Debrisoquine oxidation in a Finnish population: the effect of oral contraceptives on the metabolic ratio

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1 The debrisoquine hydroxylation phenotype was assessed in 211 healthy subjects of Finnish Caucasian origin.

2 A bimodal distribution was observed in the urinary debrisoquine/4-hydroxydebrisoquine ratio. Eleven subjects (about 5%) were poor metabolizers of debrisoquine, when a ratio of 12.6 was used as the cut-off point.

3 The mean metabolic ratio of 35 extensive metabolizers taking oral contraceptives was slightly lower than that of extensive metabolizers not taking contraceptives. This indicates that oral contraceptives may change the phenotyping near the metabolic ratio of 12.6.

4 The metabolic ratios were determined in eight subjects using both 4 h and 6 h urine collection times. The metabolic ratios corresponded well with each other indicating the usefulness of the shorter 4 h collection time.

Keywords oxidation debrisoquine population study oral contraceptives

Introduction

The polymorphic hydroxylation of debrisoquine was reported by Mahgoub *et al.* and Tucker *et al.* in 1977. Debrisoquine hydroxylation and sparteine oxidation appear to be related pharmacogenetic entities (Eichelbaum *et al.*, 1982) and these drugs have been widely used to study the prevalence of deficient oxidation capacity in different populations. The frequency of the poor metabolizer phenotype (PM) is 5–10% in Caucasian populations (Eichelbaum *et al.* 1979; Gachályi *et al.*, 1986). According to family studies, the PM phenotype is inherited as an autosomal recessive trait (Price Evans *et al.*, 1980). Recently, it has been shown that poor metabolizers have negligible amounts of the cytochrome P-450 isoenzyme metabolizing debrisoquine due to the presence of mutant alleles (Gonzalez *et al.*, 1988).

The oxidative metabolism of many other drugs e.g. metoprolol, timolol, nortriptyline and perhexiline involves the same oxidizing enzyme as debrisoquine. The same polymorphism determines the metabolism of these drugs, and deficient metabolism may result in unwanted side effects when they are used in standard doses (Jacqz *et al.*, 1986; Kalow *et al.*, 1987).

The purpose of this study was to investigate debrisoquine hydroxylation polymorphism in the Finnish Caucasian population. As the use of oral contraceptives is widespread, and may affect the disposition of other drugs, we compared the metabolic ratios of those taking oral contraceptives with the metabolic ratios of the main population. We also determined the usefulness of a shorter urine collection time in a subset of eight subjects.

Methods

Subjects

Staff and students from the Medical Faculty of the University of Turku and staff from two local pharmaceutical companies volunteered for the study. The protocol was approved by the local Ethics Committee. Informed consent was obtained from all 211 participants. They were all in good health and did not take any drugs other than oral combined low-dose contraceptives (35 subjects). One hundred and thirty were women

(age 19–62 years; mean 23.8 years) and 81 were men (age 20–42 years; mean 25.0 years).

Protocol for debrisoquine phenotyping

The volunteers fasted overnight and arrived at the Clinical Laboratory at 08.00 h. After emptying their bladders they were given a 10 mg tablet of debrisoquine (Declinax, Hoffmann-La Roche, Switzerland) with water. They were not allowed to ingest anything but water until 2 h after taking the tablet. Urine was collected for 6 h after tablet ingestion. The volume was recorded and a 30 ml aliquot of mixed urine was stored at -20°C until analyzed. For eight subjects serial urine specimens were measured in the same collection after 4 and 6 h and stored as above.

Analytical method

Debrisoquine (D) and 4-hydroxydebrisoquine (4-OH-D) concentrations were measured using the gas chromatographic method described by Lennard *et al.* (1977) with a slight modification (nomifensine as internal standard, extraction with hexane). The metabolic ratio (MR), to be

used as an index of the debrisoquine hydroxylation capacity, was determined as

$$\text{MR} = \frac{\text{amount excreted as debrisoquine}}{\text{amount excreted as 4-hydroxydebrisoquine}}$$

Subjects with a $\text{MR} > 12.6$ were classified as poor metabolizers according to Price Evans *et al.* (1980).

Statistical analysis

Student's *t*-test was used on log transformed data to assess the statistical significance of difference between two means. Pearson correlation coefficients were analysed using linear analysis of regression.

Results

A bimodal MR distribution was observed, suggesting the presence of two distinct phenotypes, as seen in the frequency histogram drawn on a semi-logarithmic scale (Figure 1). The metabolic ratios of the entire study population

Figure 1 Distribution of debrisoquine metabolic ratios (MR) among 211 Finnish subjects. □ extensive metabolisers, ■ poor metabolisers.

($n = 211$) are shown in Table 1. Eleven poor metabolizers (six women and five men) were discovered (5.2%, 95% confidence limits 2.5–9.2%). They excreted, on average, only 1/16 of the quantity of 4-OH-D excreted by the 186 extensive metabolizers (EM), whose urine volumes were known (Table 2). The total recovery of D (D + 4-OH-D) in urine, expressed as percentage of the oral dose, was about 40% higher in poor metabolizers than in extensive metabolizers ($P < 0.001$; Table 2).

The urine volumes collected in 6 h ranged between 45 and 1180 ml, with no difference between the phenotypes. In the 211 volunteers the metabolic ratios were similar in both sexes and they did not correlate significantly with age or urine volume ($r = 0.09$ and $r = 0.03$).

Thirty-five women took low-dose combined oral contraceptives containing ethinylestradiol, levonorgestrel, norethisterone and desogestrel. Two of them were phenotyped as poor metabolizers. However, the mean MR was lower in

EMs with contraceptives (0.45 ± 0.30) than in female EMs without them (0.67 ± 0.96 ; $P = 0.044$) and in male EMs (0.79 ± 0.84 ; $P = 0.002$).

The shorter collection time of 4 h did not change the classification of any of the eight studied subjects between PM or EM. However, based on the 4 h urine, the MRs of the seven extensive metabolizers were slightly higher (20%) and the MR value of the poor metabolizer was lower than the MRs of the same subjects after the 6 h collection.

Discussion

This study shows that debrisoquine hydroxylation is polymorphic also in the Finnish Caucasian population. The observed 5.2% frequency of the PM phenotype lies within the range of 5–10% reported in other Caucasian populations.

Table 1 The metabolic ratios (MR) of all subjects

		MR	
		Mean $\pm$ s.d.	Range
All subjects	EM $n = 200$	0.68 ± 0.84	0.08 – 6.75
	PM $n = 11$	42.6 ± 24.0	16.4 – 89.0
	Total $n = 211$	2.9 ± 10.7	0.08 – 89.0
Subjects taking contraceptives	EM $n = 33$	0.45 ± 0.30	0.09 – 1.22
	PM $n = 2$	48.6 ± 23.6	32.0 – 65.3
	Total $n = 35$	3.2 ± 12.0	0.09 – 65.3

Table 2 Urinary recovery of debrisoquine (D) and 4-hydroxydebrisoquine (OHD) in 6 h as percentage of the oral dose.

	EM ($n = 186$)	PM ($n = 11$)
D mean $\pm$ s.d.	12.3 ± 7.5	$47.1 \pm 9.0^*$
range	2.0 – 43.7	35.6 – 60.4
OHD mean $\pm$ s.d.	22.3 ± 6.0	$1.4 \pm 0.7^*$
range	2.0 – 38.0	0.56 – 26.6
Total mean $\pm$ s.d.	34.6 ± 8.2	$48.5 \pm 8.9^*$
range	7.6 – 57.4	37.3 – 6.2

* $P < 0.001$

Concomitantly taken drugs may affect the apparent debrisoquine MR by inducing or inhibiting the activity of the P-450-isoenzyme metabolizing D. For instance, the antipsychotic drugs thioridazine and levomepromazine inhibit the hydroxylation of D so that during treatment with these drugs many subjects simulate the PM phenotype (Syvälahti *et al.*, 1986). Enzyme induction, caused by antipyrine or rifampicin, enhances the metabolism of sparteine (Eichelbaum *et al.*, 1986), which is metabolized by the same enzyme as debrisoquine (Eichelbaum *et al.*, 1982). This induction is seen only in EMs. Cytochrome P-450 is involved in the metabolism of oestradiol and progesterone (Cheng & Schenkman, 1984). Oral contraceptives containing ethinylestradiol and progestins could therefore have some effect on debrisoquine metabolism, although the metabolism of ethinylestradiol is similar in both phenotypes (Back *et al.*, 1984). The 35 women who took oral contraceptives in this study had a similar distribution of debrisoquine phenotypes than those without contraceptives, but the mean MR of the EMs with contraceptives was significantly lower compared to female EMs without contraceptives and to male EMs. This indicates that EM women with

oral contraceptives have different metabolic ratios than other EMs. The contraceptive-induced decrease of MR in EM subjects may be a result of enzyme induction. The shorter urine collection time of 4 h for the determination of debrisoquine hydroxylation phenotype was validated also in this study. Earlier, Peart *et al.* (1986) showed that MRs after 4 h and 8 h collection times correlated significantly. The shortened collection time is of considerable value when conducting studies on an out-patient basis. In our study the MR values of seven extensive metabolizers were slightly higher after a 4 h urine collection than the MR values obtained after a 6 h collection. The poor metabolizer had a lower MR in a 4 h collection. However, the shorter collection time of 4 h did not change the phenotyping of the subjects.

The oxidative metabolism of many drugs cosegregate with debrisoquine genetic polymorphism (Jacqz *et al.*, 1986) and the metabolism of these agents is dependent on the debrisoquine hydroxylation phenotype. The deficient drug oxidation may thus have many clinical consequences e.g. in terms of higher frequency of side effects from these drugs or lower dose requirements for reaching the therapeutic response.

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