Pro198Leu Polymorphism of *GPx-1* Gene and Activity of Erythrocytic Glutathione Peroxidase and Lipid Peroxidation Products

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We studied the relationship between the *GPx-1* gene Pro198Leu polymorphism genotype and activity of free-radical processes. In patients with documented CHD, the content of lipoperoxides and MDA in LDL, activity of glutathione peroxidase in erythrocytes, and genotype of *GPx-1* gene Pro198Leu polymorphism were determined. In carriers of the minor allele, activity of glutathione peroxidase in erythrocytes was lower by 17% than in wild allele homozygotes, while the content of lipoperoxides and MDA in LDL was higher by 74 and 27%, respectively. We concluded than free-radical oxidation processes are more pronounced in carriers of the minor allele of *GPx-1* gene Pro198Leu polymorphism.

Key Words: *glutathione peroxidase; lipid peroxidation; GPx-1 gene polymorphism*

Oxidative stress plays an important role in pathophysiology of vascular diseases (hypertension, atherosclerosis, and postangioplasty restenosis [6]. Reactive oxygen species are potent oxidants; they induce the formation of lipid radicals and LPO changing the properties of cell membranes and oxidation and nitration of proteins and bond breaks in DNA molecules, which, in turn, leads to cell death [3]. Glutathione peroxidase (GPx) is a selenium-containing antioxidant enzyme reducing both inorganic and organic peroxides and participating in peroxynitrite metabolism [5]. The GPx/glutathione system is considered to be the major defense factor against activation of oxidative stress [14], therefore the decrease in GPx activity can play an important role in the development of various pathological conditions, including cardiovascular diseases. Pro198Leu (rs#1050450) polymorphism is one of the most frequent and best studied polymorphism in the gene encoding GPx.

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Here we evaluated the effect of *GPx-1* gene Pro198Leu polymorphism on activity of free-radical processes.

MATERIALS AND METHODS

The study included 69 men with angiographically documented CHD. Patients with unstable angina, myocardial infarction and stroke manifesting less than 6 months before the study, severe heart failure, severe rhythm and conduction disturbances, familial hypercholesterolemia and hypertriglyceridemia, diabetes type I mellitus or decompensated type II diabetes mellitus, tumor diseases, and hepatic and renal failure were excluded from the study. The patients received standard therapy in combination with plavix and statins.

The blood was collected into tubes with EDTA as the anticoagulant and stored at -20°C. DNA from the frozen whole blood was isolated by phenol-chloroform extraction with the use of proteinase K as described earlier [11]. PCR was performed in Mastercycler thermocycler (Eppendorf) under the following conditions: denaturation at 94°C, 5 min (1 cycle), denaturation at

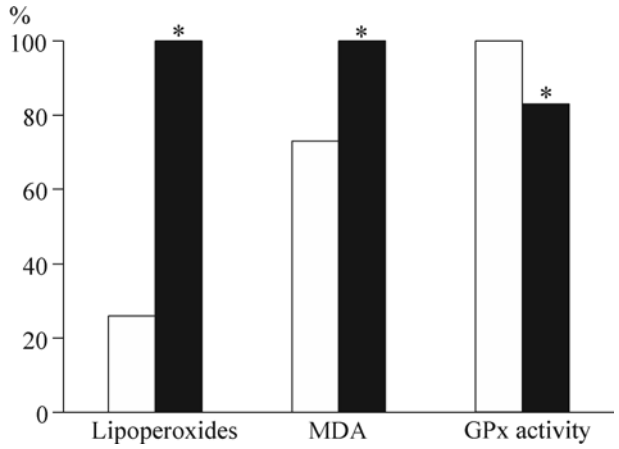

Fig. 1. Activity of oxidation processes in wild allele homozygous patients and carriers of the minor allele of *GPx-1* gene Pro198Leu polymorphism. Open bars: PP; dark bars: PL+LL. *$p<0.05$ compared to individuals with PP genotype.

94°C, 30 sec; annealing at 58°C, 30 sec and elongation at 72°C, 30 sec (36 cycles); elongation at 72°C, 5 min (1 cycle) [8]. PCR was performed in a solution (volume 30 μl) of the following composition: 100 mM tris-HCl, pH 8.3 (25°C), 50 mM KCl, 4 mM $MgCl_2$, 4 deoxynucleoside triphosphate (100 μM each), 10 pmol each primer (forward 5'-TGT GCC CCT ACG CAG GTA CA-3'; reverse 5'-CCA AAT GAC AAT GAC ACA GG-3'), 3 U Tq-polymerase, and 1 μg genome DNA. The length of PCR product was 337 b.p.

The genotype was determined by restriction fragment length polymorphism analysis based on the creation of a natural restriction site in a certain allele during PCR. After PCR, 3 μl restriction buffer (10×) and 2 U HaeIII were added to tubes and incubated for 14 h. The lengths of restriction fragments were 79 and 258 b.p. for allele P, and 337 b.p. for allele L. The restriction products were analyzed by electrophoresis in 2.5% agarose gel containing 1 μg/ml ethidium bromide. Fragment lengths were determined using a molecular weight standard (Ladder-50 bp, Fermentas)

Fasting venous blood samples were collected into tubes with 1 mg/ml EDTA. For isolation of LDL, blood plasma was twice centrifuged in NaBr density gradient at 42,000 rpm for 2 h in a 50Ti angle rotor at 4°C in a Beckman L-8 refrigerator centrifuge as described previously [13]. The content of lipoperoxides in LDL isolated from the plasma was measured by a specific colorimetrical method using the reaction of oxidation of exogenous Fe^{2+} ions followed by the analysis of the content of stechiometrically formed Fe^{3+} by the reaction with xylenol orange before and after specific reduction of organic (lipid) peroxides with triphenylphosphine [10]. MDA content (TBA-reactive products) was measured by the formation of trimethine complexes on a Hitachi 220A spectropho-

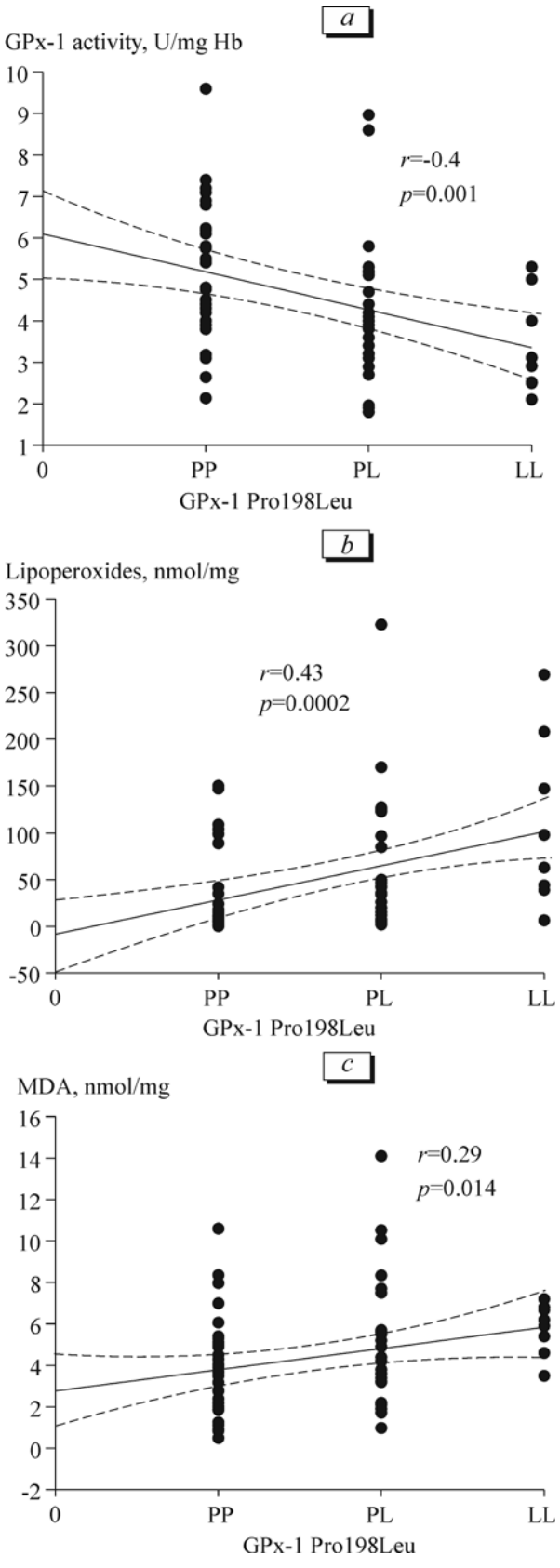

Fig. 2. Correlation of *GPx-1* gene Pro198Leu polymorphism genotype with GPx activity in erythrocytes (*a*) and content of lipoperoxides (*b*) and MDA (*c*) in LDL.

tometer at 532 nm [2]. Activity of selenium-containing GPx in human erythrocytes was measured in a coupled glutathione-peroxidase system by the rate of NADPH oxidation at 340 nm with tert-butyl as the substrate using a modified method [1] on a FP-900 chemical analyzer (Labsystems Oy) operated in a kinetic mode.

The data were processed statistically using Statistica 6.0 software. Consistency of the experimental genotype frequencies with the Hardy–Weinberg law was tested using Fisher test. Comparison by quantitative signs was performed using parametric (Student *t* test) and nonparametric (Mann–Whitney) tests. Nonparametric Spearman correlation test was applied for evaluation of correlations between the parameters. Parameters were presented as the means and confidence intervals (Mean; 95% CI; in case of normal distribution) or as the median and low and upper quartiles (Med; (LQ; HQ); if the distribution did not correspond to normal law). The differences were significant at $p<0.05$.

RESULTS

In 69 patients, genotype of *GPx-1* gene Pro198Leu polymorphism, the content of lipoperoxides and MDA in LDL, and activity of GPx in erythrocytes were determined. Genotype distribution corresponded to Hardy–Weinberg law. The frequencies of *GPx-1* gene Pro198Leu polymorphism genotypes and allele distribution were as follows: PP was found in 33 patients (47.8%), PL in 28 patients (40.6%), and LL in 8 patients (11.6%). Comparison of wild allele homozygotes (PP) and carriers of the minor allele (PL+LL) of *GPx-1* gene Pro198Leu polymorphism showed that the latter were characterized by higher intensity of free radical oxidation processes: in carriers of the minor allele, the content of lipoperoxides and MDA in LDL was higher by 74 and 27%, respectively, compared to carriers of the wild genotype (PP), while glutathione peroxidase activity (GPx) in erythrocytes was lower by 17% (Fig. 1).

At the same time, some correlations were revealed between *GPx-1* gene Pro198Leu polymorphism genotypes and parameters of free-radical oxidation processes. The minor allele was associated with reduced activity of erythrocytic GPx (r=-0.4; p=0.001), *i.e.* mean activity of the enzyme was the highest in wild allele homozygotes and the lowest in minor allele homozygotes. The minor allele was also associated with increased level of primary (r=0.43; p=0.0002) and secondary (r=0.29; p=0.014) LPO products (Fig. 2).

Previous studies of the effect of *GPx-1* gene Pro198Leu polymorphism on the development of cardiovascular diseases showed that the carriers of the minor allele are characterized by greater thickness of the intima-media complex of the common carotid artery (p=0.0028), higher incidence of cardiovascular diseases (p=0.035) and peripheral vascular pathologies (p=0.027) [7], and higher coronary artery calcium score by the data of multi-slice computed tomography (p=0.006) [9]. At the same time, GPx activity was not measured in these experiments, despite the fact that many studies demonstrated increased risk of cardiovascular complications in patients with low GPx activity and documented CHD [4,12]. Our study showed that the presence of the minor allele of *GPx-1* gene Pro198Leu polymorphism leads to a decrease in GPx activity in erythrocytes, accumulation of primary and secondary LPO products, and therefore to potentiation of oxidative stress.

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