Sparteine oxidation polymorphism: a family study

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1 Polymorphic oxidation of the pharmacogenetic probe drug sparteine was investigated in 35 parents and 29 siblings of 20 unrelated poor metabolizer (PM) probands. Phenotyping was carried out on the basis of metabolic ratio (MR) = sparteine/dehydrosparteines in the 12 h urine. The distribution of MR was bimodal: 47 relatives (20 siblings and 27 parents) had MR ranging from 0.22–12 and were defined as extensive metabolizers (EM) whereas MR ranged from 20–340 in nine siblings and eight parents thus defined as PM.

2 The 20 pedigrees confirmed that poor metabolism of sparteine is inherited as an autosomal recessive character.

3 The mean recovery of dehydrosparteines (% of dose) was 27% in 23 positively identified drug free heterozygotes compared with 37% in unrelated EM (both genotypes) ($P < 0.05$) previously phenotyped. Degrees of dominance of 73% and 77% were calculated on basis of log excretion of dehydrosparteines (% of dose in 12 h urine) and log MR, respectively.

Keywords sparteine drug oxidation family monogenic polymorphism dominance

Introduction

The oxidation of the oxytocic and antiarrhythmic drug sparteine is under diallelic, monogenic control (Eichelbaum *et al.*, 1979; Evans *et al.*, 1983). Thus, two phenotypes are clearly defined: poor metabolizers (PM), who are homozygous for a recessive allele, and an extensive metabolizer (EM) phenotype comprising both heterozygotes and homozygous dominants (Eichelbaum *et al.*, 1979; Evans *et al.*, 1983). Earlier studies demonstrated coregulation of sparteine and debrisoquine oxidation (Eichelbaum *et al.*, 1982; Inaba *et al.*, 1983) but conflicting data have recently appeared (Evans *et al.*, 1983; Eichelbaum & Woolhouse, 1985). Poor metabolism of sparteine and debrisoquine is associated with poor hydroxylation of imipramine (Brøsen *et al.*, in preparation) and likewise the oxidation of metoprolol (Lennard *et al.*, 1982), nortriptyline (Mellström *et al.*, 1981) and desipramine (Bertilsson & Åberg-Wisted, 1983) is impaired

in poor metabolizers of debrisoquine. Sparteine and debrisoquine are therefore useful probe drugs in pharmacogenetic investigations.

Phenotyping with sparteine is usually based on the 12 h urinary recovery of parent drug and its two main metabolites, 2-dehydrosparteine plus 5-dehydrosparteine (dehydrosparteines) following a single oral dose. In this context, dehydrosparteines expressed as per cent of dose appears to be as good a discriminator as the conventional metabolic ratio: sparteine/dehydrosparteines (Vinks *et al.*, 1982; Brøsen *et al.*, 1985).

However, assessment of sparteine oxidation based on the peak area MR cannot distinguish the two genotypes within the dominant phenotype (Eichelbaum *et al.*, 1979; Evans *et al.*, 1983). Combined family and population data have indicated that sparteine metabolism is impaired in heterozygotes compared with homozygous dominants (Evans *et al.*, 1983).

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The aim of the present study was to examine the urinary excretion pattern of sparteine and dehydrosparteines in the three genotypes. Therefore, siblings and parents of 20 unrelated PM probands volunteered to take a test dose of sparteine.

Methods

The probands were 20 out of the 22 PM identified in a previous population study (Brøsen *et al.*, 1985). Sixty-four first degree relatives (35 parents, 29 siblings) consented to participate in the study on the basis of verbal and written information. The mean age for parents was 56.9 years (range 45–76 years). In 16 families both parents were examined. Three fathers and one mother were dead and one father was not available. The mean age for siblings was 30.9 years (range 16–49 years). One sister (pregnant) and one brother (living abroad) were not examined.

After emptying the bladder each volunteer ingested one tablet containing 100 mg sparteine sulphate (234 μ mol free base) (Depasan®, Giuliani, FRG). Urine was collected for the following 12 h. Alcohol intake was not permitted during the test. Current drug therapy was not discontinued. The urine volume was recorded immediately and an aliquot was kept frozen (-20°C) for 1–2 months until analysis.

Urine content of sparteine, 2-dehydrosparteine and 5-dehydrosparteine was analyzed according to the gas chromatographic method described by Vinks *et al.* (1982).

Individual data are presented as % of the dose recovered as sparteine, 2-dehydrosparteine and 5-dehydrosparteine in 12 h urine. The metabolic ratio (MR) was calculated as:

$$\text{MR} = \frac{\text{sparteine}}{\text{dehydrosparteines}} \text{ in 12 h urine}$$

Student's *t*-test, the χ^2 test and Spearman's rank correlation test were used for statistical analysis.

Results

The individual data are listed in Table 1. Sparteine oxidation was polymorphic in the 64 first degree relatives (Figure 1): 47 (27 parents and 20 siblings) had MR values ranging from 0.22 to 12 and were phenotyped as EM. Seventeen (eight parents and nine siblings) had MR ranging from 20–340 and were therefore defined as PM (Figure 1). The urinary recovery of dehydrosparteines was bimodally distributed (Figure 1): the lower mode comprised all 17 PM and one EM (mother, family 6). The pedigrees

are shown in Figure 2. Sixteen EM mothers and 11 EM fathers were identified as heterozygotes. Two EM sisters and one EM brother had a PM parent and were also identified as heterozygotes. Correct genotype assignment was not possible in 17 EM siblings. Theoretically, two thirds of these are heterozygotes and one third is homozygous dominants.

Age did not correlate with the urinary recovery of parent drug or metabolites in 23 drugfree heterozygotes (Table 2), nor with sparteine recovery (% of dose) in 17 PM ($r_s = -0.37$, $P > 0.1$).

In a previous study with 301 unrelated volunteers, 22 subjects were defined as PM and 279 as EM (Brøsen *et al.*, 1985). The expected frequency of heterozygotes (Hardy Weinberg) is:

$$2(1 - \sqrt{22/301})(\sqrt{22/301}) = 0.39,$$

and the estimated number of heterozygotes and homozygous dominants is therefore 119 and 160, respectively. In order to assess the role of genetic factors for the variation in sparteine metabolism between the two genotypes within the dominant phenotype, the method described by Evans *et al.* (1980, 1983) was used. Briefly, the mean log excretion of dehydrosparteines (% of dose in 12-h urine) in 23 positively identified heterozygotes was 1.41. The degree of dominance is defined as the relative displacement of this value from the midpoint between the means of the two homozygous genotypes. The mean value of 279 EM (both genotypes) was 1.55 (Brøsen *et al.*, 1985) and the mean for homozygous dominants was calculated as follows:

$$\frac{\text{sum of values in 279 EM} - \text{sum of values in 119 heterozygotes}}{\text{estimated number of homozygous dominants}} = \frac{279 \times 1.55 - 119 \times 1.41}{160} = 1.66$$

For the homozygous recessive genotype the mean value of -0.20 in 22 unrelated PM was used (Brøsen *et al.*, 1985). Thus the midpoint (0% dominance) is 0.73 and the degree of dominance is:

$$\frac{1.41 - 0.73}{1.66 - 0.73} = 73\%.$$

If instead log MR is used as the parameter the corresponding calculated value is 77%.

Discussion

In the present study 27% of 64 first degree relatives were identified as PM. This frequency

Table 1 Individual data

Family ^a		Age (years)	Drugs	Urinary volume (ml)	SP ^b	12 h urinary excretion		MR ^c
						2-DHS ^b (% of dose)	5-DHS ^b	
1	f	56	phenprocoumon	980	17	30	7.4	0.46
	m	56		760	6.7	25	5.1	0.22
	s	31		880	17	26	6.7	0.52
	p*	29		460	44	1.0	0.43	30
2	f	67		550	44	0.40	—	110
	m	64		410	48	1.7	0.75	20
	b	37		620	49	0.42	0.12	90
	p*	34		380	61	0.43	0.24	90
3	f	67	atenolol, bendroflumethiazide, oestradiol	780	21	31	8.4	0.52
	m	64		440	14	21	5.7	0.54
	b	41		400	15	34	8.8	0.34
	s	39		390	24	27	8.7	0.67
4	p*	26		1200	72	1.3	—	57
	p**			420	53	0.36	0.12	110
	f	67		400	57	0.17	—	340
	m	64		440	16	21	7.2	0.55
5	b	36	hydralazine, bendroflumethiazide	1160	75	0.42	0.19	120
	p*	34		1400	70	0.29	—	240
	p**			560	54	0.37	0.11	110
	p**							
6	f	54	propranolol, hydroflumethiazide, prazosin	600	14	19	5.5	0.57
	m	52		440	25	0.13	—	190
	p*	28		840	48	0.36	—	130
	s	21		620	13	27	8.3	0.37
7	f	62		2380	29	8.4	2.1	2.8
	m	61		1350	21	1.5	0.21	12
	p*	36		1200	63	0.47	0.14	100
	m	45		1100	44	6.5	2.2	5.0
8	p*	25		1150	66	0.49	—	140
	s	23		740	22	31	10	0.53
	f	49		580	49	0.49	0.19	73
	m	45		1040	8.3	14	4.4	0.44
9	p*	23		930	62	0.78	—	79
	f	60		350	25	18	5.9	1.0
	m	51		310	6.7	14	4.4	0.35
	b	28		540	27	16	7.2	1.2
10	p*	24		290	64	0.30	0.12	150
	b	22		420	28	22	7.9	0.93
	f	52		800	15	20	6.0	0.53
	m	48		970	22	21	7.5	0.76
11	p*	26		900	79	1.2	—	66
	b	22		900	13	31	9.7	0.30
	s	16		880	17	21	7.9	0.61
	f	59		830	17	23	7.0	0.56
12	m	56		830	21	15	6.0	1.0
	b	34		1280	22	34	12	0.48
	p*	33		1260	65	1.6	0.47	31
	p**			870	52	0.20	—	250
13	b	22		410	46	15	4.8	2.3

Table 1 continued

Family ^a		Age (years)	Drugs	Urinary volume (ml)	SP ^b	12 h urinary excretion		
						2-DHS ^b (% of dose)	5-DHS ^b	MR ^a
12	f	60	apozepam, amylobarbitone	840	23	32	9.5	0.55
	m	54	oestradiol, oestriole norethisterone	1090	15	37	10	0.33
	b	34		770	57	0.40	0.17	100
	p*	26		480	52	0.60	0.31	57
	s	23		930	17	44	13	0.30
13	f	47		200	20	22	6.9	0.68
	m	47		930	20	25	7.9	0.60
	p*	24		470	50	0.37	0.16	96
	s	21	tetracycline	580	13	41	10	0.26
14	f	58		760	51	0.84	0.25	46
	m	47		1170	10	22	6.3	0.36
	s	24		750	60	0.56	0.22	78
	p*	22		220	28	0.16	0.07	120
	s	20		260	30	1.0	0.43	20
	b	16		410	18	33	9.7	0.43
15	m	76	gliclazide, atropine	260	8.1	13	1.5	0.53
	s	49	oestradiol	970	20	30	6.8	0.53
	b	47		480	46	11	4.2	3.0
	b	46		830	16	23	5.8	0.53
	b	42	griseofulvin	1580	34	30	9.2	0.87
	b	41		1000	80	0.71	0.43	70
	p*	39		530	56	0.33	0.21	100
	b	34		620	77	0.41	0.30	110
16	f	63		580	33	13	4.8	1.9
	m	61		760	20	27	7.5	0.58
	p*	37		315	52	0.70	0.20	57
	s	33		630	22	39	10	0.45
17	f	57		740	19	8.7	7.5	1.2
	m	55	oestradiol	620	50	0.31	0.23	94
	s	27		645	26	22	8.6	0.84
	p*	23		770	58	1.1	—	54
	p**			980	52	0.76	—	69
18	m	66		1120	20	26	11	0.53
	p*	29		1000	67	0.77	0.22	68
	p**			320	53	0.37	0.12	110
19	f	55		600	60	0.38	0.14	110
	m	47		1080	17	27	8.5	0.47
	p*	26		890	52	0.48	—	110
	p**			1225	62	0.60	—	100
	b	24		920	73	0.53	—	140
20	s	42		1770	58	0.44	0.20	90
	p*	33		870	58	0.37	—	160

α: f: father, m: mother, b: brother, s: sister, p*: proband, data from Brøsen *et al.* (1985) p**: proband, repeat sparteine test

β: SP = sparteine, 2-DHS and 5DHS = 2- and 5-dehydrosparteine

μ: metabolic ratio = $\frac{\text{sparteine}}{\text{dehydrosparteines}}$ in 12 h urine

Figure 1 Metabolic ratio = $\frac{\text{sparteine}}{\text{dehydrosparteines}}$ in 12 h urine and excretion of dehydrosparteines (% of dose in 12 h urine) in 64 parents and siblings of 20 PM probands; □ extensive metabolizers, ▨ poor metabolizers, * mother, family 6

is almost four times greater than the 7–9% seen in unrelated volunteers ($\chi^2 = 7.9$, $P < 0.005$) (Eichelbaum *et al.*, 1979; Vinks *et al.*, 1982; Evans *et al.*, 1983; Brøsen *et al.*, 1985). The pedigrees (Figure 2) strongly suggest that poor metabolism of sparteine is inherited as an autosomal Mendelian recessive character, confirming previous studies (Eichelbaum *et al.*, 1979; Evans *et al.*, 1983).

If q is the frequency of the recessive allele = $\sqrt{22/301} = 0.27$ (Brøsen *et al.*, 1985), then the predicted, relative PM risk for parents is q

and for siblings $\frac{(1+q)^2}{4}$ (Kemp, 1951). The pre-

dicted values are therefore 0.27 and 0.40, respectively, which are slightly higher than the observed values of $8/35 = 0.23$ and $9/29 = 0.31$, respectively.

Probit analysis of the log MR distribution among healthy volunteers suggested incomplete dominance: 20–27% were relative slow EM (Eichelbaum *et al.*, 1979; Vinks *et al.*, 1982; Brøsen *et al.*, 1985). It was proposed that an

overlap between the two genotypes within the EM mode could account for the remaining 12–19% heterozygotes (Vinks *et al.*, 1982). The size of this postulated transition-zone is neither theoretically nor experimentally influenced by methodology ('peak area' MR vs '% of dose' MR) (Eichelbaum *et al.*, 1979; Brøsen *et al.*, 1985).

In the present study the MR of 23 drug free heterozygotes ranged from 0.35–5.0 compared with 0.11–12.6 seen in 279 unrelated EM volunteers (both genotypes) (Brøsen *et al.*, 1985). The mean age of the former group was twice that of the latter, but age did not correlate with sparteine or dehydrosparteine excretion in heterozygotes (Table 2). The mean $\pm$ s.d. recovery of dehydrosparteines (% of dose) was significantly lower in heterozygotes: $27.2 \pm 10.1\%$ vs $37.1 \pm 10.6\%$ in 279 EM ($P < 0.05$, Student's *t*-test). However, sparteine excretion was almost identical in the two groups: $19.9 \pm 8.1\%$ and $19.7 \pm 10.4\%$ of the dose, respectively. It is therefore concluded that the difference in metabolite recovery is not accounted for by an age related

Figure 2 Pedigrees of 20 unrelated sparteine poor metabolizers. (Offspring generation: youngest to oldest from left to right). Symbols: ○ □ female, male, heterozygote or homozygous dominant; ● ■ female, male, heterozygote; ● ■ female, male, homozygous recessive (poor metabolizer); ● ■ female, male, not tested; † dead.

Table 2 12 h urinary recovery of sparteine and dehydrosparteines (% of dose) in 23 drug free heterozygotes^a

	Mean ± s.d.	r _s
Sparteine	19.90 ± 8.10	0.27*
Dehydrosparteines	27.20 ± 10.10	0.01*
Total	47.10 ± 9.30	-0.18*
MR	0.95 ± 1.04	0.27*

a: Mean age = 50.6 years (range 16–67 years)

*: Correlation with age: $P > 0.2$

decrease in kidney function. Hence impaired metabolism in heterozygotes must be considered.

Using log 'peak area' MR, Evans *et al.* (1983) found a degree of dominance of 67%, which is in close agreement with our findings using either log dehydrosparteines or log MR.

The specificity of the excretion of dehydrosparteines (% of dose) with regard to phenotyping PM was not 100% in this study (Figure 1). One subject (mother, family 6) was PM according to the metabolite recovery (1.7%) but EM according to the MR (12) (Table 1). In spite of a large urine volume, this woman had the lowest

overall drug recovery. Propranolol inhibits the metabolism of sparteine *in vitro* (Otton *et al.*, 1984), and the concomitant treatment with this drug (240 mg day⁻¹) could theoretically have led to an increase in sparteine and a decrease in the dehydrosparteine recovery. Otherwise the conflicting findings in this subject cannot be explained from the available data.

Sparteine/debrisoquine polymorphism plays a central role for the clinical outcome and toxicity of several tricyclic antidepressants (Mellström *et al.*, 1981; Bertilsson & Åberg-Wisted, 1983; Gram *et al.*, 1986). The relative disease risk among first degree relatives to manic-depressive patients is 10–15% (Pettersson, 1977). Combined with the findings in this study it is to be expected that familial occurrence of PM manic-

depressive patients are not infrequent (Gram *et al.*, 1986). Therefore, if possible siblings and parents of such patients should be offered a sparteine/debrisoquine test. Monogenic polymorphism in drug oxidation perhaps opens a new perspective: genetic counseling in clinical pharmacology.

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References

- Bertilsson, L. & Åberg-Wisted, A. (1983). The debrisoquine hydroxylation test predicts the steady state levels of desipramine. *Br. J. clin. Pharmac.*, **15**, 388–390.
- Brøsen, K., Otton, S. V. & Gram, L. F. (1985). Sparteine oxidation polymorphism in Denmark. *Acta Pharmac. Tox.*, **57**, 357–360.
- Eichelbaum, M., Spannbruckner, N., Steincke B. & Dengler, H. J. (1979). Defective N-oxidation of sparteine in man: A new pharmacogenetic defect. *Eur. J. clin. Pharmac.*, **16**, 183–187.
- Eichelbaum, M., Bertilsson, L., Säwe, J. & Zekorn, C. (1982). Polymorphic oxidation of sparteine and debrisoquine: Related pharmacogenetic entities. *Clin. Pharmac. Ther.*, **31**, 184–186.
- Eichelbaum, M. & Woolhouse, N. M. (1985). Inter-ethnic difference in sparteine oxidation among Ghanaians and Germans. *Eur. J. clin. Pharmac.*, **28**, 79–83.
- Evans, D. A. P., Mahgoub, A., Sloan, T. P., Idle, J. R. & Smith, R. L. (1980). A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J. med. Genet.*, **17**, 102–105.
- Evans, D. A. P., Harmer, D., Downham, D. Y., Whibley, E. J., Idle, J. R., Ritchie, J. & Smith, R. L. (1983). The genetic control of sparteine and debrisoquine metabolism in man with new methods of analysing bimodal distributions. *J. med. Genet.*, **20**, 321–329.
- Gram, L. F., Brøsen, K., Christensen, P. & Kragh-Sørensen, P. (1986). Pharmacokinetic considerations relevant to the pharmacodynamics of antidepressants. In *Clinical Pharmacology in Psychiatry*, eds. Dahl, S. G., Gram, L. F., Paul, S. M. & Potter, W. Z. Springer Verlag (in press).
- Inaba, T., Vinks, A., Otton, S. V. & Kalow, W. (1983). Comparative pharmacogenetics of sparteine and debrisoquine. *Clin. Pharmac. Ther.*, **33**, 394–399.
- Kemp, T. (1951). *Genetics and human disease*, pp. 123–133. Copenhagen: Munksgaard.
- Lennard, M. S., Silas, J. H., Freestone, S. & Trevethick, J. (1982). Defective metabolism of metoprolol in poor hydroxylators of debrisoquine. *Br. J. clin. Pharmac.*, **14**, 301–303.
- Mellström, B., Bertilsson, L., Säwe, J., Schulz, H. V. & Sjöqvist, F. (1981). E- and Z-hydroxylation of nortriptyline: Relationship to polymorphic debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **30**, 189–193.
- Otton, S. V., Inaba, T. & Kalow, W. (1984). Competitive inhibition of sparteine oxidation in human liver by some cardiovascular drugs. *Life Sci.*, **34**, 73–80.
- Pettersson, U. (1977). Manic-Depressive illness. A clinical, social and genetic study. *Acta Psychiat. Scand. Suppl.* 269, 61–72. Copenhagen: Munksgaard.
- Vinks, A., Inaba, T., Otton, S. V. & Kalow, W. (1982). Sparteine metabolism in Canadian Caucasians. *Clin. Pharmac. Ther.*, **31**, 23–29.

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