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CYP2D6 phenotyping with dextromethorphan

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Abstract:
Genetically determined individual differences in the ability to oxidize certain drugs have raised recently a considerable interest because of clinical importance of this problem. Determination of CYP2D6 oxidation phenotype is used to obtain more efficient pharmacotherapy and to explain lower efficacy of some drugs and presentation of adverse effects in particular patients.
The aim of this study was to identify the CYP2D6 oxidation phenotype with dextromethorphan (DM) as a probe drug. The study included 85 healthy volunteers of Polish origin. DM (40 mg) was given orally to healthy adults and 10-h urine samples were collected. DM and the metabolite dextrorphan (DX) were analyzed by the HPLC method. Phenotyping was performed using the metabolic ratio (MR) calculated as the urinary DM/DX output.
Based on the metabolic ratio, we can distinguish extensive (EM) and poor (PM) metabolizers in human population. Individuals with a dextromethorphan MR greater than 0.3 (log > –0.5) were classified as PMs.
In our study, the frequency of the PM phenotype was 9.4%, which is in the range found in other Caucasian populations (3–10%).

Key words:
dextromethorphan, CYP2D6, phenotyping

Introduction

Oxidative drug metabolism by hepatic microsomal CYP2D6 enzyme is genetically determined and has a polymorphic distribution in most populations. 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. Genetically determined variability in oxidative metabolism is partially responsible for the variability of responses to the drugs. Polymorphism of the oxidative metabolism by CYP2D6 has been studied using debrisoquine, sparteine, metoprolol and dextromethorphan (DM) as the probe drugs [3, 5]. Recently DM has most often been applied to investigate the activity of cytochrome P-450 2D6.

DM is an oral non-narcotic antitussive cure widely used in practical medicine as DM hydrobromide [8]. DM has been registered in Poland for treatment and is readily available as an OTC (over-the-counter) drug in both simple and compound pharmaceuticals. DM is very well absorbed by the digestive system and it does not bind to plasma proteins. DM is a safe cure and, therefore, can be taken even by children and pregnant women. The adverse effects of DM are revealed extremely rarely and its toxicity is quite low. The average adult dose of DM hydrobromide is 15 to 30 mg; the maximal single dose is 120 mg. Since debrisoquine and sparteine are marketed only in a limited number of countries and sometimes they cause side-effects (e.g. orthostatic hypotension) DM seems to be a safer substance than others used as probe drugs to determine the phenotype of drug oxidation.

In humans DM is mainly metabolized by CYP2D6 through O-demethylation into dextrorphan (DX). There also exists an alternative pathway of DM metabolism. Other isozymes, such as CYP 3A3/4, CYP3A5, CYP3A7, CYP2C9 and CYP2C19 catalyze biotransformation of DM to inactive 3-methoxymorphinan (MM). Both DX and MM are demethylated to hydroxymorphinan (HM) and excreted in urine, mainly as their glucoronide conjugates [4].

Phenotyping of CYP2D6 activity using DM may be carried out in urine, plasma or saliva samples and the results from these different biological samples have a good correlation [1, 4]. In the most common phenotyping method, subjects are administered a single oral dose of 15–50 mg of DM and the urine is collected over a period of 8–12 h [1].

The DM/DX ratio can be measured in urine by HPLC, GC or TLC methods [2]. Subjects with metabolic ratios higher than 0.3 are considered to be poor metabolizers (PMs). The metabolic CYP3A pathway contributes only minimally to the interindividual variability of the metabolic ratio (MR) [4].

Subjects and Methods

The study included 85 healthy volunteers of Polish origin, 58 (68.2%) females and 27 (31.8%) males with a mean age (± SD) of 36.7 ± 15.7 years (age range: 17–80 years). All subjects gave written informed consent and had been free of any medications for at least 2 weeks before the study.

The study was approved by the Ethics Committee at the Medical University of Łódź, Poland. All subject received a single oral dose of 40 mg of DM (a capsule prepared in our department with substance supplied by Sanofi-Biocom, Rzeszów, Poland). 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.

DM and its metabolite DX were determined by reversed-phase high-performance liquid chromatography (HPLC) with UV detection, using a modification of a previously reported method [10]. The liquid chromatographic system consisted of a Model 600 pump (Waters, USA), UV-VIS detector (Waters, Model 2487). Separation was performed on a Nova Pack Phenyl column (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. Under the chromatographic conditions used in this study, the retention times of DX and DM were 3.16 min and 6.3 min, respectively.

The samples were vortexed and then titrated with 1 M NaOH to pH = 11.0–11.5. The sample was extracted 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 were 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 into the HPLC system for quantification of DM and DX. Typical standard curves for DM and DX were linear and had a correlation coefficient of 0.996 and 0.999, respectively.

DM oxidation capacity was calculated as the urinary DM/DX ratio. Subjects were classified as PMs if they had a metabolic ratio greater than 0.3 (log > –0.5) [20].

The χ^2 test was used for the statistical analysis.

Results

Subjects with metabolic ratio > 0.3 were classified as PMs (n = 8 or 9.4% of the population; 95% C.I. 3.2–15.6%), and the remaining subjects with metabolic ratios < 0.3 were classified as extensive metabolizers (EMs, n = 77 or 90.6% of the population; 95% C.I. 84.4–96.8%). For 8 subjects classified as PMs, the values of log MR were between [(–0.368) and (+0.376)] (mean –0.025 ± 0.270).

The mean ± SD of DM-to-free DX MR was 0.056 ± 0.047 (range 0.004–0.207) and 1.127 ± 0.738 (range 0.428–2.377) for EMs and PMs, respectively.

In our study, 4 of 58 females (6.9%; 95% C.I. 4–13.4%) and 4 of 27 males (14.8%; 95% C.I. 10–19.6%) were PMs (Tab. 1).

The frequency distribution histogram of log MR values showed a bimodal distribution, indicating the presence of both extensive and PMs phenotypes (Fig. 1). In our population, the frequency of the PM phenotype was 9.4%, which is in the range found in other Caucasian populations (3–10%) (Tab. 2).

Tab. 1. Distribution of the urinary dextromethorphan/dextrorphan ratio in 85 healthy Polish subjects

	Sample size	PM	EM
Females	58	4 (6.9%) (95% C.I. 4–13.4%) MR = 0.612 ± 0.132 range (0.428–0.716) log MR = –0.222 ± 0.103 range (–0.368) to (–0.145)	54 (93.1%) (95% C.I. 86.6–99.6%) MR = 0.055 ± 0.050 range (0.004–0.207) log MR = –1.443 ± 0.436 range (–2.398) to (–0.684)
Males	27	4 (14.8%) (95% C.I. 10–19.6%) MR = 1.642 ± 0.740 range (0.711–2.377) log MR = 0.174 ± 0.234 range (–0.148) to (+0.376)	23 (85.2%) (95% C.I. 80.4–90%) MR = 0.06 ± 0.043 range (0.01–0.182) log MR = –1.342 ± 0.348 range (–1.921) to (–0.740)
Total	85	8 (9.4%) (95% C.I. 3.2–15.6%) MR = 1.127 ± 0.738 range (0.428–2.377) log MR = –0.025 ± 0.270 range (–0.368) to (+0.376)	77 (90.6%) (95% C.I. 84.4–96.8%) MR = 0.057 ± 0.047 range (0.004–0.207) log MR = –1.413 ± 0.412 range (–2.398) to (–0.684)

95% C.I. – 95% confidence interval, MR – metabolic ratio, PM – poor metabolizer, EM – extensive metabolizer, $\chi^2 = 2.91$ (χ^2 tab 3.84, p = 0.05)

Tab. 2. Dextromethorphan oxidation polymorphism in European countries

Country	Oxidation phenotype				References
	Sample size	Extensive	Poor	% Poor	
Czech Republic	101	95	6	5.9	Chladek et al. 2000 [4]
Estonia	210	189	21	10.0	Marandi et al. 1996 [16]
France	128	124	4	3.0	Jacqz et al. 1988 [11]
	99	95	4	3.9	Larrey et al. 1987 [14]
	216	205	11	5.1	Freche et al. 1990 [7]
Greece	102	95	7	6.9	Kimiskidis et al. 2005 [12]
Netherlands	765	723	42	5.5	Tamminga et al. 2001 [22]
	4301	3957	344	8.0	Tamminga et al. 1999 [23]
Germany	450	404	46	10.2	Hildebrand et al. 1989 [9]
Switzerland	106	98	8	7.5	Dayer et al. 1988 [6]
	245	224	10	8.6	Schmid et al. 1985 [20]
Turkey	45	42	3	6.7	Basci et al. 1988 [2]
	32	29	3	9.4	Bozkurt et al. 1996 [3]
Italy	246	235	11	4.5	Spina et al. 1994 [21]
Poland	**85**	**77**	**8**	**9.4**	**The present study**

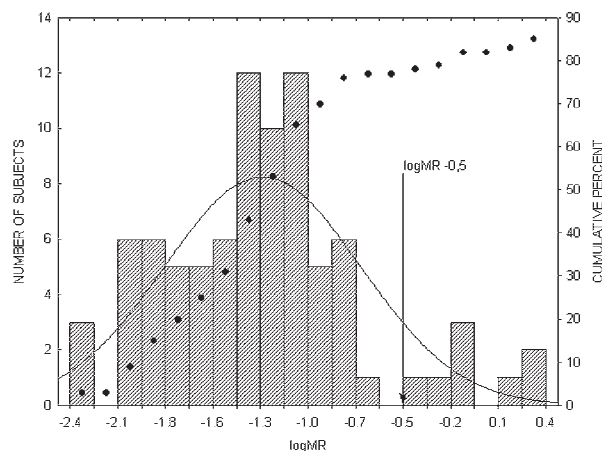

Fig. 1. Frequency distribution histogram of dextromethorphan log MR and cumulative probit plot among 85 Polish subjects

Discussion

DM is widely used as a probe agent to assess the CYP2D6 oxidation phenotype in subjects from Europe and from all over the world. Metabolic rates exhibit substantial racial and interethnic variations. Phenotyping has consistently shown that 5–10% of Caucasians from Europe and North America and about 1% of Japanese or Chinese are PMs (poor metabolizers) [17].

In European countries, studies of oxidation phenotype using DM were performed on groups ranging from 32 to 4301 subjects (Tab. 2). The results of our investigation (9.4% of PMs) have been based upon the study of 85 subjects and it showed a good agreement with the results obtained by other authors using DM as a probe drug. Our data are also in agreement with those investigations of Polish population in which different probe drugs have been used. Early Polish population studies have been based on CYP2D6 oxidation phenotyping using debrisoquine and sparteine as the probe drugs. Kunicki et al. (Warszawa and Szczecin regions) found 5.8% PMs of debrisoquine in 154 phenotyped persons and Orzechowska-Juzwenko et al. (Wrocław region) showed 8.8% PMs of sparteine in 160 subjects [13, 19].

Phenotyping is a method which has benefits and several drawbacks. It is an easy and inexpensive method. Phenotyping does not require complicated and expensive equipment. The sample may be collected from serum, urine, and saliva. For some subjects, however, this method may seem to have a complicated testing protocol. There is also a risk of adverse reactions of model drugs.

Thanks to the achievements of molecular biology, it is possible to determine the oxidation genotype by the PCR-RFLP method and allele-specific PCR [15]. Genotyping involves identification of a genetic mutation, which gives rise to the specific drug metabolism phenotype. Genotyping is the best choice for patients who are being treated, whose pharmacotherapy cannot be interrupted, and whose phenotype cannot, therefore, be determined.

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 (PM) [12].

There are studies in the literature comparing both methods. According to these data, CYP2D6 oxidation phenotyping with probe drugs shows about 98% agreement with the results of CYP2D6 genotyping [22]. In Poland, Niewiński et al. also confirmed the good concordance between genotype and phenotype in 60 randomly selected individuals [18].

Phenotyping is still a very useful method both for researching the genetic oxidation polymorphism and for individual treatment with drugs whose metabolism involves oxidative pathways.

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