Sparteine oxidation polymorphism in Greenlanders living in Denmark

K. BRØSEN

Department of Clinical Pharmacology, Odense University, Odense, Denmark

1 Sparteine oxidation appeared to be polymorphic in 185 healthy Greenlanders living in Denmark.

2 Six subjects (3.2%) were phenotyped as poor metabolizers (PM) and 179 subjects as extensive metabolizers (EM). The metabolic ratio (MR) between sparteine and 2- + 5-dehydrosparteine in a 12 h urine sample ranged from 0.06–3.12 in EM and from 30–480 in PM. The excretion of dehydrosparteines accounted for less than 2.2% of the dose in PM and ranged from 5.6%–63% in EM.

3 The urinary recovery (% of dose) of sparteine, 2-dehydrosparteine and total sparteine + dehydrosparteines was lower in Greenlander EM than in Danish EM (Brøsen *et al.*, 1985). Incomplete urine collection in a substantial proportion of the Greenlanders could explain these discrepancies.

Keywords Greenlanders Eskimos Danes drug oxidation genetic polymorphism sparteine

Introduction

The antiarrhythmic sparteine is oxidized by a distinct isozyme of cytochrome P-450 (Distlerath & Guengerich, 1984), the activity of which is rate-limiting for the appearance of the main metabolites 2- and 5-dehydrosparteine in the urine (Eichelbaum *et al.*, 1979a). Sparteine is also excreted in an unmetabolized form. Hence the sparteine versus dehydrosparteines ratio in a fixed-hour urine sample after a single test dose is a 'metabolic ratio' (MR). When this simple test was carried out in healthy volunteers a monogenic polymorphism in drug oxidation was discovered (Eichelbaum *et al.*, 1979b). In Caucasians, two phenotypes are clearly separated: poor metabolizers (PM) who make up 7–9% of the population and extensive metabolizers (EM) who make up the rest (Vinks *et al.*, 1982; Evans *et al.*, 1983; Eichelbaum & Woolhouse, 1985; Brøsen *et al.*, 1985). The PM phenotype is inherited as an autosomal Mendelian recessive trait, whereas the EM phenotype comprises

both heterozygotes and homozygous dominants (Evans *et al.*, 1983; Brøsen *et al.*, 1986a).

Both *in vitro* studies on human liver (Otton *et al.*, 1982; Boobis *et al.*, 1983; Distlerath *et al.*, 1984) and *in vivo* studies (Eichelbaum *et al.*, 1982; Inaba *et al.*, 1983; Brøsen *et al.*, 1986b) have clearly demonstrated coregulation of sparteine and debrisoquine oxidation in Caucasians, but conflicting data in a few subjects are reported (Evans *et al.*, 1983).

PM of sparteine are poor hydroxylators of imipramine and desipramine (Brøsen *et al.*, 1986b), and the oxidation of several other clinically important drugs such as nortriptyline (Mellström *et al.*, 1981; Woolhouse *et al.*, 1984), amitriptyline (Mellström *et al.*, 1983, 1986) and metoprolol (Lennard *et al.*, 1982; McGourty *et al.*, 1985) is impaired in debrisoquine PM.

Interethnic differences in gene frequencies are common (Kalow, 1982) and for a monogenic character this will almost invariably result in

Correspondence: Dr Kim Brøsen, Department of Clinical Pharmacology, Odense University, J.B. Winsløvs Vej 19, DK-5000 Odense C, Denmark

racial differences in phenotype composition. In Eskimos only 5% are genetically slow acetylators of isoniazid compared with 60% in Caucasians (Armstrong & Peart, 1960). Likewise the occurrence of the so-called 'atypical' plasma choline esterase gene is extremely rare in Eskimos, whereas the frequency of the 'silent' choline esterase gene is a thousand times greater in this racial group compared with Caucasians (Kalow, 1982).

This paper describes polymorphic sparteine oxidation in healthy Greenlanders living in Denmark.

Methods

The subjects of the study comprised 193 healthy Greenlanders living in Denmark. There were 80 women and 113 men. Their mean age was 21.6 years (range 17–54). Each subject consented to participate on the basis of verbal and written information in Danish, and the protocol was approved by the regional ethics committee. In order to minimize the impact of environmental differences all the volunteers were recruited in Denmark at the Greenlander Houses in Odense, Esbjerg, Ikast, Aarhus and Aalborg. The principle of volunteer participation was used and strict random selection was not applied. Three subjects were born in Denmark, two in East-Greenland (Angmagssalik) and the rest in West-Greenland, predominantly South-West-Greenland.

Most volunteers were staying temporarily in Denmark for educational purposes, and the mean duration of their stay prior to the sparteine test was 5.9 months (0.5–75). None had daily drug or alcohol intake. All subjects claimed that both their parents were Greenlanders born in Greenland, but no attempts were made to ascertain this via pedigrees. In most cases the physical

appearance was typical Eskimo, but some subjects had features, particularly with regard to physiognomy, that were almost indistinguishable from those of Europeans.

Each volunteer ingested 100 mg sparteine sulphate (tablet Depasan®, Giuliani) and collected urine for the following 12 h. Urine volumes were recorded immediately and an aliquot was kept frozen (–20°C) until analysis. The urinary content of sparteine (SP), 2-dehydrosparteine (2-DHS) and 5-dehydrosparteine (5-DHS) was assayed by gas chromatography (Vinks *et al.*, 1982). The lower level of detection for both dehydrosparteines (DHS) was 0.25 nmol ml^{–1}.

A metabolic ratio was calculated as:

$$\frac{\% \text{ of dose excreted as SP}}{\% \text{ of dose excreted as DHS}}$$

in the 12 h urine.

Neither SP nor DHS could be detected in the urine of four men and four women, but a repeated sparteine test was not possible. These subjects were excluded from the study.

Results

The distribution of MR was bimodal (Figure 1), and the two phenotypes were clearly separated: MR ranged from 0.06 to 3.12 in 179 EM and from 30 to 480 in six PM. The probit line for EM was curvilinear, but a clear separation of homo- and heterozygote EM was not possible (Figure 1). The mean ($\pm$ s.d.) urinary recoveries in the two phenotypes are listed in Table 1. Bimodality was also apparent in the distribution of DHS recovery (Figure 1).

Discussion

This study demonstrates polymorphic sparteine oxidation in Greenlanders. The MR antimode

Table 1 Urinary excretion (% of dose) of sparteine and dehydrosparteines in Greenlanders and Danes^a (mean $\pm$ s.d.).

	EM		PM	
	Greenlanders (n = 179)	Danes ^a (n = 279)	Greenlanders (n = 6)	Danes ^a (n = 22)
SP	13.4 $\pm$ 8.4	19.7 $\pm$ 10.4	54.1 $\pm$ 22.8 ^b	58.9 $\pm$ 10.9
2-DHS	23.8 $\pm$ 10.3	29.2 $\pm$ 8.7	0.6 $\pm$ 0.9	0.6 $\pm$ 0.4
5-DHS	8.5 $\pm$ 3.2	8.0 $\pm$ 2.3	0.1 $\pm$ 0.1	0.1 $\pm$ 0.2
Total				
SP + DHS	45.6 $\pm$ 17.6	56.9 $\pm$ 10.3	54.8 $\pm$ 23.1	59.6 $\pm$ 11.0
log MR	–0.43 $\pm$ 0.26	–0.30 $\pm$ 0.32	–	–

^aData from Brøsen *et al.* (1985)

^bSP recovery was only 7.9% in one PM. This subject had an MR of 144

Figure 1 Metabolic ratio (MR) = sparteine/dehydrosparteines in 12 h urine and excretion of dehydrosparteines (% of dose in 12 h urine) in 185 healthy Greenlanders living in Denmark. (□ and *: extensive metabolizers; ■ and o: poor metabolizers).

of 20 reported in West Germans and Danes (Eichelbaum *et al.*, 1982; Brøsen *et al.*, 1985) also seems to be valid in Greenlanders. The frequency of PM was $6/185 = 3.2\%$ compared with 7.3% found in 301 Danes (Brøsen *et al.*, 1985) ($p = 8.8\%$, Fischer's exact test). As in the Danes (Brøsen *et al.*, 1985, 1986a), the sparteine test could not distinguish the two genotypes

within the dominant phenotype. Comparative studies of families and populations (Evans *et al.*, 1983; Brøsen *et al.*, 1986a) have indicated that sparteine oxidation on the average is faster in homozygous dominants than in heterozygotes. The estimated frequencies (Hardy-Weinberg) of these genotypes were 0.67 and 0.30, respectively, in the Greenlanders compared with 0.53

and 0.40, respectively, reported in Danes (Brøsen *et al.*, 1985). Being independent of urine collection, the MR (Table 1) suggested that sparteine oxidation was faster and less variable in the Greenlandic EM (*F*-test, $P < 0.001$). This is consistent with the lower frequency of the recessive sparteine allele in Greenlanders.

Debrisoquine testing of 100 Japanese (Nakamura *et al.*, 1985) and sparteine testing of 51 Cuna Indians (Arias *et al.*, 1986) did not identify any PM, which suggests that the frequency of this phenotype is much lower in Mongoloids than in Caucasians. Genetically Greenlanders, and particularly West Greenlanders, are a mixed racial group. Earlier studies of the distribution of blood groups and HL-A tissue antigens (Gürtler *et al.*, 1970; Kissmeyer-Nielsen *et al.*, 1972) showed that Caucasian genes made up about 30% of their gene pool. However, the fraction of admixed Caucasian genes was not assessed in the 185 volunteers, and it is therefore not possible to conclude whether the recessive sparteine allele has existed in the original Eskimo population or has been imported from Europe.

In two studies of Caucasians (Vinks *et al.*, 1982; Brøsen *et al.*, 1985) there was an expected negative correlation between SP and DHS excretion in the urine. In contrast a weak positive correlation was found in the Greenlanders ($r_s = 0.30$, $P < 0.001$, $n = 185$), suggesting that some external factor overrides the impact of metabolism. Furthermore, both the SP and the 2-DHS recovery was lower in Greenlandic EM than in Danish EM (Table 1). Hence the total SP + DHS was lower and more variable in the Greenlanders (*F*-test, $P < 0.001$). Though not proven, all these discrepancies could be explained by incomplete urine collection. It should be taken into account that Danish is only a second language for Greenlanders and mis-

understandings and poor compliance may have been of importance in a substantial number of the volunteers, but the majority seemed to have followed the instruction carefully. Ethnic differences in SP and DHS excretion may reflect social and cultural barriers as well as variations in drug metabolism.

Sparteine metabolism *in vivo* is inhibited by imipramine and desipramine (Brøsen *et al.*, 1986b) and almost abolished by quinidine (Brinn *et al.*, 1986). Most likely other drugs and xenobiotics can have similar effects. All volunteers had stayed in Denmark and lived on a Danish diet for a period of time before they were sparteine-tested. It is therefore unlikely that the difference in sparteine disposition was caused by environmental factors.

The steady state kinetics of phenytoin is not subject to the same genetic variation as debrisoquine hydroxylation (Roots *et al.*, 1985). The lower steady state concentrations and faster parahydroxylation of phenytoin reported in Greenlandic epileptics (Dam *et al.*, 1977) thus cannot be explained by the greater capacity for sparteine oxidation in this racial group.

Theoretically, the lower frequency of PM in Greenlanders may offer a relative protection against the medical hazards caused by drug treatment with standard dose regimens, but general guidelines for dosing of drugs in this racial group cannot be established from the present study.

This study was supported by grants from the Lundbeck Foundation and the P. Carl Petersen Foundation.

The author wishes to express his gratitude to Mrs Ulla Høegh, Mrs Kristine Nielsen, Mr Anders Randers, Mr Niels Jørgen Jensen, Mr Apollo Lynge and Mr Finn Jørgensen for their efforts in recruiting volunteers.

References

- Arias, T. D., Jorge, L. F. & Inaba, T. (1986). No evidence for the presence of poor metabolizers of sparteine in an Amerindian group: The Cunas of Panama. *Br. J. clin. Pharmacol.*, **21**, 547–548.
- Armstrong, A. R. & Peart, H. E. (1960). Comparisons between the behaviour of Eskimos and non Eskimos to the administration of isoniazid. *Ann. Rev. resp. Dis.*, **81**, 588–596.
- Boobis, A. R., Murray, S., Kahn, G. C., Robertz, G.-M. & Davies, D. S. (1983). Substrate specificity of the form of cytochrome P-450 catalyzing the 4-hydroxylation of debrisoquine in man. *Mol. Pharmacol.*, **23**, 474–481.
- Brinn, R., Brøsen, K., Gram, L. F., Haghfelt, T. & Otton, S. V. (1986). Sparteine oxidation practically abolished in quinidine-treated patients. *Br. J. clin. Pharmacol.*, **22**, 194–197.
- Brøsen, K., Klysner, R., Gram, L. F., Otton, S. V., Bech, P. & Bertilsson, L. (1986b). Steady-state concentration of imipramine and its metabolites in relation to the sparteine/debrisoquine polymorphism. *Eur. J. clin. Pharmacol.*, **30** (in press).
- Brøsen, K., Otton, S. V. & Gram, L. F. (1985). Sparteine oxidation polymorphism in Denmark. *Acta Pharmac. Tox.*, **57**, 357–360.
- Brøsen, K., Otton, S. V. & Gram, L. F. (1986a). Sparteine oxidation polymorphism: a family study. *Br. J. clin. Pharmacol.*, **21**, 661–667.

- Dam, M., Larsen, L. & Christiansen, J. (1977). Phenytoin: Ethnic differences in plasma level and clearance. In *Antiepileptic drug monitoring*, eds. Gardner-Thorpe, T. *et al.*, pp. 73–80. London, England: Pitman Medical Publishing Co. Ltd.
- Distlerath, L. M. & Guengerich, F. P. (1984). Characterization of a human liver cytochrome P-450 involved in the oxidation of debrisoquine and other drugs by using antibodies raised to the analogous rat enzyme. *Proc. Nat. Acad. Sci.*, **81**, 7348–7352.
- 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., Spannbrucker, N. & Dengler, H. J. (1979a). Influence of the defective metabolism of sparteine on its pharmacokinetics. *Eur. J. clin. Pharmac.*, **16**, 189–194.
- Eichelbaum, M., Spannbrucker, N., Steincke, B. & Dengler, H. J. (1979b). Defective N-oxidation of sparteine in man: A new pharmacogenetic defect. *Eur. J. clin. Pharmac.*, **16**, 183–187.
- 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., 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.
- Gürtler, H., Gilberg, Aa. & Tingsgaard, P. (1971). The blood group distribution in Greenland. *The second International Symposium on Circumpolar Health*, Oulu, June 21–24, 1971 (abstract).
- Inaba, T., Vinks, A., Otton, S. V. & Kalow, W. (1983). Comparative pharmacogenetics of sparteine and debrisoquine. *Clin. Pharmac. Ther.*, **33**, 394–399.
- Kalow, W. (1982). Ethnic differences in drug metabolism. *Clin. Pharmacokin.*, **7**, 373–400.
- Kissmeyer-Nielsen, F., Kjerbye, K. E., Lamm, L. U., Jørgensen, J., Bruun Petersen, G. & Gürtler, H. (1972). *Study of the HL-A system in Eskimos. Histocompatibility testing*, pp. 317–324. Copenhagen: Munksgaard.
- Lennard, M. S., Silas, J. H., Freestone, S. & Treve-thick, J. (1982). Defective metabolism of metoprolol in poor hydroxylators of debrisoquine. *Br. J. clin. Pharmac.*, **14**, 301–303.
- McGourty, J. C., Silas, J. H., Lennard, M. S., Tucker, G. T. & Woods, H. F. (1985). Metoprolol metabolism and debrisoquine oxidation polymorphism—population and family studies. *Br. J. clin. Pharmac.*, **20**, 555–566.
- Mellström, B., Bertilsson, L., Lou, Y.-C., Säwe, J. & Sjöqvist, F. (1983). Amitriptyline metabolism: Relationship to polymorphic debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **34**, 516–520.
- Mellström, B., Bertilsson, L., Säwe, J., Schulz, H.-U. & Sjöqvist, F. (1981). E- and Z-10-hydroxylation of nortriptyline: Relationship to polymorphic debrisoquine hydroxylation. *Clin. Pharmac. Ther.*, **30**, 189–193.
- Mellström, B., Säwe, J., Bertilsson, L., Sjöqvist, F. (1986). Amitriptyline metabolism: association with debrisoquine hydroxylation in nonsmokers. *Clin. Pharmac. Ther.*, **39**, 369–374.
- Nakamura, K., Goto, F., Ray, W. A., McAllister, C. B., Jacqz, E., Wilkinson, G. R. & Branch, R. A. (1985). Interethnic differences in genetic polymorphism of debrisoquine and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin. Pharmac. Ther.*, **38**, 402–408.
- Otton, S. V., Inaba, T., Mahon, W. A. & Kalow, W. (1982). *In vitro* metabolism of sparteine by human liver: competitive inhibition by debrisoquine. *Can. J. Physiol. Pharmac.*, **60**, 102–105.
- Roots, I., Otte, F., Berchtold, C., Heinemeyer, G., Schmidt, D. & Cornaggia, C. (1985). Debrisoquine phenotyping in epileptic patients treated with phenytoin and carbamazepine. *Biochem. Pharmac.*, **34**, 447–448.
- Vinks, A., Inaba, T., Otton, S. V. & Kalow, W. (1982). Sparteine metabolism in Canadian Caucasians. *Clin. Pharmac. Ther.*, **31**, 23–29.
- Woolhouse, N. M., Adjepon-Yamoah, K. K., Mellström, B., Hedman, A., Bertilsson, L. & Sjöqvist, F. (1984). Nortriptyline and debrisoquine hydroxylations in Ghanaian and Swedish subjects. *Clin. Pharmac. Ther.*, **36**, 374–378.

(Received 21 April 1986,
accepted 13 June 1986)