Polymorphism of debrisoquine 4-hydroxylation and family studies of poor metabolizers in Chinese population

DU Yun-Long, LOU Ya-Qing

(Department of Pharmacology, Beijing Medical University, Beijing 100083, China)

ABSTRACT Debrisoquine hydroxylation capacity determined as the ratio of debrisoquine over 4-OH-debrisoquine in 8-h urine after a single dose (10 mg) was studied in 140 unrelated Chinese Han subjects and 2 families of poor metabolizers (PM) of debrisoquine. In the 140 Chinese subjects the frequency of PM was found to be 1.43% (2/140), much lower than the 5–10% in white population reported. No sex difference was shown on the hydroxylation of debrisoquine. The recoveries of debrisoquin, 4-OH-debrisoquine in 8-h urine were 16±11 and 13±6%, respectively. None of the parents in the 2 families was PMs. Phenotype distribution in each family and population was consistent with the hypothesis that debrisoquine 4-OH-hydroxylation activity is under diallelic, monogenetic control, with the PM phenotype inheriting in an autosomal recessive trait.

KEY WORDS debrisoquin; 4-hydroxy-debrisoquin; hydroxylation; pedigree; incidence studies; pharmacogenetics; Chinese population

Several genetic polymorphisms of drug oxidation have been established and the ethnic differences in drug metabolism are widely known. The debrisoquine type is the most studied polymorphism(1). The orientals seemed to have a lower frequency of debrisoquine PM(2,3). The study of debrisoquine hydroxylation is rare in native Chinese. This paper reports the results of investigation on the frequency of PM for debrisoquine and the pedigree analysis in 2 Chinese families.

METHODS AND MATERIALS

Subjects The 140 unrelated subjects chosen consisted of 85 males and 55 females, all of them were Chinese Han nationality in good health and recruited from students of Beijing Medical University. Their ages ranged from 18 to 38 yr (21.5±SD 2.5) and body weights from 42 to 80 kg (58±8). None of them consumed alcohol but a few subjects were light smokers. No drug was received recently. The families of 2 Han PM, found in present study, were investigated. Family members of the 2 PM of debrisoquine (DB) were healthy. No hepatic or renal dysfunction was found and no drug was taken recently.

Experimental procedure Each subject, after emptying the bladder, was given po 10 mg of debrisoquine sulfate (Declinax) with 100 ml water. Urine was collected for 8 h. An aliquot of 30 ml urine was put into a plastic vial and stored at -30°C.

Analytical method DB and its main metabolite, 4-OH-debrisoquine (4-OH-DB), were measured by gas chromatography with flame ionization detector (GC-FID), involving derivatisation with acetylation and extraction of the resulting pyrimidines(4). The gas chromatography was done on a Varian 3300 equipped with a quartz capillary column (20 m x 0.25 mm) combined OV-101 with SE-54. The temperatures of the injector and detector were maintained at 300°C and the column temperature was 210°C. The carrier gas was N₂ at a speed of 25 cm/s with 30 ml/min make up gas. The flow rates of H₂ and air were 20 and 200 ml/min, respectively. Split ratio was 1:30. The retention times of DB, 4-OH-DB, and guanoxan (I. S) were 5.78, 9.45, and 9.95 min, respectively. The detection limit was 1 μmol/L.
Reagents The reagents (AR) were obtained from Beijing Chemical Factory. DB, 4-OH-DB, and guanoxan (G) were kind gifts from Profs F Sjöqvist and L Bertilsson (Dept of Clinical Pharmacology, Huddinge University Hospital, Sweden). DB sulfate tablets (10 mg) were provided by Hoffmann-La Roche, Basle.

Data treatment The results of urine analysis were expressed in terms of metabolic ratio (MR); MR = DB (μmol/L)/4-OH-DB (μmol/L) in the 8 h urine.

With the antimode of Caucasians, subjects with MR <12.80 (log MR <1.10) were classified as extensive metabolizers (EM) and those with MR >12.80 were defined as poor metabolizers (PM) 

The phenotype and genotype frequencies of DB hydroxylation in population were expected from the application of the Hardy-Weinberg law. The letters p and q were used to express the frequencies of dominant and recessive alleles, respectively.

RESULTS

The frequency of distribution of urinary DB MR in 140 unrelated Chinese Han is shown in Fig 1 A. The log MR ranged from -0.77 to 1.36. Two PM were found. One of them was a male with MR 22.86. The other one was a female with DB concentration 88.82 μmol/L and no 4-OH-DB detected. The PM phenotype frequency (q²) in the present population was 1.43% (2/140). With the application of the Hardy-Weinberg law, the PM genotype frequency (q) in the present population was 0.12 and the EM genotype frequency (p) was 0.88 (p = 1 - q). Using these values, the expected genotypic frequencies within the population were 21% for heterozygous EM and 77% for homozygous EM.

The log MR of the 54 females and the 84 males were 0.04 ± 0.42, and 0.10 ± 0.35, respectively (t test, P > 0.05). The recoveries of DB, 4-OH-DB, and DB + 4-

Fig 1. Distribution histogram of debrisoquine hydroxylation in 140 (A) and 285 (B) normal Chinese Han nationality subjects.

OH-DB in the 8 h urine were 16 ± 11%, 13 ± 6%, and 29 ± 13%, respectively.

Analysis of the 2 families of PM for DB hydroxylation was shown in Fig 2. The poor metabolizer phenotype behaved as an autosomal recessive trait. Using pedigree analysis, 4 obligate heterozygotes were identified. The urinary MR in these individuals were located in the EM group, with MR of 0.86-6.51.

Fig 2. The pedigree studies of poor metabolizers (PM) of debrisoquine hydroxylation in 2 Han nationality families. Metabolic ratio was shown. OH = obligate heterozygote, PM = poor metabolizer, ND = not detected.

DISCUSSION

The present study showed that the incidence of PM for DB hydroxylation in the Chinese Han nationality (1.05%) is lower than that in white population (5-10%) 

Combining with our previous findings about the frequency of DB PM in the Chinese Han subjects, which showed that the PM
frequency was 0.69% (1/145) in 145 unrelated Chinese Han. It may be concluded that the frequency of DB PM in the Chinese Han is 1.05% (3/285) (Fig 1 B). In the Fig 1 B, the EM group was in a normal distribution. With the application of normal distribution test the 3 PMs were discrete group from the EM group (P < 0.1) and the 1.05% PM in the Chinese Han was much lower than the 8.9% PM in white population (P < 0.01). Nakamura et al (8) reported no PM for DB in 100 unrelated Japanese subjects. Our results are consistent with the hypothesis (2,3) that oriental population has a lower frequency of DB PM. The results of 1.05% PM frequency in native Chinese Han is contrary to the 30% in oversea Chinese subjects, mostly first or second generation immigrants from Hong Kong (8). A distinct bimodal was observed in the distribution histogram, indicating the DB hydroxylation being controlled by dialle.

Previous studies in Caucasian population showed that the mode of inheritance of DB PM phenotype is in an autosomal recessive trait (5,8). The present family analysis provided evidence that it is inherited in the same mode in the Chinese population. Sex linkage can be excluded by the fact that the daughter is PM but her father is EM in family B. It is possible for autosomal recessive trait that there is no PM in parents but PM appears in offspring. On the background of autosomal recessive trait, parents in the 2 families are obligate heterozygous genotype. The MR of parents ranged from 0.86 to 6.51, overlapping with the MR of EM in the Chinese population. This indicates that it is not possible to differentiate the homozygous EM from the heterozygous EM through the use of MR.

Compared with the reported 8-h urinary excretion of DB and 4-OH-DB in the Caucasian population (10,11), the Chinese population showed lower recovery of 4-OH-DB in 8-h urine and higher recovery of DB; So the distribution histogram of log MR in the Chinese shifted to right and the metabolic capacity for DB hydroxylation in the Chinese seemed slower than that in Caucasian population. Despite genetic control of DB hydroxylation, environmental factors may play a minor role in the oxidation of DB. No sex difference was shown on the hydroxylation of DB. This result is in line with the findings of Steiner et al (9).

REFERENCES

Inhibitory effects of ginsenoside Rg1 and Rb1 on rat brain microsomal Na+,K+-ATPase activity

CAO Jin, ZHENG Yu-Qun, LIU Tian-Pei, FENG Lu-Zhong

(Department of Pharmacology, Nanjing Medical College, Nanjing 210005, China)

ABSTRACT Rat brain microsomal Na+, K+-ATPase activity was inhibited significantly and rapidly by ginsenoside Rb1. The IC50 of Rb1 for Na+, K+-ATPase was 6.3 ± 1.0 μmol/L. The inhibition was enhanced with increasing the concentration of Rb1 or decreasing that of Na+ and K+. Kinetic analysis revealed that ginsenoside was an uncompetitive inhibitor with respect to ATP. The inhibitory effect of Rg1 on rat brain microsomal Na+, K+-ATPase was much weaker than that of Rb1.

KEY WORDS ginseng; saponins; brain; microsomes; sodium, potassium adenosine triphosphatase

Received 1988 Nov 23 Accepted 1989 Aug 14

Project supported by National Natural Science Foundation of China, No. 3860695

Panax ginseng CA Meyer improved both stimulating and inhibiting processes of central nervous system (CNS), and the active component enhancing the former might be panaxatriol and that enhancing the latter, panaxadiol. Mammal brain and nervous tissues contain rich Na+, K+-ATPase, which plays an important role in maintaining the excitation and conduction. The inhibitory effects of some drugs on CNS such as anisodamine and chloropromazine are partially related to their inhibition of Na+, K+-ATPase activity. We have demonstrated in previous reports that the antistress action of ginsenoside was related to central transmitters, and ginsenoside inhibited Na+, K+-ATPase activity in rat kidney. In this paper, we study the effects of Rb1 and Rg1, the pure glycosides.