. The area under the plasma concentration-time curve to infinite time, AUC, was obtained by adding to AUC(0, th) the area extrapolated from the last sampling time assuming monoexponential decay. Statistical analysis on MMDL pharmacokinetics was performed using the Tukey test for multiple comparisons. Since no pharmacokinetic parameters could be calculated for MDL in subjects that were PM of debrisoquine, the comparison between MDL pharmacokinetic parameters in the other two groups was performed using Student's t-test for unpaired data. Differences were considered to be significant if P was <0.05.

Table 1 Pharmacokinetic parameters of MMDL and MDL after a single, oral 30 mg dose of nicergoline, given to three groups of healthy volunteers

Five EM of debrisoquine and mephenytoin

		MMDL				*MDL*				
Subject	*MR*	C_{max} (*nmol* l^{-1})	t_{max} (*h*)	t_z (*h*)	*AUC*(0, th) (*nmol* $l^{-1}h$)	C_{max} (*nmol* l^{-1})	t_{max} (*h*)	t_z (*h*)	AUC (*nmol* $l^{-1}h$)	$t_{\frac{1}{2}}$ (*h*)
1	0.23	37	1	4	101	147	4	48	2033	12.0
8	0.13	24	0.5	2	26	185	4	72	3297	29.1
9	0.41	45	0.5	4	120	219	4	48	3109	16.6
10	0.65	99	0.5	8	212	184	2	48	2481	17.4
15	0.65	147	0.5	4	234	203	2	48	2506	19.3
Mean	0.41	71	0.5[b]	4.4	139	188	4[b]	52.8	2685	18.9
s.d.	0.24	51		2.2	85	27		10.7	513	6.3

Five EM of debrisoquine and PM of mephenytoin

		MMDL				*MDL*				
Subject	*MR*	C_{max} (*nmol* l^{-1})	t_{max} (*h*)	t_z (*h*)	*AUC*(0, th) (*nmol* l^{-1})	C_{max} (*nmol* $l^{-1}h$)	t_{max} (*h*)	t_z (*h*)	*AUC* (*nmol* $l^{-1}h$)	$t_{\frac{1}{2}}$ (*h*)
2	0.57	9	4	4	21	145	4	48	2182	19.8
3	0.80	53	0.5	8	208	206	2	48	2849	14.8
5	0.36	50	0.5	4	89	225	2	48	2303	14.2
6	0.99	120	0.5	8	415	220	2	48	3017	13.5
11	0.20	10	1	1	7	94	4	48	2501	37.5
Mean	0.58	48	0.5[b]	5.0	148	178	2[b]	48.0	2570	20.0
s.d.	0.32	45		3.0	169	57		0	355	10.1

Five PM of debrisoquine and EM of mephenytoin

		MMDL						*MDL*	
Subject	*MR*	C_{max} (*nmol* l^{-1})	t_{max} (*h*)	t_z (*h*)	*AUC*(0, th) (*nmol* $l^{-1}h$)	*AUC* (*nmol* $l^{-1}h$)	$t_{\frac{1}{2}}$ (*h*)	C_{max} (*nmol* l^{-1})	t_{max} (*h*)
4	190	418	4	72	9517	10422	21	14	4
7	180	394	2	72	12235	13650	21	n.d.	*
12	140	249	8	72	7728	8752	23	n.d.	*
13	200	381	2	72	8559	9123	19	n.d.	*
14	90	339	8	72	9318	10611	24	n.d.	*
Mean	160	356	4[b]	72	9471[a]	10512	21.5		
s.d.	45	66		0	1698	1929	2.2		

t_z = time of last detectable concentration. MR = debrisoquine/4-hydroxydebrisoquine metabolic ratio. n.d. = below 7 nmol l^{-1} (limit of quantitation). * not calculable. [a] Significantly higher ($P<0.001$) than in the two groups of EM of debrisoquine. [b] t_{max} is median value.

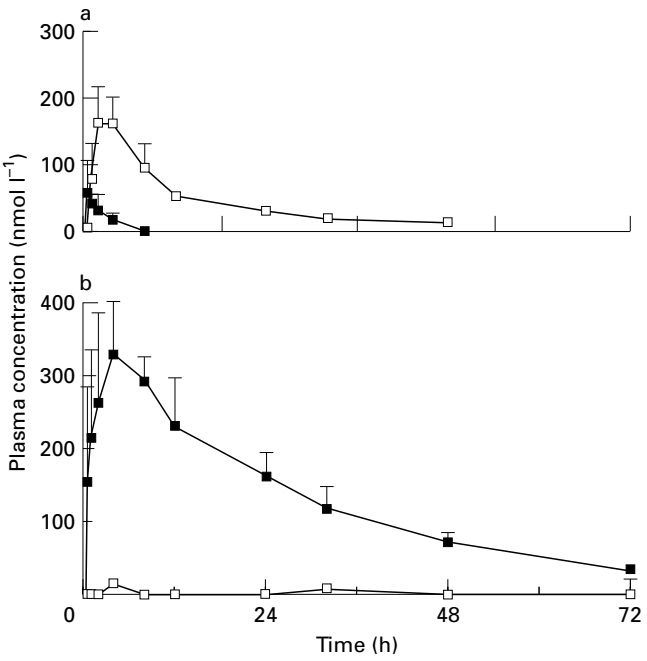


Figure 2 Plasma concentrations of MMDL (■) and MDL (□) in a) EM ($n=10$) and b) PM ($n=5$) of debrisoquine after a single, oral 30 mg dose of nicergoline.

Results

All subjects completed the study. Nicergoline was well tolerated, and no adverse events that could be ascribed to the drug were reported. ECG traces before the study and at follow-up were normal. Haematological and blood chemical analyses showed no clinically significant divergencies on either pre- or post-study testing. Volunteers did not report any significant discomfort due to study procedures.

Individual pharmacokinetic parameters of MDL and MMDL are reported in Table 1. All parameters in the five subjects that were EM of both debrisoquine and S-mephenytoin and in the five subjects that were EM of debrisoquine but PM of S-mephenytoin were similar and did not differ statistically (Table 1). Plasma concentrations of MMDL were much higher (mean AUC(0, *t*h) 9471 nmol l^{-1}h)($P<0.001$) in PM of debrisoquine than those in the two EM of debrisoquine groups (mean AUC(0, *t*h) 139 and 148 nmol l^{-1}h, respectively) and in the combined EM group (Figure 2). In the group of PM of debrisoquine, four out of the five subjects showed plasma concentrations of MDL below the limit of quantitation at all sampling times, and very low concentrations were detected at two time points (4 and 32 h) in the fifth subject. Accordingly, the mean concentrations of the *N*-demethylated metabolite MDL were very low in PM compared with EM of debrisoquine (Figure 2). No statistical comparisons were needed to support this evidence.

Discussion

The findings indicate a major role for CYP2D6 in the metabolism of nicergoline, and represent the first example of the *N*-demethylation of an *N*-methylindole derivative to be catalysed by this enzyme. Previously the *N*-demethylation of the aromatic amine derivative amiflamine has been shown to be catalysed by CYP2D6 [9]. A remarkable characteristic of CYP2D6 is that, in contrast to other CYP enzymes, it only accepts basic compounds as substrates. The distance between the basic nitrogen atom in position 6 in nicergoline and the oxidation site, the carbon atom of the methyl group in position 1, was estimated using X-ray data from the Crystallographic Cambridge Databank [10], and was found to be 7.46 Ångström (Dr S. Mantegani, personal communication), which is in reasonable agreement with the value (5–7 Å) for classical CYP2D6 substrates [11, 12].

The present study also shows that CYP2C19 is not involved in nicergoline metabolism to any detectable extent. Since the differences in MMDL and MDL concentrations between EM and PM of debrisoquine are large, with almost no MDL appearing in PM, it can be concluded that the *N*-demethylation of MMDL is almost exclusively catalysed by CYP2D6. This is probably the most striking example of a CYP2D6 enzyme specificity and it opens up the possibility of using nicergoline as a probe drug for CYP2D6 phenotyping [13]. In particular, it is well tolerated and could seemingly be given at a very low dose with a minimal risk of side-effects. However, the optimal experimental conditions for such a procedure need to be determined and further safety evaluation concerning the effect of the high concentrations of MMDL in the PM of debrisoquine is warranted. In this single dose study in healthy volunteers, no side effects were noted in the poor metabolisers of debrisoquine.

Knowing that CYP2D6 is involved in the metabolism of nicergoline could be helpful in evaluating adverse outcomes of treatment and, if nicergoline inhibits the metabolism of other CYP2D6 substrates, in predicting and treating drug-drug interactions. Drug interactions could be particularly important as senile dementia is mostly a disease of the elderly, in whom multiple drug use is common. The influence of debrisoquine polymorphism on nicergoline efficacy and tolerability remains to be established, as does the contribution of MDL and MMDL to the pharmacological activity of nicergoline.

This study was supported in part by the Swedish Medical Research Council (3902), the Bank of Sweden Tercentenary Foundation, EU Biomed 1 (BMH 1-CT94–1622) and Biomed 2 (BMH 4-CT96–0291).

References

1 Arrigo A, Moglia A, Borsotti, L, *et al.* A double-blind, placebo-controlled cross-over trial with nicergoline in patients with senile dementia. *Int J Pharm Res* 1982; **2** (Suppl.1): 33–41.

2 Nicergoline Cooperative Group. A double-blind randomised study of two ergot-derivatives in mild to moderate dementia. *Curr Ther Res* 1990; **48**: 597–612.

3 Battaglia A, Bruno G, Ardia A, Sacchetti G. Nicergoline in mild to moderate dementia: A multicenter, double-blind

placebo controlled study. *J Am Geriatric Soc* 1989; **37**: 295–302.

4 Bellotti V, Jannuzzo MG, Moro E, Jori C, Dubini A, Strolin Benedetti M. Plasma pharmacokinetics of nicergoline metabolites in healthy volunteers. *Eur J Clin Pharmacol* 1989; **36** (Suppl.): A150.

5 Johnston A, Abrams SM, Patel M, *et al.* Bioequivalence of controlled release nicergoline 60 mg once daily *vs* the plain form 30 mg twice daily in 24 male subjects. *Br J Clin Pharmacol* 1993; **35**: 80P.

6 Poggesi I, Strolin Benedetti M, Berti CA, Dubini A, Pelluzzoni C, Dostert P. Does nicergoline N-demethylation follow a bimodal distribution? *J Basic Clin Physiol Pharmacol* 1992; **3** (Suppl.): 255.

7 Alvan G, Bechtel P, Iselius L, Gundert-Remy U. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* 1990; **39**: 533–537.

8 Bertilsson L, Lou Y-Q, Du Y-L, *et al.* Pronounced differences between native Chinese and Swedish populations in the polymorphic hydroxylations of debrisoquine and S-mephenytoin. *Clin Pharmacol Ther* 1992; **51**: 388–397.

9 Alvan G, Graffner C, Gustavsson LL, *et al.* Tolerance and pilot pharmacokinetics of amiflamine after increasing single oral doses in healthy subjects. *Clin Pharmacol Ther* 1986; **40**: 81–85.

10 Foresti E, Sabatino P, Riva di Sanseverino L, Fusca R, Tosi C, Tonani R. Structure and molecular orbital study of ergoline derivatives. 1-(6-Methyl-8 beta-ergolinyl-methyl) imidazolidine-2,4-dione (I) and 2-(10-methoxy-1,6-dimethyl-8 beta-ergolinyl)ethyl 3,5-dimethyl-1H-2-pyrrole-carboxylate toluene hemisolvate (II) and comparison with nicergoline (III). *Acta Crystallogr B* 1988; **44**: 307–315.

11 Meyer UA, Gut T, Kronbach T, *et al.* The molecular mechanisms of two common polymorphisms of drug oxidation—evidence for functional changes in cytochrome P-450 isozymes catalysing bufuralol and mephenytoin oxidation. *Xenobiotica* 1986; **16**: 449–464.

12 Koymans L, Vermeulen NP, van Acker SA, *et al.* A predictive model for substrates of cytochrome P450-debrisoquine (2D6). *Chem Res Toxicol* 1992; **5**: 211–219.

13 Dayer P. Advantages and drawbacks of probe drugs for the assessment of phenotypic expression of cytochrome P-450 db1 (P450 IID6). In *European Consensus Conference on Pharmacogenetics*, eds Alvan G, Balant LP, Bechtel PR, Boobis AR, Gram LF, Pithan K, Commission of the European Communities, Luxembourg, 1990: 33.

(*Received 22 February 1996, accepted 25 June 1996*)