SPARTEINE METABOLISM IN A NIGERIAN POPULATION

J.C. Ritchie*, S.C. Mitchell** and R.L. Smith

Pharmacology and Toxicology, Imperial College School of Medicine at St. Mary's, Norfolk Place, London W2 1PG, U.K.
**Present address: SmithKline Beecham Pharmaceuticals Transnational Regulatory Affairs and Compliance New Frontiers Science Park, Third Avenue, Harlow Essex CM19 5AW, U.K.*

SUMMARY

The oxidative metabolism of sparteine has been investigated in a Nigerian population. The distribution of metabolic capacities was shown to be skewed with two subjects (2/97, 2.1%) being relatively deficient in their ability to produce the dehydrometabolites. These observations afford evidence that sparteine oxidation is under polymorphic control in Nigerians.

KEY WORDS

sparteine, oxidation, polymorphism, Nigerians

INTRODUCTION

It is now well established that the biotransformation of sparteine, a naturally occurring quinolizidine alkaloid with cardio-stimulatory /1/ and oxytocic properties /2/, exhibits wide interindividual variability /3, 4/. In European populations the oxidative metabolism of sparteine (to its dehydrometabolites) and debrisoquine (to 4-hydroxydebrisoquine) are under a common genetic control /5/ and are mediated via cytochrome P-4502D6, the gene for which (CYP2D) has been localized in the long arm of chromosome 22 (22q11.2-q12.2) /6/. Numerous investigations have pointed to ethnic differences in the sparteine/debrisoquine oxidation polymorphism /7/ and the reported frequency of

**Author for correspondence

occurrence of 'poor metabolisers' of sparteine has varied from zero in Ghanaians /8/, South African Venda /9/ and Cuna (Panama) Indians /10/ to 8.3% in Canadian Caucasians /11/. Despite such a wealth of information, only one study concerning sparteine has been reported in black Nigerians /12/; the present investigation provides additional data on the polymorphic metabolism of sparteine in this African population.

MATERIALS AND METHODS

Human volunteers

Ninety-seven healthy black Nigerians (78 male, 19 female; age 19 to 29 years [23.2 ± 2.3, mean ± s.d.]) were recruited from the student population of the University of Ife, Ile-Ife, Nigeria. All subjects were in good health, had no history of major organ disease and had not received any recent drug medication. Local ethical approval and informed consent were obtained before participation in the study. Following a light breakfast, each volunteer emptied the bladder and then received sparteine sulphate (100 mg, Aldrich Chemical Co. Ltd., Dorset, UK) orally in a gelatin capsule together with 150 ml water. Thereafter all urine was collected for the next 0-12 hours. Total urine volumes were recorded and aliquots (20 ml) taken and stored at -20°C until they were transported over dry-ice to London for analysis.

Analysis of sparteine and metabolites

Each urine sample was allowed to thaw and an aliquot (1 ml) applied to a glass column (5 x 0.5 cm i.d.) containing the nonionic polymeric adsorbent Amberlite XAD-2 (Aldrich Chemical Co.) previously prepared by successive washings with distilled water (3 ml), methanol (3 ml) and water (3 ml). The column was washed with distilled water (3 x 1 ml), the excess fluid being drained off and discarded, and then eluted with methanol (3 x 1 ml). This eluate was evaporated under reduced pressure (<36°C) and the dried residue dissolved in a small volume (50-100 μl) of methanol before examination by chromatography. The recovery of known amounts of sparteine added to urine within the working range from Amberlite XAD-2 columns was 73.2 ± 3.0% (mean ± s.d.).

Thin-layer chromatography (t.l.c.) was performed on precoated aluminium-backed silica gel 60 plates (0.2 mm thick; Merck,

Darmstadt, Germany) and developed in a solvent system consisting of ethyl acetate/methanol/aq. NH_4OH (sp. gr. 0.88) (10/1/1 by vol) /13/. The plates were subsequently dried in a current of warm air to remove any remaining solvent and alkaloid related compounds were visualised by dipping in iodoplatinate reagent /13-15/.

After drying, the plates were assessed visually and then scanned at 540 nm employing reflection-absorption photometry using a Shimadzu CS930 dual wavelength chromatoscanner (V.A. Howe and Co. Ltd., London, UK). The use of automatic background compensation and scanning in a zig-zag fashion permitted the integration of the moving average of data from the individual sampling points (spikes) and enabled the calculation of the volume of chromatospots (zone volume). In contrast to analysis with solutions, a simple linear relationship cannot be obtained between absorbance and concentration of material on a thin-layer surface owing to the scattering of light by the adsorbing agent within the plate. Fortunately, this relationship between absorption and concentration can be linearized mathematically by employing the Kubelka-Munk equations /16/.

The application of known amounts of sparteine in urine to t.l.c. plates, subsequent development, visualisation and quantification permitted the construction of a simple semilogarithmic plot which gave a straight line calibration curve between 10 and 250 μg (sparteine free base) which encompassed the working range for the urine samples under analysis. This calibration curve did not pass through the origin, giving a lower limit of detection equivalent to 5 μg sparteine (free base) on the t.l.c. plate. Results obtained from several determinations of the same urine samples, spiked with sparteine, showed coefficients of variation of 5.0 ± 0.9% (n=6; 20 μg on plate) and 4.8 ± 0.8% (n=6, 200 μg on plate). The variability observed over a period of two months between aliquots of the same spiked urine samples analysed at weekly intervals resulted in a maximum coefficient of variation value of 5.6% (n=8).

Unfortunately, insufficient amounts of material prevented similar procedures being undertaken for the metabolites and relative extinctions could not be measured. Consequently, metabolites were quantified as sparteine equivalents and absolute recoveries could not be determined. However, the semi-quantitative measurements undertaken enabled the expression of a relative ratio (sparteine/2-dehydrosparteine + 5-dehydrosparteine) and thus permitted the ranking of individuals with respect to their relative urinary metabohte excretion.

RESULTS AND DISCUSSION

Sparteine (Rf 0.71) and its two major dehydrogenated metabolites, 2-dehydrosparteine (Rf. 0.33) and 5-dehydrosparteine (Rf 0.11), were visualised as black spots on a light pink ground together with several other unidentified minor metabolites from which they were clearly resolved. The pink background fades to white (2-3 days) but the black spots are permanent (at least 12 years in the dark). This has an advantage over the use of iodine vapour (fades in 30 minutes) previously advocated by other workers /17/. In addition, both major metabolites, unlike sparteine, gave a red colouration with an alkaline nitroprusside reagent, known to furnish red or violet-red products with steroids containing unsaturated lactone rings and previously noted for Δ^5 and Δ^{11} dehydro compounds /13,15/. Although lack of authentic compounds prevented an absolute confirmation of identity, these metabolites possessed the same relative front values (ref. sparteine) on thin-layer plates and subsequent extracts provided gas chromatographic peaks in accordance with data from previous workers /4,8,11,13,18/.

From visual examination of the chromatograms it could be seen clearly that whilst the majority of individuals excreted relatively large amounts of the two dehydro products amidst other unidentified metabolites, a few produced only small amounts of these metabolites and two subjects (both female) had only sparteine detectable in their urine, traces of the dehydro-metabolites eventually being identified only after urine concentration (alkalinization then diethyl ether extraction). The presence of these two 'poor metabolisers' was confirmed by repeat testing at a later date. Interestingly, those individuals who produced little or no observable dehydro-metabolites also showed a lack of the other unidentified metabolites, perhaps suggesting the route to these products may be through the dehydro-compounds /19/.

When the data calculated for each individual were presented in the form of a histogram relating frequency of occurrence and the common (Briggsian) logarithm (base 10) of the sparteine metabolic ratio, it could be seen that the distribution was skew (skewness 1.22; 0.25 with the two outliers removed) with a tail extending towards the higher values in the positive direction from the median. For such distributions the value of the arithmetic mean (ratio 6.0, $\log_{10}$ 0.778) is usually greater than the median (ratio 0.7, $\log_{10}$ -0.155). A mathematical measure of non-normality called kurtosis gave a positive value (4.68; 0.40 with the two outliers removed) indicating an excess density in the tails of the distributions (it is zero for a normal distribution) (Fig. 1). The

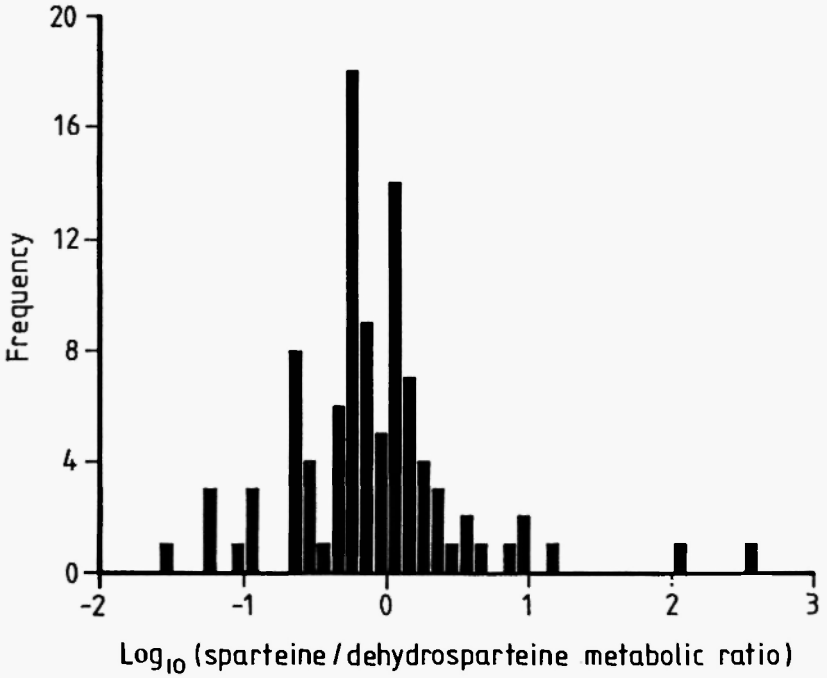

Fig. 1: Frequency distribution of the $\log_{10}$ 0-12 hour urinary ratios of sparteine/dehydrosparteine after 100 mg dose of sparteine sulphate in an unrelated black Nigerian population (n=97).

present identification of two subjects (2/97, 2.1%) with very high sparteine/dehydrosparteine metabolite ratios agrees with a previous study in which four such individuals (4/106, 3.8%) were identified /12/, and affords additional evidence that sparteine oxidation is under polymorphic control in black Nigerians. As previously mentioned, the reported frequency of sparteine 'poor metabolisers' has varied from zero to 8.3%, depending upon the population group examined, and this variation is reflected in studies concerning 'poor metabolisers' of debrisoquine (another cytochrome P-4502D6 probe) /20,21/. Such ethnological differences require closer examination before a full understanding can be approached /22/.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P.A.F. Dixon, Department of Pharmacology, University of Ile Ife, Nigeria, for organising the sparteine dosing and urine collection.

REFERENCES

1. Raschack M. Wirkungen von Spartein und Sparteinderivaten auf Herz und Kreislauf. Arzneimittel-Forsc 1974; 24: 753-761.
2. Brazeau P. The Pharmacological Basis of Therapeutics. New York: MacMillans, 1965; 886-887.
3. Eichelbaum M, Spannbrucker N, Dengler HJ. N-Oxidation of sparteine in man and its interindividual differences. Naunyn-Schmiedebergs Arch Exp Path Pharmak 1975; 287 Suppl P: R94.
4. Eichelbaum M, Reetz KP, Schmidt EK, Zekorn C. The genetic polymorphism of sparteine metabolism. Xenobiotica 1986; 16: 465-481.
5. Evans DAP, Harmer D, Downham DY, Whibley E, Idle J, Ritchie J, Smith RL. The genetic control of sparteine and debrisoquine metabolism in man with new methods of analysing bimodal distributions. J Med Genet 1983; 20: 321-329.
6. Gonzalez FJ, Vilbois F, Hardwick JP, McBride OW, Nebert DW, Gelboin HV, Meyer UA. Human debrisoquine 4-hydroxylase (P450 II D I); cDNA and deduced amino acid sequence and assignment of the CYP2D locus to chromosome 22. Genomics 1988; 2: 174-179.
7. Eichelbaum M, Gross AS. The genetic polymorphism of sparteine/debrisoquine metabolism - clinical aspects. Pharm Therap 1990; 46: 377-394.
8. Eichelbaum M, Woolhouse NM. Inter-ethnic difference in sparteine oxidation among Ghanaians and Germans. Eur J Clin Pharmacol 1985; 28: 79-83.
9. Somers DeK, Moncrieff J, Avenant JC. Absence of polymorphism of sparteine oxidation in the South African Venda. Hum Exp Toxicol 1991; 10: 175-178.
10. Arias TD, Jorge LF, Lee D, Barrantes R, Inaba T. The oxidative metabolism of sparteine in the Cuna Amerindians of Panama. Absence of evidence of deficient metabolisers. Clin Pharm Ther 1988; 43: 456-465.
11. Vinks A, Inaba T, Otton SV, Kalow W. Sparteine metabolism in Canadian Caucasians. Clin Pharmacol Ther 1982; 31: 23-29.
12. Lennard MS, Iyun AO, Jackson PR, Tucker GT, Woods HF. Evidence for a dissociation in the control of sparteine, debrisoquine and metoprolol metabolism in Nigerians. Pharmacogenetics 1992; 2: 89-92.
13. Cho YD, Martin RO. Resolution and unambiguous identification of microgram amounts of 22 lupin alkaloids by sequential use of thin layer and gas-liquid chromatography and mass spectrometry. Anal Biochem 1971; 44: 49-57.

14. Barnsley EA, Thompson AER, Young L. Biochemical studies of toxic agents. The biosynthesis of ethylmercapturic acid and sulphoxide. Biochem J 1964; 90: 588-596.
15. Elliot DC. Methods for the detection of biochemical compounds on paper. In: Dawson RMC, Elliott DC, Elliott WH, Jones KM, eds. Data for Biochemical Research. London: Clarendon Press, 1959; 210-272.
16. Yamamoto H, Kurita T, Suzuki J, Hira R, Nakano K, Makabe H, Shibata K. Dual-wavelength point zig-zag scanning of zones on thin-layer chromatograms as a tool for quantitative assay. J Chromatogr 1976; 116: 29-41.
17. Ebner T, Meese CO, Eichelbaum M. Thin-layer chromatography screening test for polymorphic sparteine oxidation. Ther Drug Monit 1989; 11: 214-216.
18. Ritchie JC, Mitchell SC, Smith RL, Zhang AQ. Use of site-specified tritium-labelling to confirm the formation of 17-oxosparteine as a minor urinary metabolite of sparteine in man. Xenobiotica 1996; in press.
19. Pospísil J, Patzelová V, Máca B. New evidence of sparteine metabolites in humans. Drug Metab Dispos 1992; 20: 330-332.
20. Evans DAP. Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics. Cambridge: Cambridge University Press, 1993; 54-101.
21. Relling MV, Cherrie J, Schell MJ, Petros WP, Meyer WH, Evans WE. Lower prevalence of the debrisoquine oxidative poor metaboliser phenotype in American black versus white subjects. Clin Pharm Ther 1991; 50: 308-313.
22. Gonzalez FJ, Meyer UA. Molecular genetics of the debrisoquine-sparteine polymorphism. Clin Pharm Ther 1991; 50: 233-238.