BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Mar; 17 (2): 119 - 121

Genetic polymorphism of 4′-hydroxylation of *S*-mephenytoin in 148 Chinese of Han nationality

RUAN Zou-Rong, CHENG Yuan-Shen, ZHOU Jun-Fu, ZHAO Yi, PAN Yi-Zheng, DING De-Yun
(*Institute of Clinical Pharmacology, Zhejiang Medical University, Hangzhou 310009, China*)

KEY WORDS *S*-mephenytoin; 4′-hydroxymephenytoin; hydroxylation; phenotype; pedigree; pharmacogenetics; population; drug metabolic detoxication

AIM: To study genetic polymorphism of *S*-mephenytoin (*S*-Mep) 4′-hydroxylation in the Chinese population of Han nationality. **METHODS**: The lg metabolic ratio (MR) and lg hydroxylation index (HI) in the urine (0 - 12 h) after oral administration with 100 mg of racemic Mep tablet were determined by HPLC method in 148 consangeously unrelated native Chinese subjects and 21 individuals of 5 families. **RESULTS**: The lg MR and lg HI showed a bimodal distribution with an antimode of -1.00 and 1.50, respectively. The occurrence of poor metabolizers (PM) was 13.5 % in the population. The pedigree analysis in 5 families indicated that deficient *S*-Mep hydroxylation was an autosomal recessive trait. **CONCLUSIONS**: The occurrence of PM for *S*-Mep 4′-hydroxylation in Chinese was higher than that of the Caucasians, and both genetic modes were of autosomal recessive trait.

Individual variations in drug metabolisms are often observed in humans, and are closely related to the genetic polymorphism of metabolism[1-3]. One of the most widely studied genetic polymorphism of drug oxidation is the metabolism of debrisoquine/sparteine[4]. The recently discovered polymorphic 4′-hydroxylation of *S*-mephenytoin (*S*-Mep) is independent of the type of debrisoquine/sparteine hydroxylation[5]. The frequency distribution of metabolic capacity of converting *S*-Mep to 4′-hydroxymephenytoin (4′-OH-Mep) shows a clear-cut bimodality and is expressed with 2 distinct phenotypes: extensive metabolizers (EM) and poor metabolizers (PM)[5]. The orientals seem to have a higher frequency of *S*-Mep PM[6-8]. This study was to explore the frequency of PM for *S*-Mep with hydroxylation index (HI) and metabolic ratio (MR) and the genetic mode of PM in native Chinese.

Received 1994-07-16 Accepted 1995-06-12

MATERIALS AND METHODS

Subjects Consanguineously unrelated, healthy native Chinese subjects (*M* 90, *F* 58; age 19 - 22 a, 54 ± *s* 7 kg) were studied with their informed consent. They were students of Zhejiang Medical University. No subject consumed alcohol or tobacco. No drugs were taken for at least 2 wk before and during the study. No subject had any abnormalities on physical examination or any biochemical evidence of renal or hepatic dysfunction.

Drugs and reagents Racemic Mep tablets (100 mg, Mesantoin®), Mep and 4′-OH-Mep were obtained from Sandoz (Basel, Switzerland). Phenobarbital (internal standard, IS) and β-glucuronidase were purchased from Sigma. All other reagents and solvents were of AR.

Phenotyping procedure After an overnight fast, each subject emptied the bladder and took a single oral dose of 100 mg of racemic Mep tablet with 100 mL water. Urine samples were collected for 12 h. Aliquots (10 mL) were frozen at -20 ℃ until analyzed. The measurement of urinary creatinine served as an index for assessment of the completeness of urinary recovery. An incomplete urine collection was assumed when urinary creatinine was < 50 mg[10].

Drug assay Urinary Mep and 4′-OH-Mep were determined by HPLC with an ultraviolet detector[9].

Family studies Five families (21 members) were studied. The probands were PM idetified in the population studies. The phenotype determination was the same as the population study.

Data analysis For each subject an MR and HI were calculated:

$$\mathrm{HI} = \frac{\text{Mep dose (}S\text{-Mep enatiomer)}}{\text{4′-OH-Mep in urine 0}\rightarrow\text{12 h}}$$

$$\mathrm{MR} = \frac{\text{Mep in urine 0}\rightarrow\text{12 h}}{\text{4′-OH-Mep in urine 0}\rightarrow\text{12 h}}$$

The *frequency distribution histograms* were constructed using the logarithm of the individual MR and HI of Mep. Each of antimodes was decided using probit analysis.

RESULTS

Population study None of the subjects excreted < 75 mg creatinine in the 12-h urine,

which indicated that the identification of PM phenotype was validated. The frequency distributions were bimodal and the histograms discriminated with a large visual gap between the PM and EM. By probit analysis method the presence of at least 2 modes with an antimode was indicated at lg MR −1.00 and lg HI 1.50. Therefore, 21 (M 13, F 8) of the 148 subjects were classified as the PM, corresponding to the frequency of 13.5 % (95 % confidence interval: 8.5 % to 19.4 %) (Fig 1)

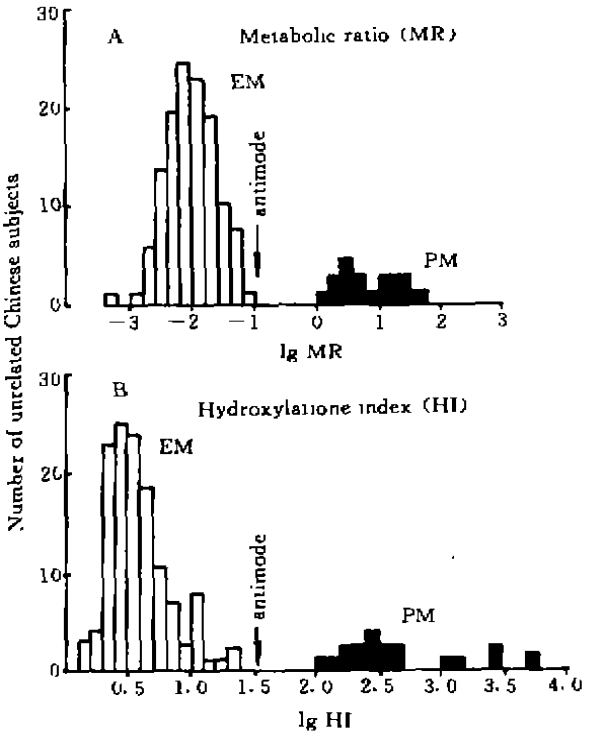

Fig 1. Frequency distribution of 12-h urinary mephenytoin/4′-hydroxymephenytoin metabolic ratio (MR) and hydroxylation index (HI) in 148 Chinese.

The Mep, 4′-OH-Mep, MR, and HI values for the EM and PM in the 148 native Chinese subjects were summarized in Tab 1.

There was no difference between the sexes in *S*-Mep 4′-hydroxylation capacity: 8/58 females (13.8 %) and 12/90 males (13.3 %) were PM ($P > 0.05$).

Family study The pedigree analysis of 5 families (21 members) indicated that the deficient trait for *S*-Mep 4′-hydroxylation was inherited in an autosomal-recessive Mendelian fashion which was determined by at least 2 alleles at a single gene locus. Accordingly, the PM phenotype represented the homozygous recessive genotype and the EM population was a mixed distribution of heterozygous and homozygous dominant genotypes (Fig 2).

Tab 1. Mep, 4′-OH-Mep, and metabolic capacity (MR and HI) in 12-h urine of 148 Chinese after *po* 100 mg Mep tablet.

	EM	PM
n	128	20
Mep/mp	0.18 ± 0.16	0.49 ± 0.25
4′-OH-Mep/mg	16.4 ± 7.4	0.12 ± 0.11
lg HI	0.57 ± 0.24	2.76 ± 0.54
lg MR	−2.02 ± 0.39	0.80 ± 0.46

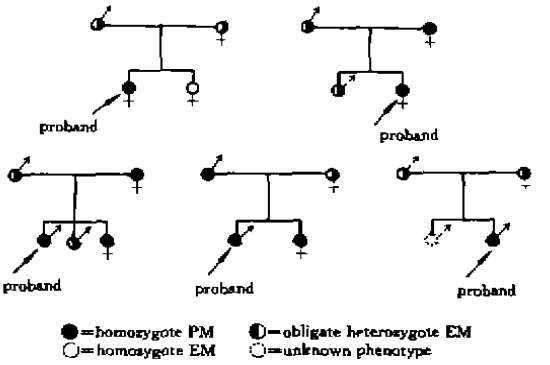

Fig 2. Pedigrees for *S*-mephenytoin 4′-hydroxylation polymorphism from 5 families.

Adverse effect None of these subjects suffered any intolerable adverse effects after Mep ingestion. However, a drowsiness was complained by 14/20 (70 %) PM and 4/128 (3 %) EM ($P < 0.01$).

DISCUSSION

S-Mep 4′-hydroxylation metabolism phenotype has been linked to the oxidative metabolism of other kinds of drugs, such as imipramine [10], diazepam [11], propranolol [12], omeprazole [13]. This paper classified the *S*-Mep 4′-hydroxylation of 148 native Chinese with the method of measuring individual: HI and MR. S-Mep 4′-hydroxylation metabolism was bimodal distribution, which may be classified into 2 kinds of phenotype: EM and PM; the frequency of deficiency of *S*-Mep 4′-hydroxylation was 13.5 %, which was similar to Japanese

(17 %)[6] and Korean (12.6 %)[7], and Chinese (14.6 %)[8], but different from that of Caucasian (only 3.5 %)[5]. Compared with S-Mep, the debrisoquine which was another probe drug for the study of pharmacogenetics showed higher frequency of deficiency of hydroxylation metabolism in Caucasian than in Orientals (Japaneses, Koreans and Chineses)[5-8]. It suggested that there was a difference between Orientals and Caucasians in polymorphism of drug oxidative metabolism.

The pedigree analysis of S-Mep 4′-hydroxylation metabolism for 5 families showed that the deficiency of metabolism in native Chinese was an autosomal recessive trait which was determined by at least two alleles at a single gene locus. This result was coincident with the reports of Caucasian[14] and Japanese[15].

REFERENCES

1 Gonzalez FJ, Skoda RC, Kimura S, Umeno M, Zanger UM, Nebert DW, *et al*. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. Nature 1988; **331**: 442 - 6.

2 Mitchell SC, Waring RH. The deficiency of sulfoxidation of *S*-carboxymethyl-*L*-cysteine. Pharmacol Ther 1989; **43**: 237 - 49.

3 Veronese ME, Miners JO, Randles D, Gregov D, Birkett DJ Validation of the tolbutamide metabolic ratio for population screening with use of sulfaphenazole to produce model phenotypic poor metabolizers. Clin Pharmacol Ther 1990; **47**: 403 - 11.

4 Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of CYP2D6 in a European population DNA Cell Biol 1991; **10**: 545 - 58.

5 Alvan G, Bechtel P, Iselius L, Gundert-Remy U. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. Eur J Clin Pharmacol 1990; **39**: 533 - 7.

6 Nakamura K, Goto F, Ray WA, McAllister CB, Jacqz E, Wilkinson GR, *et al*. Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. Clin Pharmacol Ther 1985; **38**: 402 - 8.

7 Sohn DR, Kusaka M, Ishizaki T, Shin SG, Jang IJ, Shin JG, *et al*. Incidence of S-mephenytoin hydroxylation deficiency in a Korean population and the interphenotypic differences in diazepam pharmacokinetics. Clin Pharmacol Ther 1992; **52**: 160 - 9

8 Lou YQ, Bertilsson L, Du YL, Sjöqvist F. S-Mephenytoin hydroxylation polymorphism and its relation to debrisoquine hydroxylation phenotype in native normal Chinese subjects. Chin J Clin Pharmacol 1992; **8**: 32 - 6.

9 Ruan ZR, Cheng YS, Ding DY. Determination of mephenytoin and 4′-hydroxymephenytoin in urine by high-performance liquid chromatography Acta Pharm Sin 1994; **29**: 624 - 8.

10 Skjelbo E, Gram LF, Brsen K. The *N*-demethylation of imipramine correlated with the oxidation of S-mephenytoin (S/R-ratio). A population study. Br J Clin Pharmacol 1993; **35**: 331 - 4.

11 Bertilsson L, Henthorn TK, Sanz E, Tybring G, Sawe J, Villen T. Importance of genetic factors in the regulation of diazepam metabolisam: relationship to *S*-mephenytoin, but not debrisoquin, hydroxylation phenotype. Clin Pharmacol Ther 1989; **45**: 348 - 55

12 Ward SA, Walle T, Walle UK, Wilkinson GR, Branch RA. Propranolol's metabolism is determined by both mephenytoin and debrisoquin hydroxylase activities. Clin Pharmacol Ther 1989; **45**: 72 - 9.

13 Andersson T, Regardh CG, Dahl-Puustinen ML, Bertilsson L. Slow omeprazole metabolizers are also poor S-mephenytoin hydroxylators. Ther Drug Monit 1990; **12**: 415 - 6.

14 Inaba T, Jurima M, Kalow W Family studies of mephenytoin hydroxylation deficiency. Am J Hum Genet 1986; **38**: 768 - 72.

15 Ward SA, Goto F, Nakamura K, Jacqz E, Wilkinson GR, Branch RA S-Mephenytoin 4-hydroxylase is inherited as an autosomal-recessive trait in Japanese families. Clin Pharmacol Ther 1987; **42**: 96 - 9.

中国汉族人 *S*-美芬妥英 4′-羟化代谢的遗传多态性

阮邹荣，程源深，周君富，赵奕，潘以正，丁德云
(浙江医科大学临床药理研究所，杭州 310009，中国)

关键词 S-美芬妥英；4′-羟美芬妥英；羟化；表型；系谱；药物遗传学；人群；药物代谢解毒

目的：研究我国汉族人 S-美芬妥英(S-Mep) 4′-羟化代谢的遗传多态性. **方法**：148 名互无血缘关系的汉族健康志愿者和 5 个家族 21 名成员，口服美芬妥英 100 mg 后，用 HPLC 法测定 0 - 12 h 尿中 S-Mep 4′-羟化代谢的代谢比值(lg MR)和羟化指数(lg HI). **结果**：lg MR 和 lg HI 均呈两态性分布，分型点(antimode)分别为 - 1.00 和 1.50，羟化代谢缺陷的频发率为 13.5 % (20/148). 系谱分析表明 S-Mep 4′-羟化代谢缺陷为常染色体隐性遗传. **结论**：S-Mep 4′-羟化代谢缺陷频发率东方人高于高加索人，遗传方式均为常染色体隐性遗传.