

Dextromethorphan metabolic phenotyping in a Chinese population

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KEY WORDS dextromethorphan; polymorphism (genetics); cytochrome P-450 CYP2D6; phenotype; Chinese; pharmacogenetics; debrisoquin; hydroxylases

AIM: To establish a phenotyping of dextromethorphan (DM) oxidation polymorphism in a native Chinese population. **METHODS:** The urine concentrations of DM and its metabolite dextrophan (DX) were assayed by HPLC and metabolic ratios (MR) were calculated in 120 unrelated native Chinese subjects after ingestion of DM 20 mg. **RESULTS:** The incidence of poor metabolizers was 0.8 % (one in 120 subjects). There were distinct dimodal distributions which divided extensive metabolizers into 43 intermediate metabolizers and 76 very extensive metabolizers. The 0-8 h urinary recoveries of DM and DX were $0.4 \% \pm s 0.5 \%$ and $26 \% \pm s 13 \%$, respectively. There was no difference in 0-8 h urinary recoveries between male and female subjects. **CONCLUSION;** DM metabolic phenotyping provides a new information for debrisoquine 4-hydroxylation (CYP2D6) polymorphism in native Chinese.

Debrisoquine 4-hydroxylase (CYP2D6) is the most well-characterized P-450 polymorphism⁽¹⁻⁴⁾. The CYP2D6 polymorphism has a large impact because many of its drug substrates have a narrow range of therapeutic plasma concentrations, and would result in serious clinical consequences if patients' CYP2D6 phenotypes were not identified. It has been argued that determination of a patient's CYP2D6 phenotype should be a prerequisite for treatment with antipsychotic drugs. Previous phenotyping of CYP2D6 which utilized debrisoquin as a probe drug is of limited clinical use because of its potential toxicity⁽⁵⁾. Dextromethorphan (DM), a common ingredient in many over-the-counter preparation, is a newer marker for CYP2D6. Its metabolism is to cosegregate well

with debrisoquin 4-hydroxylation⁽⁵⁻⁷⁾. The purpose of this study was to explore DM as a new marker to CYP2D6 phenotype in native Chinese population.

MATERIALS AND METHODS

Drugs and reagents DM hydrobromide tablets (10 mg) were purchased from Shanghai Huanghe Liya Pharmaceutical Co (expire 1997-01-30). DM and its demethylation metabolite dextrophan (DX) were provided by Hoffman-La Roche Inc (Nutley NJ, USA). Heptane sulfonic acid and β -glucuronidase (Type H-3, from *Helix pomatia*, 125 MU · L⁻¹) were purchased from Sigma (St Louis MO, USA). All other chemicals were reagent grade.

Subject One hundred and twenty healthy unrelated native Chinese (M 64, F 56) were enrolled. The average age was $32 \pm s 10$ a (18-57 a) and weight $60 \pm s 9$ kg (41-90 kg). They were all of Han nationality except one of Man nationality, and born in 18 different provinces in China. All subjects were non-smokers. Subjects were excluded for any history of abnormal hepatic or renal functions, pregnancy, alcohol abuse, drug allergy, and intoxication. No medication was ingested within the last 7 d. A written informed consent was signed by each subject before study.

Phenotyping Subjects were asked to empty their bladders to collect baseline urine before bedtime. DM (20 mg) was ingested with 200 mL water. Urine was collected over the next 8 h. A 15-mL aliquot of urine was stored at -20 °C until HPLC assay. Subject urinary molar metabolic ratio (MR) was calculated by use of the equation:

$$MR = DM (\text{mg} \cdot \text{L}^{-1}) / DX (\text{mg} \cdot \text{L}^{-1}) \times 0.948$$

Phenotype was made on the basis of MR value according to the method of Schmid⁽⁵⁾. Any subject with an $MR > 0.3$ ($\lg MR > -0.52$) was categorized as a poor metabolizer (PM), and an $MR \leq 0.3$ ($\lg MR \leq -0.52$) as an extensive metabolizer (EM). A mathematical model of Gram-Charlier series⁽⁸⁾ was applied to dissect the mixed sample of EM. Based on the nonlinear least squares, a quasi-Newton optimization method was used to estimate iteratively the parameters of the model by HTATHTICA software. Chi-square test was used to determine the goodness-of-fit of the model. Mann-Whitney rank sum test was applied to infer whether the 2 samples would come from the same population.

HPLC assay A reverse-phase HPLC with fluorescence detection⁽⁹⁾ was employed with modification to quantitate urine concentrations of DM and DX in duplicate. Briefly, urine 1 mL

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was incubated with β -glucuronidase 20 μ L in acetate buffer (pH 5.0) 1 mL at 37 $^{\circ}$ C for 18 h. The solution was adjusted to pH 11 and extracted with *n*-butanol-hexane (1:9) 5 mL. The organic layer was back-extracted into 200 μ L of HCl 0.01 mol \cdot L $^{-1}$. The aqueous layer 20 μ L was injected onto the HPLC column (ZORBAX Phenyl column, 4.6 mm \times 25 cm). The HPLC system comprised of a 114 M Beckman pump (1.2 mL \cdot min $^{-1}$), an RF-10A Shimadzu fluorescence detector with λ_{ex} 280 nm and λ_{em} 310 nm, and a C-R1B Shimadzu integrator. Mobile phase was acetonitrile/heptane sulfonic acid 0.01 mol \cdot L $^{-1}$ in KH $_2$ PO $_4$ 0.01 mol \cdot L $^{-1}$ (pH 4.0) (35:65). The limit of detection for DM (23 μ g \cdot L $^{-1}$) was lower than that (0.10 mg \cdot L $^{-1}$) in literature^[9]. The average intraday and interday CV % were 2.72 and 3.74, respectively.

Analysis of data Results were expressed as $\bar{x} \pm s$. The *t* test was used for group comparisons.

RESULTS

Only one subject (0.8 %) was a PM of CYP2D6. For the rest of subjects, there seemed to be bimodal distributions among EM if one PM was excluded. A quasi-Newton optimization of original EM showed that a mixture of 2 skewed distributions come from different populations ($Z = 9.2488$, $P < 0.001$). The goodness-of-fit $\chi^2 = 7.56$, degree of freedom = 12, and $P = 0.82$. A new antimode of DM/DX lg MR value was calculated at -1.81 (MR = 0.015) to divide original EM into 76 very extensive matabolizers (VEM) and 43 intermediate metabolizers (IM) (Fig 1).

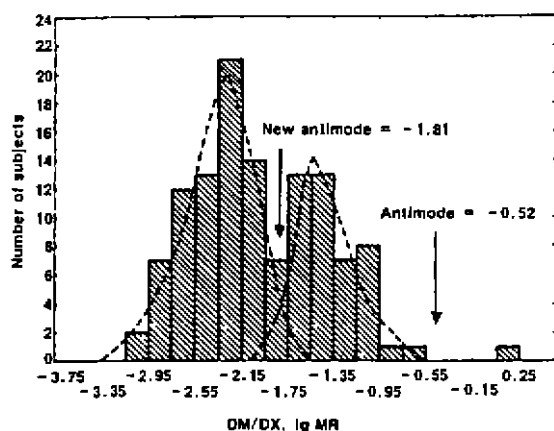


Fig 1. Frequency distribution of urinary dextromethorphan/dextrophan molecular metabolic ratio (lg MR) in 120 native Chinese. "-----" represents the predicted distributions obtained from a quasi-Newton optimization in EM group.

The differences of mean MR and lg MR between VEM and IMs were highly significant (Tab 1).

Tab 1. Metabolic molar and lg molar ratios of 120 Chinese after *po* dextromethorphan 20 mg. * $P < 0.01$ vs VEM.

	EM (n = 119)		PM (n = 1)
	VEM (n = 76)	IM (n = 43)	
MR	0.0056 \pm 0.0038	0.0487 \pm 0.0356 ^c	1.185
lg MR	-2.35 \pm 0.31	-1.39 \pm 0.26 ^c	0.074

The mean DM and DX concentrations were 0.1 mg \cdot L $^{-1}$ and 17 mg \cdot L $^{-1}$ for VEM, 0.4 mg \cdot L $^{-1}$ and 11 mg \cdot L $^{-1}$ for IM, and 1.8 mg \cdot L $^{-1}$ and 1.4 mg \cdot L $^{-1}$ for PM, respectively. Two DM levels in VEM were below the limits of detection. The MR values were calculated with use of the lowest measurable DM concentration for each subject. The urinary recoveries of DM and DX during 0-8 h after ingestion of DM were 0.4 % \pm s 0.5 % and 26 % \pm s 13 %, respectively. There were no differences in 0-8 h of urinary recoveries between male and female subjects (Tab 2).

Tab 2. The urinary recoveries of DM and DX during 0-8 h after *po* dextromethorphan 20 mg in 120 native Chinese subjects. * $P > 0.05$ vs male.

	Male & female (%) (n = 120)	Male (%) (n = 64)	Female (%) (n = 56)
DM	0.4 \pm 0.5	0.5 \pm 0.6	0.4 \pm 0.6 ^c
DX	26 \pm 13	29 \pm 14	24 \pm 11 ^c

After ingestion of DM 20 mg, the PM subject experienced heart palpitations. There were 11 subjects (26 %) in the IM group who complained of dizziness, lethargic, vomiting, and xerostomia. Only 6 subjects (8 %) in the VEM group suffered similar adverse reactions.

DISCUSSION

Debrisoquin has long been used as a probe drug in the phenotyping of CYP2D6 polymorphism. However, it is no longer used clinically because of its severe adverse effects, such as orthostatic hypotension. Thus, debrisoquin is not available in hospitals for routine screening of CYP2D6 polymorphism.

Dextromethorphan, however, is safe and readily available in many countries. Therefore, once the metabolism of DM was shown to genetically cosegregate with that of debrisoquin, it has been used widely to determine the CYP2D6 phenotype of subjects. However, it was the first attempt of using DM as a marker to phenotype native Chinese CYP2D6 polymorphism in mainland China. We found that DM was relatively safe and acceptable to subjects during the period of study.

The incidence of DM poor metabolizers in our study was 0.8%. This is comparable with other studies in Chinese^[3-4], but much lower than that of Caucasians (5% - 10%)^[3,5]. Interestingly, previous work using debrisoquin as a marker has found that EM in Chinese were actually a mixture of two normal distributions and MR were shifted to the right compared with those of Caucasian^[3] and Japanese^[10].

With the remarkable increase in HPLC sensitivity for DM, we could obtain a histogram which divided Chinese EM into bimodal distributions more clearly. By a quasi-Newton optimization of 119 EM data, we could obtain a new antimode of -1.81 and divide EM group into 76 subjects (63%) with lower MR values and 43 subjects (36%) with higher MR values. It is similar to the proportion of 44% IM phenotype in 27 Chinese subjects as compared with only 13.8% IM in 29 Caucasians in a previous study^[11].

CYP2D6 genetic polymorphism appears to have clinical consequences in the use of a large number of therapeutic drugs. Therefore, characterization of IM from original Chinese EM is by no means unnecessary because Chinese subjects are more sensitive than white subjects to some drug adverse reactions in spite of very much lower incidence of PM. DM metabolic phenotyping provides a new information for debrisoquin 4-hydroxylation (CYP2D6) polymorphism in native Chinese.

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441-444

中国人右美沙芬代谢的表型分型

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关键词 右美沙芬; 多态性(遗传学); 细胞色素 P-450 CYP2D6; 显型; 中国人; 遗传药理学; 异喹啉; 羟化酶类

目的: 建立中国人右美沙芬(DM)氧化代谢多态性的表型分型。 **方法:** 120名无血缘关系的中国人口服 DM 20 mg 后, 测定其尿中 DM 及其代谢物右啡烷(DX)浓度, 并计算代谢比值(MR)。 **结果:** 氧化代谢缺陷率为 0.8% (1人); 快代谢者可明显区分为极快代谢者(76人)和中速代谢者(43人)。 0-8 h 内 DM 和 DX 的尿排百分率分别为

0.4 % \pm s 0.5 % 和 26 % \pm s 13 % , 但两者无性别差异。结论: 右美沙芬代谢的表型分型为中国

人异喹肼 4-位羟化 (CYP2D6) 多态性提供了新的信息。

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Anionic subsite of active center of *Torpedo* acetylcholinesterase constructs a part of its conformational epitope¹

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KEY WORDS *Torpedo*; acetylcholinesterase; monoclonal antibodies; epitope; structure-activity relationship; pralidoxime compounds

AIM: To study the structure-activity relationship of *Torpedo* acetylcholinesterase (AChE) and explore whether the anionic subsite of the active center is a constituent of the conformational epitope of enzyme.

METHODS: Using ELISA and enzyme inhibition test to examine the effect of 1-methyl-2-hydroxyiminomethylpyridium chloride (2-PAM), an anionic subsite probe of AChE, on the immunoreactivity between *Torpedo* AChE and its monoclonal antibody (McAb) 3F3. **RESULTS:** McAb 3F3 did not react with 2-PAM-AChE complex. 2-PAM decreased the inhibitory rate of McAb 3F3 on AChE in a concentration-dependent fashion, but did not dissociate the McAb 3F3-AChE complex. **CONCLUSION:** Anionic subsite of the active center of *Torpedo* AChE constructs a part of its conformational epitope.

Acetylcholinesterase (EC 3. 1. 1. 7, AChE) is normally associated with the breakdown of acetylcholine in the synapse and the neuro-muscular junction. Among monoclonal antibodies (McAb) against *Torpedo* AChE^[1], only McAb 3F3 was able to

combine with the conformational epitope of AChE active center^[2]. 2-PAM is a good probe for the anionic subsite of AChE owing to its hydrophobic pyrimidine ring and N-CH₃ substituent causing molecular orbit interaction via $\pi - \pi$ charge translocation with aromatic residues lining the surface of the active site gorge. In this paper the effect of 2-PAM on the binding of McAb 3F3 with AChE was studied to find whether the anionic subsite of the active center of *Torpedo* AChE constructs a part of its conformational epitope.

MATERIALS

Torpedo AChE was purified from electric organ of *Torpediniforms Torpedo torpedo* by affinity chromatography^[3]. McAb 3F3 in the ascites fluids produced in Balb/c mice injected with its corresponding hybridoma cells was purified by protein-A affinity-chromatography^[1,4]. Horseradish peroxidase-labeled goat-anti-mouse IgG was purchased from Beijing Biotinge Biomedicine Co.

Acetylcholine bromide (ACh·Br) was purchased from Beijing Chemical Plant. 2-PAM was synthesized in Institute of Pharmacology and Toxicology (purity 98 %). Other chemicals were all of AR.

METHODS AND RESULTS

Influence of 2-PAM on the immunoreaction between McAb 3F3 and AChE Aliquots (100 μ L/well) of *Torpedo* AChE (5 mg \cdot L⁻¹) in *Torpedo* saline (phosphatidylcholine 0.01 %, NaCl 1

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