THESIS FOR THE DEGREE OF Ph.D.

PARAOXONASE EFFECT ON OXIDATIVE DNA DAMAGE

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INTRODUCTION

Cardiovascular disease causes more premature deaths and long term disability than any other disorder in industrialized countries, therefore studying the pathomechanism of atherosclerosis is especially important. Clinical and epidemiological studies have provided that individuals with elevated LDL show an increased risk for cardiovascular diseases. The earliest stage of atherosclerotic process is fatty streak formation induced by oxidative modification of LDL. Phospholipase A2 promoted LDL oxidation. Apolipoprotein B100, the major protein of circulating LDL is taken up and degraded by macrophages and macrophage like cells' scavenger receptors rapidly producing lipid-laden foam cells. The oxidized LDL is able to activate the transcription factor NFkβ, the result of which is the enhanced expression of chemotactic peptides and adhesion molecules of monocytes.

There are several endothelial protecting mechanisms. NO is capable to inhibit the LDL oxidation, the adhesion of the monocytes and its' penetration into the vessel wall. Lipid soluble antioxidants (ubiquinol-10, alpha-tocopherol, and beta-carotene) are able to protect the apoB against lipid peroxidation. The various components of HDL can intervene at different stages of atherosclerotic process; induction of monocyte adhesion molecules, removal of excess cholesterol by reverse cholesterol transport and prevention of LDL oxidation.

The antioxidant effect of HDL is due to the influence of an HDL associated esther-hydrolase, the paraoxonase, which is mainly band to apoA1 and apoJ component. PON is able to inhibit copper-catalyzed LDL oxidation in vitro and the inflammatory response induced by oxidized LDL in the arterial wall. Recent studies showed that in diseases with disturbed lipid

metabolism such as diabetes mellitus, chronic renal failure, renal transplantation and familial hypercholesterinemia the activity of HDL associated paraoxonase is reduced. Lipid peroxidation is a chain reaction which results in peroxy, hydroperoxy, alcoxy and alkyl radicals, then aldehydes, ketones or hydroxy-acids from free radical attack on polysaturated fatty acids. All of these compounds are highly reactive and have been shown to interact directly with DNA and caused genetic damage. The PON1 gene is located at q21 to q22 on the long arm of chromosome 7 with other members of its supergene family and encodes for a 43-45 kDa protein, that contains up to three carbohydrate chains. There are two common DNA polymorphisms within the PON1 gene. One is $Leu_{55} \rightarrow$Met (TTG$\rightarrow$ATG), the other is $Gln_{192} \rightarrow$Arg (CAA$\rightarrow$CGA). The dominant effect on activity is exerted by the PON1-192 polymorphism. However, the PON1-55 polymorphism also has a smaller, but significant effect on activity. The reason for polymorphism-associated differences in PON1 activity is unclear. Both polymorphisms have been identified as independent risk factors for cardiovascular disease in diabetic and nondiabetic patients.

How does the reduced antioxidant capacity and the large amount of lipids in the sera of hyperlipidemic patients influence the DNA damage induced by oxidative stress? Preventive effect of paraoxonase on oxidative DNA damage have not been previously investigated. To date, genotyping for PON1 is performed by PCR, followed by digestion with restriction enzymes and restriction fragment length polymorphism (RFLP) analysis. These methods are widely used, though they are time-consuming and require optimization of the PCR reaction to eliminate nonspecific PCR products. It is necessary to establish new, high-speed and easy to perform genotyping for the two most significant PON1 polymorphisms.

OBJECTS

1. Determination of oxidative DNA damage induced by hydrogen-peroxide in lymphocytes of hyperlipidemic patients and controls using comet-assay.
2. Determination of superoxide anion generation in resting and stimulated granulocytes of these patients and its correlation to the level of oxidative DNA damage.
3. Detection of serum levels of lipids, TBARS (characteristic for lipid peroxidation), NO, and another non-enzymatic antioxidant, α-tocopherol, and its correlation correlation to the level of oxidative DNA damage.
4. Determination of HDL associated antioxidant paraoxonase activity, and its correlation correlation to the level of oxidative DNA damage.
5. Developing a new mutation detection method in the PON1 gene to identify the two common paraoxonase polymorphisms $Leu_{55} \rightarrow$Met and $Gln_{192} \rightarrow$Arg by real-time fluorescence PCR and melting curves.

MATERIAL AND METHOD

Study design and patients

Inclusion criterias were: age between 21 and 70 years, previously untreated type II/a hyperlipidemia (triglyceride <2.2 mmol/l, LDL cholesterol >4.2 mmol/l). Only non-smoking patients were recruited into the study. Patients with hepatic disorder, endocrinological or renal disorders (serum creatinine level >130 μmol/l), alcoholism, drug dependence, gallstones, malignancy, pregnancy or lactation, or treated with anticoagulant or lipid-lowering therapy were excluded from the study. The patients were kept on National Cholesterol Education Program (NCEP) step 1 diet. The main markers of infection (C-reactive protein (CRP), leukocyte number, sedimentation rate, blood smear), and Lp(a) were normal. The control population consisted of 7 apparently healthy people not taking any medication, who attended a routine health check in our outpatient clinic. They gave written, informed consent to participate, and the study was performed according to the requirements of the Ethical Committee of Medical and Health Science Center, University of Debrecen.

Blood Sampling

After overnight fasting 10 ml venous blood was drawn. Hemoglobin, hematocrit, white blood cell count, sedimentation rate, liver enzymes, urea, creatinine, creatinine kinase, fibrinogen, CRP, bilirubin, uric acid, serum glucose, cholesterol, HDL cholesterol, LDL cholesterol, apoA-I, apolipoprotein B (apo B), triglyceride levels, serum paraoxonase activity and concentration were measured. The lipid parameters were determined from fresh sera. Sera for paraoxonase activity measurements were kept at -20°C before analysis.

Lipid measurements

Serum cholesterol and triglyceride were assayed using a Boehringer Mannheim GmbH Diagnostic enzyme kit, while HDL cholesterol was investigated by the phospho-tungstic-magnesium precipitation method. The LDL cholesterol fraction was calculated indirectly using the Friedewald equation. Apolipoprotein examination was performed by the immuno-nephelometric assay in which the Orion Diagnostic kit was used.

Determination of α-tocopherol

LDL (500 μg protein/ml) was extracted after addition of ethanol and methanol (1:1 v/v) by n-hexane. The organic layer was evaporated under N_2, and redissolved in methanol, and injected into HPLC (Hitachi-Merck) coupled to a fluorescence detector (F1050) using 292 nm for excitation and 325 nm for emission.

Analysis of paraoxonase activity

Paraoxonase activity was determined using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma Chemical Co.) as substrate, and measuring the increase in the absorbance at 412 nm due to formation of 4-nitrophenol. Activity was measured by adding 50 μl serum to 1 ml Tris/HCl buffer (100 mmol/l, pH=8.0) containing 2 mmol/l $CaCl_2$ and 5.5 mmol/l paraoxon. The rate of generation of 4-nitrophenol was determined at 412 nm, 25°C, using of a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated from the molar extinction coefficient 17100 $M^{-1}cm^{-1}$. One unit of paraoxonase activity is defined as 1 nmol of 4-nitrophenol formed per minute under the assay conditions mentioned above.

Arylesterase assay

Arylesterase activity was measured spectrophotometrically. The assay contained 1 mM phenylacetate in 20 mM Tris-HCl pH=8.0. The reaction

was started by the addition of the serum, and the increase in absorbance at 270 nm was read. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using the molar extinction coefficient 1310 $M^{-1}cm^{-1}$. Arylesterase activity is expressed in U/ml; 1 U is defined as 1μmol phenylacetate hydrolyzed per minute.

ELISA for serum PON concentration

Serum paraoxonase concentration was determined by enzyme-linked immunosorbent assay (WAK-Chemie Medical Gmbh, Germany). Serum concentration of PON was determined by reference to a standard curve constructed with purified PON.

NO

The reaction of NO with oxygen and reactive oxygen radicals resuls in the production of nitrite and nitrate. We measured the sum of nitrite and nitrate production in the plasma according to the method of Griess. The fresh plasma was treated with $ZnSO_4$ and Cd suspension. After centrifugation the supernatant was incubated with 1% sulfonamide and 0.1% naphtylethylendiamine for 10 min at 60^{o}C. The colorful result of the reaction was determined using spectrophotometer at 546 nm.

TBARS

After the SDS treatment (pH=3.5), the human plasma was incubated with thiobarbituric acid at 95^{o}C for 60 min. Then the optical density of the organic phase was detected using spectrophotometer at 532 nm after extraction with *n*-butanol:pyridine.

Isolation of PMNLs

Polymorphonuclear leukocytes (PMNLs) were separated by Ficoll-Hypaque density gradient centrifugation according to the method of Boyum. The cell suspensions were 95% pure for PMNLs as judged by

morphological criteria, and 96% were viable by Trypan blue dye exclusion test.

Measurement of superoxide anion generation

The superoxide anion production was measured in PMNLs using superoxide dismutase inhibitable reduction of ferricytochrome C (Sigma). The amount of oxidized ferricytochrome C was measured at 550 nm. Results are presented as nmol/min/10^6 cells. The superoxide anion production was determined in resting cells and after 10^{-8} M formyl-Met-Leu-Phe (FMLP) and 10^{-8} M phorbolmiristate acetate (PMA) stimulation.

Peripheral blood lymphocyte preparation

500 μL of whole blood was carefully layered over 500 μL Ficoll and centrifuged at 500 *x g* for 15 min. The buffy coat was removed and the cells were washed with PBS and then collected by 15 min. centrifugation at 500 *x g*, than resuspended in 1% low melting point (LMP) agarose for comet analysis. The whole procedure was conducted without any explosure to light.

Single cell gel electrophoresis (SCGE)

Isolated human lymphocytes were suspended in 600 μL of a 1% solution of LMP agarose in phosphate-buffered saline (PBS), pH 7.4, at 37°C and immediately pipetted onto a frosted glass microscope slide precoated with 1% normal melting point (NMP) agarose in PBS. The agarose was allowed to set for 10 min. at 4°C and the slides were incubated in PBS containing 50 μM H_2O_2 for 25 min. Then the slides were placed in lysis solution (2.5 M NaCl, 10 mM Tris, 100 mM Na_2EDTA, NaOH to pH 10.0 and 1% Triton X-100) for 1 h at 4°C to remove cellular proteins. After lysis, the slides were aligned in horizontal electrophoresis tank containing buffer (1 mM Na_2EDTA, 0.3 M NaOH, pH 10.0) for 30 min. before electrophoresis at 25

V and 300 mA for 20 min. at 4°C, temperature of running buffer was 15°C. The slides were washed three times for 3 min. at 4°C in neutralizing buffer (0.4 M Tris-HCl, pH=7.5) and stained with 50 μg/ml ethidium bromide, than covered with coverslip.

Analysis of the slides

Visual score (VS) Ethidium bromide stained nucleotides were evaluated with a Zeiss fluorescence microscope. Visual scoring of the comet assay was evaluated by two independent persons in order to increase the reliability of data. A total of 100 comets on each slide were scored visually as belonging to one of five classes according to tail intensity. Values of 0, 1, 2, 3 or 4 were given with (0) = undamaged and (4) = maximum damage (4). Consequently, the total score was in the range from 0 to 400**.**

Preparation of genomic DNA

We have used genomic DNA isolated from EDTA blood from healthy individuals using QIAamp Blood Kit (Qiagen).

Primers and hybridization probes for polymerase chain reaction

The primer sequences for PCR and sequences of the hybridization probes are shown in Table 1. The antisense primer for PON1-55 and the sense primer for PON1-192 polymorphism were previously published by Akhmedova and Mackness at al., respectively. Primers and fluorescently labeled hybridization probes were synthesized by TIB MOLBIOL (Berlin, Germany).

Table 1. PCR primers és hybridization probes.

Primers	Sequence[a]	Orientation	PCR product
PCR primers			
PON1-55F	ATTCTGAACCTATTAAAGAAGAGTGATG	Sense	240 bp
PON1-55R	CTTAAACTGCCAGTCCTAGAAAACG	Antisense	
PON1-192F	TATTGTTGCTGTGGGACCTGAG	Sense	199 bp
PON1-192R	GACATACTTGCCATCGGGTGAA	Antisense	
Detection probes			
PON1-55DA	GCTCTGAAGACATGGAGATA-F[b]		
PON1-192DA	TGACCCCTACTTACAATCCT-F		
Anchor probes			
PON1-55A	LCRed 640-GCCTAATGGACTGGCTTTCATTAGCTCTGT-ph[b]		
PON1-192A	LCRed 640-GAGATGTATTTGGGTTTAGCGTGGTCGTAT-ph		

[a]Sequences given 5` to 3` (accession number: AC004022). The detection probe of PON1-55 is derived from the mutant allele, the mutation site is underlined.
[b]F: fluorescein, ph: phosphoryl group.

Real-time PCR and melting curve analysis

The detection probe of PON1-55 is derived from the mutant allele. PCR and melting curve determination were performed in 20-μL volumes in glass capillaries (Roche Molecular Biochemicals). For PON1-55 and 192 genotyping, the following pipeting scheme was used: 9μL of H_2O, 1μL of 25 mmol/L $MgCl_2$, 2 μL each of 5 pmol/μL PON1-55F and PON1-55R or PON1-192F and PON1-192R, 1 μL each of 4 pmol/μL PON1-55DA and PON1-55A or PON1-192DA and PON1-192A, and 2 μL of DNA-Master Hybridization Probes (Roche Molecular Biochemicals), containing Taq polymerase, reaction buffer, and dNTP mixture, and 10 mmol/L $MgCl_2$ as a 10× concentrate. Genomic DNA (2 μL, 50-200 ng) was used for amplification. The cycling program for PON1-55 genotyping consisted of initial denaturation at 94°C for 600 s, followed by 50 cycles of denaturation 94°C for 0 s, annealing at 50°C for 5 s, and extension at 72°C for 2 s, with a

ramping rate of 20°C/s. The fluorescence was monitored at the end of each 5-s annealing phase. After the products were amplified, we generated melting curves by denaturing the reaction at 94°C for 150 s, holding the sample at 45°C for 30 s, and then slowly heating the sample to 75°C with a ramp rate of 0.2°C/s, simultaneously monitoring the decline in fluorescence with respect to temperature (-dF/dT) against temperature. Cycling conditions for PON1-192 genotyping were the following. Initial denaturation was performed at 94°C for 600 s, followed by 50 cycles of denaturation 94°C for 0 s, annealing at 57°C for 5 s, and extension at 72°C for 2 s, with a ramping rate of 20°C/s. The fluorescence was monitored at the end of each 5-s annealing phase. After the products were amplified, we generated melting curves by holding the sample at 45°C for 3 min, and then slowly heating the sample to 75°C with a ramp rate of 0.2°C/s, simultaneously monitoring the decline in fluorescence with respect to temperature (-dF/dT) against temperature. Amplification and detection occurred in the same closed tube in less than 40 minutes.

Statistical analysis

The statistical analysis was performed by the SAS for Windows 6.12 computer program. Data are presented by descriptive analysis (mean±SD). The comparisons between groups were performed by the paired *t*-test and ANOVA. Significance was accepted at the $p<0.05$ level. The relations between the parameters were determined by regression and correlation analysis. A value of $r>0.5$ was considered to be statistically significant.

RESULTS

15 hyperlipidemic patients were involved in the study (11 female, 4 male, mean age 54.6±10.25 years, mean BMI 26.30±3.26 kg/m^2, cholesterol 7.63±0.90 mmol/l, triglyceride 2,09±0,79 mmol/l, HDL 1.48±0.25 mmol/l, LDL 5.2±0.85 mmol/l). 7 normolipidemic subjects served as controls (5 female, 2 male, mean age 53.4±8.07 years, mean BMI 25.70±2.15 kg/m^2, cholesterol 3.56±0.44 mmol/l, triglyceride 1.07±0.39 mmol/l, HDL 1.43±0.31, LDL 2.6±0.6 mmol/l). The level of the α-tocopherol was similar in control and hyperlipidemic groups (control 30.9±5.2 μM, hyperlipidemic 36.77±6.8 μM)(Table 2.).

Table 2.

	Control			Patients		
Sex	2 males, 5 females			4 males, 11 females		
Age (years)	53.4	±	8.07	54.60	±	10.25
BMI (kg/m^2)	25.7	±	2.15	26.30	±	3.26
Cholesterol (mmol/l)	4.71	±	0.89	7.63	±	0.9*
Triglyceride (mmol/l)	1.06	±	0.52	2.09	±	0.11*
HDL chol (mmol/l)	1.43	±	0.31	1.48	±	0.25
LDL chol (mmol/l)	2.6	±	0.6	5.20	±	0.85*
Alpha-tocopherol (uM)	30.9	±	5.2	36.77	±	6.80

BMI=Body mass index. Values are mean ± SD. *p<0.05

Superoxid anion production

Superoxid anion production of resting granulocytes were 1.17±0.47 nmol/10^{6}cells/min. Stimulating the granulocytes with FMLP, a chemotactic peptide activating the G protein on a specific cell surface receptor, the production of superoxid anion was significantly elevated. Protein kinase activator PMA induced superoxid anion production was more prominent.

The stimulability with PMA and FMLP were reduced comparing to the healthy controls.

TBARS and NO levels

TBARS characteristic for the lipid peroxidation was significantly higher in the sera of hypercholesterinemic patients comparing to the controls (0.36±0.25; 0.79±0.31 μM, $p<0.01$). Endothelium protector NO level was significantly reduced comparing to the control group (19.2±14.9; 58.1±12.7 μM, $p<0.001$).

Serum paraoxonase activity

Paraoxonase activity was significantly reduced in hyperlipidemic group (186.38±44.7; 108.01±49.0 U/l; $p<0.001$). Salt stimulated paraoxonase activity characteristic for the maximal enzyme activity was also significantly reduced (441±71.2; 220.33±136.06 U/l; $p<0.001$). Arylestherase activity typical of the quantity of the enzyme was significantly reduced (129±31.2; 80.34±42.09 U/l; $p<0.001$). Examining the PON/HDL ratio a significant reduction was found in the hyperlipidemic group (182.42±64.4; 77.81±43.08 U/l; $p<0.001$).

Oxidative DNA damage

Analyzing the DNA damage by Comet assay significant elevation was found in the hyperlipidemic group (visual score 289.5±29.49; 350.97±31.31; $p<0.001$). Making correlation analysis between the PON activity and the degree of DNA damage negative correlation was found ($r=-0.469$). Positive correlation was found between the superoxid anion production of resting granulocytes and the degree of DNA damage ($r=0.517$). There was also positive correlation between superoxid anion production after PMA and FMLP stimulation and the degree of DNA damage ($r=0.326$; 0.525). The cholesterol level in sera was also correlated

positively to the DNA damage (r=0.38). There were no connection between the concentration of NO (r=0.098) and TBARS (r=0.061) in plasma and the degree of DNA damage.

Detection of the Two Common Paraoxonase Polymorphisms $Leu_{55} \rightarrow Met$ and $Gln_{192} \rightarrow Arg$ by Real-Time Fluorescence PCR and Melting Curves

When the hybridization probes for the 192 or 55 isotype were used, fluorescence increased constantly in the samples with DNA, whereas no fluorescence was detected in the H_2O control. The melting point (T_m) of the PON1-55 homozygous mutant type sample was at 60.5°C, the heterozygous sample showed a two-phase melting behavior, whereas the T_m of the wild type sample was at 54.7°C. The T_m of the PON1-192 wild type sample was at 60.4°C, whereas the heterozygous sample produced two melting peaks at 53.9°C and 60.4°C. The melting point of the homozygous mutant sample was 53.9°C. Analysis of PCR products on agarose gels revealed the presence of the specific 240 and 199 bp PCR products. We have validate the technology by analyzing 50 healthy individuals for PON1-192 and PON1-55 polymorphisms, the genotypes determined with the LightCycler were identical to those obtained with conventional PCR and restriction fragment length analysis.

DISCUSSION

It is well known that the oxidation of lipoproteins has been proposed as a biological process that indicates and accelerates atherosclerosis development. Superoxide anion and H_2O_2 support metal-catalyzed lipid peroxidation through their participation in redox reactions. Previous studies showed that the superoxide anion production of resting granulocytes was enhanced in the elderly and in hyperlipidemic patients. In the present study, according to previous results of our laboratory, increased resting superoxide anion production was found in granulocytes, while the effects of stimulation with PMA and FMLP were reduced in hyperlipidemic patients compared to healthy controls. Lipid peroxidation serves as a marker of cellular oxidative stress and has long been recognized to contribute to oxidative damage in chronic diseases such as atherosclerosis and cancer. The level of TBARS characteristic for the degree of lipid peroxidation was elevated, while the activity of HDL-associated PON was significantly reduced in hyperlipidemic patients compared to the controls. In the hyperlipidemic group enhanced lipid peroxidation and reduced HDL associated natural antioxidant capacity can promote the atherosclerotic process. We also measured the concentration of the NO, an antiatherogenic agent, and found significant reduction in the hyperlipidemic group. Both antioxidant and pro-oxidant roles for NO in the development of atherosclerosic plaques have been reported. NO has been shown to be efficient in scavenging lipid peroxides. However, NO reacting with superoxide anions can form peroxi-nitrite, which can seriously damage the endothelia. Vitamin E is the major lipid peroxidation chain-breaking antioxidant in cell membranes and may provide protection against the damaging effects of polyunsaturated fatty acids**.** In the present study, there were no apparent differences in plasma alpha-tocopherol levels between

hyperlipidemic and control patients. These findings suggest that the importance of alpha-tocopherol as a protective factor against oxidative DNA damage may be not crucial. Beside the reduced antioxidant capacity, the enhanced oxidative process can cause DNA damage as well and can be detected by Comet assay. The inverse relation of PON activity to the degree of DNA damage indicates that the natural HDL-associated antioxidant system can play an important role not only in the prevention of atherogenic process but also by impeding DNA damage. To our knowledge, inverse relationship between serum PON activity and oxidative DNA damage have not been previously reported. Increased oxidative stress and lipid peroxidation causing DNA damage and disturbance of cell signaling pathways, are being implicated in human cancers, neurodegenerative diseases and atherosclerosis. It is known from recent studies that free radicals interact with cells generate an oxidative chain reaction via electron transfer and that the final result could be deleterious to DNA. Our speculation is that the free radicals produced in part by granulocytes oxidize fatty acids forming conjugated dienes, lipid hydroperoxides that can generate further oxidative processes. The PON hydrolyzing these lipid hydroperoxides practically inhibits the development of further oxidative processes reducing the degree of oxidative stress in cells and preventing the development of DNA damage.

In the last few years, several laboratories have reported the result of their studies investigating the relationship between PON1 polymorphisms and the presence of cardiovascular and other diseases using RFLP analysis. To our knowledge, genotyping of PON1-192 and PON1-55 polymorphisms using real-time fluorescence PCR and melting curves have not been previously reported. This technology is less time-consuming and more accurate and cost-effective than traditional PCR analysis.

NEW RESULTS

1. Analyzing the DNA damage by Comet assay induced by hydrogen peroxide in lymphocytes of the hyperlipidemic group significant elevation was found.
2. Increased resting superoxide anion production was found in granulocytes, while the effects of stimulation with PMA and FMLP were reduced in hyperlipidemic patients compared to healthy controls. There was also positive correlation between superoxid anion production after PMA and FMLP stimulation and the degree of DNA damage.
3. The level of the α-tocopherol was similar in control and hyperlipidemic groups. The cholesterol level in sera was also correlated positively to the DNA damage. There were no connection between the concentration of NO and TBARS in plasma and the degree of DNA damage.
4. Paraoxonase, salt stimulated paraoxonase and arylestherase activity was significantly reduced in hyperlipidemic group. Making correlation analysis between the PON activity and the degree of DNA damage negative correlation was found.
5. We have established high-speed and easy to perform genotyping for the two most significant PON1 polymorphisms $Leu_{55} \rightarrow$Met and $Gln_{192} \rightarrow$Arg, by use of the LightCycler technology and melting curves.

Publication the thesis is based on

1. **Harangi M**, Remenyik É, Seres I, Varga Z, Katona E, Paragh G: Determination of DNA damage induced by oxidative stress in hyperlipidemic patients. ***Mutat Res*** 2002; 513:17-25 **IF: 1.624**
2. **Harangi M**, Remenyik É, Seres I, Varga Zs, Balogh Z, Kakuk Gy, Paragh Gy: Oxidatív stressz hatására kialakuló DNS károsodás meghatározása Comet-assay-vel hyperlipidaemiás betegekben. ***Magyar Belorvosi Archivum*** 2001; 53:393-396
3. **Harangi M**, Aslanidis C, Paragh G, Schmitz G: High-speed detection of the two common paraoxonase polymorphisms $Leu_{55}\rightarrow$Met and $Gln_{192}\rightarrow$Arg by real-time fluorescence PCR and melting curves. ***Clin Chem Lab Med*** 2002; 40:337-340 **IF: 1.595**

Other publication

1. Paragh Gy, Asztalos L, Seres I, Balogh Z, Lőcsey L, Kárpáti I, Mátyus J, Katona E, **Harangi M**, Kakuk Gy: Serum paraoxonase activity changes in uremic and kidney transplantated patients. *Nephron* 1999; 83:126-131 IF:**1.696**

2. Paragh G, Balogh Z, Seres I, **Harangi M**, Boda J, Kovacs P: Effect of gemfibrozil on HDL-associated serum paraoxonase activity and lipoprotein profile in patients with hyperlipidemia. *Clin Drug Invest* 2000; 19:277-282 IF: **0.888**

3. Balogh Z, Seres I, **Harangi M**, Kovacs P, Kakuk G, Paragh G: Gemfibrozil increases paraoxonase activity in type 2 diabetic patients. A new hypothesis of beneficial action of fibrates? *Diab Met* 2001; 27:604-610 IF: **1.624**

4. Balogh Z, Fülöp P, Seres I, **Harangi M**, Katona E, Kosztáczky B, Paragh G: Effects of simvastatin on serum paraoxonase activity. *Clin Drug Invest* 2001; 21:505-510 IF: **0.846**

5. Paragh Gy, Balogh Z, Seres I, **Harangi M**, Boda J: Gemfibrozil hatása a HDL-hez kötött paraoxonáz aktivitására és a lipoprotein szintek alakulására. *Háziorvosi Továbbképző Szemle* 1999; 4:73-77

6. Balogh Z, Bacsó J, Seres I, Brúgós B, **Harangi M**, Paragh Gy: A haj szeléntartalmának változása hiperkoleszterinémiás betegekben. *Magyar Belorvosi Archívum* 2000; 53:209-213

7. Seres I, Varga Zs, Balogh Z, **Harangi M**, Fülöp P, Paragh Gy: Hiperkoleszterinémiás betegek szérum paraoxonáz aktivitása, fenotípus megoszlása és E vitamin szintjei. *Magyar Belorvosi Archívum* 2000; 53:115-117

8. Balogh Z, Paragh Gy, Seres I, **Harangi M**, Kovács P, Kakuk Gy: Gemfibrozil hatása 2-es típusú cukorbetegek szérum paraoxonáz aktivitására. *Magyar Belorvosi Archívum* 1999, 3:347-352

9. Paragh Gy, Seres I, Balogh Z, Lőcsey L, Asztalos L, **Harangi M**, Kárpáti I, Mátyus J, Kakuk Gy: A serum paraoxonáz aktivitás változása vesetranszplantáció után. *Hypertonia és Nephrológia* 2000; 4:31-36

10. Paragh Gy, Balogh Z, Seres I, **Harangi M**, Katona E, Fülöp P, Kakuk Gy: A szimvasztatin hatása a szérum lipidszintekre és a paraoxonáz aktivitására. *Magyar Belorvosi Archívum* 1999; 52:255-258

11. Paragh Gy, Balogh Z, Kovács P, Wórum F, **Harangi M**, Czuriga I, Illyés L, Kakuk Gy: A szekunder prevenció szerepe korai myocardialis infarctust követően. *Cardiologia Hungarica* 2000; 28:115-121

12. Paragh Gy, Seres I, Balogh Z, **Harangi M**, Ilyés L, Boda J, Varga Zs, Kovács P: A mikronizált fenofibrát hatása a szérum paraoxonáz aktivitására coronaria betegségben szenvedő hyperlipidaemiás egyénekben. *Magyar Belorvosi Archívum* 2000; 53:227-232

13. Paragh Gy, **Harangi M**: A HDL szerepe a kardiovaszkuláris események megelőzésében. *Orvosi Hetilap* 2001; 142:121-126

14. Paragh Gy, **Harangi M**: A lipidek szerepe az atherogenesisben a primer, szekunder és regressziós tanulmányok alapján. *Családorvosi Fórum* 2001; 2:43-51

15. Paragh Gy, **Harangi M,** Balogh Z: Diabetes mellitus és paraoxonáz. *Diabetologia Hungarica* 2001; 9:221-228

16. Paragh Gy, **Harangi M,** Balogh Z: Multimetabolikus syndroma és hypertonia. *Granum* 2001; 1:26-28

17. **Harangi M**, Katona E, Remenyik É, Paragh Gy: Sclerosis tuberosa. *Hypertonia és Nephrológia* 2001; 5:144-149

18. **Harangi M**, Seres I, Varga Zs, Remenyik É, Emri Gabriella, Paragh Gy: Az atorvastatin-kezelés hatása a paraoxonáz aktivitásra és az oxidatív DNS-károsodásra. *Magyar Belorvosi Archívum* 2002; 55:83-88

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Abstracts published in interational journals

1. Paragh Gy, Asztalos L, Seres I, Balogh Z,Lőcsey L, Kárpáti I, Mátyus J, Katona E, **Harangi M**, Kakuk Gy: Serum paraoxonase activity changes in uremic and kidney transplanted patients. ***Atherosclerosis*** 1999, 144(Suppl 1) 117.

2. Paragh Gy, Balogh Z, Seres I**, Harangi M**, Boda J: Gemfibrozil effect on HDL-associated serum paraxoxonase activity and lipoprotein profile in patients with hyperlipidemia. ***Atherosclerosis***, 1999, 146(Suppl 1) S28.

3. Seres I, Varga Zs, Balogh Z, **Harangi M**, Fülöp P, Kakuk Gy, Paragh Gy: Paraoxonase activity and α-tocopherol levels in hypercholesterinemic patients. ***Atherosclerosis***, 1999, 146(Suppl 1) S35.

4. Paragh Gy, Seres I, Balogh Z, **Harangi M**, Illyés L, Boda J, Varga Zs, Kovács P: Micronised fenofibrate effect on serum paraoxonase activity in patients with coronary heart disease. ***Atherosclerosis***, 2000; 151(Suppl 1) S263.

5. **Harangi M**, Remenyik É, Seres I, Varga Z, Balogh Z, Paragh G: Determination of DNA damage induced by oxidative stress in

hyperlipidemic patients using Comet assay. ***Atherosclerosis*** 2001, (Suppl 2) S120.

Oral presentations and posters presented on international and hungarian congresses

European Atherosclerosis Society, 2000 EAS Workshop, „Low HDL in Cardiovascular Diseases"
Istambul, Turkey 2000.április 7-8.
Paragh Gy, Balogh Z, Seres I, Harangi M, Boda J: Gemfibrozil effect on HDL-associated serum paraxoxonase activity and lipoprotein profile in patients with hyperlipidemia

Debreceni Nephrológiai Napok
1999.05., Debrecen
A HDL-hez kötött paraoxonáz szerepe vesebetegségekben

DE OEC ÁOK Ph.D. és TDK Tudományos diáktalálkozója 1999/2000.
2000.04.3-6., Debrecen
Harangi M: Gemfibrozil hatása a HDL-hez kötött paraoxonáz aktivitásra és a lipoprotein szintek alakulására

Magyar Atherosclerosis Társaság XIII. Kongresszusa
2000.10.12-14., Sopron
Harangi M, Remenyik É, Seres I, Varga Zs, Balogh Z, Paragh Gy:
DNS károsodás meghatározása oxidatív stressz hatására hyperlipidaemiás betegekben Comet-assay-vel

A MNT Gyermeknephrológiai Szekció Tudományos ülése
2000.11.10., Budapest, SOTE I.sz. Gyermekklinika
Paragh Gy, Harangi M: A leptin klinikai jelentősége

DE OEC ÁOK Ph.D. és TDK Tudományos diáktalálkozója 2000/2001.
2000.02.19-22., Debrecen
Harangi M: Oxidatív stressz hatására kialakuló DNS károsodás meghatározása Comet-assay-vel hyperlipidaemiás betegekben

A Magyar Laboratóriumi Diagnosztikai Társaság 51. Nagygyűlése 2002.
2002.08.28-31., Gyula
Paragh Gy, Harangi M: Időskori lipidabnormalitások

A Magyar Belgyógyász Társaság Északkelet-Magyarországi Szakcsoportjának Tudományos Ülése
2002.10.3-5., Eger
Harangi M, Seres I, Varga Zs, Emri G, Remenyik É, Paragh Gy: Az atorvastatin kezelés hatása az oxidatív DNS károsodásra és a HDL-hez kötött paraoxonáz aktivitásra

Magyar Belgyógyász Társaság 38. Nagygyűlése
2000.11.16-18., Budapest
Harangi M, Remenyik É, Seres I, Varga Zs, Balogh Z, Paragh Gy: Oxidatív stressz hatására kialakuló DNS károsodás meghatározása Comet-assay-vel hyperlipidaemiás betegekben (poszter)

72nd European Atherosclerosis Society Congress
2001.05.20-23., Glasgow, UK
Harangi M, Seres I, Varga Z, Emri G, Remenyik E, Paragh G: Determination of DNA damage induced by oxidative stress in hyperlipidemic patients using comet assay (Poster)