The Paraoxonase Polymorphism in a Korean Population

Dong-Ryul Sohn, Jun-Tack Kwon, Hyung-Kee Kim

Department of Clinical Pharmacology, Soonchunhyang University College of Medicine, Chonan, Korea

Background : Human serum paraoxonase is a polymorphic enzyme that is capable of catalyzing the hydrolysis of paraoxon and other organophosphates, some carbamates and certain aromatic carboxylic acid ester. We have studied interindividual differences in the activity of the serum paraoxonase in a Korean population.

Methods : The paraoxonase polymorphism was analyzed in the serum of 183 native, unrelated and healthy Korean subjects. The phenotyping method used was one based on the ratio of paraoxonase/arylesterase.

Results : The distribution of the ratio of paraoxonase/arylesterase in the sample was trimodal and it provided a good resolution for the identification of three phenotypes : A-homozygotes with low activity; B-homozygotes with high activity; AB-the corresponding heterozygotes with intermediate activity. Gene frequencies were 0.437 for the allele A with low activity and 0.563 for the allele B with high activity.

Conclusion : The findings indicate that the Korean subjects have a greater incidence of allele B with high activity compared with that reported from white subjects.

KEY WORDS : Paraoxonase · Polymorphism · Koreans.

Although the natural substrate of the human serum paraoxonase is unknown, it has been suggested that the enzyme is capable of catalyzing the hydrolysis of a number of substrates including organophosphates, carbamates and certain aromatic carboxylic acid esters. This enzyme may play a protective role against poisoning due to chronic exposure to these toxic environmental chemicals¹⁻³⁾.

Recent population studies have revealed that bimodal distribution of serum paraoxonase levels existed in various Caucasian populations⁴⁻⁶⁾. This polymorphic enzyme is determined by two allelic genes at one autosomal locus^{5,7-9)}. These alleles are referred to as the A allele responsible for the low paraoxonase activity,

and B, the allele responsible for the high paraoxonase activity; AB is the corresponding heterozygote^{6,10)}.

However, the distribution of serum paraoxonase in a number of different ethnic groups, including Nigerian, Senegales and Oriental populations show unimodal and the clear identification of phenotypes is more difficult¹¹⁾. Thus, it can be assumed that the interracial differences will be existed in the activity of paraoxonase. Furthermore, it has recently been reported that the pharmacogenetic characteristics of debrisoquine oxidation and S-mephenytoin hydroxylation would differ within the similar ethnic populations residing in the same geographic region¹²⁻¹⁴⁾.

No pharmacogenetic information on paraoxonase polymorphism has been available from a native Korean population. Only one study¹⁵⁾ has revealed that a low ac-

Department of Clinical Pharmacology, Soonchunhyang University College of Medicine, Chonan 330-090

tivity group was 19.6% in a Koreans. We, however, are unable to say whether they were residing in Korea. Thus the aim of this study was to explore the polymorphism of paraoxonase in a native Korean subject using the serum paraoxonase/arylesterase ratio.

Materials and Methods

1. Chemicals

All chemicals were purchased from Sigma(St. Louis, MO, U.S.A.).

2. Subjects

One hundred and eighty three unrelated healthy Korean subjects(72 females and 111 males, whose ages, weights, and heights ranged from 20 to 41 years, 43 to 85kg, and 150 to 188cm, respectively) have been included in the study. They were residing in the Chonan area of Korea and were members of laboratory staffs or were medical or nursing school students. No subject had recent illness, and none had taken drugs for at least 1 week before the study. No subject had any abnormalities on physical examination or on clinicopathologic examination. The study protocol was approved by the Ethics Committee of the Soonchunhyang University Hospital, Chonan, Korea. Each subject consented to participate in the study on the basis of verbal and written information.

Heparinized venous blood(10ml) was collected from each subject and the plasma separated by centrifugation and stored at -20°C until analyzed.

3. Paraoxonase activity

The activity of paraoxonase was measured spectrophotometrically by the method of Eckerson *et al.*¹⁶⁾. The rate of hydrolysis of paraoxon was assessed by monitoring the formation of 4-nitrophenol spectrophotometrically at 412 nm using 1.0mM paraoxon in 0.05M glycine buffer, pH 10.5, with 1.0ml CaCl_2 , with or without 1.0M NaCl at 25°C . Enzyme activity was expressed as nM 4-nitrophenol formed/ml serum/min.

4. Arylesterase activity

The modified method of Eckerson *et al.*⁶⁾ and Fur-

long *et al.*¹⁷⁾ was used for the assay of arylesterase. Phenylacetate was the substrate and the rate of formation of phenol(at 25°C) was monitored spectrophotometrically at 270 nm in a reaction mixture containing 1.0mM phenylacetate, 20 μl of 1:50 dilution of serum(in water) and 0.9mM CaCl_2 in 3ml 9mM, pH 8.0, Tris/HCl buffer. One unit of the enzyme was defined as the rate of hydrolysis of 1 μmol of phenylacetate per min.

5. Phenotyping method

To determine individual phenotypes, two parameters were used: (1) The degree of stimulation of paraoxonase activity by 1M NaCl¹⁶⁾; (2) The ratio of paraoxonase activity to arylesterase activity⁶⁾.

Results

There were no significant sex-related differences in paraoxonase activity and no significant linear connection between age and paraoxonase activity in the subjects studied. The paraoxonase activities ranged from 58 to 410 nmol 4-nitrophenol formed per mL of serum per min in the 183 subjects and the distribution of paraoxonase activities is shown in Fig. 1.

Fig. 2 demonstrates the distribution of the ratios of paraoxonase+salt/arylesterase activity in which a trimodality is apparent. The ratios of paraoxonase+salt/arylesterase activity(Fig. 2) allowed a better definition of the phenotypic groups than the basal paraoxonase (Fig. 1). Using the antimodes between groups at 3.0 and 6.5, individuals were assigned to three possible phenotypes. The first mode in Fig. 2 corresponds to individual homozygotes for the A allele with low activity; the last mode corresponds to homozygotes for the B allele with high activity; the intermediate mode corresponds to the AB heterozygotes. There were 35 individuals of the A phenotype(19.1%), 107 of the heterozygous AB phenotype(58.5%) and 41 of the B phenotype(22.4%) in the Korean subjects studied.

From the frequency of the A phenotype the gene frequencies were estimated as 0.437 and 0.563 for the A and B alleles, respectively. Using these gene frequencies and assuming the population to be in Hardy-

Weinberg equilibrium the expected phenotype numbers were calculated as A: 35.0, AB: 90.0, B: 58.0.

The characteristics of the serum paraoxonase: arylesterase of the three phenotypes are shown in Table 1.

Discussion

In this study we have shown that there is con-

siderable interindividual variation(16-fold, Table 1) in the activities of the serum paraoxonase and arylesterase. Several modifications to the original method⁴⁾ have been proposed to improve the detection of paraoxonase phenotypes. One of these modifications was the addition of 1M NaCl to the assay, because the enzymes determined by allele A and B are stimulated by salt in different degrees¹⁶⁾. Other modifications include the use of chelators of calcium^{5,9,17,18)}, chlorpromazine¹⁹⁾ and the ratio paraoxonase/arylesterase⁶⁾. Eckerson *et al*⁶⁾ pro-

Fig. 1. Frequency distribution histograms of paraoxonase activities(nmol of 4-nitrophenol/ml/min)in a Korean population.

Fig. 2. The distribution of the ratio of paraoxonase+salt/arylesterase activity in a Korean population.

Table 1. Characteristics of the paraoxonase/arylesterase phenotypes of the 183 Korean subjects

	Paraoxonase/arylesterase phenotype		
	A	AB	B
Number of subjects(male/female)	35(20/15)	107(68/39)	41(25/16)
Age(yr)	23.9 ± 5.8	23.1 ± 6.5	23.8 ± 5.2
Paraoxonase activity(nmol/min/ml)			
- NaCl	98 ± 35	203 ± 76	317 ± 86
+ NaCl	111 ± 42	429 ± 152	983 ± 274
Arylesterase activity(μmol/min/ml)	49 ± 10	102 ± 23	120 ± 31
Paraoxonase+salt/arylesterase ratio	2.3 ± 0.5	4.3 ± 0.8	8.2 ± 0.7

The data are expressed as mean ± SD.

posed the use of the ratio of activity with two substrates for identification of paraoxonase phenotypes. This is a ratio between: a) the activity with paraoxon, measured in presence of salt; and b) the arylesterase activity, measured with phenylacetate as the substrate. Evidence that both activities are properties of the same enzyme is presented in Gan *et al*²⁰. Activity of this enzyme with other substrates is discussed in Furlong *et al*¹⁷, and Smolen *et al*²¹. The ratio of paraoxonase/arylesterase activities has been used successfully to identify paraoxonase phenotypes in several different populations^{5,6,8,22,23}. In those samples, it provided a much better discrimination of the paraoxonase phenotypes than the determination of paraoxonase alone, the so called basal paraoxonase activity.

The frequency distribution of paraoxonase activity in 183 unrelated Korean subjects, assayed without added salt(basal activity; Fig. 1), was not bimodal. When the same subjects were assayed for paraoxonase activity with 1M NaCl and arylesterase activity, the ratio of paraoxonase activity+salt/arylesterase activity separated these subjects into three classes and A, AB and B phenotypes could be clearly identified(Fig. 2). Our data are consistent with that the clear identification of phenotypes of paraoxonase polymorphism was more difficult in Orientals¹¹ and Nigerian populations¹¹. In addition, the frequency of the A allele determined in this study is close to the frequencies observed in other Oriental populations^{15,24}. Thus it can be postulated that the ratio of paraoxonase+salt/arylesterase activities is useful method for phenotyping the paraoxonase polymorphism in Orientals.

Although the average levels of paraoxonase and arylesterase activity in our study were about 40% lower than those of La Du *et al*⁶, the ratio of paraoxonase+salt/arylesterase activities was similar for AB and B but not for A. The absolute levels of serum basal paraoxonase, salt stimulated paraoxonase, and arylesterase in the present samples are, however, lower than in the Caucasian subjects reported^{3,6,22}. The same was observed in population from Japan¹⁵, Vietnam¹⁵ and Turkey²³: the samples had lower levels of the paraoxonase and arylesterase activities, but a similar distribution of

the ratio paraoxonase/arylesterase, compared to the Caucasians. The gene frequency for the B allele in our population was found as 0.563. The frequencies, however, reported for other Caucasian populations were ranged from 0.270 to 0.317^{5,6,7,22}. It is apparent from the above indicated gene frequencies for the B allele that our result is relatively high. The data presented above are important because of the concept that serum paraoxonase plays a major role in the metabolism of organophosphorous compounds including at least parathion, chlorpyrifos, diisopropylfluorophosphate, soman, and sarin^{21,26}. Also as suggested by Furlong *et al*¹⁷, La Du³ and Playfer *et al*⁵, that individuals with low serum paraoxonase activity are more susceptible to poisoning by toxic organophosphorous oxons than individuals with higher serum enzyme levels.

The importance of the polymorphism of the human serum paraoxonase for the paraoxon detoxification in man has been discussed by several investigators. Butler *et al*²⁵, were able to show that covalent binding sites did not contribute significantly to the clearance of paraoxon from the whole animal. Hydrolysis of paraoxon by tissue paraoxonases, in addition to that catalysed by paraoxonase in the blood, had a major role in the disappearance of paraoxon in the rabbit. This suggests that susceptibility of people to chronic parathion exposure may vary, according to their inherited level and type of serum paraoxonase.

The identification of paraoxonase alleles using the serum phenotyping method is of limited value, compared with the greater degree of discrimination possible with the genotyping method using DNA. The measurement of the serum enzyme, however, provides very useful information about the level and type of esterase activity characteristic of the different alleles. We believe that a combination of both methods will be the best way to study the paraoxonase polymorphism in the future.

Acknowledgments

This work is supported in part by the Soonchunhyang University Grants 95-2201.

References

- 1) La Du BN, Eckerson HW : *Could the human paraoxonase polymorphism account for different responses to certain environmental chemicals?* In : Omenn GS, Gelboin HV, eds. *Banbury report 16 : genetic variability in response to chemical exposure*. Cold Spring Harbor, New York : Cold Spring Harbor Laboratory 1984 ; 167-176
- 2) Origoza-Ferado J, Richter R, Furlong C, Motulsky AG : *Biochemical genetics of paraoxonase*. In : Omenn GS, Gelboin HV, eds. *Banbury report 16 : genetic variability in response to chemical exposure*. Cold Spring Harbor, New York : Cold Spring Harbor Laboratory 1984 ; 177-178
- 3) La Du BN : *The human serum paraoxonase/arylesterase polymorphism*. *Am J Hum Genet* 1988 ; 43 : 227-229
- 4) Krisch K : *Enzymatische hydrolyse von diathyl-p-nitrophenylphosphat(E600) durch menschliches serum*. *Z Klin Chem Klin Biochem* 1968 ; 1 : 41-45
- 5) Playfer JR, Elze LC, Bullen MF, Evans DAP : *Genetic polymorphism and interethnic variability of plasma paraoxonase activity*. *J Med Genet* 1976 ; 13 : 337-342
- 6) Eckerson HW, Wyte CM, La Du BN : *The human serum paraoxonase/arylesterase polymorphism*. *Am J Hum Genet* 1983 ; 35 : 1126-1138
- 7) Geldmacher v. Mallinckrodt M, Lindorf HH, Petenyi M, Flügel M, Fisher T, Hiller T : *Genetisch determinierter polymorphismus der menschlichen serumparaoxonase(E.C.3.1.1.2)*. *Humangenetik* 1973 ; 17 : 331-335
- 8) Geldmacher v. Mallinckrodt M, Hommel G, Dumbach J : *On the genetics of the human serum paraoxonase(E.C. 3.1.1.2)*. *Hum Genet* 1979 ; 50 : 313-326
- 9) Mueller RF, Hornung S, Furlong CE, Anderson J, Giblett ER, Motulsky A : *Plasma paraoxonase polymorphism : a new enzyme assay, population, family, biochemical and linkage studies*. *Am J Hum Genet* 1983 ; 35 : 393-408
- 10) La Du BN, Adkins S, Bayoumi RA-L : *Analysis of the serum paraoxonase/arylesterase polymorphism in some Sudanses families*. In : Kalow W, Goedde HW, Agarwal DP eds. *Ethnic differences in reactions to drugs and xenobiotics*. New York : Alan R. Liss 1986 ; 87-98
- 11) Geldmacher v. Mallinckrodt M, Diepgen TL, Duhme C, Hommel G : *A study of the polymorphism and ethnic distribution differences of human serum paraoxonase*. *Am J Phys Anthropol* 1983 ; 62 : 235-241
- 12) Sohn D-R, Kusaka M, Ishizaki T, Shin S-G, Jang I-J, Shin J-G, Chiba K : *Incidence of S-mephenytoin hydroxylation deficiency in a Korean population and the interphenotypic differences in diazepam pharmacokinetics*. *Clin Pharmacol Ther* 1993 ; 52 : 160-169
- 13) Sohn D-R, Shin S-G, Park C-W, Kusaka M, Chiba K, Ishizaki T : *Metoprolol oxidation polymorphism in a Korean population : comparison with native Japanese and Chinese populations*. *Brit J Clin Pharmacol* 1991 ; 32 : 504-507
- 14) Horai Y, Nakano M, Ishizaki T, Ishikawa K, Zhou H-H, Zhou B-J, Liao C-L, Zhang L-M : *Metoprolol and mephenytoin oxidation polymorphisms in Far Eastern Oriental subjects : Japanese versus mainland Chinese*. *Clin Pharmacol Ther* 1989 ; 46 : 198-207
- 15) Diepgen TL, Geldmacher v. Mallinckrodt M, Goedde HW : *The interethnic differences of the human serum paraoxonase polymorphism analysed by a quantitative and a qualitative method*. *Toxicol Environ Chem* 1987 ; 14 : 101-110
- 16) Eckerson HW, Romson J, Wyte CM, La Du BN : *The human serum paraoxonase/arylesterase polymorphism : Identification of phenotypes by their response to salts*. *Am J Hum Genet* 1983 ; 35 : 214-227
- 17) Furlong CE, Richter RJ, Seidel SL, Motulsky AG : *Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon*. *Am J Hum Genet* 1988 ; 43 : 230-238
- 18) Carro-Ciampi G, Gray S, Kalow W : *Paraoxonase phenotypes distribution in Canadian Indian and Inuit populations*. *Can J Physiol Pharmacol* 1983 ; 61 : 336-340

- 19) Eckerson HW, Stern S, La Du BN : *Chlorpromazine : a differential inhibitor of human serum arylesterases A and B*. *Am J Hum Genet* 1982 ; 34 : 50A
- 20) Gan KN, Smolen A, Eckerson HW, La Du BN : *Purification of human serum paraoxonase/arylesterase. Evidence for one esterase catalyzing both activities*. *Drug Metab Dispos* 1991 ; 19 : 100-106
- 21) Smolen A, Eckerson HW, Gan KN, Hailat N, La Du BN : *Characteristics of the genetically determined allozymic forms of human serum paraoxonase/arylesterase*. *Drug Metab Dispos* 1991 ; 19 : 107-112
- 22) Carro-Ciampi G, Kadar D, Kalow W : *Distribution of serum paraoxon hydrolyzing activities in a Canadian populations*. *Can J Physiol Pharmacol* 1981 ; 59 : 904-907
- 23) Karakaya A, Suzen S, Sardas S, Karakaya AE, Vural N : *Analysis of the serum paraoxonase/arylesterase polymorphism in a Turkish population*. *Pharmaco-genetics* 1991 ; 1 : 58-61
- 24) Geldmacher v. Mallinckrodt M, Diepgen TL : *The human serum paraoxonase polymorphism and specificity*. *Toxicol Environ Chem* 1988 ; 18 : 79-196
- 25) Butler EG, Eckerson HW, La Du BN : *Paraoxon hydrolysis versus covalent binding in the elimination of paraoxon in the rabbit*. *Drug Metab Dispos* 1985 ; 13 : 640-645
- 26) Furlong CE, Richter RJ, Siedel SL, Costa LG, Motulsky AG : *Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase*. *Anal Biochem* 1989 ; 180 : 242-247

= 국 문 초 록 =

한국인에서의 Paraoxonase 대사 다형성

손동렬 · 권준택 · 김형기

순천향대학교 의과대학 임상약리학교실

배 경 : 혈청 paraoxonase는 paraoxon, 유기인제 및 carbamate를 가수분해 시키는데 촉매역할을 하는 효소로서 개체에 따라 다양한 활성도를 나타내며 인구집단에서 유전적인 다형성을 보인다. 본 연구에서는 한국인에서의 혈청 paraoxonase 다형성을 확인하고자 하였다.

방 법 : 183명의 건강한 자원자에서 혈액을 채취하여 분광흡도계를 이용하여 paraoxonase 및 arylesterase의 활성도를 측정하였다. 이를 바탕으로 paraoxonase/arylesterase의 비를 계산하여 표현형을 결정하였다.

결 과 : 183명의 혈청 paraoxonase/arylesterase 비의 분포는 삼봉성을 나타내었으며 3종류의 표현형을 확인할 수 있었다. 활성도가 낮은 A homozygote는 19.1%, 활성도가 높은 B homozygote는 22.4% 이었고 AB heterozygote는 58.5% 이었다. 이상의 표현형으로 A allele의 유전자 빈도는 0.437, B allele의 유전자 빈도는 0.563임을 알 수 있었다.

결 론 : 한국인에서는 parathion 등을 대사시키는 효소인 paraoxonase의 활성도가 높은 B allele가 백인 보다는 높은 빈도를 보였으며 다른 동양인들과 비교 했을 때는 거의 같은 빈도를 보임을 알 수 있었다.