PARAOXONASE AND ACETYLCHOLINESTERASE ACTIVITIES IN HUMANS EXPOSED TO ORGANOPHOSPHOROUS COMPOUNDS

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ABSTRACT: Organophoshorous compounds (OP) are used as pesticides in Turkish agriculture. OP inhibit acetylcholinesterase (AchE) activities on the other hand serum paraoxonase (PON1) hydrolyzes the toxic metabolites of a variety of OP. AChE and PON1 activities in serum were measured spectrophotometrically. The individuals were classified for PON1 phenotypes as A, AB and B types. A positive correlation was found between AChE activities and percent of PON1 stimulation. This study suggests that individuals with phenotype A might be more sensitive to OP induced toxicity.

KEY WORDS: Paraxonase; Acetylcholinesterase; Organophosphorous compounds.

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INTRODUCTION

Organophosphorous compounds are used worldwide in increasing quantities for the control of insects affecting agriculture. Suicidal, accidental or occupational exposure to OP is common in developing countries. Intoxication may occur following absorption via the gasrointestinal system, the respiratory tract or the skin [10, 11].

Measurement of acetylcholinesterase activities has been used as an index factor in order to determine the level of human absorbtion of OP and exposure [10]. OP are bioactivated in vivo via oxidative desulfuration to form its oxygen analogues and active metabolites (-oxon forms, chlorpyrifos-oxon, para-oxon, mala-oxon, etc.) which are potent inhibitors of the enzyme AchE. These metabolites are responsible for the cholinergic crisis seen in of OP poisoning. Active metabolites (-oxon forms) are detoxified predominantly by hydrolysis, catalyzed by paraoxonase [3]. Human serum PON1 is a polymorphic enzyme and is capable of catalyzing the hydrolysis of a number of substrates including OP, carbamates and certain carboxylic acid esters. Serum PON1 may play a protective role against poisoning caused by chronic exposure to these toxic environmental chemicals [8, 9, 13].

Using enzymatic analysis, humans can be divided into three serum PON1 phenotypes: A (low activity), AB (intermediate activity) and B (high activity). Studies on a large number of people of Asian, European and African origin indicated significant inter-ethnic differences in the distribution of PON1 phenotypes [1, 3, 9, 15].

Growing interest in PON1 arises from the hypothesis that individuals with low serum activity of this enzyme would be expected to have a diminished ability to metabolize -oxon forms and, therefore might be more sensitive to the toxicity of OP. These individuals also might require a longer recovery time after any given exposure [3]. Previous studies in animals showed that exogenous PON1 protected them against cholinesterase inhibition by -oxon forms [2, 3, 7].The present study was undertaken to determine whether there is a correlation between AchE and PON1 activities in humans exposed to OP.

METHODS

Subjects

The study group consisted of 18 agricultural male workers who were exposed to a variety of OP in Turkey. Males were chronically exposed to OP (azinphos-methyl, chlorpyriphos, malathion etc.) for 1 to 240 months (mean ± SD, 126 ± 77 months), during spraying, transportation and storage of cereals. OP are applied once a year onto cereals at the end of winter sesion. Since all of them worked in each of these areas by a rotation programme, they are assumed to be exposed to the same amount of the same OP. A workday was 8 hours, with a 1 hour lunch. Ages ranged from 20 to 56 years (mean ± SD, 40.3 ± 9.9 years). Blood samples (10 ml) were obtained by venipuncture, the sera were kept frozen at –70°C until assay. The investigation confirms with the principles outlined in the Declaration of Helsinki.

Assay of red cell AChE activity

The activity of red cell AChE were measured spectrophotometrically using Cholinesterase Kit. Into each 0.2 ml of serum 0.2 ml sodium chloride solution (0.15 mol/l) was added. Blank tubes were placed in a 60°C water bath for 10 minutes to inactivate the enzyme. Then, 3 ml water, 2 ml nitrophenol solution (0.75 g/l) and 0.2 ml acetylcholine chloride solution (150 mg/ml) were added. Exactly 30 minutes later, absorbance values at 420 nm versus water were read. DA values were calculated ($DA = A_{Blank} – A_{Test}$). The calibration curve showing Cholinesterases activity (Rapport Units/ml) versus D absorbance values was plotted [18].

Assay of paraoxonase activity

PON1 activities were measured either without any addition of NaCl (basal activity) or with the addition of 1M NaCl (salt-stimulated activity). The rate of hydrolysis of

paraoxon was assessed by measuring the liberation of p-nitrophenol at 412 nm at 30°C. The basal assay mixture included 1.0 mM paraoxon and 1.0 mM $CaCl_2$ in 0.05 M Tris-HCl buffer, pH = 10.5 and salt-stimulated PON1 included 1.0 M NaCl in addition to these buffer. One unit was defined as the amount of PON1 producing 1nmol of p-nitrophenol per min per ml serum. The percent stimulation of PON1 was calculated as:

$$\frac{\text{Paraoxonase activity with 1 M NaCl - Basal paraoxonase activity}}{\text{Basal paraoxonase activity}} \times 100$$

The individuals were classified for PON1 phenotypes using the antimode of histogram of PON1 as proposed by Eckerson et al (1983). Below of the 60% stimulation were accepted for A phenotypes, between the 60%–200% for AB phenotypes and above of the 200% stimulation for B phenotypes [6].

Statistical analysis

Multiple range tests, Student Newman-Keuls test was used to compare the differences between AChE activity and PON1 phenotypes: differences among groups were regarded as significant if p values were less than 0.05. Correlations were made using the Pearson product moment correlation test.

RESULTS

AChE activities and basal and salt-stimulated PON1 activities according to PON1 phenotypes are shown in Table I. The individuals with phenotype A had the lowest AChE activities. There was a significant difference in AChE activity according to PON1 phenotypes. There was a positive correlation between AChE activities and the percent stimulation of PON1 (Figure 1, r = 0.6493, p = 0.009). AChE and PON1 activities in the serum were unaffected by the exposure period.

TABLE I. SERUM ENZYME PROFILE OF OP EXPOSED HUMANS

PON1 phenotypes	A phenotype (n = 6)	AB phenotype (n = 5)	B phenotype (n = 7)
Acetylcholin esterase			
Mean ± SD	35.2±8.25	61.8±15.7*	60.8±13.1*
Median	36.0	58.0	58.0
Basal PON1			
Mean ± SD	29.7±13.8	54.2 ± 35.3	55.3 ± 35.4
Median	27.5	36.0	39.0

		Salt-stimulated PON1	
Mean ± SD	38.0 ± 14.7	169.2 ± 104.0*	130.1 ± 86.8*
Median	37.5	108.0	106.0

Data are mean ± SD, number of subjects shown in parenthesis.
*Significantly different from phenotype A ($p < 0.05$).

DISCUSSION

Several recent studies suggest that serum PON1 is protective against poisoning by OP. In 1953, Aldrige classified esterases into two groups depending on their interaction with OP acting as anticholinesterases: "A" esterases hydrolyzed such compounds whereas "B" esterases (which include the carboxylesterases and cholinesterases) are inhibited by them [14]. A-esterase (PON1) can detoxify OP but the role this enzyme plays

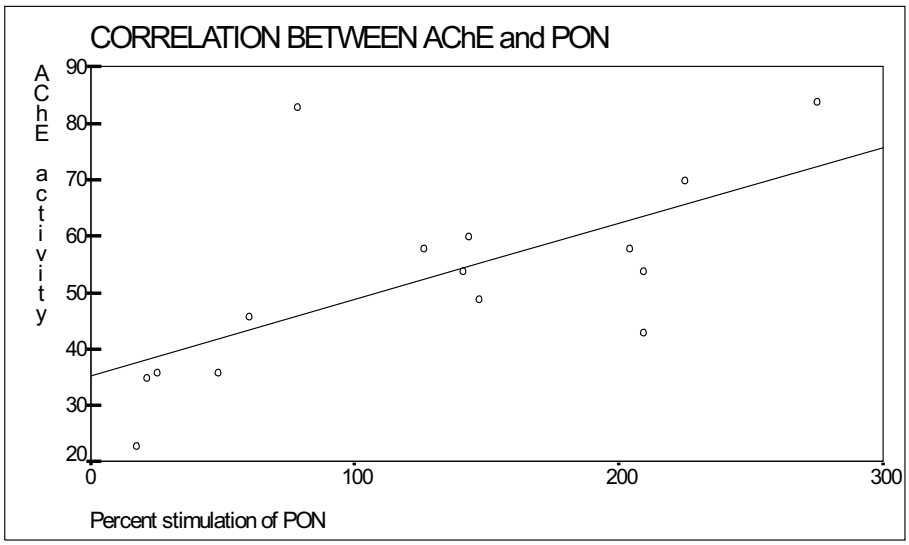

Fig. 1. Correlation between AChE activity and the percent stimulation of PON1 in humans exposed to OP ($r = 0.6493$, $p = 0.009$).

in toxicity of these compounds is not well understood.

Some animal studies have shown that a difference in serum PON1 activity can influence selective toxicity to OP [3]. Birds that have very low levels of paraoxon hydrolyzing activity in their sera are very susceptible to parathion poisoning [12]. Several OP are also 10 to 100 times more toxic to birds than mammals, and this differential sensivity is attributed to differences in serum A-esterase (PON1) activity [17]. Rabbits which have a sevenfold higher enzyme level compared with rats, have a fourfold higher resistance to

OP poisoning [12]. Costa and Manzo [4] reported that serum PON1 activity modulates the toxicity of OP insecticides in mammals. Toxicity studies have shown that raising rat plasma PON1 levels by intravenous administration of partially purified rabbit PON1 protected against cholinesterase inhibition by paraoxon and chlorpyriphosoxon. Protection was correlated with the relative rates of hydrolysis of these two compounds [8]. Li et al. [13] showed that administration of PON1 after exposure to several OP abolished cholinergic signs and significantly protected against cholinesterase inhibition.

Less attention has been given to the role of serum PON1 activity in the differential sensitivity of OP toxicity in mammals, especially in humans. The genetically determined large variation in serum PON1 activity in the human population is known [9, 14]. Based on our results it was proposed that human subjects with phenotype A are probably more susceptible to OP poisoning due to low AChE activity than those with AB or B phenotypes. Another interesting finding in this study was the correlation between the percent stimulation of PON1 and AChE activities. Li et al. [12] also proposed that serum PON1 status may serve as a biomarker for OP susceptibility to toxicity in humans. In healthy human subjects, Mackness et al. [16] showed that PON1 polymorphism may provide a method for identifying those individuals most at risk for OP poisoning. On the other hand, Drevenkar at al. [5] found no correlation between AChE and PON1 activities in a study with 97 agricultural workers in Croatia, however PON1 phenotypes were not investigated.

It is well known that cholinesterase activity measurements are the most common way of determining OP exposure in humans. Data suggest that, in addition to cholinesterase activity measurements, PON1 activities of the individuals should also be investigated.

References:

1. Abbott C. A., Mackness M. I., Kumar S., Boulton A. J., Durrington P. N., Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins, *Arteriosclerosis Thrombosis and Vasculer Biology* 1995, vol. 15, pp. 1812–1818.
2. Aldrige W. N., Serum esterases 2: an enzyme hydrolysing diethyl p-itrophenylphosphate E600 and its identity with the A-esterase of mammalian sera, *Biochemical Journal* 1953, vol. 53, pp. 117–124.
3. Costa L. G., McDonald B. E., Murphy S. D., Omenn G. S., Richter R. J., Motulsky A. G., Furlong C. E., Serum paraoxonase and its influence on paraoxon and chlorpyrifos-oxon toxicity in rats, *Toxicology and Applied Pharmacology* 1990, vol. 103, pp. 66–76.
4. Costa L.G., Manzo L., Biochemical markers of neurotoxicity: research strategies and epidemiological applications, *Toxicology Letters* 1995, vol. 77, pp. 137–144.
5. Drevenkar V., Radic Z., Vasilic Z., Reiner, R., Dialkylphosphorous metabolites in the urine and activities of esterases in the serum as biochemical indices for human absorption of organophosphorous pesticides, *Archives of Environmental Contamination and Toxicology* 1991, vol. 20, pp. 417–422.

6. Eckerson H. W., Wyte C. M., La Du B. N., The human serum paraoxonase/arylesterase polymorphism, *American Journal of Human Genetetics* 1983, vol. 35, pp. 1126–1138.
7. Furlong C. E., Richter R. J., Seidel S. L., Motulsky A. G., Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyriphos oxon and paraoxon, *American Journal of Human Genetics* 1988, vol. 43, pp. 230–238.
8. Furlong C. E., Costa L. G., Hassett C., Richter R. J., Sundstrom J. A., Adler D. A., Disteche C. M., Omiecinski C. J., Chaplin C., Crabb J.V. [et al.], Human and rabbit paraoxonases: purification, cloning, sequencing, mapping and role of polymorphism in organophosphate detoxification, *Chemical and Biological Interactions* 1993, vol. 87, pp. 35–48.
9. Karakaya A., Suzen S., Sardas S., Karakaya A. E., Vural N., Analysis of the serum paraoxonase/arylesterase polymorphism in a Turkish population, *Pharmacogenetics* 1991, vol. 1, pp. 58–61.
10. Karalliedde L., Senanayake N., Organophosphorous insecticide poisoning, *British Journal of Anesthesiology* 1989, vol. 63, pp. 736–750.
11. Klette K. L., Levine B., Dreka C., Smith M. L., Goldberger B. A., Cholinesterase activity in postmortem blood as a screening test for organophosphate chemical weapon exposure, *Journal of Forensic Sciences* 1993, vol. 38, pp. 950–955.
12. Li W. F., Costa L.G., Furlong C. E., Serum paraoxonase status: a major factor in determining resistance to organophosphates, *Journal of Toxicology and Environmental Health* 1993, vol. 40, pp. 337–346.
13. Li W. F., Furlong C. E., Costa L. G., Paraoxonase protects against chlorpyrifos toxicity in mice, *Toxicology Letters* 1995, vol. 76, pp. 219–226.
14. Mackness M. I., Harty D., Bhatnager D., Winocour P. H., Arrol S., Ishola M., Durrington P. N., Serum paraoxonase activity in familial hypercholesterolemia and insulin-dependent diabetes mellitus, *Artherosclerosis* 1991, vol. 86, pp. 193–199.
15. Mackness M. I., Mackness B., Durrington P. N., Connelly P. W., Hegele R. A., Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins, *Current Opinion in Lipidology* 1996, vol. 7, pp. 69–76.
16. Mackness B., Mackness M. I., Arrol S., Turkie W., Durrington P. N., Effect of the molecular polymorphism of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon, *British Journal of Pharmacology* 1997, vol. 122, pp. 265–268.
17. Walker C., Mackness M. I., "A" esterases and their role in regulating the toxicity of organophosphates, *Archives of Toxicology* 1987, vol. 60, pp. 30–33.
18. Witter R. F., Measurement of blood cholinesterase, *Archives of Environmental Health* 1963, nr 6, p. 537.