

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

**Statin-paraoxonase interactions during statin
treatment**

by Hossein Z. Mirdamadi, M.D.



UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF HEALTH SCIENCES
DEBRECEN, 2009.

THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

**Statin-paraoxonase interactions during statin
treatment**

by **Hossein Z. Mirdamadi, M.D.**

**Supervisors: György Paragh, M.D., D.Sc.
Ildikó Seres, Ph.D.**



UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF HEALTH SCIENCES
DEBRECEN, 2009.

Advisors:

György Paragh, M.D., D.Sc.

Ildikó Seres, Ph.D.

Head of the Examination Committee:

Róza Ádány, M.D.,D.Sc

Members of the Examination Committee:

István Ilyés, M.D. Ph.D.

Gyula Pados, M.D., Ph.D.

The Examination takes place at:

The Library of the 1st Department of Medicine, Institute of Internal Medicine,
Medical and Health Science Center, University of Debrecen

at 12.00 on July 9, 2009

The Committee of the Public Defense:**Head of the Defense Committee:**

Róza Ádány, M.D.,D.Sc.

Reviewers:

Anna Oláh, CSc.,Ph.D.

László Márk, M.D., Ph.D.

Members of the Defense Committee:

István Ilyés, M.D., Ph.D.,

Gyula Pados, M.D., Ph.D.

The Ph.D. Defense takes place at

The Lecture Hall of 1st Department of Medicine Institute of Internal
Medicine, Medical and Health Science Center, University of Debrecen

at 14.00 on July 9, 2009

INTRODUCTION

High-density lipoprotein (HDL) cholesterol has been demonstrated to be a strong, independent, and inverse predictor of coronary heart disease (CHD) risk and therefore has emerged as a potential therapeutic target.

HDL facilitates the process known as reverse cholesterol transport, in which cholesterol from the peripheral tissues binds to the HDL and returns to the liver in order to get excreted into the bile. The first step of HDL formation is the secretion of a HDL precursor, namely the nascent HDL. Discoidal HDL is a small particle consisting apolipoprotein (apo)A1, A2 and possibly apoE, that are embedded in a lipid monolayer constituted of phospholipids and free cholesterol. Nascent HDL is a good acceptor of unesterified cholesterol. The transformation of unesterified cholesterol to cholesterol ester is catalyzed by the lecithin-cholesterol acyltransferase enzyme (LCAT). This process leads to the formation of a lipid core, transforming the nascent HDL to a small spherical particle called HDL3, which continues to acquire more unesterified cholesterol. Further enlargement of the lipoprotein particle generates a particle called HDL2a. Cholesterol ester transfer protein (CETP) is assumed to mediate the heteroexchange of triglycerides and cholesterol esters primarily between HDL2a and the apoB-containing lipoproteins. This neutral lipid exchange results in the formation of HDL2b, which subsequently becomes enriched in triglyceride and poor in cholesterol ester. HDL2b is then transformed back into HDL3 by the hepatic lipase that hydrolyses triglycerides and phospholipids.

Human paraoxonase-1 (PON1) is a calcium-dependent esterase associated with apoA1 and apoJ-containing HDL particles. PON1 hydrolyses mainly organophosphates and arylesters. Previous studies indicated that PON1 could prevent low-density lipoprotein (LDL) oxidation by hydrolyzing lipid peroxides, and therefore might protect against atherosclerosis. PON1 activity variations have been related mainly to some common polymorphisms in the coding and promoter regions. The coding region contains two common polymorphisms, a leucine (L) to methionine (M) substitution at codon 55 (L55M) and a glutamine (Q) to arginine (R) substitution at codon 192 (Q192R). The latter has the most significant impact on the enzyme activity. The efficacy of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also named as statins in decreasing serum cholesterol levels is well documented. In addition to their lipid lowering effect, statins and their metabolites may have an antioxidant effect. Several clinical studies proved the beneficial effect of statins on the PON1 activity in hyperlipidemic patients, although the results of these studies are conflicting. The effect of statins on the paraoxonase activity seems to be independent of HDL-cholesterol (HDL-C) elevation.

Only a few studies focused on the possible modulatory role of PON1 genotypes in statin treatment, however, the role of PON1 phenotypes has not been investigated yet. Some of our unpublished previous observations suggested that PON1 phenotype estimating the PON1-192 genotype might modulate the effect of statins on PON1 activity.

Recently, increased attention has been paid to the concentration and distribution of LDL and HDL subfractions, since they may be more sensitive indicators of CHD risk than total LDL-cholesterol or HDL-cholesterol levels.

It has been shown that apoA1 containing HDL particles are mainly found within the HDL2 subclasses, while apoA1 and A2 containing particles dominate the HDL3 fraction. Recently, Moren et al. have reported that apoA1/A2 containing HDL particles have a greater capacity to stabilize secreted PON1 and exhibit greater resistance to inactivation arising from oxidation *in vitro* compared to HDL particles containing only apoA1.

Previous studies have investigated the effect of statin treatment on HDL and/or LDL subfractions in heterogeneous patient populations, but the results are contradictory. In a previous study, we found that atorvastatin treatment significantly increased PON1 and LCAT activities in hyperlipidemic patients, while CETP activity was significantly decreased. Correlations between the PON1, CETP and LCAT activity and HDL subclasses after atorvastatin treatment have not yet been investigated.

OBJECTIVES

Our goal was to answer these previously uninvestigated, but clinically still relevant questions:

- How statins influence PON1 activity in humans and what is the putative mechanism of the effect of statins on the PON1 activity?
- How PON1 phenotype might influence the effect of different statins (simvastatin, atorvastatin and extended-release fluvastatin) on the serum lipid parameters, especially on the HDL-C levels?
- Does the PON1 phenotype have an impact on the paraoxonase-activating and lipid-lowering effect of different types of statins?

- Is any clinically important difference between the effect of the three statins on PON1 activities due to their structural and pharmacokinetic differences?
- How can the atorvastatin treatment influence the LDL particle size and HDL subfraction profile?
- What is the role of the enzymes involving HDL remodelling (LCAT, CETP) in the PON1 activity changes?
- Is any correlation between the changes of HDL composition pattern and the activity of the antioxidant PON1, LCAT and CETP?

METHODS

Study population

The study was carried out in accordance with the Helsinki Declaration (2000) and approved by the Ethics Committee of the University of Debrecen Medical and Health Science Center. Subjects participated in the study after their written informed consent was obtained, explaining the nature and the purpose of the study. Subjects between 21 and 70 years of age, nonsmokers, with previously untreated type II/b hyperlipidemia were enrolled in this study. Exclusion criteria were: the presence of a significant endocrine disorder, active liver disease or hepatic dysfunction defined as alanine aminotransferase or aspartate aminotransferase >1.5 times the upper limit of normal range, renal disease, malignancy, diabetes mellitus, coronary artery disease, blood pressure >160/100 mm Hg, cerebral vascular disease, triglyceride level >4.5 mmol/l, alcoholism, smoking, drug dependence, infectious disease, significant inflammation, pregnancy or lactation,

anticoagulant, lipid lowering, glucocorticoid, oral contraceptive or sex hormone replacement medication.

After six weeks on the National Cholesterol Education Program (NCEP) step 1 diet, patients were randomly divided into three groups: the first group received 10 mg atorvastatin daily (ATO), the second group got 10 or 20 mg simvastatin daily (SIM), and the third group was treated by 80 mg extended-release fluvastatin daily (FLU) for three months. Atorvastatin and simvastatin were given at commonly used low doses. In the case of fluvastatin, an extended release form was used, which was available only in 80 mg dose. These statin doses are able to reduce LDL-cholesterol levels similarly, approximately by 35-39%. Based on these data these were equivalent doses.

Blood sampling

After 12 hours of fasting, 10 ml venous blood sample was taken between 7.30 and 8.00 in the morning. The lipid parameters were determined from fresh serum. The sera for paraoxonase and arylesterase activity measurements were kept at -70°C before analysis. LCAT, CETP and HDL subfraction analysis were carried out from frozen serum samples.

Lipid measurements

Serum cholesterol and triglyceride levels were measured by using enzymatic, colorimetric tests (GPO-PAP, Modular P-800 Analyzer, Roche/Hitachi), while HDL-C and LDL-C were assessed by homogenous, enzymatic, colorimetric assays (Roche HDL-C plus 3rd generation, Roche LDL-C plus 2nd generation). Apolipoprotein examination was performed by immunoturbidimetric assay Tina-Quant APO A (Version 2, Roche), Tina-Quant APO B (Version 2, Roche).

Analysis of paraoxonase activity

Briefly, we set up the following enzymatic reaction using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate, Sigma) as substrate and the generation of 4-nitrophenol was followed spectrophotometrically: 50 μ l serum was dissolved in 1 ml Tris/HCl buffer (100 mmol/l, pH=8.0) containing 2 mmol/l CaCl_2 and 5.5 mmol/l paraoxon. We measured the absorbance at 412 nm (25 $^\circ\text{C}$), using Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient $17100 \text{ M}^{-1}\text{cm}^{-1}$. One unit of PON1 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. Briefly, the assay contained 1 mM phenylacetate (Sigma) in 20 mM Tris-HCl, pH=8.0. The reaction was started by the addition of the serum, and then the absorbance was monitored at 270 nm. Blanks were included to correct for the spontaneous hydrolysis of phenylacetate. Enzyme activity was calculated using the molar extinction coefficient $1310 \text{ M}^{-1}\text{cm}^{-1}$. Arylesterase activity is expressed in U/ml; 1 U is defined as $1 \mu\text{mol}$ phenylacetate hydrolyzed per minute.

Paraoxonase phenotyping

The phenotype distribution of PON1 was determined by the dual substrate method. The genetic polymorphism at codon 192 Q->R is responsible for the presence of two isotypes: A (low activity) and B (high activity). The ratio of the hydrolysis of paraoxon in the presence of 1 M NaCl (salt-stimulated PON1 activity) to the hydrolysis of phenylacetate was used to assign individuals to one of the three possible (AA, AB, BB) phenotypes. Cut-off

values between phenotypes were as follows: type AA, ratio < 3.0; type AB, ratio 3.0-7.0; and type BB, ratio > 7.0. AA represents low; AB intermediate; and BB high enzyme activity.

Measuring the LCAT activity

We determined the LCAT activity by a commercially available kit (Roar Biomedical Inc.). Plasma was incubated with a fluorescent substrate, and the fluorescence intensity of the intact substrate was measured at 470 nm by a Hitachi F-4500 Fluorescence Spectrophotometer. A monomer was formed as the substrate was hydrolyzed by LCAT. This was detectable at 390 nm. The LCAT activity was calculated by measuring the change in 470/390 nm emission intensity. The intra-assay and inter-assay coefficients of variation were <5%.

Measuring the CETP activity

CETP activity was measured by a CETP Activity kit (Roar Biomedical Inc.). The kit contains donor (synthetic phospholipids and cholesteryl ester) and acceptor (VLDL) particles. A 6 μ L plasma and a 20 μ L donor/acceptor mixture were added to a 1 mL buffer (150 mM NaCl, 10 mM Tris and 2 mM EDTA, pH 7.4). The cholesterol ester transfer from the donor to the acceptor molecule mediated by CETP was quantified by measuring the increased fluorescence intensity using a Hitachi F-4500 Fluorescence Spectrophotometer. The excitation was conducted at 465 nm and the emission at 535 nm. The intra-assay and inter-assay coefficients of variation were <3%.

Quantification of the oxidized LDL (oxLDL)

The oxLDL was quantified by sandwich ELISA. We measured antibodies against oxLDL with the Wak-Chem-Med (Berlin, Germany) kit. In this test, plasma oxLDL reacted with mouse monoclonal antibody. After washing, the antibody conjugated with peroxidase against apolipoprotein B recognized the oxLDL fixed to the solid fraction. The fixed conjugation was detected by the tetramethylbenzidine reaction and measured spectrophotometrically. Intra- and inter-assay coefficients of variance (CV) were 5.4% and 8.3%, respectively.

Determination of the ratios of the HDL subclasses

Briefly, lipoproteins in plasma were separated based on their size, using nondenaturing 3% to 30% polyacrylamide gradient gels. Gels were stained with bromophenol-blue (Sigma, St Louis, Mo) and scanned with a laser densitometer (LKB-Ultrosan LX, LKB Instruments Inc, Paramus, NJ). Molecular weights were determined using thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin as standards (High Molecular Weight Electrophoresis calibration Kit, Pharmacia). HDL absorbance profiles were analyzed by fitting curves representing the generally accepted HDL subclasses, as suggested previously. Curves were fit to HDL3 (<8.8 nm), HDL2a (8.8 to 9.8 nm), and HDL2b (\geq 9.8 nm).

Determination of the LDL diameter

The LDL peak diameter was determined from whole plasma by lipid-stained, non-denaturing gel electrophoresis using 3% to 16% polyacrylamide gradient gels. Gels were stained for lipid with Sudan black (Sigma, St Louis, Mo) and scanned with a laser densitometer (LKB-Ultrosan LX, LKB Instruments Inc, Paramus, NJ). Size of LDL was estimated from calibration curves that were constructed using latex beads (Bangs Laboratories, Fishers, IN, USA) and

high molecular-weight standards (Pharmacia Biotech, St.Albans, England). The between-run coefficient of variation was 0.95% for the internal control sample included in every gel, and 0.54% for 20 blinded duplicate samples interspersed throughout the samples in the study.

Statistical analysis

SAS for Windows 6.12 (SAS Institute Inc.) computer program was used for the statistical analysis. Normality of distribution of data was tested by Kolmogorov-Smirnov test. Non-normally distributed parameters were transformed logarithmically to correct their skewed distributions. Data were expressed as means \pm S.D. in case of normal distribution, and medians and 95% confidence interval for median, in case of non-normal distribution. To evaluate the effect of treatment, we analyzed changes with repeated measures ANOVA and with post hoc Newman–Keuls critical ranges test. Correlations between continuous variables were assessed by calculation of linear regression using Pearson's test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Paraoxonase phenotype modifies the effect of statins

Lipid parameters

The initial cholesterol and LDL-C levels were significantly higher in the FLU group compared to the ATO group (7.61 ± 0.98 mmol/l vs. 6.98 ± 0.90 mmol/l, 5.17 ± 0.78 mmol/l vs. 4.70 ± 0.81 mmol/l, respectively, $p < 0.05$). We could not find significant differences between the FLU, ATO, and SIM groups in the other lipid parameters before treatment.

The statin treatment significantly lowered the total cholesterol and the apoB levels in the whole study population (-24% and -22%, $p<0.05$) and in the three statin groups (ATO:-39% and -42%, SIM:-5% and -15%, FLU:-29% and -27%, $p<0.05$). Significantly decreased triglyceride levels were found in the whole study population (-16%) and in the ATO group (-29%, $p<0.05$). The LDL-C level significantly decreased in the whole study population (-31%) and in the ATO (-43%) and FLU (-43%, $p<0.05$) groups. The HDL-C levels did not change significantly. A significant increase in the apoA1 levels was found in the SIM group (+9.5%, $p<0.05$).

Paraoxonase and arylesterase activities

The paraoxonase activity significantly increased after the statin treatment in the whole study population (+14%) and in all three statin groups (ATO:+13%, SIM:+11%, FLU:+15%, $p<0.05$). There was a significant increase in the arylesterase activity in the whole study population (+7%) and in the SIM (+7%) and FLU (+9%, $p<0.05$) groups.

Paraoxonase phenotype distribution

The allelic frequencies were in accordance with the results of our previous studies and the literature, following the Hardy-Weinberg equilibrium ($p<0.05$). PON1 phenotype distribution of the three groups did not differ significantly ($p=0.1005$).

Responses of patients to statin treatment according their phenotypes

Because of the low number of patients with BB phenotypes the study population was divided into AA and AB+BB phenotype groups.

Changes in lipid levels in the AA and in the AB+BB phenotype groups

There was no significant difference between the PON phenotypes in initial lipid levels. The statin treatment decreased more effectively the triglyceride

levels in the AB+BB group compared to the AA group in the whole study population (-24.11% vs. -3.13%, $p<0.05$) and in the SIM group (-17.80% vs. -3.63%, $p<0.05$). Although similar tendencies were found in the ATO and FLU groups, the differences between the different phenotype groups were not significant. The change in HDL-C level was consequently higher in the AB+BB phenotype group in the whole study population and in the three statin groups, but the differences between the two phenotype groups were not significant. The atorvastatin treatment was significantly more effective on apoB levels in the AB+BB phenotype compared to the AA phenotype group (-50.37% vs. -34.83%, $p<0.05$).

Changes in paraoxonase and arylesterase activities in the AA and in the AB+BB phenotype groups

In patients with AB+BB phenotype the statin treatment was significantly more effective on the paraoxonase activity in the whole study population (+13.65%, $p<0.001$) and in all the three statin groups (ATO:+14.7%, $p<0.001$; SIM: +12.2%, $p<0.001$; FLU: +14.6%, $p<0.001$). In the AA phenotype group there was no significant change in the paraoxonase activity in the whole study population (+8.8%) and in the ATO (+7.1%) and SIM (+8.4%) groups. In the AB+BB phenotype group, a significant increase in the PON activity was found in all groups. In the FLU group, we could find a significant increase in both phenotype groups (+10.5%, $p<0.05$), however, in the AB+BB group the change was more prominent (+14.7%, $p<0.001$).

Similar results were found when we examined the changes in arylesterase activity with the exception of atorvastatin.

Effect of the atorvastatin on the distribution of HDL subfractions and human paraoxonase activity

Changes in the distribution of HDL subfractions

The atorvastatin treatment significantly increased the ratio of HDL3 by 8.13%, while the ratios of HDL2a and 2b were significantly decreased by 1.57% and 6.55%, respectively. The HDL2/HDL3 ratio was significantly decreased by 28.72% ($p < 0.05$).

Changes in the size of LDL

The mean LDL size significantly increased after the atorvastatin treatment, by 3.29%. We could not find significant correlations between the size of LDL and the activities of PON1, LCAT and CETP when we pooled the results of before and after atorvastatin treatment. The changes in LDL diameter did not correlate significantly with the changes in the activities of these enzymes.

Correlation between HDL subclasses and CETP activity

A significant positive correlation ($r = 0.32$, $p < 0.01$) was found between the CETP activity and the HDL2b ratio when we pooled the results of before and after atorvastatin treatment, while we found a significant negative correlation ($r = -0.28$, $p < 0.05$) between CETP activity and HDL3 ratio. Similarly, there was a negative correlation between CETP activity and HDL2a ratio, the association being on the margin of statistical significance ($r = -0.25$, $p = 0.05$). There was a significant negative correlation between the change in CETP activity and the change in HDL2a ratio ($r = -0.39$, $p < 0.05$).

Correlation between HDL subclasses and LCAT activity

We did not find significant correlations between LCAT activity and HDL subclasses either before or after the atorvastatin treatment, or in the pooled data. However, there was a significant positive correlation between the change of LCAT activity and the change in HDL2a ratio ($r=0.41$, $p<0.05$). There was no significant correlation between the LCAT/CETP ratio and HDL subclasses.

Correlation between HDL subclasses and PON1 activity

We did not find significant correlations between PON1 activity and HDL subclasses either before or after the atorvastatin treatment, or in the pooled data.

HDL subclasses in subjects with different PON1 phenotypes

Since only a few subjects belonged to the BB phenotype, only phenotypes AA and AB+BB could be compared. No significant associations were found between PON1 activity and HDL2b, HDL2a and HDL3 in subjects with AB+BB phenotypes. Similarly, we could not find a significant correlation between PON1 activity and HDL2b, HDL2a and HDL3 in subjects with AA phenotypes.

DISCUSSION

Paraoxonase phenotype modifies the effect of statins

It has been reported recently that patients who were homozygous for the -107C allele showed a significant increase in PON1 activity and protein concentration after 2 months of simvastatin treatment compared to -107TT homozygotes. This study indicated that only a portion of the population could benefit from this pleiotropic effect of simvastatin. On the other hand, other

PON1 polymorphisms may also exert a beneficial effect on the response to statin treatment, although the supporting findings are scanty.

A previous study with fluvastatin demonstrated that the increase in paraoxonase activity was larger in the R allele carriers compared to the QQ homozygotes, although the difference was not significant. Others reported similar results with simvastatin treatment. Our results support the initial hypothesis that the PON1 phenotype has a modulatory effect on paraoxonase activity: we found that the change in paraoxonase activity after statin treatment was significantly higher in the AB+BB phenotype group. The difference between the two phenotype groups was significant in each statin groups, nevertheless PON1 activity represents a great inter-individual variability. The effect of statins were similar on arylesterase activities, however, the difference between the two phenotype groups was not significant in the atorvastatin-treated group. Each statin has different pharmacokinetic properties, bioavailability and hydrophilicity. These individual properties, like affinity to cytochrome P450 isoenzymes may influence the effect of statins on PON1 activity. Diverse study populations, different types of hyperlipoproteinaemia and various types of statin dosages could contribute to the discrepancies found in the literature.

A previous study reported that PON1 genotype could modify the effect of statins on the lipid parameters. Pravastatin increased the apoA1 concentration and tended to increase the HDL-C concentration in the R allele carriers but not in QQ homozygotes. Although the change in total cholesterol and HDL-c levels was higher in the AB+BB phenotype group, the study was not powered to detect this difference at a statistically significant level. On the other hand, others found that the Q192R variants of PON1 are not associated with plasma

lipid levels and response to treatment with 40mg/d fluvastatin. The results of another study showed that in R allele carriers the decrease in triglyceride levels was larger compared to the QQ homozygotes, although the difference was not significant. We also found an association between PON1 phenotype and response of triglyceride levels to simvastatin treatment. Although the exact mechanism is not clarified, in a previous study we found that in diabetic patients there was a significant positive correlation between paraoxonase and lipoprotein lipase (LPL) activities. Moreover, the PON1 paraoxonase activity of the AA phenotype group did not correlate with LPL activity, while in the AB+BB phenotype group there was a significantly positive correlation between paraoxonase and LPL activity. It is also known that the activity of LPL inversely correlated with triglyceride concentration. Presumably, the activity of LPL may influence the HDL remodeling, and consequently, modifies the activity of the HDL-associated PON1. Based on these data, higher paraoxonase activity (AB+BB phenotypes) results in increased LPL activity, and consequently, reduced triglyceride level.

There were no significant differences between phenotypes in initial lipid levels. We also found that statins were more effective on HDL-C in the AB+BB phenotype group, which represent the R allele carriers, although the difference between the two phenotype groups was not significant. A previous study showed that simvastatin treatment tended to be more effective on total cholesterol, LDL-c, triglyceride and apoB levels in the R carriers, but the differences were not significant. We also found that changes in total cholesterol levels tended to be higher in the AB+BB phenotype group.

Effect of the atorvastatin on the HDL and LDL subfractions

Data regarding the predictive ability of HDL subfractions for CHD risk are not conclusive, although the concept is that larger HDL particles may be associated with greater protection against atherosclerosis has been widely accepted in the last decades.

The metabolic and clinical significance of HDL subclasses is still not fully understood. In the past, it was widely accepted that the HDL2 subfraction was responsible for the beneficial health effects of high HDL-C . Some other studies, however, have reported that HDL3, and not HDL2, is negatively associated with the progression of atherosclerosis. Recently, the relationship of HDL subclasses with the Framingham risk score in middle-aged asymptomatic individuals has been investigated. The authors could not prove any significant association between the calculated risk and HDL subfractions, although they found a notable incidence of small size HDL phenotypes (which included HDL3 particles) in subjects categorized as low-risk. Therefore, further studies will be necessary to evaluate the benefits of the increased HDL3 ratio after atorvastatin treatment found in our study. Similarly, in our results, increased HDL3 levels were found in hyperlipidemic patients after simvastatin and fluvastatin treatment using ultracentrifugal methods.

CETP is a glycoprotein that is physically associated with HDL particles. It facilitates the transport of cholesterol ester from HDL to apolipoprotein B-containing lipoproteins. Simultaneously, an energy-neutral transfer of triglycerides also occurs in the opposite direction. Additionally, CETP is also a part of a remodeling cascade, required for the effective interaction of HDL with the scavenger receptor B1 (SR-B1) and selective uptake of cholesteryl

esters by the liver. Furthermore, CETP may be involved in intracellular cholesterol metabolism. It was demonstrated that CETP inhibitors increased apoA-I synthesis. Previously, decreased CETP activity was found after atorvastatin treatment in different patient populations. In the present study, we also found similar results.

A previous study found that CETP could promote the formation of HDL3 particles at the expense of HDL2a. A negative correlation has been reported between CETP activity and HDL2a levels, and a positive correlation between CETP and HDL3b levels. Contrarily, others found a negative correlation between CETP activity and HDL3b. We also found significant negative correlations between CETP activity and HDL3, and between CETP activity and HDL2a, while there was a significant positive correlation between CETP activity and HDL2b.

Previously, in healthy individuals LCAT activity showed a significant negative correlation with large HDL2a, and in patients with higher LCAT/CETP ratio had a greater relative proportion of small HDL3. In this study, we could not find a correlation between LCAT activity and the ratio of HDL subfractions. Moreover, we found a significant positive correlation between the change of LCAT activity and the change of HDL2a ratio after atorvastatin treatment.

Only a few studies investigated the effect of statins on HDL subclasses, and the results are not concordant. Unfortunately, different statins have been administered in different doses for various patient populations. In addition, different assays have been used. Therefore the comparison of these results is strongly problematic. Yet, most of these studies demonstrated that statins increase the levels of the smaller HDL subfractions, but not all of them.

Surprisingly, only a few studies investigated the effect of atorvastatin on LDL and HDL subclasses, and the results are not in concordance. It has been found that atorvastatin treatment increased the ratio of large HDL2b by 25%, while the medium and small HDL3 did not change significantly, and the drug decreased each LDL subfraction in CHD patients and control subjects. It has been reported that atorvastatin did not significantly alter the overall LDL subfraction pattern in dyslipidemic patients, however, in the isolated hypercholesterolemia group, the proportion of LDL - presented as the small dense fraction - increased by 23%. In patients with atherogenic dyslipidemia, the sdLDL (small dense LDL) preponderance was not significantly decreased. Others treated patients with combined hyperlipidemia, and found that atorvastatin treatment reduced the levels of all LDL subspecies, but the reduction in dense LDL levels was the most prominent. Total HDL mass was not modified by atorvastatin; however, the relative proportion of HDL2a was significantly increased, whereas the HDL3c ratio was decreased. The HDL3a ratio was not significantly increased. Another group investigated the effect of a maximal dose of atorvastatin on LDL subfractions in hyperlipidemic patients, and reported a significant decrease in small dense LDL levels after treatment. Others found that in CHD patients, using five different statins, atorvastatin was the most effective in modifying the HDL subfraction profile, by increasing the concentration of large, cholesterol rich, apoA1 containing HDL2b fraction and decreasing the concentration of the small, ApoA1/A2 containing HDL3 subpopulation. In patients with low initial HDL levels atorvastatin treatment alone did not significantly affect the distributions of large and small HDL subclasses. We also found that atorvastatin increased the diameter of LDL particles, which may reflect a decrease in the sdLDL

fraction. Interestingly, in our study a significant increase was found in HDL3 percentage, while the HDL2a and 2b were decreased. Probably, lower doses of atorvastatin used in our study were not able to restore the physiological proportions of the HDL subclasses.

Previous studies showed that there could be a strong variation among different statin agents regarding their effects on other lipoprotein subclasses. Statins potentially lower all LDL subclasses, thus, their net effect on LDL subclasses or size is often moderate. However, analyses of published studies suggested a very limited role of pravastatin and simvastatin in modifying LDL size and their subclasses, while fluvastatin and atorvastatin seemed to be much more effective agents. Finally, rosuvastatin seemed to be promising in altering LDL subclasses towards less atherogenic particles. Similarly to their various effect on LDL subfractions, different statins may exert different effect on HDL subclasses. Therefore, further studies will be necessary to clarify the role of different statins in modifying HDL subfractions.

Previously it has been found that PON1 levels and activities significantly modified the HDL-C increment in familial hypercholesterolaemia (FH) patients undergoing atorvastatin or simvastatin therapy. Recently, the subfraction distribution of HDL-associated PON1 enzyme activity and stability has been analyzed in hamster cell culture. PON1 was equally distributed between apoA1 and apoA2 containing HDL particles. Particles containing both apoA1 and A2 showed significantly greater resistance to PON1 inactivation arising from oxidation. ApoA1 and A2 were independent determinants of serum PON1 concentration and activity. It is also known that a higher proportion of PON1 is located in the apoJ containing HDL fraction;

although, not all of the plasma paraoxonase is associated with apoJ. It has been also reported that PON1 activity is highest in the denser HDL3 and ApoJ containing HDL particles, which are present only in the HDL3 fraction. In agreement with most of the previous studies, we found that PON1 activity significantly increased after atorvastatin treatment. Although, we did not find any correlation between the changes in PON1 activity and HDL subclasses; the significant increase in HDL3 fraction (which consists of the majority of apoJ and apoA1/A2 containing HDL particles) may have contributed to the augmented PON1 activity.

SUMMARY

Human serum paraoxonase-1 (PON1) protects lipoproteins against oxidation by hydrolyzing lipid peroxides in oxidized LDL, therefore it may protect against atherosclerosis. Changes in the ratio of HDL subfractions may alter the stability and antioxidant capacity of PON1. PON1 activity variations have been also related to some common polymorphisms in the coding and promoter regions. The PON1-192 polymorphism has the most significant impact on enzyme activity, and its prevalence can be estimated by phenotype distribution analysis.

The aim of the study was to examine the effect of atorvastatin treatment on the distribution of HDL subfractions, LDL size, cholesteryl ester transfer protein (CETP), lecithin:cholesterol acyltransferase (LCAT) and human serum paraoxonase-1 (PON1) activity, and to clarify the role of PON1 phenotypes on the effect of three different statins on paraoxonase

activity and lipid parameters in patients with type IIa and IIb hypercholesterolemia.

Three months of 20 mg/day atorvastatin treatment significantly increased the HDL3 and decreased the HDL2a and HDL2b subfractions. The mean LDL size was significantly increased. The PON1 activity was augmented by the atorvastatin treatment. The CETP activity positively correlated with the HDL2b and negatively correlated with the HDL3 and HDL2a levels. Three months of statin (10 mg/day atorvastatin, 10/20 mg/day simvastatin and 80 mg/day extended-release fluvastatin) treatment significantly increased the paraoxonase activity in every statin-treated group. In patients with AB+BB phenotype the statin treatment was significantly more effective on paraoxonase activity than in the AA group. The statin treatment more effectively decreased the triglyceride levels in the AB+BB group compared to the AA group in the whole study population and in the simvastatin-treated group. The atorvastatin treatment was significantly more effective on apoB levels in patients with AB+BB phenotype than in the AA phenotype group.

These results confirm that atorvastatin normalizes lipid levels and preferentially increases HDL. Our data are consistent with the concept that changes in CETP and LCAT activities during statin treatment may directly alter the ratio of HDL subclasses. Data on PON1 activity and HDL subfraction alterations suggest that the increase in HDL3 ratio may also be responsible for the enhanced PON1 activity after atorvastatin treatment. Our results indicate that the PON1 phenotype may be a novel predictive factor for the effectivity of statin treatment on PON1 activity and serum lipid levels;

however, different types of statins may exert different effects on these parameters.

NEW RESULTS

We reported for the first time that PON1 phenotypes have an impact on the paraoxonase-activating and lipid-lowering effect of three different types of statins:

- All the statins (atorvastatin, simvastatin and fluvastatin) included in this study were able to effectively increase the serum paraoxonase activity and decrease triglyceride levels, however this response seemed to be more significant in patients with AB+BB PON1 phenotype than in patients bearing AA PON1 phenotype.
- Furthermore, the apoB-lowering effect of atorvastatin was also found to be PON1 phenotype-dependent.

We were the first ones to find correlations between the CETP and LCAT activities and HDL subclasses after atorvastatin treatment:

- We found a significant positive correlation between the CETP activity and the HDL2b ratio, while we found a significant negative correlation between CETP activity and HDL3 ratio.
- There was a significant negative correlation between the change in CETP activity and the change in HDL2a ratio.
- We found a significant positive correlation between the change of LCAT activity and the change of HDL2a ratio after atorvastatin treatment.

PUBLICATIONS

Publications serving as the basis of the dissertation:

1. **Mirdamadi HZ**, Sztanek F, Derdak Z, Seres I, Harangi M, Paragh G. The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters. *Br J Clin Pharmacol.* 2008;66(3):366-74. (IF: 3.128)
2. Harangi M, **Mirdamadi HZ**, Seres I, Sztanek F, Molnár M, Kassai A, Derdák Z, Illyés L, Paragh G. Atorvastatin effect on the distribution of high-density lipoprotein subfractions and human paraoxonase activity. *Transl Res.* 2009;153(4):190-8. (IF:1.984)

Other publications:

1. Kassai A, Illyés L, **Mirdamadi HZ**, Seres I, Kalmár T, Audikovszky M, Paragh G. The effect of atorvastatin therapy on lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein and the antioxidant paraoxonase. *Clin Biochem.* 2007;40(1-2):1-5. (IF:2.072)
2. Kassai A, **HZ Mirdamadi**, Seres I, Kalmár T, Paragh Gy: Az atorvastatin kezelés hatása a lecitin-koleszterin-acil-transzferázra és koleszterin-észter-transzfer-proteinre. *Metabolizmus* 2006; IV. 1:7-11

Quotable abstracts linked to the topic of the dissertation:

1. Paragh G, Kassai A, Sztanek F, Illyés L, **Mirdamadi HZ**, Seres I. The effect of atorvastatin therapy on HDL subfractions, lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein and human paraoxonase-1 *Atherosclerosis Supplements* 2007; 8(1) 203

Abstracts not directly linked to the topic of the dissertation:

1. I Seres, G Paragh, B Kosztáczky, T Kalmár, **HZ Mirdamadi**, A. Kassai, G. Fóris: Disturbed CA2+ transport in neutrophils of obese patients
Atherosclerosis Supplements 2006. 7:229.
2. G. Paragh, I. Seres, M. Harangi, Z. Pocsai, **HZ Mirdamadi**, L. Asztalos, L. Locsey, L. Kardos, E. Varga, R. Adany: Discordance between phenotypic and genotypic determinations of human paraoxonase-1 in uremic and kidney transplanted patients.
Atherosclerosis Supplements 2006. 7:292.

Lectures and posters at Hungarian and international conferences:

1. I Seres, G Paragh, B Kosztáczky, T Kalmár, **HZ Mirdamadi**, A. Kassai, G. Fóris: Disturbed CA2+ transport in neutrophils of obese patients. Poster XIV International Symposium on Atherosclerosis Rome, Italy, 2006. június 18-22.
2. G. Paragh, I. Seres, M. Harangi, Z. Pocsai, **HZ Mirdamadi**, L. Asztalos, L. Locsey, L. Kardos, E. Varga, R. Adany: Discordance between phenotypic and genotypic determinations of human paraoxonase-1 in uremic and kidney transplanted patients. Poster XIV International Symposium on Atherosclerosis Rome, Italy, 2006. június 18-22.
3. Köbling T., **HZ Mirdamadi**, Seres I., Paragh Gy.: Lipidcsökkentők hatása a paraoxonáz aktivitásra. Magyar Atherosclerosis Társaság XVI. Kongresszusa, Sopron, 2006. okt.12-14
4. Seres I., Paragh Gy., Kosztáczky B., **HZ Mirdamadi**, Kassai A., Fóris G.: A CA2+ transzport zavara elhízott betegek neutrofiljeiben. Magyar Atherosclerosis Társaság XVI. Kongresszusa, Sopron, 2006. okt.12-14
5. Sztanek Ferenc, **HZ Mirdamadi**, Kassai A., Seres I., Köbling T., Balogh Z., Harangi M., Paragh Gy.: Az atorvastatin hatása a HDL

összetételére és a paraoxonáz aktivitásra Magyar Atherosclerosis Társaság XVI. Kongresszusa, Sopron, 2006. okt.12-14

6. Paragh G, Kassai A, Sztanek F, Illyés L, **Mirdamadi HZ**, Seres I. The effect of atorvastatin therapy on HDL subfractions, lecithin:cholesterol acyltransferase, cholesteryl ester transfer protein and human paraoxonase-1 76thEuropean Atherosclerosis Society Congress, Helsinki, 2007
7. György Paragh, **HZ Mirdamadi**, Mariann Harangi, Zoltán Balogh, Ildikó Seres: Effect of lipid lowering drugs on paraoxonase activity 2nd International Conference on Paraoxonases, Hajdúszoboszló, 2006. szept.7-10
8. György Paragh, Mariann Harangi, **HZ Mirdamadi**, Ferenc Sztanek, Zoltan Derdak, Ildiko Seres: The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters 3rd International Conference on Paraoxonases University of California Los Angeles, USA, 2008. szept.7-10
9. Seres Ildikó, Sztanek F, **HZ Mirdamadi**, Harangi M., Derdák Z., Paragh Gy.: A humán paraoxonáz fenotípusa befolyásolja a statinok hatását a lipid paraméterekre Magyar Atherosclerosis Társaság XVII. Kongresszusa, Sopron, 2008.okt.9-11
10. Harangi Mariann, Seres I., Illyés L., **H.Z. Mirdamadi**, Molnár M., Kassai A., Derdák Z., Paragh Gy.: Az atorvastatin kezelés hatása a HDL szubfrakciók megoszlására és a humán paraoxonáz-1 aktivitásra Magyar Atherosclerosis Társaság XVII. Kongresszusa, Sopron, 2008.okt.9-11

Cumulative impact factor of *in extenso* publications: 7.184