Trakia Journal of Sciences, Vol. 10, Suppl. 1, 99-103, 2012

Available online at:
http://www.uni-sz.bg

ISSN 1313-7050 (print)
ISSN 1313-3551 (online)

INVESTIGATION OF CHANGES IN THE SERUM LEVELS OF PARAOXONASE AND ARYLESTERASE ACTIVITY OF PON1 IN EXPERIMANTALLY INDUCED DIABETES

T. Tacheva[1*], A. Anastasov[1], M. Yakovlieva[2], S. Mihaylova[2], A. Tolekova[2], T. Vlaykova[1]

[1]Dept. of Chemistry and Biochemistry, Medical Faculty, Trakia Univesity, Stara Zagora, Bulgaria
[2]Dept. of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia Univesity, Stara Zagora, Bulgaria

ABSTRACT

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar, either because pancreas does not produce enough insulin, or because cells do not respond to the insulin. It has long been known that diabetes complications are associated with oxidative stress. In the plasma of diabetic patients higher levels of lipid peroxidation products are related to enhanced plasma lipoprotein oxidation and decrease of plasma antioxidant defenses.

Human paraoxonase 1 (PON1) is HDL-associated enzyme , bound to apolipoprotein A–I (apo A-I) proven to protect both LDL and HDL from oxidative modifications.

In this study were enrolled 38 healthy rats, divided in three groups: a) controls: b) study group treated with streptozotocin, and c) study group treated with streptozotocin and feed with fructose. The blood samples were collected on the 7th day under anesthesia via cardiac puncture. We measured paraoxonase and arylesterase activity of PON1 in the plasma of all the groups. There was found a significant difference in both activities of PON1 between control group and streptozotocin-treated group. Interestingly the levels of PON1 activity were higher in the group of animals with higher blood glucose levels (streptozotocin-fructose). We suppose it is associated with fructose admission: perhaps fructose leads to lower oxidative stress.

Key words: diabetes, PON1, paraoxonase activity, arylesterase activity, streptozotocin.

INTRODUCTION

Diabetes is a metabolic disease; however, it has long been known that its complications are associated with oxidative stress [1]. It has been observed that lipid disorders can play important role in the development of late diabetic complications [2, 3]. Several factors may take part in these changes. Firstly, oxidative stress is accelerated and thus lipid peroxidation may contribute to vascular wall impairment. Secondly, glycation of proteins including enzymes may decrease their activities in diabetes. When HDL were incubated at very high concentrations of glucose (1 mol/l), the esterolytic PON1 activity was preserved. In contrast, HDL incubated in normal (5 mmol/l) or elevated (up to 100 mmol/l) glucose concentrations caused a loss of the esterolytic PON1 activity [4]. A suggestion was made that in the plasma of diabetic patients higher levels of lipid peroxidation products could be related to a higher susceptibility of plasma lipoproteins of diabetic patients to oxidation and to a decrease of plasma antioxidant defenses [5-7]. Studies have shown that serum paraoxonase(PON) activity is reduced in patients whit diabetes mellitus [8 , 9]. PON has been identified as an independent genetic risk factor for vascular disease, particularly in diabetes mellitus patients. It is closely associated with HDL, and may contribute to the antiatherogenicity of HDL by preventing the oxidation of LDL containing the “bad” cholesterol [10].

PON1 is a calcium-dependent glycoprotein, synthesized mainly in the liver. While over the years enzymatic activity has been named with regard to the substrates metabolized, the same

***Correspondence to**: *Tanya Tacheva, Dept. of Chemistry and Biochemistry, Medical Faculty, Trakia Univesity , 11 Armeiska str., Stara Zagora, Bulgaria, E-mail: tanya.ta4eva@abv.bg*

enzyme has been shown to exhibit nearly the whole arylesterase and paraoxonase activities. It contains 337 amino acid residues, has a molecular weight of approximately 43 kD, and a serum concentration of about 50 mg/L [11-14, 15 , 16]. Human paraoxonase-1 (PON-1) is HDL-assosiated and bounded to apolipoprotein A–I (apo A-I), protecting it and LDH from oxidative modifications. PON hydrolyzes some phospholipid oxidative products, such as isoprostenoidcarboxyl and aldehyde esters, phosphatidylcholine hydroperoxides, and lipid peroxidaton products, demonstrating phospholipase-A2-like activity. Paraoxonase hydrolyses the toxic oxon metabolites (paraoxon, diazoxon) of the organophosphates, aromatic esters primly of acetic acid (phenylacetate, thiophenylacetate, 2-naphtylacetate). The enzyme has also lactonase activity and hydrolyzes a variety of aromatic and aliphatic lactones, such as homogentisic acid lactone, dihydrocoumarin, γ-butyolactone, homocysteine thiolactone etc. PON also metabolizes the lactones of hydroxyl derivates of arachidonic and docosahexaeonic acid, platelet-activating factor (PAF) – one of the important pro-inflamatory mediators. Most recently it has been reported, that PON metabolizes estrogen esters [12, 17].

There are three izoenzyme forms of PON (PON1, PON2, PON3) and PON1 is one of them. As a result of polymorphisms in the gene encoding PON-1, there are found to exist different variant forms of it (alloenzymes) [18, 19]. So far there have been described two common SNPs in the coding sequences of the gene: Gln(Q)/Arg(R) substitution at position 192, and Leu(L)/Met(M) substitution at position 55. Differences between the 192Q/R PON1 alloforms have been established in regard to their efficiency in retarding of LDL oxidation: the variant R alloenzyme is less effective than the wide-type Q alloform, probably due to the lower activity of the former one in hydrolysis of lipid hydroperoxides. The L/M polymorphism at position 55 does not affect catalytic activity, but it has been found to influence the plasma PON1 protein level, as $PON1_{M55}$ being associated with low plasma PON1 concentration.

About 200 new SNPs, both in coding and in introns and regulatory regions of the gene have been identified recently [20].

Many (but not all) studies in human populations have suggested that these plymorphisms may be risk factors for atherosclerosis. The serum concentrations of PON across the general population is highly variable and there is some debate as to whether genotype or phenotype is most accurately associated whit risk of disease development [21].

Because of the growing importance in the protection against oxidative stress, in our study we evaluate the levels of paraoxonase and arylesterase activity of PON 1 in rats whit diabetes induced by streptozocin and streptozotocin+frictose.

MATERIALS AND METHODS

Patients

In this study were enrolled 38 healthy rats at the age of three months divided in three groups: a) controls (8 male and 7 female), b) study group treated with streptozotocin (3 male and 3 female) and c) study group treated with streptozotocin and feed with fructose (3 male and 10 female). The control group was treated with citrate buffer applied by intraperitoneal injection in doses 0.2 ml. Streptozotocin and streptozotocin and fructose treated group were injected intraperitoneally with streptozotocin desolved in citrate buffer by in doses 40mg/kg. Furthermore the second group was given 10% fructose per os in the water with free acces. All the animals from the same gender were equal to weight (male 300 g, female 250 g). During the experiment the animals were kept under standard conditions and free access to food and water. The blood samples were collected on the 7^{th} day under anesthesia via cardiac puncture.

Methods

The current study was conducted in the Laboratory of Biochemistry of tumor growth in Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora.

Paraoxonase activity (PON activity)

The method for assessment of serum activity of PON1 toward the substrate paraoxon (paraoxonase activity, PON activity) is kinetic and it is based on the velocity of hydrolysis of the paraoxon by the PON1 enzyme. The changes in the optical density (OD) of the resulted after the hydrolysis *p*-nitrophenol at 37^0C for 5 minutes, was recorded at 405 nm. The concentration of PON1 in serum was

presented as U/l. The paraoxonase activity (PON activity) of 1 U was defined as 1μmol *p*-nitrophenol formed per minute. The molar extinction coefficient of *p*-nitrophenol is 18 053 $(mol/l)^{-1}.cm^{-1}$ at pH 8.5.

Arylesterase activity (ArEs activity)

The method described by Tomas et al [22] is based on a spectrophotometrical analysis in UV range: the velocity of hydrolysis of phenyl acetate into phenol and acetic acid was measured as the increase of the optical density (OD) of the phenol at 270 nm was recorded for 3 minutes after incubation at 37^0C. The concentration of PON1 in serum was presented in kU/l (U/ml).

RESULTS AND DISCUSSION

In our study there were enrolled healthy 38 rats. When the values of the paraoxonase activity of PON1 was measured there was found a significant difference only between control group and streptozotocin-treated group (223.283±135.438 vs. 73.738±78.368, p=0.006, Mann-Whitney U test). Such difference was not observed between the controls and streptozotocin+fructose treated group (223.283±135.438 vs. 164.763±84.503, p=0.224) and also between the streptozotocin and streptozotcin+fructose treated groups (p=0.092) **(Table 1)**. Similar outcome was observed in arylesterase activity between control group and streptozotocin–treated group (controls vs. streptozotocin: 8.770±7.226 vs. 3.238±3.177, p=0.020; controls vs.streptozotocin+fructose: 8.770±7.226 vs. 5.482±4.668, p=0.128; streptozotocin vs. streptozotocin+fructose p=0.344). **(Table 2)**

Table 1. *Paraoxonase activity of PON1*

	mean±st.dev.	**range**	**median**
Controls	223.283 ± 135.438	21.049 - 465.518	266.770
Streptozotocin	73.738 ± 78.368	3.545 -259.680	36.448
Strep.+ fructose	164.763 ± 84.503	62.704 – 381.543	148.009

Table 2. *Arylesterase activity of PON1*

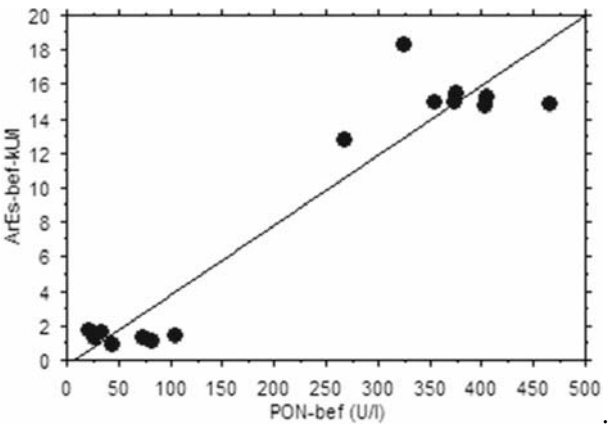
	means±st.dev.	**range**	**median**
Controls	8.770 ± 7.226	0.967 – 18.372	12.774
Streptozotocin	3.238 ± 3.177	1.282 – 11.715	2.148
Strep.+ fructose	5.482 ± 4.668	1.181 – 15.379	3.847

We evaluated the possible correlations between both enzyme activities of PON1 and we found that there was a positive correlation between them. This is most pronounced in the control and streptozotocin-treated group **(Figure 1A and 1B)**, while in the group of treated with streptozotocin and fructose only a tendency was observed **(Figure 1C)**.

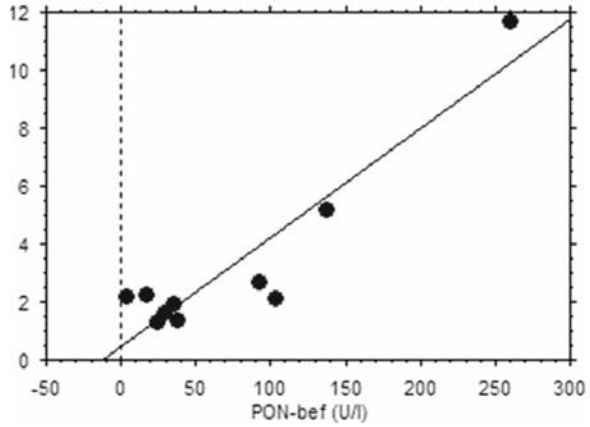
Interestingly the rats treated with streptozotocin and fructose developed diabetes with higher levels of blood sugar in short period of time compared with streptozotocin treated animals. Moreover, in this group the decrease of PON1 activities were less pronounced than in the the group of animals treated only with streptozotocin. Based on previously reported studies by other research teams, we hypothesize that fructose might minimize the effect of streptozotocin on the oxidative damage. Furthermore, it has been reported that dietary fructose has weaker stimulatory effect on insulin secretion.

The current investigation based on streprozotozin and streptozotocin-fructose treated rats shows that paraoxonase and arylesterase activity of PON1 decrease in

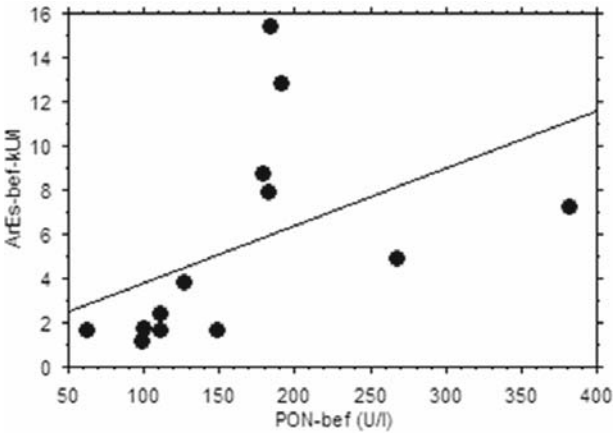
diabetes induced by streptozotocin. This does not apply to streptozotocin-fructose induced diabetes. Interestingly the levels of PON1 activities are higher in the group of animals with higher blood glucose levels (streptozotocin-fructose). We suppose it is associated with fructose admission: perhaps it leads to lower oxidative stress.



1A.



1 B.



1C.

REFERENCES

1. Celik VK, Sahin ZD, Sari I, Bakir S: Comparison of oxidant/antioxidant, detoxification systems in various tissue homogenates and mitochondria of rats with diabetes induced by streptozocin. *Exp Diabetes Res* 2012, 2012:28.
2. Wegner M, Piorunska-Stolzmann M, Araszkiewicz A, Zozulinska-Ziolkiewicz D, Wierusz-Wysocka B: Evaluation of paraoxonase 1 arylesterase activity and lipid peroxide levels in patients with type 1 diabetes. *Pol Arch Med Wewn* 2011, 121:448-454.
3. Kopprasch S, Pietzsch J, Kuhlisch E, Graessler J: Lack of association between serum paraoxonase 1 activities and increased oxidized low-density lipoprotein levels in impaired glucose tolerance and newly diagnosed diabetes mellitus. *J Clin Endocrinol Metab* 2003, 88:1711-1716.
4. Mackness B, Durrington PN, Abuashia B, Boulton AJ, Mackness MI: Low paraoxonase activity in type II diabetes mellitus complicated by retinopathy. *Clin Sci* 2000, 98:355-363.
5. Merzouk S, Hichami A, Sari A, Madani S, Merzouk H, Yahia Berrouiguet A, Lenoir-Rousseaux JJ, Chabane-Sari N, Khan NA: Impaired oxidant/antioxidant status and LDL-fatty acid composition are associated with increased susceptibility to peroxidation of LDL in diabetic patients. *Gen Physiol Biophys* 2004, 23:387-399.
6. Ferretti G, Bacchetti T, Busni D, Rabini RA, Curatola G: Protective effect of paraoxonase activity in high-density lipoproteins against erythrocyte membranes peroxidation: a comparison between healthy subjects and type 1 diabetic patients. J *Clin Endocrinol Metab* 2004, 89:2957-2962.

7. Leonhardt W, Hanefeld M, Muller G, Hora C, Meissner D, Lattke P, Paetzold A, Jaross W, Schroeder HE: Impact of concentrations of glycated hemoglobin, alpha-tocopherol, copper, and manganese on oxidation of low-density lipoproteins in patients with type I diabetes, type II diabetes and control subjects. *Clin Chim Acta* 1996, 254:173-186.
8. Abbott CA, Mackness MI, Kumar S, Boulton AJ, Durrington PN: Serum paraoxonase activity, concentration, and phenotype distribution in diabetes mellitus and its relationship to serum lipids and lipoproteins. *Arterioscler Thromb Vasc Biol* 1995, 15:1812-1818.
9. Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN: Serum paraoxonase activity in familial hypercholesterolaemia and insulin-dependent diabetes mellitus. *Atherosclerosis* 1991, 86:193-199.
10. Valabhji J, McColl AJ, Schachter M, Dhanjil S, Richmond W, Elkeles RS: High-density lipoprotein composition and paraoxonase activity in Type I diabetes. *Clin Sci* 2001, 101:659-670.
11. Agachan B, Yilmaz H, Ergen HA, Karaali ZE, Isbir T: Paraoxonase (PON1) 55 and 192 polymorphism and its effects to oxidant-antioxidant system in turkish patients with type 2 diabetes mellitus. *Physiol Res* 2005, 54:287-293.
12. Anastasov A, Larouche M, Doneva-Basheva K, Dincheva V, Peeva P, Habib T, Vlaykova T: Serum activity of PON1 in patients with skin malignant melanoma. In Youth scientific conference "KLIMENT`S DAYS" 22-23 NOVEMBER, 2011 *Conference proceedings Sofia,* Bulgaria. 2011
13. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN: Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest* 1998, 101:1581-1590.
14. Kuo CL, La Du BN: Comparison of purified human and rabbit serum paraoxonases. *Drug Metab Dispos* 1995, 23:935-944.
15. 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.
16. Eckerson HW, Wyte CM, La Du BN: The human serum paraoxonase/arylesterase polymorphism. *Am J Hum Genet* 1983, 35:1126-1138.
17. Riedmaier S, Klein K, Winter S, Hofmann U, Schwab M, Zanger UM: Paraoxonase (PON1 and PON3) Polymorphisms: Impact on Liver Expression and Atorvastatin-Lactone Hydrolysis. *Front Pharmacol* 2011, 2:27.
18. Liu YL, Yang J, Zheng J, Liu DW, Liu T, Wang JM, Wang CN, Wang MW, Tian QB: Paraoxonase 1 polymorphisms L55M and Q192R were not risk factors for Parkinson's disease: A HuGE review and meta-analysis. *Gene* 2012, 2012:9.
19. Bayrak A, Bayrak T, Tokgozoglu SL, Volkan-Salanci B, Deniz A, Yavuz B, Alikasifoglu M, Demirpence E: Serum PON-1 Activity but not Q192R Polymorphism is Related to the Extent of Atherosclerosis. *J Atheroscler Thromb* 2011, 2011:22.
20. Flekac M, Skrha J, Zidkova K, Lacinova Z, Hilgertova J: Paraoxonase 1 gene polymorphisms and enzyme activities in diabetes mellitus. *Physiol Res* 2008, 57:717-726.
21. Deakin SP, James RW: Genetic and environmental factors modulating serum concentrations and activities of the antioxidant enzyme paraoxonase-1. *Clin Sci* 2004, 107:435-447.
22. Tomas M, Senti M, Garcia-Faria F, Vila J, Torrents A, Covas M, Marrugat J: Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. *Arterioscler Thromb Vasc Biol* 2000, 20:2113-2119.