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Activities of Serum Esterases in Patients with Hyperlipidaemia

Elsa Reiner,[a] *Elizabeta Pavković,*[a] *Vera Simeon-Rudolf,*[a] *Mate Sučić*[b] *and Vaskresenija Lipovac*[b]

[a]*Institute for Medical Research and Occupational Health, Ksaverska cesta 2, P.O.B. 291, 10001 Zagreb, Croatia*

[b]*Vuk Vrhovac Institute, University Clinic for Diabetes, Endocrinology and Metabolic Diseases, Dugi dol 4A, Faculty of Medicine, University of Zagreb, 10000 Zagreb, Croatia*

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Esterases were studied in serum samples from 141 patients with hyperlipidaemia and 27 patients with hyperlipidaemia and non-insulin-dependent diabetes mellitus. The control group consisted of 322 non-diseased individuals. Paraoxon, beta-naphthylacetate and phenylacetate were used as substrates. Enzyme activities were measured in the absence and presence of EDTA in order to differentiate the EDTA-sensitive (*v*-sen) from the EDTA-insensitive (*v*-ins) enzymes. Activity ranges for a given enzyme and substrate grossly overlapped between all the studied groups. However, sera from the control group had significantly higher median v-sen and *v*-ins activities for paraoxon, and higher *v*-sen and *v*-ins activities for PA, than the two groups of patients. HDL-cholesterol, total cholesterol, triglycerides and lipidograms were determined in serum samples from the two groups of patients. Serum samples with type V lipidogram had the lowest *v*-sen and *v*-ins activities for paraoxon, the lowest HDL-cholesterol and the highest triglyceride concentrations.

INTRODUCTION

Paraoxonases (EC 3.1.8.1) are defined as enzymes that hydrolyze organophosphorus compounds (such as paraoxon) including esters of phosphonic and phosphinic acids.[1] Mammalian sera contain two paraoxonases: one that can be inhibited by EDTA (EDTA-sensitive) and the other EDTA-insensitive.[2–4] The

physiological role of paraoxonases is not known. However, very low or even no paraoxonase activities were found in patients with Fish eye disease or Tangier disease, and in patients after myocardial infarction.[5,6] These diseases are accompanied by lipid metabolism disorders. It has been suggested that paraoxonase might be involved in the protection of low-density lipoprotein against oxidative modification.[7,8]

It is known that paraoxonases are associated with high density lipoproteins and it has been reported that patients with low HDL-cholesterol have lower paraoxonase activities than control subjects.[9–14] These findings refer to the total paraoxonase activity, which includes the EDTA-sensitive and EDTA-insensitive enzymes. The aim of this paper was to evaluate separately the EDTA-sensitive and EDTA-insensitive enzymes, and to include serum esterases related to paraoxonase into the study. Three substrates were used: paraoxon (POX), beta-naphthylacetate (BNA) and phenylacetate (PA). Paraoxon is the characteristic substrate of paraoxonases. BNA is a substrate of several esterases and it was shown that the EDTA-sensitive BNA activity correlates well with the EDTA-sensitive paraoxonase.[15] PA is the characteristic substrate of arylesterase (EC 3.1.1.2).[1] Paraoxonases and arylesterases have been reported to have overlapping substrate specificities, particularly concerning the hydrolysis of PA.[2,15,16]

The present study also included measurements of total cholesterol (TC), HDL-cholesterol (HDL-C) and triglyceride (TG) in patients with hyperlipidaemia, as well as evaluation of lipidograms, in order to correlate lipid levels with the activity of serum esterases.

SUBJECTS

Patients with hyperlipidaemia and patients with hyperlipidaemia and non-insulin-dependent diabetes mellitus were studied. The number of subjects, their gender, age, body mass index and the concentration of total cholesterol, HDL-cholesterol and triglyceride are given in Table I. The two studied groups were not further subdivided according to age or gender, because the number of subjects was too small.

All patients were attending the Vuk Vrhovac Institute (Zagreb, Croatia). They were selected on the basis of their fasting TC and TG concentrations, which were 6.5 and 2.0 mmol/L resp. at the time of admission to the Institute. At the time of this study, patients were receiving Fenofibrate and/or Probucol treatment, or were on diet only.

Patients with non-insulin dependent diabetes mellitus (NIDDM) were above 35 yrs old at the onset of diabetes and their body mass index was above 25 (for females) and 27 kg/m^2 (for males) at the time of diagnosis. At the time of this study, patients were either on diet only or diet combined with oral hypoglycaemic agents.

TABLE I

Number (*N*) of male (m) and female (f) patients, their median age, body mass index, and serum concentrations of TC, HDL-C and TG

Group	*N* (m, f)	Age (Range) yrs	Body mass index (Mean ± SD)/(kg/m^2)
Hiperlipidaemia only	141 (92, 49)	51 (17–81)	27.4 ± 3.4
Hyperlipidaemia and NIDDM	27 (20, 7)	53 (39–74)	27.7 ± 3.6

Group	Median (Range)/(mmol/L)		
	TC	HDL-C	TG
Hiperlipidaemia only	7.0 (2.7–20)	1.1 (0.3–2.5)	2.8 (0.7–58)
Hyperlipidaemia and NIDDM	6.9 (4.0–20)	1.0 (0.4–2.0)	3.9 (1.0–47)
	3.88–7.24*	0.97–1.92*	0.84–1.96*

* Reference values from the Biochemical Laboratory of the Vuk Vrhovac Institute

Esterase activities in patients' sera were compared to esterase activities in sera of a non-diseased population (control group): 151 females and 171 males, age = 16–82 yrs, median age = 34 yrs.

METHODS

Blood sampling

Blood was drawn in the morning, after a 12 hour fast. Blood was drawn once from 109 patients and 2–4 times from 59 patients. The total number of serum samples was 250. The analysis of lipids was done on the same day, while serum samples were stored at –18 °C, up to one year, before enzyme analysis. The enzyme activities remained stable over that period (unpublished data).

Enzyme assays

Enzyme activities were measured in 0.1 M Tris/HCl buffer pH = 7.4 at 37 °C by spectrophotometric methods (optical path 1.0 cm; thermostated LKB spectrophotometer) with POX,[4] BNA[17] or PA[18] as substrates.

Stock solutions of POX were prepared in buffer, BNA in methanol and PA in 40% methanol in water (*v/v*). Final concentrations of the substrates were 5.0 mM for POX and PA, and 1.0 mM for BNA. The final methanol concetration was 1.0% for BNA and PA. Stock solutions of EDTA were prepared in buffer and the final concetration was 1.0 mM.

Enzyme activities were expressed in micromoles of released product per minute per millilitre serum. Activities were corrected for spontaneous substrate hydrolysis.

Lipids and lipidograms

TC concetrations were measured by CHOD-PAP, and TG concentrations by the GPO-PAP enzymatic kit methods (Boehringer Mannheim, Germany). HDL-C concentration was determined using the method of Kostner *et al.*.[19] Agarose gel electrophoresis of lipoproteins was carried out at pH = 8.6 in barbital buffer and stained with Fat Red 7 B.[20] Visual interpretation of lipoprotein electrophoresis was used in conjuction with quantitative lipid measurements. Control samples with normal TC, TG and HDL-C demonstrate distinct alpha and beta bands, and a faint pre-beta band. Lipidograms were evaluated according to the method of Fredrickson[21] which is based on TC and TG concentrations, and a quantitative electrophoretic analysis.

Statistical analysis

The within-run imprecision was calculated from measurements done on the same day (in duplicates or triplicates) and the betwee-run imprecision from measurements done on two different days (each time in duplicates or triplicates).[4,22] The intraindividual variation was calculated from measurements done on serum samples drawn 2–4 times from the same subject. The results were expressed as coefficients of variation (CV). Comparison of populations concerning the studied parameters was made by the Wilcoxon two sample rank sum test (Mann-Whitney form).[23] The critical significance level was chosen to be $P = 0.05$. The asymmetry of the activity distribution curves was quantified in terms of skewness coefficients.[23]

RESULTS

The activities of serum esterases in patients with hyperlipidaemia and in the control group are given in Table II. The activity measured in the presence of EDTA (*v*-ins) refers to the EDTA-insensitive enzyme. The difference between the activity measured in the absence of EDTA (*v*-tot) and in the presence of EDTA (*v*-ins) refers to the EDTA-sensitive enzyme (*v*-sen). Under our experimental conditions, *v*-ins amounted only to a small proportion of *v*-tot ; this applies to the hydrolysis of all the three substrates.

TABLE II

Activities of serum esterases in patients and controls
(n is the number of serum samples)

Substrate (n)	Activity/(μmol min^{-1} mL^{-1})	
	Median	(Range)
	Hyperlipidaemia only	
POX (166)	v_{sen}= 0.0656	(0.00592–0.427)
	v_{ins}= 0.00493	(0.000722–0.0688)
BNA (190)	v_{sen}= 11.0	(2.88–28.8)
	v_{ins}= 2.54	(0.713–5.24)
PA (182)	v_{sen}= 138	(44.4–292)
	v_{ins}= 1.50	(0.148–2.70)
	Hyperlipedaemia and NIDDM	
POX (35)	v_{sen}= 0.0643	(0.00461–0.456)
	v_{ins}= 0.00310	(0.000893–0.0110)
BNA (43)	v_{sen}= 12.0	(3.68–29.8)
	v_{ins}= 2.34	(1.29–4.22)
PA (39)	v_{sen}= 130	(68.4–258)
	v_{ins}= 1.47	(0.51–2.70)
	Control	
POX (322)*	v_{sen}= 0.114	(0.015–0.762)
	v_{ins}= 0.00741	(0.00077–0.0322)
PA(322)**	v_{sen}= 155	(41–370)
	v_{ins}= 1.7	(0.15–3.1)

* Unpublished data
** Mean activities; Ref. 4.

For any given substrate, the activity ranges grossly overlapped between all the studied groups of patients and controls (*cf.* Table II). However, median v-sen and v-ins activites for POX were significantly higher ($P < 0.01$) in the control group than in both groups of patients with hyperlipidaemia; v-sen and v-ins activites for PA were also higher in the control group than in both groups of patients.

In patients with hyperlipidaemia, the EDTA-sensitive activities of POX were polymodally distributed and those of PA and BNA unimodally, but skewed towards higher values (skewness coefficients 0.5 and 0.8, resp.).

TABLE III

Median activities of serum esterases and median lipid levels relative to the type of lipidogram in patients with hyperlipidaemia (n is the number of serum samples)

Lipidogram (n)	Lipids mmol L^{-1}		Substrate	Activity μmol min^{-1} mL^{-1}	
				v_{sen}	v_{ins}
Type IIa (21)	TC	7.96	POX	0.0845	0.00585
	HDL-C	1.25	BNA	12.3	2.65
	TG	1.62	PA	139	1.41
Type IIb (79)	TC	7.83	POX	0.102	0.00350
	HDL-C	1.01	BNA	12.3	2.57
	TG	3.13	PA	144	1.58
Type IV (85)	TC	5.75	POX	0.0621	0.00418
	HDL-C	1.05	BNA	10.6	2.50
	TG	3.20	PA	137	1.47
Type V (37)	TC	8.06	POX	0.0156	0.00303
	HDL-C	0.67	BNA	10.2	2.19
	TG	13.02	PA	129	1.66
Normal (28)	TC	5.45	POX	0.0481	0.00637
	HDL-C	1.34	BNA	9.83	2.76
	TG	1.49	PA	131	1.43

The EDTA-insensitive activities of POX had a unimodal, but skewed, distribution (skewness coefficient 5.3), while the distribution of PA and BNA was unimodal and symmetrical.

The activities of serum esterases relative to the type of lipidogram are presented in Table III. Sera with the type V lipidogram had the lowest v-sen and v-ins activities for POX ($P < 0.01$), and the lowest HDL-C ($P < 0.01$) and the highest TG ($P < 0.01$) concentrations as compared to sera of all other lipidogram types. Activities for PA and BNA in type V were neither consistently higher nor lower than the corresponding activities in the sera of other lipidogram types.

Intraindividual variations in enzyme activities and in lipid concentrations were evaluated from repeated blood sampling in 59 patients (Table IV). Time intervals between samplings were between 15 days and one year (median = 3 months). The intraindividual variations of enzyme activities and lipid concentration ranged from 13 to 40%. The within-run imprecisions of measurement were between 2 and 5%, while the between-run imprecisions were higher, particularly for v-ins enzyme activities (19–27%).

TABLE IV

Enzyme activities and lipid levels: intraindividual variations for 59 patients with hyperlipidaemia, and imprecision of with-run (WR) and between-run (BR) measurements (all data are expressed in percentage as coefficients of variation (CV))

		$CV_{intraindividual}$	CV_{WR}	CV_{BR}
Enzyme	Activities			
POX	v_{tot}	35	3.3	15
	v_{ins}	36	4.6	27
BNA	v_{tot}	17	4.0	6.0
	v_{ins}	25	3.8	20
PA	v_{tot}	16	2.5	10
	v_{ins}	23	2.2	19
Lipids				
TC		13	3.0	–
HDL-C		16	4.1	–
TG		40	2.9	–

DISCUSSION

It is known that *v*-tot paraoxonase activities are polymodally distributed in almost all ethnic population groups.[2] In European populations only the low activity mode is well separated and, on average, 53% of individuals have activities in that mode. This is the mean value for non-diseased individuals in 18 European countries, including Croatia.[2,4] When activities are measured under conditions in which *v*-ins amounts only to a small proportion of *v*-tot, the above findings apply equally to *v*-tot and *v*-sen activities. In the present study, 75% of patients with hyperlipidaemia had *v*-sen activities in the low activity mode. This agrees with data reported by Mackness *et al.*[11] that 72% patients with familial hypercholesterolaemia had *v*-tot activities in the low activity mode. The distribution profile of *v*-ins paraoxonases in non-diseased individuals is unimodal[2] but asymmetric;[24] the same was found in patients with hyperlipidaemia.

Distribution profiles of *v*-sen and *v*-ins PA activities in non-diseased populations are unimodal and symmetric.[4] In this study, patients with hyperlipidaemia also had a unimodal distribution profile for PA; however, the distribution of *v*-sen activities was skewed, while the *v*-ins activities were symmetrically distributed. No data seem available for the distribution profiles of BNA hydrolysis in non-diseased populations. In this study, the distribution profiles of *v*-sen and *v*-ins BNA activities in patients with hyperlipidaemia were the same as for the PA activities.

When enzyme activities were analyzed with respect to the type of hyperlipidaemia, only subjects with type V hyperlipidaemia had consistently lower activities for POX than the other groups.

It might be assumed that paraoxonase activities could be used for diagnostic purposes. The present results do not seem to support such an assumption. Although median activities for a given group of patients differ from those in the controls, the activity ranges grossly overlap. Therefore, it would not be possible to attribute a given enzyme activity to a hyperlipidaemic patient. Furthermore, the intraindividual variations of enzyme activities in patients with hyperlipidaemia were high, while those in control groups were much lower (about 4% for the activities measured with either POX or PA[4]). However, the present data confirm that lipid metabolism disorders are accompanied by changes in paraoxonase activity, indicating a possible link between lipid metabolism and the activity of the paraoxonases.

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SAŽETAK

Aktivnosti serumskih esteraza u bolesnika s hiperlipemijom

Elsa Reiner, Elizabeta Pavković, Vera Simeon-Rudolf, Mate Sučić i Vaskresenija Lipovac

Aktivnosti esteraza mjerene su u serumima 141 bolesnika s hiperlipemijom i 27 bolesnika s hiperlipemijom i *diabetes mellitus* (bez inzulinske terapije). Kontrolnu skupinu sačinjavale su 322 osobe. Kao supstrati esteraza uporabljeni su paraokson, beta-naftilacetat i fenilacetat. Da bi se razlikovali enzimi koji se inhibiraju s EDTA (*v*-sen) od onih koji na nju nisu osjetljivi (*v*-ins), aktivnost enzima mjerena je u odsutnosti i u prisutnosti EDTA. Rasponi aktivnosti u uzorcima seruma kontrolne skupine preklapaju se s onima dobivenim na serumima bolesnika. Serumi kontrolne skupine imali su značajno veći medijan obiju paraoksonaznih aktivnosti (*v*-sen i *v*-ins) i veću aktivnost za fenilacetat (*v*-sen i *v*-ins) od odgovarajućih aktivnosti u dvjema skupinama bolesnika. HDL-kolesterol, ukupni kolesterol, trigliceridi i lipidogrami mjereni su u serumima bolesnika. Serumi s lipidogramom tipa-V imali su najniže aktivnosti paraoksonaze (*v*-sen i *v*-ins), najniži HDL-kolesterol i najviše trigliceride.