

POLYMORPHISM OF DEXTROMETHORPHAN OXIDATION IN POLISH POPULATION

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Abstract: Determination of oxidation phenotype is used to obtain the best results of pharmacotherapy and to explain a lower efficiency of some drugs in particular patients. The purpose of the present study was to determine the distribution of CYP2D6 metabolizer status in subjects from central Poland, using dextromethorphan as the probe drug. The study included 104 healthy Polish volunteers. All subjects received a single oral dose of 40 mg of dextromethorphan and the complete urine output was collected over a period of 10 h. DM and the metabolite dextrophan (DT) were analyzed by HPLC method. The results of a Polish population study revealed a bimodal distribution of the dextromethorphan metabolic ratio and showed the existence of two oxidation phenotypes as extensive (EM) and poor (PM) metabolizers. In our population, the frequency of the PM phenotype was 9.6%. The results obtained (9.6% of PM phenotype) were in accordance with other results obtained in Poland and other Caucasian populations.

Keywords: dextromethorphan, CYP2D6, oxidation polymorphism, Polish population

Oxidation is one of the basic metabolism ways of exogenic substances including drugs. Cytochrome P-450 and its microsomal enzymes play the key role in the oxidation of drugs. The activity of isozyme CYP2D6 is genetically determined. Polymorphism of the oxidative metabolism has been examined in many populations (1-5).

In the 1970s two research teams independently discovered genetic deficiencies in the metabolism of debrisoquine and sparteine (6-9). These deficiencies were revealed through adverse reactions to these cures. Since then, different probe drugs have been used for CYP2D6 phenotyping. The first phenotyping studies were performed with debrisoquine and sparteine. Later, dextromethorphan and metoprolol were also used for phenotyping CYP2D6 activity (10-12).

On the basis of many population studies we may distinguish two main phenotypes of oxidation. Subjects with functional CYP2D6 are classified as extensive metabolizers (EM); those with defective CYP2D6 are classified as poor metabolizers (PM). The frequency of both phenotypes (EM and PM) is bimodal. It is characteristic for particular races and ethnic groups. The following metabolic rates have been found in Caucasians: 20 for sparteine,

0.3 for dextromethorphan, and 12.6 for debrisoquine/metoprolol (13). The PM phenotype is found in 3-10% Caucasians and, for comparison, in 1-2% Asians (14).

Many clinically important drugs are known to be substrates of the enzyme CYP2D6, e.g., tricyclic antidepressants, many neuroleptics, antiarrhythmics, lipophilic β -blockers and codeine (15). Genetically determined variability in oxidative metabolism is partially responsible for the variability of responses to the drugs. Several clinically significant adverse reactions to these drugs were found to be much more frequent in poor metabolizers (PM) (16). On the contrary, in extensive metabolizers (EM) pharmacotherapy might turn out to be ineffective. Knowledge of CYP2D6 status may be clinically important: it could become the basis for the individualization of drug treatment (3). Phenotyping of oxidation may be also economically important: it allows a more rational approach to drug prescription.

In Poland, genetic oxidation polymorphism was determined in Polish volunteers from the region of Wrocław, using sparteine as a model drug, and from the Szczecin and Warszawa regions, using debrisoquine as the test substance (17, 18).

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EXPERIMENTAL

The study included 104 healthy Polish volunteers (17-80 years old, including 35 males and 69 females). The subjects were nonsmokers and were taking no medication. The exclusion criteria were a history of renal insufficiency, liver disease, cardiovascular disease, and current drug or alcohol abuse. The study was approved by the Ethics Committee at the Medical University of Łódź, Poland. All subjects received a single oral dose of 40 mg of dextromethorphan (capsule prepared in our department with substance supplied by Sanofi-Biocom, Rzeszów, Poland), and the complete urine output was collected over a period of 10 h. The total volume of the urine was measured, and a 20 mL aliquot was kept at -20°C for further analysis.

The analysis of DM and DX in urine was performed using HPLC assay with slight modifications (19). The liquid chromatographic system consisted of a Model 600 pump (Waters, USA), and a UV-VIS detector (Waters, Model 2487). Separation was performed on a Nova Pack Phenyl (3.9 × 150 mm, Waters). The mobile phase consisted of acetonitrile/10 mM phosphate buffer, pH 4.0 (55:45 v/v) with a flow rate of 1 mL/min and pressure of 1200 psi. The assay was performed with the UV detector operating at 280 nm. The retention times were: dextrophan, 3.16 min; dextromethorphan, 6.3 min.

The samples were vortexed and then titrated with 1 M NaOH to a pH of 11.0 to 11.5. The sample was then eluted sequentially with 10 mL and 5 mL volumes of 10% n-butanol in hexane. To the combined eluent 300 µL of 0.01 M HCl was added. The sample was then shaken for 20 min and centrifuged at 2000 rpm for 15 min. The aqueous phase of each sample was injected for high-performance liquid chromatography. Typical standard curves for dextromethorphan and dextrophan were linear and had a correlation coefficient of 0.996 and 0.999, respectively.

Phenotype was assigned using the metabolic ratio MR calculated as:

$$MR = \frac{\text{0-10 h urinary output of dextromethorphan (DM)}}{\text{0-10 h urinary output of dextrophan (DX)}}$$

On the basis of the urinary dextromethorphan/dextrophan ratios, subjects with metabolic ratios of 0.3 and above were classified as poor

metabolizers (PM), and those with ratios below 0.3 were classified as extensive metabolizers (EM) (20).

For the statistical analysis the χ^2 test was used.

RESULTS

The metabolic ratio values of all subjects are separated into extensive and poor metabolizers on the basis of white antimode (MR = 0.3; log MR = -0.5). No statistically significant age difference was observed between extensive metabolizers (37.2 ± 15.4 years, n = 94) and poor metabolizers (36.1 ± 16.2 years, n = 10).

Descriptive statistics of 0-10 h urinary percentile recovery of dextromethorphan and free dextrophan, MR and log MR are shown in Table 1. Dextromethorphan urinary output was < 6.72 mmol/10 h in extensive metabolizers and > 0.66 mmol/10 h in poor metabolizers. Dose recovery in urine as dextromethorphan was < 5.9% in extensive metabolizers and > 0.58% in poor metabolizers. Dextrophan output was > 1.12 mmol/10 h in extensive metabolizers and < 14.748 mmol/10 h in poor metabolizers. Dose recovery in urine as dextrophan was > 0.98% in extensive metabolizers and < 13.02% in poor metabolizers. p value was testing the difference between extensive and poor metabolizers using NIR-test. The limit of significance accepted for all statistical analyses was p = 0.05.

Ninety four subjects (90.4% of the population) whose log MR was between -3.0 and -0.68 (mean -1.458 ± 0.468) were classified as extensive metabolizers. Ten subjects (9.6% of the population) with a log MR between -0.398 and +0.376 (mean -0.027 ± 0.292) were classified as poor metabolizers. Figure 1 shows the semilogarithmic frequency distribution histogram of MR values in all subjects.

Our results could also be supported by classification based on metabolic ratios. 94 subjects whose metabolic ratio was between 0.001 - 0.207 (mean 0.055 ± 0.048) were classified as extensive metabolizers. Ten subjects with metabolic ratio between 0.4 - 2.377 (mean 1.151 ± 0.765) were classified as poor metabolizers. Only 5 of the 69 females (7.2%; C.I. 0.01-0.13) and 5 of the 35 males (14.3%; C.I. 0.03-0.26) were poor metabolizers (Table 2).

The mean values of urinary outputs of dextromethorphan and dextrophan and dose recoveries found in extensive and poor metabolizers were significantly different (p < 0.05).

In our population, the frequency of the PM phenotype was 9.6%, which is in the range found in other Caucasian populations (3-10%).

Table 1. Urinary output (0-10 h) of dextromethorphan and dextrorphan and metabolic ratio (MR) in extensive and poor metabolizers after the oral administration of 40 mg dextromethorphan hydrobromide.

	Extensive metabolizers (EM)	Poor metabolizers (PM)	p value
Number of subjects	94	10	
Age Mean \pm SD (range)	37.2 \pm 15.4 (17 – 80)	36.1 \pm 16.2 (18 – 63)	0.837
Dextromethorphan output mmol/10 h Mean \pm SD (range)	0.54 \pm 0.82 (0.01 – 6.72)	6.30 \pm 4.97 (0.66 – 13.80)	< 0.01
Dose recovery as dextromethorphan (%) Mean \pm SD (range)	0.48 \pm 0.72 (0.02 – 5.9)	5.55 \pm 4.37 (0.58 – 12.15)	0.06
Dextrorphan output mmol/10 h Mean \pm SD (range)	11.82 \pm 10.44 (1.12 – 78.05)	7.22 \pm 5.25 (0.32 – 14.78)	< 0.01
Dose recovery as dextrorphan (%) Mean \pm SD (range)	10.4 \pm 9.19 (0.98 – 68.7)	6.35 \pm 4.62 (0.28 – 13.02)	0.17
Log MR Mean \pm SD (range)	-1.458 \pm 0.468 (-3.0) to (-0.684)	-0.027 \pm 0.292 (-0.398) to 0.376	< 0.01
MR Mean \pm SD (range)	0.055 \pm 0.048 (0.001 – 0.207)	1.151 \pm 0.765 (0.40 – 2.377)	< 0.01

Table 2. Distribution of the urinary dextromethorphan/dextrorphan ratio in 104 healthy Polish subjects.

Total groups	Oxidation phenotype		
	Sample size	PM (poor metabolizers)	EM (extensive metabolizers)
Females	69	5 (7.2%) C.I. 0.01-0.13	64 (92.8%) C.I. 0.87-0.99
Males	35	5 (14.3%) C.I. 0.03-0.26	30 (85.7%) C.I. 0.74-0.97
Total	104	10 (9.6%) C.I. 0.04-0.15	94 (90.4%) C.I. 0.85-0.96

95% confidence interval (C.I.)

DISCUSSION AND CONCLUSION

CYP2D6 polymorphism is the most extensively studied oxidation polymorphism in humans and exhibits substantial interethnic variation (21). For appropriate dosing of the drugs metabolized by the polymorphic enzymes, it is important to know the distribution of the activity of these enzymes and the frequency of subjects with genetically determined enzyme deficiency.

The individual status of CYP2D6 activity can be probed with several drugs such as debrisoquine,

sparteine, metoprolol, and dextromethorphan. The genetic polymorphism of oxidative metabolism was first reported for debrisoquine and sparteine and has been extensively studied in various populations (10, 14). The incidence of a defective CYP2D6 is about 3-10% in North American and European populations (22). Dextromethorphan (DM) is widely used as a probe drug to assess *in vivo* the activity of cytochrome P450 2D6. Drug oxidation phenotyping with dextromethorphan is in complete concordance with the results of phenotyping with debrisoquine and sparteine (23). DM is an oral non-narcotic anti-

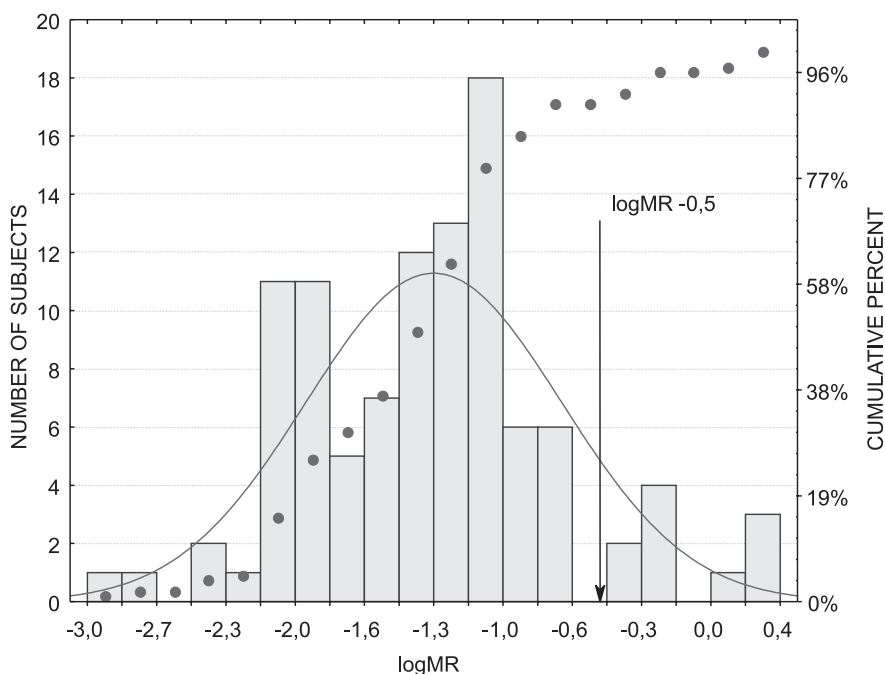


Figure 1. Frequency distribution histogram of dextromethorphan log MR and cumulative probit plot among 104 Polish subjects

tussive cure often used in practical medicine as DM hydrobromide. The adverse effects of DM are revealed extremely rarely and its toxicity is quite low. Dextromethorphan seems to be a safer substance than others used as probe drugs to determine the phenotype of drug oxidation (24, 25). In the population of Poland oxidation polymorphism was mainly determined using debrisoquine and sparteine as model drugs. The frequency of PM phenotype was 8.8% for subjects from the region of Wrocław using sparteine and 5.8% for subjects from the Szczecin and Warszawa regions using debrisoquine (13, 20). In our study of genetically-controlled drug oxidation capacity dextromethorphan was used as a test compound in 104 healthy Polish subjects (35 males, 69 females). The results obtained (9.6% of PM phenotype) were presented at The Fifth Multidisciplinary Conference on Drug Research (26). These results were also in accordance with other results obtained in Poland and other Caucasian populations (25).

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