PARAOXONASE PHENOTYPE DISTRIBUTION IN A HEALTHY POPULATION GROUP FROM THE SLAVONSKI BROD AREA

Dubravka Juretić1, Milena Tadijanović2, Branka Rekić3, Roberta Petlevski1, Vera Simeon Rudolf4

1Faculty of Pharmacy and Biochemistry, Division of Medical Biochemistry and Hematology, Zagreb, Croatia
2“Dr. Josip Benčević” General Hospital, Slavonski Brod, Croatia
3“INA” Health Centre, Zagreb, Croatia
4Institute for Medical Research and Occupational Health, Zagreb, Croatia

ABSTRACT - Objective: Paraoxonase (EC 3.1.8.1) is an esterase that hydrolyzes organophosphate compounds. The enzyme is associated with HDL and could protect LDL against peroxidation, which suggests a possible involvement of paraoxonase in the antiatherogenic properties of HDL. Several studies have shown that paraoxonase levels in humans have a distribution characteristic of two alleles, one with low (AA) and the other with high activity (BB). Paraoxonase also has arylesterase activity. We investigated paraoxonase and arylesterase activity of the enzyme as well as phenotype distribution of paraoxonase in sera of volunteer blood donors from the area of Slavonski Brod, Croatia.

Methods: The paraoxonase activity was measured with 2.0 mM paraoxon in the absence and in the presence of 1 M NaCl, and with 2.0 mM phenylacetate. Both activities were measured spectrophotometrically at 37°C in 0.1 M Tris-HCl buffer pH 8.0 containing 2 mM CaCl2.

Results: The distribution of paraoxonase activities in the absence of NaCl was polymodal and the activities ranged between 55 and 611 U/L while NaCl-stimulated activities ranged between 93 and 1437 U/L. The arylesterase activities ranged between 28 and 215 kU/L and the distribution was unimodal. Bz using the double substrate method to assign phenotypes on 145 healthy individuals selected among the blood donor volunteers, 39% individuals were classified as AA homozygous low paraoxonase activity group, 48% individuals as AB heterozygous intermediate activity group, and 13% individuals belonged to BB homozygous high activity group.

Conclusion: Distribution of paraoxonase phenotypes was similar to that of other European populations.

Key words: Lipoproteins, HDL; Phenotype; Croatia

INTRODUCTION

Paraoxonase (aryldialkylphosphatase, EC 3.1.8.1) is a serum esterase that is synthesized in the liver and hydrolyzes organophosphorus compounds such as paraaxon, including esters of phosphonic and phosphinic acids. More recently paraoxonase was shown to also hydrolyze aromatic carboxylic acid esters, such as phenylacetate which is a characteristic substrate of the arylesterase (EC 3.1.1.2). The enzyme was originally found to be responsible for the hydrolysis of paraaxon (O,O-diethyl-O-p-nitrophenylphosphate), a catabolite of the insecticide parathion.

Paraoxon is toxic to humans because of its irreversible inhibition of acetylcholinesterase (1).

Paraoxonase exhibits two sequence polymorphisms, Arg(192)→Gln and Met(55)→Leu, the former being responsible for distinct catalytic activity of the two corresponding allozymes against paraaxon. The amino acid Arg at position 192 of the protein specifies high activity paraoxonase (B), whereas Gln at that position specifies low-activity paraoxonase (A). They are in Hardy-Weinburg equilibrium and therefore give rise to three phenotypes: phenotype homozygous AA (low activity), heterozygous AB (intermediate activity) and the least common homozygous BB (high activity). Paraoxonase also has arylesterase activity, which does not exhibit activity polymorphism (2, 3).
Paraoxonase circulates in blood as a component of HDL in humans and other vertebrates, thus acting as its carrier and the site of action. Paraoxonase is tightly bound to apoA-I with the hydrophobic N-terminal domain of the enzyme (4,5). The presence of paraoxonase in HDL may be a major contributor to the antiatherogenicity of this lipoprotein. Paraoxonase isolated from human HDL in liposomes has been shown to decrease the susceptibility of LDL to lipid peroxidation. This suggests a potential role for paraoxonase in the detoxification of lipid peroxides and suggests that individuals with a low paraoxonase activity may have a greater risk of developing a disease such as atherosclerosis than high-activity individuals (6-13).

The aim of this study was to investigate the serum paraoxonase activity and phenotype distribution in healthy individuals of the Slavonski Brod area.

MATERIALS AND METHODS

Subjects

The population comprised 145 apparently healthy people: 78 males; 67 females; mean age: 36 (range 14-77) not taking medication, who were volunteer blood donors. This study was approved by the Research Ethics Committee of the “Dr. Josip Benčević” General Hospital, Slavonski Brod.

Serum samples

Blood samples from fasting volunteers were collected by venipuncture. Blood was centrifuged, and 2 mL aliquots of serum were frozen at -20 °C before further analysis, usually within 5 weeks.

Enzyme activity assays

Paraoxonase assays were performed in the absence of NaCl (basal activity) and in the presence of 1M NaCl (salt-stimulated activity) (10,14). The rate of hydrolysis of paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co, London, United Kingdom) was determined by measuring liberated p-nitrophenol at 405 nm at 37 °C on a Technicon RA-1000 autoanalyzer (Bayer, Milan, Italy).

The basal assay mixture included 2.0 mM paraoxon and 2.0 mM CaCl₂ in 0.1 M Tris-HCl buffer, pH 8.0. 10 μL of serum was added to 350 μL of reagent mixture. For the salt-stimulated assay, 1M NaCl was added into the above mixture. Percent activation of paraoxonase by 1M NaCl was expressed as: [(paraoxonase activity with 1M NaCl – basal paraoxonase activity)/basal paraoxonase activity]*100.

Arylesterase activity was measured using phenylacetate as the substrate (15). Initial rates of hydrolysis were determined spectrophotometrically by following the increase in phenol concentration at 270 nm. The reaction mixture contained 2.0 mM phenylacetate (Sigma Chemical Co, London, United Kingdom) and 2.0 mM CaCl₂ in 0.1 mol/L Tris-HCl buffer, pH 8.0.

Enzyme activities are expressed in international units (or kunits) per liter of serum.

Paraoxonase phenotype distribution

The phenotype distribution of paraoxonase activity was determined by the double substrate method (16). The ratio of the hydrolysis of paraoxon in the presence of 1M NaCl to the hydrolysis of phenylacetate was calculated for each individual. The ratio was used to assign individuals to one of the three possible phenotypes: AA (homozygous low activity), AB (heterozygous intermediate activity) or BB (homozygous high activity). The phenotypes were also assigned based on basal or salt-stimulated paraoxonase activity distributions by visual evaluation of the first antimode (AA phenotype) and using the Hardy-Weinberg equation for evaluation of the others (17).

Statistical analysis

Comparison between unmatched groups were made by Student’s unpaired t test. Statistical analysis was also undertaken with the use of Student’s paired t test after the groups were matched for age and sex. Spearman’s rank correlation was used to examine the strength of the association between different variables. P values that were lower than 0.05 were considered significant.

RESULTS

The group studied consisted of 78 males and 67 females. The Student’s t test showed no difference between gender concerning paraoxonase and arylesterase activities, and therefore we evaluated paraoxonase phenotypes from data obtained on all 145 individuals.

The distribution of basal paraoxonase activities (in the absence of 1M NaCl) was polymodal.
and the activities ranged between 55 to 611 U/L (Fig. 1A). Salt-stimulated paraoxonase activities were between 93 and 1437 U/L and the distribution was polymodal (Fig. 1B). The arylesterase activities ranged between 28 and 213 kU/L and the distribution was unimodal but skewed (skewness coefficient=0.26) (data not shown). The ratio of the salt-stimulated paraoxonase vs. arylesterase activities was clearly trimodal dividing the populations at antimodes (3.48 and 8.96) (Fig. 1C). According to these antimodes, 57 individuals were classified as AA phenotype (homozygous-low group), 70 individuals as heterozygous AB phenotype (heterozygous high group), and 18 individuals as BB phenotype (homozygous high group). Linear regression of the salt-stimulated paraoxonase activity versus arylesterase activity was highly significant in each group (homozygous-low r=0.906, P<0.05; heterozygotes r=0.850, P<0.05; homozygous-high r=0.710, P<0.05).

We compared the results obtained by the double substrate method with another method for separation of phenotype groups based only on paraoxonase activities. The low activity AA phenotype was visually segregated from plots shown in Figs. 1A and 1B and the AB and BB phenotypes were then calculated by the Hardy-Weinberg equation (17). The assessed antimodes were 200 and 500 U/L for the basal and salt-stimulated paraoxonase, respectively. The number of individuals assigned to mode AA was 48% and 53% respectively. These two numbers are similar, but they are higher than obtained by the double substrate method which assigned 39% individuals to the AA phenotype (Table 1).

Both methods depend on personal evaluation of at least one antimode in the enzyme distribution profile. The double substrate method appeared to be more reliable because it segregated well all three enzyme modes. We therefore applied the classification obtained by this method in our further calculations.

Table 2 shows the paraoxonase and arylesterase activities of the AA, AB and BB phenotypes. Paraoxonase activities and the ratios of salt-

![Figure 1](image1.png)

**Figure 1.** Distribution profiles of paraoxonase and ratios of paraoxonase/arylesterase activities in healthy individuals.
Frequency of basal paraoxonase activities (A), NaCl-stimulated paraoxonase activities (B) and ratios of NaCl-stimulated paraoxonase vs. arylesterase activities (C). The assessed antimodes are indicated with arrows.

**Slika 1.** Raspodjela aktivnosti paraoksonaze i omjer aktivnosti paraoksonaze i arilesteraze u 145 zdravih ispitanika.
Učestalost bazalne aktivnosti paraoksonaze (A), aktivnosti paraoksonaze stimulirane NaCl (B) i omjer aktivnosti paraoksonaze i arilesteraze (C). Antimodovi su označeni strelicama.
Paraoxonase phenotype distribution in a healthy population group...

D. Juretić et al.

5


stimulated paraoxonase vs. arylesterase were significantly different in the three groups (P<0.05). Arylesterase activities were significantly different in group BB. Paraoxonase activation by 1M NaCl was similar in groups AB and BB, but lower in group AA. The distribution of any activity in the three groups was not symmetrical. The skewness coefficients ranged from –1.34 to +1.55. The mean and median enzyme activities were therefore not identical in all phenotype groups.

**DISCUSSION**

Interpopulation differences in serum paraoxonase specific activity have been shown previously in healthy individuals of European and non-European populations. The gene frequency for A allele is quite similar for Caucasian populations while higher frequency of allele B with high activity has been found in the Japanese population, (18-20).

The range and distribution profiles of paraoxonase and arylesterase activities in this study were similar to enzyme activities and distributions reported for many Caucasian populations (11, 16, 18). For 13 European countries, the number of individuals in the AA group was reported to range from 45% to 61% (mean 53%) (18). One report for the population of Zagreb, Croatia, attributed 60% individuals to group AA based on visual estimation of basal paraoxonase activity distribution. This estimation fits into the above range (21). Our data obtained from the analysis

---

**Table 1.** Number of individuals (%) classified into paraoxonase AA, AB and BB

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Antimode</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl-stimulated paraoxonase vs arylesterase activity ratio</td>
<td>1.63-23.1</td>
<td>3.48 ans 8.96</td>
<td>39</td>
<td>48</td>
<td>13</td>
</tr>
<tr>
<td>Basal paraoxonase activity (U/L)</td>
<td>55-611</td>
<td>200</td>
<td>48</td>
<td>43*</td>
<td>9*</td>
</tr>
<tr>
<td>NaCl-stimulated paraoxonase activity (U/L)</td>
<td>93-1437</td>
<td>500</td>
<td>53</td>
<td>40*</td>
<td>7</td>
</tr>
</tbody>
</table>

* Calculated by the Hardy-Weinberg equation.

Classification was based on the distribution profile of indicated parameters. The total number of individuals was 145.

**Table 2.** Paraoxonase and arylesterase activities of the indicated paraoxonase phenotypes

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Paraoxonase Phenotype</th>
<th>Mean ± standard deviation</th>
<th>Median (Range)</th>
<th>Skewness coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal paraoxonase activity (U/L)</td>
<td>AA</td>
<td>126 ± 41</td>
<td>126 (55-260)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>313 ± 119</td>
<td>309 (119-611)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>408 ± 124</td>
<td>436 (116-594)</td>
<td>-0.54</td>
</tr>
<tr>
<td>NaCl-stimulated paraoxonase activity (U/L)</td>
<td>AA</td>
<td>245 ± 87</td>
<td>245 (93-579)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>691 ± 285</td>
<td>687 (241-1437)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>903 ± 288</td>
<td>980 (323-1387)</td>
<td>-0.53</td>
</tr>
<tr>
<td>Percent activation by 1M NaCl (%)</td>
<td>AA</td>
<td>93 ± 11</td>
<td>94 (53-123)</td>
<td>-0.87</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>118 ± 13</td>
<td>122 (82-135)</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>120 ± 9.1</td>
<td>121 (96-131)</td>
<td>-1.34</td>
</tr>
<tr>
<td>Arylesterase activity (kU/L)</td>
<td>AA</td>
<td>105 ± 32</td>
<td>108 (37-213)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>AB</td>
<td>113 ± 36</td>
<td>116 (28-183)</td>
<td>-0.30</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>75 ± 29</td>
<td>71 (36-125)</td>
<td>0.36</td>
</tr>
<tr>
<td>NaCl-stimulated paraoxonase vs arylesterase activity ratio</td>
<td>AA</td>
<td>2.32 ± 0.34</td>
<td>2.28 (1.63-3.48)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

The phenotypes were segregated by the double substrate method. AA, AB and BB groups comprised 57, 70 and 18 individuals, respectively.
Paraoxonase phenotype distribution in a healthy population group...

of paraoxonase activity profiles (48% and 53%) also fit into the above range (Table 1). However, only 39% of individuals were attributed to group AA by applying the double substrate method (Table 1). This, on the other hand, agrees with data by Nevin et al. (22) based on paraoxonase genotyping, who attributed 42% individuals to the AA group. This agreement further supports our conclusion that the double substrate method seems to be the most reliable for differentiation of phenotypes when genotyping is not possible.

The ratios of salt-stimulated paraoxonase vs. arylesterase activities (Table 2) were higher than data reported in the literature, as for instance by Abbott (8) who found ratios 1.21, 4.68 and 8.36 for groups AA, AB and BB, respectively. This difference is probably due to different experimental conditions used by different authors.

Recent reports have indicated that paraoxonase genotype is a major determinant of serum lipid and lipoprotein concentrations, particularly HDL-associated parameters (5,9,14). Dietary habits, such as lipid levels in the diet, were reported to affect paraoxonase activities (23-25). Our study involved an area in Croatia which has a high prevalence of endemic nephropathy (26). A study on paraoxonase activities and phenotypes reported in this paper for healthy individuals from the same area provided a required base-line for comparison.

REFERENCES


SAŽETAK — Cilj: Ispitati katalitičku aktivnost paraoxonaze te učestalost fenotipova AA, AB i BB paraoxonaze u serumu zdravih ispitanika iz okolice Slavonskog Broda, područja poznatog po endemskoj nefropatiji.

Metode: U skupini ispitanika bilo je 145 zdravih osoba (67 žena i 78 muškaraca), dobrovoljnih davatelja krvi, prosječne starosti 36 godina (14-77). Uzorci venske krvi su centrifugirani 15 minuta na 3000 okretaja/minuti, serum odvojen od stanica i smrznut na –20 °C do izvođenja analize. Aktivnost paraoxonaze mjerenja je s paraoxonom na biokemijskom analizatoru Technicon RA-1000. Koncentracije reagensa u reakcijskoj smjesi za određivanje bazalne aktivnosti paraoxonaze bile su: 2.0 mM paraoxon i 2.0 mM CaCl₂.

Bazalne aktivnosti paraoxonaze u serumu ispitanika izmjerene u reakcijskoj smjesi bez dodatka 1M NaCl nalaze se u rasponu od 55 – 61 U/UL, dok se aktivnosti stimulirane s NaCl nalaze u rasponu od 93 do 1437 U/UL, a raspodjela aktivnosti je polimodalna. Primjenom metode dvostrukog supstrata dobivena je trigonalna raspodjela omjera aktivnosti paraoxonase, sa različitim razmacima za AA, AB i BB.

Rezultati: Bazalna aktivnost paraoxonaze u serumu ispitanika izmjerena u reakcijskoj smjesi bez dodatka 1M NaCl nalaze se u rasponu od 55 – 61 U/UL, dok se aktivnosti stimulirane s NaCl nalaze u rasponu od 93 do 1437 U/UL, a raspodjela aktivnosti je polimodalna. Primjenom metode dvostrukog supstrata dobivena je trigonalna raspodjela omjera aktivnosti paraoxonase, sa različitim razmacima za AA, AB i BB.

Zaključak: Raspodjela fenotipa paraoxonaze u skupini zdravih ispitanika iz okolice Slavonskog Broda ne razlikuje se značajno od datih prethodno izvijesti.

Ključne riječi: Lipoproteini, HDL; Fenotip; Hrvatska

BIOCHEMIA MEDICA god. 14, br. 1-2, 2004. 55


