Polymorphism of dextromethorphan oxidation in a French population

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Genetically-controlled drug oxidation capacity was studied using dextromethorphan, an anti-tussive drug, as the test compound in 103 healthy white French subjects (61 males and 42 females). Phenotyping was performed using the metabolic ratio (MR) calculated as $MR = 0-10 \text{ h urinary output of dextromethorphan} / 0-10 \text{ h urinary output of dextrorphan}$, after oral administration of 40 mg (113.6 μmol) of dextromethorphan hydrobromide. The log MR was bimodally distributed: 99 subjects (96.1%) were phenotyped as extensive metabolizers; they had a log MR between -3.1 and -1.1 , a urinary output of dextromethorphan below $5 \mu\text{mol } 10 \text{ h}^{-1}$ and a urinary output of dextrorphan above $20 \mu\text{mol } 10 \text{ h}^{-1}$. Four subjects (3.9%) were phenotyped as poor metabolizers; they had a log MR between -0.5 and $+0.7$, a urinary output of dextromethorphan above $5 \mu\text{mol } 10 \text{ h}^{-1}$ and a urinary output of dextrorphan below $20 \mu\text{mol } 10 \text{ h}^{-1}$.

Keywords dextromethorphan oxidation phenotyping population study

Introduction

The influence of genetic control on hepatic drug oxidation has gained renewed interest since the description of a genetic polymorphism in the capacity to 4-hydroxylate debrisoquine (Mahgoub *et al.*, 1977). Two phenotypes have been identified: most subjects are 'extensive metabolizers' (EM) whereas a minority of subjects are 'poor metabolizers' (PM) (Mahgoub *et al.*, 1977). The impairment of debrisoquine 4-hydroxylation is inherited as an autosomal recessive trait (Evans *et al.*, 1980) and is related to the deficiency of a particular liver cytochrome P-450 (Larrey *et al.*, 1984; Distlerath *et al.*, 1985). The PM phenotype is found in 3–10% of European and American Caucasians (Küpfer & Preisig, 1983). Its prevalence in France is unknown. Therefore, we determined the proportion of subjects genetically deficient for this oxidative pathway in a white French population by using dextromethorphan, an anti-tussive drug, recently proposed as a test compound for oxidation phenotyping (Schmid *et al.*, 1985). Dextromethorphan is *O*-demethylated to dextrorphan,

its main metabolite. Drug oxidation phenotyping with dextromethorphan is in complete concordance with results of phenotyping with debrisoquine (Küpfer *et al.*, 1984; Schmid *et al.*, 1985). Dextromethorphan has the advantages over debrisoquine of being ubiquitously available and of being innocuous (Schmid *et al.*, 1985). Moreover, we have developed a simple high performance liquid chromatographic (h.p.l.c.) assay for dextromethorphan and dextrorphan in urine.

Methods

Subjects

The study was performed in 103 unrelated white French volunteers (mostly staff and students from Hospital Beaujon, Clichy) 20 to 73 years old, including 61 males and 42 females. All subjects gave their informed consent. The study was approved by the local Ethics Committee. The subjects were judged as healthy, did not

abuse alcohol, and had taken no drug for at least 1 week before the study, except for females receiving oral contraceptives which have no significant influence on dextromethorphan disposition (Schmid *et al.*, 1985).

Phenotyping

The subjects took two capsules of dextromethorphan hydrobromide (20 mg/capsule kindly supplied by Laboratoire Norgan, France) at 22.00 h and urine was collected for 10 h thereafter. Phenotype was assigned using the metabolic ratio (MR) calculated as:

$$\text{MR} = \frac{0-10 \text{ h urinary output of unchanged dextromethorphan}}{0-10 \text{ h urinary output of dextrophan}}$$

Analytical procedures

Dextromethorphan hydrobromide, dextrophan, 3-hydroxymorphinan, 3-methoxymorphinan and 3-methoxy-17-methyl morphinan 10-one were kindly provided by Laboratoire Norgan. Dextromethorphan and dextrophan were measured in urine by an h.p.l.c. assay. Hydrolysis of dextrophan conjugates and extraction of dextrophan and dextromethorphan in ether were performed as described by Schmid *et al.*, (1985) with minor modifications: a larger urine sample (2.5 ml) was used and 10 µg 3-methoxy-17-methyl morphinan 10-one was added as the internal standard. The drug residue obtained after extraction into ether and evaporation was dissolved in 100 µl ethanol and an aliquot of 10 µl was injected into the chromatograph. The assay was performed with the detector operating at 210 nm. A CN precolumn and a 10 cm × 8 mm (inside diameter) µ Bondapack phenyl analytical column (Waters Associates) housed in a radial compression Z module (Waters Associates) were used. The mobile phase was 10 mM monobasic potassium phosphate-acetonitrile (65:35 v/v) adjusted to pH 5.5 with orthophosphoric acid. The flow rate was 1.6 ml min⁻¹. The retention times were: dextrophan, 7.2 min; dextromethorphan, 14.7 min; and internal standard, 19.3 min. Other metabolites of dextromethorphan did not interfere with the assay: hydroxymorphinan, 6.3 min; methoxymorphinan, 12.1 min. Dextromethorphan and dextrophan gave linear responses over concentrations ranging from 0.1 to 500 µmol l⁻¹. Sensitivity was generally sufficient to detect both compounds. However, in some cases, dextromethorphan concentration was very low and its measurement required a 10-fold concentration of the sample by taking up the drug residue in 10 µl of ethanol instead of 100 µl.

Inter-assay variation was less than 10%. Initial studies showed that the extraction efficiencies of dextromethorphan, dextrophan and the internal standard from urine ranged from 50 to 80%. These compared with values of 90–100% reported by Schmid *et al.*, (1985). Extraction efficiencies were similar for these three compounds. In the routine assay, standard curves were prepared by mixing dextromethorphan, dextrophan and the internal standard in ethanol. The concentrations of dextromethorphan and dextrophan in ethanol, after their extraction from urine, were calculated from the standard curves in ethanol. Their initial concentrations in urine were then calculated after allowing for the extraction efficiency of the internal standard. This allowed us to measure by a simplified method both the concentrations of dextromethorphan and dextrophan in urine, and the extraction efficiency for each subject.

Results

The frequency distribution histogram of the dextromethorphan log metabolic ratio in 103 white French subjects is shown in Figure 1. Two distinct phenotypes were apparent. Ninety-nine subjects (96.1%) had a log metabolic ratio between -3.1 and -1.1 and were classified as extensive metabolizers (EM). Four subjects (3.9%), three men and one female, with a log metabolic ratio between -0.5 and +0.7 were poor metabolizers (PM). A probit plot also separated two groups of subjects (Figure 1). The mean values of urinary outputs of dextromethorphan and dextrophan and dose recoveries found in EM and PM were significantly different (Table 1). Moreover, there was no overlap between EM and PM for each parameter (Table 1): dextromethorphan urinary output was < 4.73 µmol 10 h⁻¹ in EM and > 5.95 µmol 10 h⁻¹ in PM; dose recovery in urine as unchanged dextromethorphan was < 4.2% in EM and > 5.2% in PM; dextrophan urinary output was > 20.5 µmol 10 h⁻¹ in EM and < 18.8 µmol 10 h⁻¹ in PM; dose recovery in urine as dextrophan was > 18.1% in EM and < 16.4% in PM.

Discussion

This study demonstrated the polymorphism of dextromethorphan *O*-demethylation to dextrophan in a white French population. No subject had a log MR between -1.1 and -0.5. These results are in agreement with those of Schmid *et al.*, (1985) in which the antimode separating EM and PM phenotypes was MR = 0.3 (i.e. log MR = -0.5). This distinction is further substantiated

Table 1 Urinary output (0–10 h) of dextromethorphan and dextrorphan, dose recovery and metabolic ratio (MR) in extensive and poor metabolizers after the oral administration of 40 mg (113.6 μmol) dextromethorphan hydrobromide (mean $\pm$ s.d. results)

	Extensive metabolizers	Poor metabolizers
Number of subjects	99	4
Age (years)	28.3 $\pm$ 8.6	26.0 $\pm$ 4.7
Dextromethorphan output ($\mu\text{mol } 10 \text{ h}^{-1}$) (range)	0.71 $\pm$ 0.77 (0.06 to 4.73)	12.97 $\pm$ 5.91 (5.95 to 19.01)
Dose recovery as dextromethorphan (%) (range)	0.6 $\pm$ 0.7 (0.1 to 4.2)	11.4 $\pm$ 5.2 (5.2 to 16.7)
Dextrorphan output ($\mu\text{mol } 10 \text{ h}^{-1}$) (range)	48.7 $\pm$ 20.9 (20.5 to 89.2)	8.7 $\pm$ 6.9 (3.4 to 18.8)
Dose recovery as dextrorphan (%) (range)	43.5 $\pm$ 18.7 (18.1 to 78.6)	7.7 $\pm$ 6.1 (3.0 to 16.5)
MR = $\frac{\text{Dextromethorphan output}}{\text{Dextrorphan output}}$ (range)	0.0162 $\pm$ 0.0175 (0.0009 to 0.090)	3.409 $\pm$ 0.658 (0.318 to 4.307)
log MR (range)	-1.788 $\pm$ -1.757 (-3.036 to -1.045)	+0.533 $\pm$ -0.182 (-0.498 to +0.635)

Figure 1 The frequency distribution histogram of the dextromethorphan log metabolic ratio in 103 white French subjects.

by the absence of overlap in values of 0–10 h urinary outputs of unchanged dextromethorphan and dextrorphan between phenotypes. The mean values of urinary output of dextromethorphan and dextrorphan observed in this study are higher than those found by Schmid *et al.*, (1985). This may be explained by the higher dose of dextromethorphan hydrobromide (40 mg *vs* 25 mg) used in our study in order to facilitate the measurement of dextromethorphan urinary output. Despite these differences, we found similar

mean values in percent dose recovery in urine as dextrorphan and in mean MR (Table 1; Schmid *et al.*, 1985). In our population, the frequency of the PM phenotype was 3.9%, which is in the range found in other Caucasian populations (3–10%) using sparteine or debrisoquine as the test compound (Küpfer & Preisig, 1983). These results may serve as a basis to test relationships between drug oxidation polymorphism and drug-induced adverse reactions or diseases of unknown aetiology in the French population.

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