Eur J Clin Pharmacol (1990) 39: 533–537

European Journal of Clinical Pharmacology

Originals

Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations

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Received: February 1, 1990/Accepted: July 16, 1990

Summary. European data on the polymorphic metabolism of debrisoquine, sparteine, dextromethorphan and mephenytoin have been collected.

No significant difference in phenotype frequencies was found between the separate series for debrisoquine, sparteine and dextromethorphan, which supports the claim that these probe drugs reflect the same enzyme polymorphism.

The mean frequency of the phenotype slow debrisoquine metaboliser was 7.65% based on 5005 determinations. The overall mean reflecting all three drugs and 8764 determinations was 7.40%. This is consistent with a gene frequency of 0.27 (95% confidence interval 0.26–0.28).

The overall mean of the phenotype slow metaboliser of mephenytoin was 3.52% corresponding to a gene frequency of 0.19 (confidence interval 0.17–0.20).

The incidence of slow metabolism of debrisoquine and possibly also of S-mephenytoin was homogeneous in the samples from European populations. This is of considerable interest as interethnic differences are now being found both in the phenotypic characters as well as the genotypes of polymorphic drug oxidation.

Key words: Genetic polymorphism, debrisoquine, pharmacogenetics, sparteine, dextromethorphan, mephenytoin, oxidative drug metabolism, meta-analysis

Variability and selection is nature's strategy to ensure adaptation to the wide variety of demands that are imposed on all living beings. It is rather the rule than the exception that genetically controlled characters are modified in quantal steps. One extreme is a character solely regulated by the presence or absence of a certain allele at a certain gene locus in the genome (monogenic trait), and the other extreme is the contribution of several genes (polygenic trait) and perhaps environmental factors, too, in producing a certain quality of phenotype. Systems have also been identified where several major genes contribute significantly to the presence of a certain quality.

A genetic polymorphism is a monogenic trait that appears in two or more phenotypes. It is the result of the action of different alleles at a single gene locus (Vogel and Motulsky 1986). The influence of a single gene on variability among individuals may sometimes be visualised in frequency distributions that exhibit more than one mode. If the least common phenotype appears less often than in 1% of the individuals, it is arbitrarily defined as a rare trait rather than a polymorphism, although there is no particular difference other than frequency between the two entities.

Table 1. Collected data on the Debrisoquine metabolic ratio

Sample size	rapid	slow	% slow	reference
258	235	23	8.9	Evans et al. 1980
268	245	23	8.6	Schmid et al. 1985
107	101	6	5.6	Syvälahti et al. 1986
100	90	10	10.0	Gachalyi et al. 1986
221	198	23	10.4	Küpfer and Preisig 1984
100	92	8	8.0	Szórády and Santa 1987
377	352	25	6.6	Benitez et al. 1988
99	95	4	4.0	Benitez et al. 1988[a]
167	155	12	7.2	Leclercq et al. 1987
92	85	7	7.6	Boobis et al. 1988[a]
104	96	8	7.7	Feely 1988[a]
155	150	5	3.2	Arvela et al. 1988
1049	969	80	7.6	Siest 1988[a]
757	716	41	5.4	Steiner et al. 1988
163	154	9	5.5	Bechtel et al. 1988[a]
61	54	7	11.5	Leopold 1986
309	286	23	7.4	Ciba-Geigy 1988[a]
106	94	12	11.3	Rominger 1988[a]
164	153	11	6.7	Hollman 1988[a]
205	187	18	8.8	Sanz et al. 1989[a]
143	131	12	8.4	McGourty et al. 1985
sum	sum	sum	mean (sd)	
5005	4638	367	7.65 (2.17)	

[a] personal communications

Table 2. Collected data on the metabolic ratio of sparteine

Sample size	rapid	slow	% slow	reference
301	279	22	7.3	Brøsen et al. 1985
172	159	13	7.6	Schellens et al. 1986
130	124	6	4.6	Schellens et al. 1988[a]
983	913	70	7.1	Eichelbaum 1988[a]
210	194	16	7.6	Rosenkrantz 1988[a]
231	220	11	4.8	Wingender et al. 1988[a]
358	325	33	9.2	Drohse et al. 1988[a]
71	67	4	5.6	Neugeboren 1988[a]
121	109	12	9.9	Paar et al. 1989
sum	sum	sum	mean (sd)	
2577	2390	187	7.08 (1.83)	

[a] personal communications

Table 3. Collected data on the metabolic ratio of dextromethorphan

Sample size	rapid	slow	% slow	reference
268	245	23	8.6	Schmid et al. 1985
50	47	3	6.0	Siest et al. 1988[a]
106	98	8	7.5	Dayer 1988[a]
103	99	4	3.9	Larrey et al. 1987
73	66	7	9.6	Jonkman 1988[a]
450	404	46	10.2	Hildebrand et al. 1988[a]
132	128	4	3.0	Jacqz et al. 1988
sum	sum	sum	mean (sd)	
1182	1087	95	6.98 (2.78)	

[a] personal communications

Table 4. Collected data on the metabolic ratio of mephenytoin

Sample size	rapid	slow	% slow	reference
358	349	9	2.5	Drohse et al. 1989
253	246	7	2.8	Sanz et al. 1989
221	209	12	5.4	Küpfer et al. 1984
172	168	4	2.3	Breimer et al. 1990
132	124	8	6.1	Jacqz et al. 1988
sum	sum	sum	mean (sd)	
1136	1096	40	3.26 (1.46)	

It is very common that both polymorphically regulated characters and rare traits differ in frequency in ethnic groups living in geographically separate areas. Textbooks in medical genetics (eg Vogel and Motulsky 1986) give numerous examples. The main explanations for such inequalities are natural selection and differences occurring by chance alone (genetic drift).

In an early study, Harris et al. 1968 found 6 out of 18 investigated enzymes to be polymorphic, which would imply that polymorphic enzymes were by no means rare. The outstanding example of a polymorphic drug metabolising enzyme is the acetyltransferase responsible for acetylation of isoniazid (Evans et al. 1960) and certain other drugs, including hydralazine and some sulphonamides. Although many scientists were prepared to find polymorphisms in oxidative drug metabolism, too, it took several years to discover the polymorphic metabolism of debrisoquine (Mahgoub et al. 1977) and sparteine (Eichelbaum et al. 1975, 1979), which are now considered to reflect the activity of the same enzyme. The possibility of detecting a polymorphism in drug metabolism is highly dependent on which drug and which pharmacokinetic index are selected for investigation (Jackson et al. 1986).

There is now a number of drugs whose metabolism, or at least one of whose metabolic pathways, seems to be associated with that of debrisoquine (for review see Brøsen and Gram 1989). There are also seemingly separate polymorphisms for other drugs that are metabolized by oxidation. Although those polymorphisms are now being elucidated at the molecular level they still have to be defined operationally by the actual probe drugs used for their ascertainment.

Material and methods

After more than 10 years of research on the debrisoquine polymorphism, there are many reports on the distribution of the debrisoquine metabolic ratio in population samples. For a definition and discussion of the meaning of this experimental index refer to Mahgoub et al. (1977), Inaba et al. (1983), Jackson et al. (1986) and Steiner et al. (1987).

We have collected all published data on the frequencies of slow and rapid metabolizers of debrisoquine in samples from European populations and we also received unpublished observations. The collection of data ceased in mid 1989. Care was taken to include, as far as possible only data from subjects recruited in such a way that would give no bias of the distribution. The possibility of continuous extension of series in successive publications was also considered.

Available data on debrisoquine are shown in Table 1. Table 2 contains the corresponding data for sparteine. The cut off point for attribution as a slow metaboliser of debrisoquine used by all workers is > 12.6, as assessed by Evans et al. (1980). This limit appears unambigously to separate Caucasian population samples into two modes. Eichelbaum (1979) suggested that > 20 was a suitable limit to characterise a slow oxidizer of sparteine. The collected data for dextromethorphan and mephenytoin are shown in Tables 3 + 4 respectively. The tables also contain data obtained as personal communications, which are indicated by [a].

Statistical analysis

Heterogeneity tests between different populations were performed using chi square tests on 2 x n – contingency tables. The different test drugs were each analysed separately. Estimates of gene frequencies were obtained assuming Hardy-Weinberg equilibrium.

Results

There was no significant heterogeneity between populations for any of the three debrisoquine polymorphism test drugs (Table 5). The percentage of slow metabolisers did not show a significant difference between the three test drugs ($\chi^2_2 = 0.81$, $P = 0.67$). The data for the three drugs were combined to obtain an overall estimate of 7.40% slow metabolisers in the European population. Since the slow metabolising phenotype is due to an autosomal recessive gene, the estimate of the gene frequency (q) is $\sqrt{0.0740} = 0.272$ with standard error (SEM):

$$\sqrt{\frac{1-q^2}{4 \cdot N}} = \sqrt{\frac{1-0.0740}{4 \cdot 8764}} = 0.0051$$

Table 5. Summary and heterogeneity tests for debrisoquine, sparteine and dextromethorphan metabolism

Drug	Total Number	Number of rapid metabolisers	Number of slow metabolisers	% slow metabolisers	Heterogeneity χ^2
Debrisoquine	5005	4638	367	7.33	21.96 (20df)
Sparteine	2577	2390	187	7.26	7.17 (8df)
Dextromethorphan	1182	1087	95	8.03	10.45 (6df)
All	8764	8115	649	7.40	0.81[a] (2df)

[a] Heterogeneity test between the three test drugs

where N = total number of individuals tested (Emery 1986). The 95% confidence interval for the gene frequency is q ± 1.96 SEM = 0.262 – 0.282. For mephenytoin there was no significant heterogeneity between populations ($\chi^2_4 = 7.09$ $P = 0.13$), and the percentage of slow metabolisers at 3.52% corresponded to a gene frequency of 0.188 (SEM 0.015), giving a confidence interval (0.173 – 0.203) for the gene frequency.

Discussion

This compilation and analysis show that there are no significant differences between the European population samples with regard to the frequency of the slow debrisoquine metaboliser phenotype. The range between the extreme estimates of debrisoquine metabolic ratio presented in Table 1 is 3.2 – 11.5, the extreme values being obtained in small samples. The overall mean, based on data from 8764 determinations of the metabolic ratios of debrisoquine, sparteine and dextromethorphan is 7.40% slow metabolisers of debrisoquine. The reported frequencies of debrisoquine metabolic ratios are shown in association with their geographical origins in Fig. 1 to indicate the lack of systematic differences in the proportion of slow metabolisers.

No difference was found between data reported for the three most commonly investigated probe drugs debrisoquine, sparteine and dextromethorphan. This finding supports the general assumption that metabolic indices obtained with these drugs consistently reflect the genotype. There is strong evidence that this not always be the case, e.g. in Africans (Woolhouse et al. 1985, Iyun et al. 1986) and Asians (Yue et al. 1989). The genetic background of the debrisoquine polymorphism is being explored, and RFLP analysis can now account for 25% of the mutated allele related to slow debrisoquine hydroxylation (Skoda et al. 1988). The present analysis shows that there is homogeneity with regard to the slow metaboliser phenotype in European populations. Interestingly, a RFLP genotype associated with slow metabolism in Caucasians has recently been found in Chinese phenotyped as rapid metabolisers of debrisoquine (Yue et al. 1989). The frequency of slow metabolisers in Asian samples is about 1% using the antimode of 12.6 established in Caucasian populations (cf Bertilsson L 1989). These discrepancies indicate that several isozymes of the cytochrome may play a role in the metabolism of probe drugs. However, since the distribution of the debrisoquine metabolic ratio in the Chinese population is shifted to the right, i.e. towards slower metabolism, the European cut off point may not be relevant.

The family studies carried out on the debrisoquine metabolic ratio (Evans et al. 1980, Steiner et al. 1985) have unequivocally shown that a monogenic system regulates the capacity of the debrisoquine 4-hydroxylation reaction. Both groups estimated the degree of dominance to be 0.3, which means that the trait is between recessive and additive and far from dominant. The practical consequence, as shown by Steiner et al. (1985), is that heterozygotes cannot be revealed by the debrisoquine test. This was also demonstrated in simulations performed by Jackson et al. (1986). In addition to the major gene, which accounted for 71% of the total variability in debrisoquine metabolic ratio, there was another genetic component explaining 8% of the variability as assessed by path analysis (Steiner et al. 1985). A genetic heritability explaining 79% of a measured character, in this case a compounded pharmacokinetic index, is remarkable, and a similar high degree of genetic heritability is known for only a few other characters, e.g. body height.

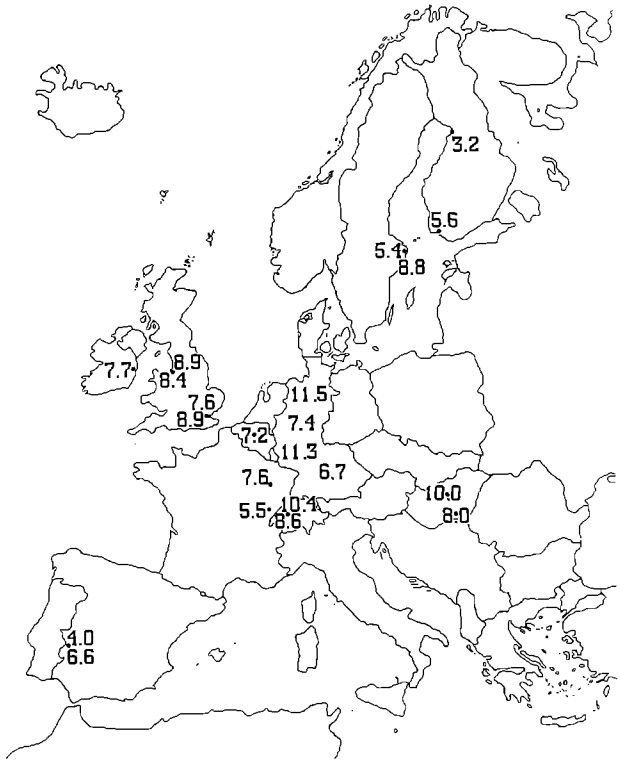

Fig. 1. Geographical distribution of percentage of slow metabolisers of debrisoquine in collected European series

Only 6% of the total variability could be ascribed to environmental factors, including consumption of food, caffeine, alcohol etc. Low inducability is probably a prerequisite for the persistence of a bimodal distribution of a drug metabolic pharmacokinetic index. Eichelbaum et al. (1986) concluded that enzyme induction only exerted a marginal effect on the debrisoquine/sparteine metabolism in a study in which rifampicin was administered to rapid and slow metabolisers of debrisoquine. In line with those results, Bechtel et al. (1986) found no difference in the proportion of slow metabolisers of debrisoquine among 200 epileptic patients who were treated with a variety of drugs, while Steiner et al. (1987) suggested that there might be a slight shift to the left in the distribution of the debrisoquine metabolic ratio in 72 epileptic patients compared to controls.

This meta-analysis of samples characterising the debrisoquine and mephenytoin oxidation polymorphisms indicates that there is considerable homogeneity among European populations in these phenotypic characters, which are also known to be stable in the individual.

Acknowledgements. The colleagues who have generously shared their data with us are cordially thanked for their assistance.

The review was prepared within a European collaborative group of the Centre for Co-operation in the field of Scientific and Technical Research (COST) of the European Communities (EC), Project B1. Supported by the Swedish MRC (3902), The Swedish National Board for Technical Development, the French MRC (86C 0856) and UK CRC 9672-17.

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