

Activity of Paraoxonase 1 and Lipid Profile in Healthy Children

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Received April 3, 2006

Accepted May 26, 2006

On-line available June 22, 2006

Summary

Paraoxonase 1 (PON1) seems to have a relevant role in detoxifying processes and in atherosclerosis. The aim of this study was to determine PON1 activity, the total antioxidant capacity, as well as entire lipid profile in children for screening of possible risk of atherosclerosis development. Serum PON1 arylesterase/paraoxonase activities were determined spectrophotometrically. The total antioxidant capacity of the serum was measured by TEAC method. Parameters of lipid profile were analyzed by routine laboratory methods. It has been shown that PON1 arylesterase/paraoxonase activities were very similar to values found in adults. In children, no significant correlation between PON1 arylesterase activity and HDL was observed. PON1 paraoxonase activity correlated only with atherogenic index. PON1 arylesterase activity was significantly higher in girls than in boys. The antioxidant capacity was inversely related to the body mass index. In this study, PON1 activity was determined in healthy children aged 11 to 12 years and we found a similarity in PON1 activities of children and adults. Moreover, the results of our study support the hypothesis that higher body weight of children may contribute to a greater risk for development of atherosclerosis in which oxidative stress plays a role.

Key words

Atherosclerosis • HDL • Lipid peroxides • Paraoxonase 1 • TEAC

Introduction

The association between organophosphate pesticides, paraoxonase 1 (PON1) and atherosclerosis is not manifest in a majority of people. Organophosphate pesticides are sulphur derivatives widely used in agriculture. These nontoxic compounds are transformed in the liver to toxic oxon forms that are further detoxified

by several enzymes such as PON1, transferase or monooxygenase. In mammals, any oxon form that escapes hepatic detoxification can be hydrolyzed in the blood by serum paraoxonase before it reaches the brain (Mackness *et al.* 1998).

PON1 is a dimer with molecular weight in the range of 43000-45000. Its gene is located on the long arm of chromosome 7 (Mackness *et al.* 2002a,b) and was

detected besides the serum and liver also in other tissues such as kidneys or small intestine. Major part of this enzyme in the serum is associated with HDL particles, but a low level of PON1 was also observed in VLDL and postprandial chylomicrons (Aviram and Rosenblat 2004, Fuhrman *et al.* 2005). No PON1 is bound to LDL particles (James and Deakin 2004), although PON1 plays a key role in LDL protection. It is believed that PON1 has two active sites because it acts as a detoxifying enzyme hydrolyzing oxygen analogs of several organophosphorus insecticides as well as nerve agents but also as an antiatherogenic enzyme, capable of attenuating plaque formation. This antiatherogenicity comes from its ability to protect lipoprotein particles from free radical oxidation because it can hydrolyze oxidized cholesteryl esters, phosphatidylcholine core aldehydes, and degrade hydrogen peroxide (Aviram *et al.* 2000, Ahmed *et al.* 2001, Aviram and Rosenblat 2004). Concerning the physiological substrate for PON1, this question is still open.

The beneficial role of this enzyme on atherosclerosis has been supported by several authors directly or indirectly showing that PON1 reduces oxidative stress. Shih *et al.* (2000) observed in apolipoprotein E/PON1 double mutant mice increased lipoprotein oxidation in comparison to apolipoprotein E-mutant controls. In addition, decreased activity of PON1 and its other isoform has been reported by 25-45 % in apolipoprotein E-deficient mice vs. controls (Aviram and Rosenblat 2004). Further studies have demonstrated that PON1 activity is reduced in human subjects prone to develop atherosclerosis such as in individuals with diabetes mellitus type 1 and 2, or hypercholesterolemia (Mackness *et al.* 2000, 2001, 2002a,b).

Taken together, PON1 seems to be responsible for inhibition of oxidative processes. This finding indicates that the determination of PON1 activity could already be of benefit for children in order to protect them from atherosclerosis in the future.

Because it is known that every fourth child in Slovakia has high cholesterol levels, the purpose of our study was to determine PON1 activity, lipid profile and total antioxidant capacity in children aged 11 and 12 years.

Methods

Patients and sample preparation

Seventy healthy children (mean age 11.62±0.06;

range 11-12 years) joined this study after written consent of their parents. Blood samples were collected from veins into serum commercial tubes (S-Monovette[®], Sarstedt). After centrifugation (1200 xg, 10 min), serum was frozen and kept at -80 °C until use.

Determination of parameters of lipid metabolism

Serum total cholesterol, HDL-cholesterol and triacylglycerols (TAG) were analyzed by routine biochemical procedures on Hitachi 721 (Roche, Switzerland). LDL-cholesterol was calculated according to the formula of Friedewald *et al.* (1972).

Determination of PON1 arylesterase activity

PON1 arylesterase activity in serum was determined spectrophotometrically by using synthetic substrate – phenylacetate (Sigma, USA) according to Gan *et al.* (1991) with a slight modification. After adding 100 µl of 10 mmol/l substrate solution to assay mixture containing 5 µl serum and 1 mmol/l CaCl₂ (Sigma, USA) in 50 mmol/l Tris buffer pH 8 (AppliChem, Biochemica, Germany), production of phenol was detected after 2 min at 270 nm (Biochrom 4060, Cambridge, UK). PON1 arylesterase activity was determined in triplicate and all results are presented in µmol per min per ml. Mean intra- and inter-assay coefficients of variation for this analysis were 4.2 % and 7.1 %, respectively.

Determination of PON1 paraoxonase activity

PON1 paraoxonase activity in serum was measured spectrophotometrically at 412 nm according to Beltowski *et al.* (2002) with some modifications. Determination of PON1 paraoxonase activity was performed by using paraoxon (Sigma, USA). Reaction mixture contained 10 µl serum and 1 mmol/l CaCl₂ (Sigma, USA) in 20 mmol/l Tris buffer, pH 8 (AppliChem, Biochemica, Germany). The reaction was initiated by adding 3.27 mmol/l paraoxon to assay mixture. The production of p-nitrophenol was detected after 3 min by Biochrom 4060 spectrophotometer (Cambridge, UK). PON1 paraoxonase activity was monitored in triplicate and all results are presented in nmol per min per ml. Intra- and inter-assay coefficients of variation were 13.3 % and 10.9 %.

TEAC (trolox equivalent antioxidant capacity) assay

The antioxidant capacity of serum was assessed by TEAC assay using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt tablets

(ABTS) (Sigma, USA) according to Re *et al.* (1999). The method is based on the production of blue-green ABTS^{•+} radical cation formed by potassium persulfate (Lachema, Czech republic) that was subsequently reduced in the presence of blood antioxidants into colorless form. ABTS^{•+} radical solution was prepared 12-16 h before each assay. The reaction mixture was prepared with a slight modification because ABTS^{•+} radical solution was diluted with water instead of phosphate buffered saline solution. Reaction was initiated by adding 10 µl of sample to the reaction mixture. The reduction of absorbance was read after 10 min and quantification was performed on the basis of the dose-response curve for reference antioxidant Trolox – a water-soluble form of vitamin E (Sigma, USA). Results are presented in mmol of Trolox per liter. The intraassay coefficient of variation for this analysis was lower than 4.6 %.

Statistical analysis

All results are presented as mean ±S.E.M. Student's t-test was used for the analysis of data with a Gaussian distribution. The data with non-Gaussian distribution were compared with Mann-Whitney U-test. $P < 0.05$ value was considered to be statistically significant. The Pearson's product moment and Spearman's correlation coefficients were used to determine the relationships between the studied parameters. Logistic regression analysis was used to determine the odds ratios (OR) with 95 % confidence intervals (CI) between TEAC and BMI, as well as between TEAC and TAG. The correlation analysis was performed by using StatsDirect® 2.3.7 (StatsDirect Sales, Sale, Cheshire M33 3UY, UK).

Results

Personal and biochemical parameters of children are shown in Table 1. All lipid parameters were in the reference ranges for children determined in our laboratory. Surprisingly, we found out that 24.3 % children from the study group had total cholesterol levels above 4.5 mmol/l. Total cholesterol levels above 4.5 mmol/l are considered harmful to health for this age group.

The PON1 arylesterase activity was determined towards phenylacetate as a substrate. Serum PON1 arylesterase activity in children was 80.97 ± 2.10 µmol/min/ml ($n=63$) and ranged from 30.88 to 120.50 µmol/min/ml. This activity was very similar to the mean

of PON1 arylesterase activity determined in 30 healthy adults (86.04 ± 3.26 µmol/min/ml) with the mean age of 41.3 years (unpublished results).

PON1 arylesterase activity in children was not correlated with any other lipid parameters such as total, LDL- or HDL-cholesterol and TAG (Table 2). The relationship between PON1 arylesterase activity and the total cholesterol/HDL-cholesterol ratio was also non-significant.

For determination of PON1 paraoxonase activity, synthetic compound – paraoxon as a substrate was used and the production of p-nitrophenol was detected. Serum PON1 paraoxonase activity in children was 97.55 ± 9.50 nmol/min/ml ($n=63$) and ranged from 2.73 to 274.26 nmol/min/ml. This activity did not differ from PON1 paraoxonase activity (93.83 ± 14.38 nmol/min/ml) determined in 30 adults with a mean age of 41.3 years (unpublished results).

As shown in Table 3, serum PON1 activity towards paraoxon was inversely related to atherogenic index (total cholesterol/HDL ratio) ($p=0.027$). No further associations with other lipid parameters were observed.

Antioxidant ability of the serum was expressed in Trolox equivalents, exhibiting the same antioxidant ability as the sample. Mean antioxidant capacity (TEAC) in the serum of children between the age of 11 and 12 years, was 1.64 ± 0.07 mmol/l ($n=63$) with the range from 0.71 to 3.35 mmol/l.

Logistic regression was used to determine whether the measurements of BMI or TAG are related to the presence of TEAC deficiency, i.e. whether BMI, TAG and some other measures are predictive of having decreased serum total antioxidant defense. BMI and TAG (Fig. 1) were found to be significant risk factors for total antioxidant deficiency ($p=0.019$ and $p=0.008$, respectively). Odds ratio for BMI was 1.14 (95% CI: 1.02-1.27) and for TAG 4.97 (95% CI: 1.51-16.33).

No significant correlations were found between TEAC and the parameters of lipid metabolism such as total cholesterol ($r=-0.070$, $p=0.584$, $n=63$), LDL-cholesterol ($r=-0.010$, $p=0.936$, $n=63$) or HDL-cholesterol ($r=0.213$, $p=0.107$, $n=58$) that were expressed by the Pearson's product moment and Spearman's correlation coefficients.

Figure 2 shows that girls had significantly higher serum PON1 arylesterase activity (85.84 ± 3.65 µmol/min/ml; $n=27$) than boys (77.31 ± 2.32 µmol/min/ml; $n=36$, $p=0.044$). PON1 paraoxonase activity in 27 girls was 112.92 ± 15.96 nmol/min/ml, while in 36 boys it was

Table 1. Characteristics of studied subjects

Parameter	Mean \pm SEM (n)	R. V.
Gender(M/F)	29/41 (70)	
Age	11.63 \pm 0.06 (70) (11 – 12)	
BMI (kg /m ²)	19.85 \pm 0.60 (69)	
Serum cholesterol		
Total	4.11 \pm 0.07 (70)	<4.5
HDL	1.35 \pm 0.04 (65)	<1.42 for boys <1.68 for girls
LDL	2.54 \pm 0.07 (70)	<3.4
Triacylglycerols (mmol/l)	0.98 \pm 0.08 (69)	<1.3
AI	3.21 \pm 0.10 (65)	

AI (atherogenic index) means total cholesterol/HDL cholesterol; n means the number of subjects in our study group. R. V. means reference values for children determined in our laboratory

Table 2. PON1 arylesterase activity and its relationship to parameters of lipid profile of children.

	r	n	Significance level (p)
Cholesterol			
Total	0.130	63	0.309
HDL	0.189	58	0.153
LDL	-0.040	63	0.756
Triacylglycerols	0.038	62	0.767
AI	-0.131	58	0.325

AI (atherogenic index) means total cholesterol/HDL-cholesterol; n means the number of subjects in our study group.

Table 3. PON1 paraoxonase activity and its relationship to parameters of lipid profile of children

	r	n	Significance level (p)
Cholesterol			
Total	- 0.116	63	0.364
HDL	0.126	58	0.344
LDL	- 0.204	63	0.109
Triacylglycerols	- 0.147	62	0.253
AI	- 0.290	58	0.027*

* P<0.05, AI (atherogenic index) means total cholesterol/HDL-cholesterol; n means the number of subjects in study group.

lower by 24 % (86.02 \pm 11.35 nmol/min/ml) but this difference did not reach the level of significance (p=0.227).

Moreover, no significant difference was observed in the concentration of circulating HDL-

cholesterol between both gender groups (girls: 1.31 \pm 0.05 mmol/l, n=28 and boys: 1.36 \pm 0.05 mmol/l, n=37; p=0.542). The levels of total and LDL-cholesterol were also not different between both gender groups (girls 4.16 \pm 0.12 mmol/l, n=29 vs. boys 4.08 \pm 0.10 mmol/l,

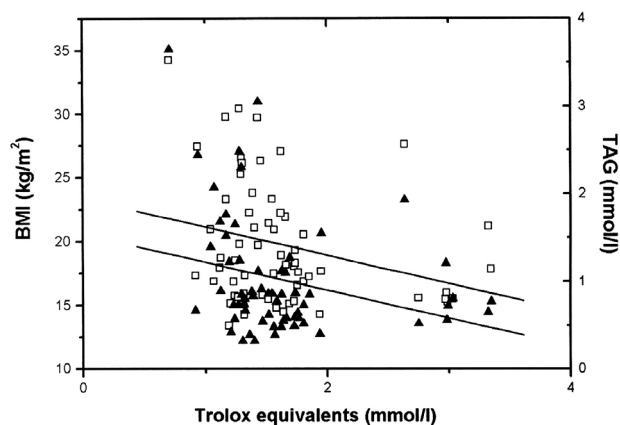


Fig. 1. Associations between serum antioxidant capacity quantified by the TEAC assay as Trolox equivalents and BMI (□, $p = 0.019$, $n = 62$) or TAG (▲, $p = 0.008$, $n = 62$) in healthy children.

$n=41$, $p=0.619$ for total cholesterol and for LDL: girls 2.57 ± 0.11 mmol/l, $n=29$ vs. boys 2.51 ± 0.08 mmol/l, $n=41$; $p=0.596$).

Regarding the gender, no significant differences were observed in TAG (1.09 ± 0.14 mmol/l, $n=29$ in girls vs. 0.91 ± 0.09 mmol/l, $n=40$ in boys; $p=0.366$) and in atherogenic index (girls: 3.32 ± 0.19 , $n=28$ vs. boys: 3.14 ± 0.11 , $n=37$; $p=0.688$).

The antioxidant capacity of serum was similar in both gender groups (1.59 ± 0.08 mmol/l, $n=36$ for boys and 1.68 ± 0.13 mmol/l, $n=27$ for girls; $p=0.807$).

Discussion

It is generally accepted that high total cholesterol level is linked with a greater risk for development of coronary artery disease and other organ complications. Taking into account all recent studies it has been shown that every fourth child in Slovakia aged 11 to 12 years has increased cholesterol levels (above 4.5 mmol/l) (Nagyová 2004). We can confirm this assertion because we found that 17 children out of 70 participants had total cholesterol over 4.5 mmol/l.

PON1 activity has already been determined in children suffering from chronic renal failure (Ece *et al.* 2005) or bronchial asthma exacerbation (Gornicka *et al.* 2002) and it was found to be decreased in comparison to the controls. In this study, we determined PON1 activity as well as its association with lipid parameters in healthy children between the age of 11 and 12 years. We found that PON1 activities in children were very similar to corresponding PON1 activities in adults. This finding is in accordance with the assertion that adult PON1 levels

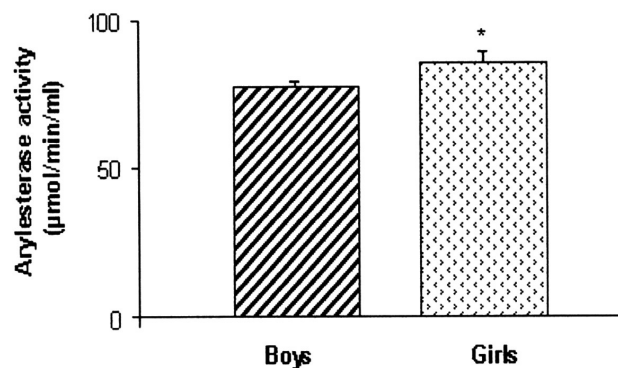


Fig. 2. PON1 arylesterase activity ($\mu\text{mol}/\text{min}/\text{ml}$) determined in children (27 girls and 36 boys), * $p = 0.044$.

are reached approximately 1 year after birth (Mackness *et al.* 1998) and then they tend to stay constant until menopause in women or middle aged men.

Our data showed no relationship between PON1 arylesterase activity and HDL or other parameters of lipid metabolism. Similarly, PON1 activity towards paraoxon was not related to cholesterol-associated parameters such as total-, LDL- or HDL-cholesterol. The only observed relationship was that with atherogenic index which is usually used for predicting of coronary artery disease in humans.

According to our findings, boys had significantly lower PON1 arylesterase activity than girls. This finding is in accordance with our previous results reported in adults, whose PON1 arylesterase activity was also marginally affected by gender ($p=0.056$) (unpublished results). Our findings are controversial to the results of Tomás *et al.* (2000) who found that PON1 arylesterase activity in normocholesterolemic subjects was unaffected by gender. Therefore, further studies are needed.

As expected, PON1 paraoxonase activity in children was not affected by gender. Our results did not also show any significant differences in individual parameters of lipid profile or the antioxidant capacity between both gender groups.

It is alarming that about 25 % children randomly selected for this study had increased total cholesterol levels (>4.5 mmol/l), which indicated a higher risk of heart disease or heart attacks in adult age. Moreover, we have determined activity of the PON1 enzyme, another marker or predictor of possible atherosclerotic changes, in blood vessels in 11- and 12-year-old children. We have found that their PON1 activity was very similar to PON1 activity in adults.

Interesting observations of our study concern correlations between total serum antioxidant capacity and

BMI or TAG (Fig. 1). Our findings imply that BMI is a risk factor for the decrease in total antioxidant ability of the organism when determined by the TEAC method. It was found with 95 % confidence that for every additional unit of BMI (measured in kg/m²) increases the risk of decrease of total antioxidant capacity in children below the norm by 1.14 times. From this point of view, the decrease of body mass could contribute to the reduction of damaging oxidative processes in the organism and thus it can be an important factor in preventing the development of diseases in which oxidative stress plays a role.

Furthermore, high body mass index (overweight) in children is associated with a decrease of total serum antioxidant defense. The decrease in serum antioxidants could be linked to elevated production of oxidatively modified molecules and could therefore cause a greater risk for increased oxidative stress and for heart and

vascular diseases such as atherosclerosis.

Acknowledgements

This work was supported by VEGA grant of Ministry of Education of SR 1/1157/04 and 1/3037/06, APVV grant 51-027, grant of Comenius University UK/28/2004 and Mind&Health-civil association. Authors wish to thank Dr. Bianca Fuhrman from Lipid Research Laboratory, Technion Faculty of Medicine and Rambam Medical Center, Haifa, Israel for her useful comments and help with introduction of the PON1 method.

Abbreviations:

AI, atherogenic index; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PON1, paraoxonase1; TAG, triacylglycerols; OR, odds ratio

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