中国药理学报 *Acta Pharmacologica Sinica* 1990 Sep; 11 (5) : 385-388

Debrisoquine hydroxylation and sulfamethazine acetylation in a Chinese population

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ABSTRACT Both debrisoquine hydroxylation and sulfamethazine acetylation phenotypes were studied in the same native Chinese population. Debrisoquine hydroxylation status was determined by HPLC assay of debrisoquine metabolic ratio in urine after a single oral dose of 10 mg debrisoquine. Three poor metabolizers were found in 220 subjects (1.36%). One hundred and one subjects of this population previously debrisoquine phenotyped were also tested for acetylation phenotyping on a seperate occasion. Their acetylation status were determined by HPLC assay of "% acetylation" after a single oral dose of 1 g sulfamethazine. Twenty (19.8 %) slow acetylators were found. There were no significant association between the 2 metabolic pathways.

KEY WORDS debrisoquin; sulfamethazine; hydroxylation; acetylation; high pressure liquid chromatography; population

Interindividual and interethnic differences in drug metabolism occur not infrequently. They can influence both therapeutic and toxic drug response. One best established example of genetic polymorphism in drug-metabolizing capacity is acetylation. Metabolism of the antihypertensive drug, debrisoquine is also genetically determined. Nowadays, debrisoquine hydroxylation, mediated by cytochrome P-450 enzyme (P-450 IIDI), has been used as a metabolic marker of oxidation status for an increasing list of up to 24 drugs[1]. Drugs are commonly metabolized by more than one steps, and some drugs, such as procainamide, hydralazine, phenelzin, dapsone and certain sulphonamides may undergo both acetylation and oxidation. Either defects of the 2 metabolic pathways has an autosomal recessive mode of inheritance,[2,3]. Coincidentally, preliminary evidences showed that the frequencies of deficiency in acetylation[4] as well as debrisoquine hydroxylation[5,6] in native Chinese population were much lower than those in the Caucasians. However, the studies were performed in different regions and therefore were heterogenous. The purpose of the present study was to undertake the first survey of both sulfamethazine acetylation and debrisoquine hydroxylation metabolism in the same native Chinese population. In addition, any qualitative or quantitative relationship between the 2 metabolic status was explored.

Received 1989 Oct 20 Accepted 1990 Jun 6

METHODS

Debrisoquine hydroxylation phenotying 220 unrelated native Chinese generated university staff and students from Shanghai Medical University participated in the study. One hundred and 20 were ♂ aged 19-36 yr (24.3 ± 3.4) and 100 ♀, aged 18-40 yr (22.1 ± 2.8). They were not taking any medication, smoking or alcohol and have been judged in good health by physical examination and laboratory tests including blood and urine routine tests, liver and kidney function tests. All the volunteers in our study had similar diet from the campus canteen. Before going to bed, each subject ingested a single oral dose of 10 mg debrisoquine tablet immediately after voiding the bladder. Urine was collected for the next 8 h over night. The volume and pH of each urine sample were measured. Aliquots of urine were stored at -20℃. The urinary debrisoquine (D) and 4-hydroxy debrisoquine (HD) concentrations were measured by HPLC described previously[6].

described previously[6]. Assignation of the phenotype was based on the debrisoquine metabolic ratio (MR). MR = (% dose eliminated as D) / (% dose eliminated as HD)

Sulfamethazine acetylation phenotyping

Among 220 subjects who had previously been debrisoquine phenotyped, 101 subject who had no history of sensitivity to sulphonamides volunteered for this study. This group included 57 ♂ and 44 ♀. Each subject had a single oral dose of 1 g sulfamethazine capsule together with 150 ml water at approximately 14:00 h on the day of the test. Six h later, 20 μl capillary blood sample was collected by finger-tip puncture and assayed by a modified HPLC method of Weber[10]. The sample was added to 100 μl internal standard solution (Sulfadiazine in 0.9% sodium chloride solution, 5 mg / L) in microcentrifuge tube. The content was mixed and centrifugated. Of the supernatant 100 μl was taken, mixed with 50 μl acetonitrile and centrifugated at 10000 g for 5 min. The supernatant was injected into the HPLC column (ODSC 18 150 × 6 mm) eluted with a 3 / 10 (vol / vol) mixture of acetonitrile and acetate buffer (pH 4.0) at 1.2 ml / min. The column oven was set at 40℃, and UV detector wavelength at 270 nm. Sulfamethazine and acetylsulfamethazine concentrations in the blood were quantitated by relating sulfamethazine / sulfadiazine and acetylsulfamethazine / sulfadiazine peak-area ratios to the standard calibration curves. The within and between day precision (coefficients of variation) was between 1.5–5.0 % for the assay. Sulfamethazine acetylation phenotype was determined by the % of sulfamethazine acetylated in the 6 h blood sample:

% acetylation = (*N*-acetylsulfamethazine) / (*N*-acetylsulfamethazine Sulfamethazine) × 100%

Comparison of acetylation status with debrisoquine hydroxylation status was performed by Chi square analysis and Spearman rank correlation test.

RESULTS

Debrisoquine hydroxylation phenotyping

The distribution of the logarithmically transformed data of debrisoquine MR (log MR) of the 220 subjects appeared to be bimodel (Fig 1). The majority (217) ranged in debrisoquine log MR from −1.52 to 0.95. Three subjects had log MR sepearated from the main group. No subjects had metabolic ratio falling in the range from log MR = 1.00 to log MR = 1.50, in agreement with the same antimode (log MR = 1.10) for differentiating poor metabolizers (PM) and extensive metabolizers (EM) in Caucasians[3]. Hence the frequency of PM was 1.36%. The PM were checked by biological repetition. Two nuclear families of the 3 PM probands identified in the population study were also studied. No more PM was found in their family members. MR of their obligate heterozygous parents were between 0.61 and 2.96.

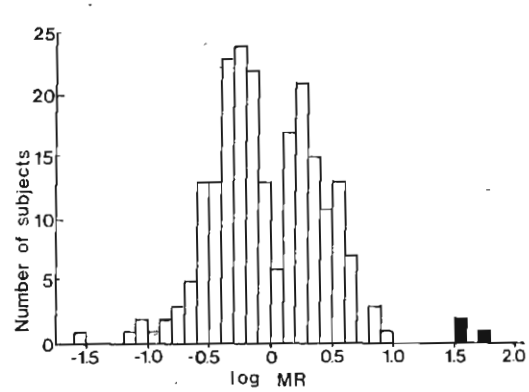

Fig 1. Frequency distribution of log metabolic ratio (MR) for debrisoquine in 220 unrelated Chinese subjects.

Sulfamethazine acetylation phenotyping

Twenty of the 101 volunteers who had encountered both debrisoquine phenotyping were slow acetylators (19.8%). They had % of sulfamethazine acetylation from 10.8–28.6.

No subjects had % of sulfamethazine acetylation between 30.0 and 47.5 corresponding to 40 % acetylation which serves as the marker between slow and fast acetylator in the blood collected 6 h after dosing[8,9].

Comparison between debrisoquine hydroxylation and sulfamethazine phenotypes. Both Chi square analysis (Tab 1) and Spearman rank correlation test (Fig 2) showed no significant association between debrisoquine hydroxylation and sulfamethazine acetylation metabolic pathways.

Tab 1. Debrisoquine hydroxylation and sulfamethazine acetylation phenotypes in 101 subjects. EM : Extensive metabolizer PM: Poor metabolizer (Chi square test, $P<0.001$)

		Debrisoquine hydroxylation		Total
		EM	PM	
Sulfamethazine acetylation	Fast	79	2	81
	Slow	20	0	20
Total		99	2	101

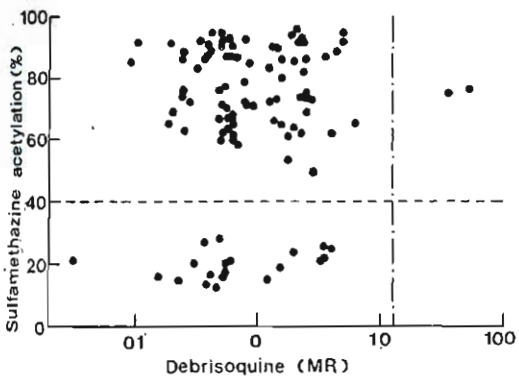

Fig 2. Lack of correlation between debrisoquine hydroxylation and sulfamethazine acetylation in 101 subjects. (– • – •): Division between debrisoquine poor metabolizers and extensive metabolizers; (——): Division between slow and fast sulfamethazine acetylator.

DISCUSSION

The frequency of 1.36 % in the present study is still within the 95% confidence limit of our previous preliminary report[6] of a low frequency (1%) of slow debrisoquine hydroxylator in Chinese population. The family studies of PM probands have revealed no more PM in their nuclear family members, indicating that the gene frequency of deficiency in debrisoquine oxidation is quite low in native Chinese population. The obligate heterozygotes vary widely in their MR, thus, it may not be possible to differentiate between the two EM genotypes by their MR values.

In the present study of blood sulfamethazine acetylation status through HPLC assay,19.8 % were classified as slow acetylators. This frequency is higher than those found by the colorimetric urine analysis of sulfamethazine and is consistent with the frequencies of slow isoniazid acetylation[4,10]. The reason of this discrepancy is partially due to the authenticity of HPLC blood analysis which avoids the misclassification of sulfamethazine acetylation status when using urine analysis and the colormetric assay.

In the crossover study of debrisoquine hydroxylation and acetylation status in 101 subjects, no correlation between the two metabolic pathways could be found (Tab 1, Fig 2). This verifies that the gene loci for the two characteristics are not linked[11,12]. In fact, sulfamethazine *N*–acetylation is mediated by an acetyltransferase which does not belong to cytochrome P–450 family. In our ongoing study about amitryptyline metabolising rate, out of our 220 subjects who were debrisoquine phenotyped, 7 subjects (including 1 PM) participated in the study on pharmacokinetics of amitryptyline and its metabolites (10–hydroxyamitriptyline, nortriptyline and 10–hydroxynortryptyline). Unlike sulfamethazine, 10–hydroxylation metabolism of amitriptyline and its demethylated active metabolite nortriptyline is highly correlated with debrisoquine hydroxylation (unpublished). Although the mechanisms responsible for interindividual and interethnic differences in drug metabolism may be multiple and complex, the probe drug

probe drug as debrisoquine that can be used to predict the metabolic rate of other drugs cosegregating with it may still be of clinical and research implications.

ACKNOWLEDGMENT The authors thank Dr Keith ARNOLD for supplying debrisoquine as a gift.

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中国人的异喹胍羟化代谢与磺胺二甲嘧啶乙酰化代谢

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提要 在同一个中国人群中作异喹胍(D)羟化与磺胺二甲嘧啶(SM_2)乙酰化代谢遗传表型的分型.分别以HPLC法测定口服D后8h尿中D与4-OH-D的比率,及口服SM_2后第6 h指尖采血中SM_2乙酰化%.220个志愿者中3人为羟化慢代谢型,占1.36%;101人中20人为乙酰化慢代谢型,占19.8%.计数和计量资料均未显示二者间有统计学相关性.

关键词 异喹胍; 磺胺二甲嘧啶; 羟化作用; 乙酰化; 高压液相色谱法; 人口

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