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Short Communication

Metabolism of the new MAO-A inhibitor brofaromine in poor and extensive metabolizers of debrisoquine*

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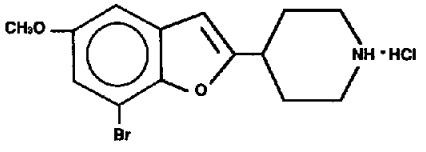
Keywords: *Brofaromine; MAO-A inhibitor; metabolism; polymorphism of debrisoquine; cytochrome P450IID6.*

Introduction

Brofaromine hydrochloride (Fig. 1) is a new specific and reversible inhibitor of monoamine oxidase type A. Several phase I studies showed high safety of the drug [1, 2]. Its effectiveness and good tolerability in depressed patients have been described recently [3]. Earlier pharmacokinetic investigations revealed that oxidative *O*-demethylation plays a key role in the biotransformation of brofaromine in man [4]. The predominant primary metabolite of brofaromine is *O*-desmethyl brofaromine (CGP 35 748). It is extensively conjugated and renally excreted. In plasma of young healthy subjects treated with an oral ^{14}C-labelled preparation about 40% of the total plasma radioactivity represented *O*-desmethyl brofaromine. The major part was present in the conjugated form. Unchanged brofaromine accounted for another 40%; 5% was identified as conjugated brofaromine [4]. Oxidative *O*-demethylation of brofaromine is probably catalysed by isoenzymes of the cytochrome P450 family. One of these isoenzymes, P450IID6, is also involved in the 4-hydroxylation of debrisoquine [5, 6]. Deficiency in P450IID6 may be responsible for the large inter-individual variability in the pharmacokinetic profile of brofaromine reported in a previous study [7]. The aim of the present trial was to investigate to what extent the metabolism of brofaromine is affected by deficiency of the debrisoquine hydroxylating enzyme.



Brofaromine
4-(7-bromo-5-methoxy-2-benzofuranyl)piperidine HCl

Figure 1
Chemical structure of brofaromine.

Experimental

Human subjects

Seven extensive (EM) and six poor (PM) metabolizers of debrisoquine were used in the study. The phenotype assignment was confirmed by a repeat debrisoquine test. Thirteen volunteers were used in the study (eight female, five male). The age range was 25–40 years and the body weight ranged from 49 to 75 kg. Written informed consent was obtained and the design and procedures of the study were reviewed by the Ethics Committee of the Human Pharmacology Institute of Ciba-Geigy GmbH, Tübingen, Germany.

Treatment

The trial was performed in an open design with a fixed sequence of treatments. After a 12 h fasting period all subjects received:

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On study day 1: a single oral dose of 10 mg (equals 46 μmol) debrisoquine hemisulphate (tablet); 0–12 h urine was collected for the determination of the metabolic ratio of debrisoquine.

On study day 2: a single oral dose of 75 mg (equals 216 μmol) brofaromine HCl (trilongette). Two hours after drug administration the volunteers received a standard breakfast. To measure total (unchanged and conjugated) brofaromine and total *O*-desmethyl brofaromine, blood samples were drawn before and 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 24, 32, 48 and 72 h after administration of brofaromine. Urine fractions were collected before and from 0–6, 6–12, 12–24, 24–36, 36–48 and 48–72 h. Between study days 1 and 2, an interval of at least 1 week was observed.

Methods

Metabolic ratio of debrisoquine. The metabolic ratio (MR) was calculated as the ratio of debrisoquine–4-hydroxy debrisoquine measured in 0–12 h urine. Subjects with MR ≥ 12.6 were considered to be PM; those with MR ≤12.6 as EM [8]. Debrisoquine and its metabolite were quantified by HPLC with fluorometric detection. A 1 ml volume of urine, spiked with 5 μg guanoxan hemisulphate (internal standard) was incubated with 0.75 ml saturated $NaHCO_3$ solution, 0.75 ml methanol and 0.25 ml acetylacetone at 50°C for 16 h. The mixture was extracted with 5 ml diethyl ether (15 min, 200 rpm). The organic phase was back-extracted with 1 ml 1 N HCl and 5 ml diethyl ether, the aqueous phase separated by freezing at −70°C and reextracted with 1.5 ml 1 N NaOH and 5 ml cyclohexane (15 min, 200 rpm). The organic phase was evaporated to dryness (N_2, 30°C), the residue redissolved in mobile phase and injected into the HPLC. Chromatographic separation was carried out on a 10 μ Bondapak C18 column (150 × 4 mm). The mobile phase consisted of acetonitrile–water–phosphate buffer (25 mM) (30:30:40, v/v/v) (pH 2.0). Fluorometric detection was performed at 192 nm (excitation) and 340 nm (emission). The limit of quantitation for both compounds was 446 nmol l^{-1}.

Determination of brofaromine and O-desmethyl brofaromine in plasma and urine. Total brofaromine and its *O*-desmethyl metabolite were measured by gas chromatography with electron capture detection, after enzymatic hydrolysis of plasma or urine [9]. The limit of quantitation for both compounds was 60 nmol l^{-1}.

Pharmacokinetic calculation and statistics. Peak plasma concentration (C_{max}) and the time to reach peak (t_{max}) were taken directly from the measured data. Area under plasma concentration–time curve ($AUC_{(0-72h)}$) was calculated according to the trapezoidal rule. $AUC_{(0-\infty)}$ was calculated as $AUC_{(0-\infty)} = AUC_{(0-72h)} + C_{72h}/k_{el}$, where C_{72h} is the last measured plasma concentration (72 h after administration) and k_{el} is the elimination constant. The terminal half-life of elimination ($t_{½}$) and k_{el} were estimated from the last descending part of the semi-logarithmic plasma concentration–time curves by linear regression. Total clearance was calculated as $Cl = f \times dose/AUC_{(0-\infty)}$, the bioavailability factor was assumed to be $f = 0.9$ (M. Jedrychowski, unpublished data). The metabolic ratio of brofaromine (MR_{br}) was calculated as the molar ratio of brofaromine–*O*-desmethyl brofaromine measured in 0–72 h urine. Student's unpaired *t*-test was used to compare C_{max}, AUC, $t_{½}$, Cl and cumulative renal elimination of PM and EM. Values $P < 0.05$ were considered to be statistically significant.

Results and Discussion

The metabolic ratio of debrisoquine in EM ranged from 0.07 to 0.75 and in PM from 38.13 to 112.27 (Table 1). PM eliminated brofaromine from plasma slower than EM. The mean elimination half-life was significantly longer in this group (25.1 ± 9.1 vs 11.0 ± 3.4 h); the mean clearance was significantly lower (77.4 ± 32.2 vs 172.5 ± 76.9 ml min^{-1}). Correspondingly, higher plasma concentrations of brofaromine were measured in PM (Fig. 2). The mean $AUC_{(0-\infty)}$ was twice as high as in EM, whereas the mean C_{max} was not different (Table 1). This suggests that there are only small differences in the first-pass metabolism between the two groups. On the other hand, PM showed significantly lower plasma levels of the metabolite (Fig. 3). The mean $AUC_{(0-72h)}$ in PM was about two times and the mean C_{max} about three times lower than in EM (Table 2). The peak concentration of metabolite was reached later in PM (11 h), than in

Table 1
Pharmacokinetic data of brofaromine after 75 mg (216 μmol) brofaromine hydrochloride p.o.

Vol. no.	Sex	MR	MR_{br}	C_{max} (μmol l^{-1})	$AUC_{(0-\infty)}$ (μmol × h l^{-1})	$t_{1/2}$ (h)	Cl (ml min^{-1})
Extensive metabolizers							
1	M	0.36	0.74	1.54	16.89	10.29	192.60
2	F	0.07	1.99	1.38	18.29	8.78	177.48
3	F	0.21	1.81	2.28	46.74	12.59	69.48
4	F	0.28	0.50	0.96	11.03	8.51	294.30
5	F	0.75	0.94	2.17	25.35	15.20	128.07
6	F	0.22	0.74	1.84	29.20	15.01	111.15
7	M	0.30	0.32	1.65	13.84	6.27	234.45
Mean		0.31	1.01	1.69	23.05	10.95	172.53
±SD		0.21	0.64	0.46	12.21	3.42	76.86
Poor metabolizers							
8	F	38.13	12.83	1.58	42.66	17.46	76.05
9	M	58.71	4.55	1.19	29.83	23.40	108.70
10	M	54.00	3.17	1.80	26.50	16.71	122.49
11	F	50.82	8.54	2.16	61.28	21.20	52.92
12	M	112.27	3.83	1.54	79.52	40.05	40.77
13	F	42.11	2.68	1.40	51.03	31.88	63.63
Mean		59.34	5.93	1.61	48.47*	25.12†	77.43*
±SD		27.02	3.97	0.34	19.99	9.13	32.19

MR, metabolic ratio of debrisoquine; MR_{br}, metabolic ratio of brofaromine.
* $P = 0.02$; † $P = 0.01$.

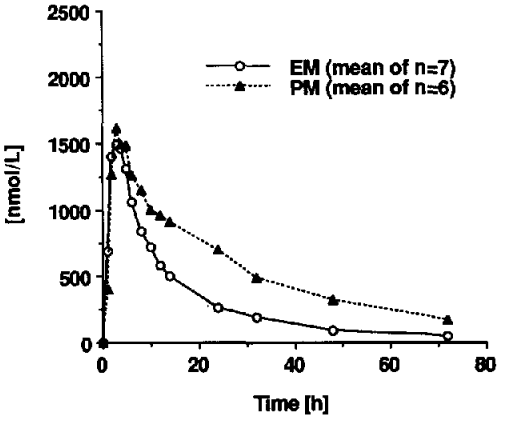

Figure 2
Plasma concentrations of total brofaromine after 75 mg (216 μmol) brofaromine hydrochloride p.o.

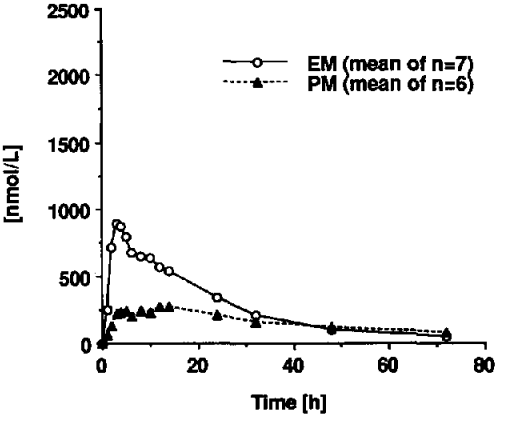

Figure 3
Plasma concentrations of total *O*-desmethyl brofaromine after 75 mg (216 μmol) brofaromine hydrochloride p.o.

EM (3 h after administration; median values). The observed phenotypic differences in plasma kinetics of brofaromine display clearly an impairment of biotransformation in PM. The data on urinary excretion support this finding. The mean cumulative excretion of brofaromine was four times higher in PM than in EM (3.6 vs 1.0 μmol 72 h^{-1}). During the same time interval PM excreted significantly less *O*-desmethyl brofaromine than EM (59.3 vs 98.7 μmol per 72 h, $P = 0.003$). The time course of excretion was markedly different in both groups: while EM excreted brofaromine within 24 h after administration, in PM considerable amounts of drug were found for up to 72 h (Fig. 4). EM eliminated the main part of *O*-desmethyl brofaromine during 36 h after administration; in PM excretion continued up to at least 72 h (Fig. 5).

The metabolic ratio of brofaromine calculated from the urinary data ranged from 0.3 to 2.0 in EM and from 2.7 to 12.8 in PM (Table 1). The phenotype assignment established with brofaromine was in each subject the same as

Table 2
Pharmacokinetic data of *O*-desmethyl brofaromine after 75 mg (216 μmol) brofaromine hydrochloride p.o.

Vol. no.	Sex	C_{max} (μmol l^{-1})	$t_{½}$ (h)	$AUC_{(0-72h)}$ (μmol × h l^{-1})
Extensive metabolizers				
1	M	0.82	4.00	19.24
2	F	1.18	3.00	22.53
3	F	0.54	10.00	14.29
4	F	0.98	3.00	19.12
5	F	0.77	2.00	14.42
6	F	1.33	4.00	25.79
7	M	1.20	3.00	21.61
Mean		0.97		19.57
±SD		0.28		4.21
Median			3.0	
Poor metabolizers				
8	F	0.39	14.00	16.30
9	M	0.21	5.00	7.81
10	M	0.47	12.00	16.30
11	F	0.27	12.00	9.28
12	M	0.19	8.03	11.12
13	F	0.22	10.03	8.58
Mean		0.29*		11.57†
±SD		0.11		3.83
Median			11.02	

$P = 0.0002$; †$P = 0.005$.

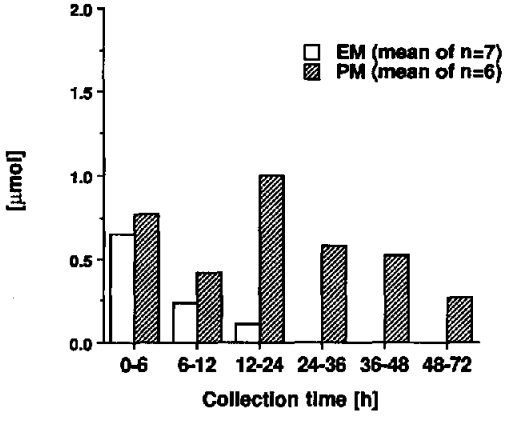

Figure 4
Renal elimination of total brofaromine after 75 mg (216 μmol) brofaromine hydrocholoride p.o.

with debrisoquine; PM of debrisoquine were also PM of brofaromine. Thus, the association of debrisoquine oxidative polymorphism with oxidative demethylation of brofaromine has been established. For all drugs whose metabolism has been shown to be controlled by the sparteine–debrisoquine polymorphism, the differences in $t_{½}$ and clearance between PM and EM have always been substantial [10–12]. Although the results of the present study demonstrate pronounced differences in these

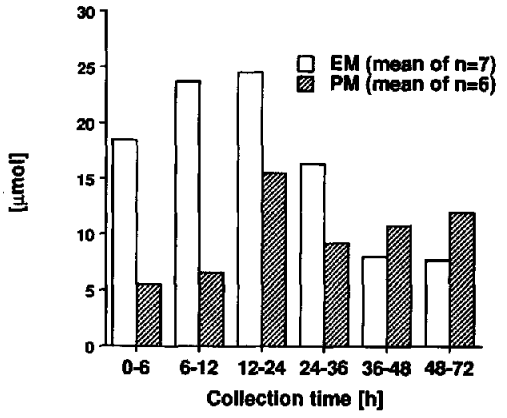

Figure 5
Renal elimination of total *O*-desmethyl brofaromine after 75 mg (216 μmol) brofaromine hydrochloride p.o.

parameters, no clear correlation between either $t_{½}$ or clearance and the metabolic ratio of debrisoquine for individual subjects could be established. In light of our results, it can be assumed that cytochrome P450IID6 might not be the only factor involved in the metabolism of brofaromine. Since *O*-demethylation is not completely abolished in PM, it is suggested that in PM this metabolic pathway is predominantly governed by alternative forms of P450.

Conclusions

This study has shown that poor metabolizers of debrisoquine eliminate brofaromine slower than extensive metabolizers. During long-term treatment higher plasma concentrations of brofaromine may be expected in these subjects. Brofaromine seems to be metabolized by more than one *O*-demethylase. One of them is the genetically variable isoenzyme cytochrome P450IID6.

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