The human paraoxonase-1 phenotype modifies the effect of statins on paraoxonase activity and lipid parameters

Hossein Z. Mirdamadi, Ferenc Sztanek, Zoltan Derdak,¹ Ildiko Seres, Mariann Harangi & György Paragh

First Department of Medicine, University of Debrecen Medical and Health Science Centre, Debrecen, Hungary and ¹Division of Gastroenterology and Liver Research Center, Brown Medical School and Rhode Island Hospital, Providence, RI, USA

Correspondence

Dr Ildiko Seres, PhD, First Department of Medicine, University of Debrecen, Medical and Health Science Centre, Nagyerdei krt. 98, H-4012 Debrecen, Hungary.
Tel/Fax: + 36 5244 2101
E-mail: seres@internal.med.unideb.hu

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- It has been suggested that the human paraoxonase-1 (PON1) genotype is an important determinant of the therapeutic response given to statin treatment.
- It is also known that the PON1 activity status is a better predictor of coronary heart disease risk than any of the known PON1 genotypes.
- Our goal was to answer this previously uninvestigated, still clinically relevant question: does the PON1 phenotype have an impact on the paraoxonase-activating and lipid-lowering effect of different types of statins.

WHAT THIS STUDY ADDS

- All the statins (atorvastatin, simvastatin and fluvastatin) included in this study were able to increase serum paraoxonase activity and decrease triglyceride levels effectively; however, this response seemed to be more significant in patients with AB+BB PON1 phenotype than in those bearing AA PON1 phenotype.
- Furthermore, the apolipoprotein B-lowering effect of atorvastatin was also found to be PON1 phenotype-dependent.
- Our results indicate that the PON1 phenotype may be a novel predictive factor for the effectiveness of statin treatment on PON1 activity and serum lipid levels; however, different types of statins may exert different effects on these parameters.

Aims

Human serum paraoxonase-1 (PON1) protects lipoproteins against oxidation by hydrolysing lipid peroxides in oxidized low-density lipoprotein, therefore it may protect against atherosclerosis. One of the two common PON1 gene polymorphisms within the PON1 gene is the Q192R, whose prevalence can be estimated by phenotype distribution analysis. The goal of this study was to clarify the role of PON1 phenotypes on the effect of three different statins on paraoxonase activity and lipid parameters.

Methods

One hundred and sixty-four patients with type IIb hypercholesterolaemia were involved in the study. We examined the effect of 10 mg day⁻¹ atorvastatin, 10/20 mg day⁻¹ simvastatin and 80 mg day⁻¹ extended-release fluvastatin treatment on lipid levels and paraoxonase activity in patients with different PON1 phenotypes. The phenotype distribution of PON1 was determined by the dual substrate method.

Results

Three months of statin treatment significantly increased paraoxonase activity in every statin-treated group. In patients with AB+BB phenotype, statin treatment was significantly more effective on paraoxonase activity than in the AA group. Statin treatment more effectively decreased triglyceride levels in the AB+BB group compared with the AA group in the whole study population and in the simvastatin-treated group. Atorvastatin treatment was significantly more effective on apolipoprotein B levels in patients with AB+BB phenotype than in the AA phenotype group.

Conclusions

The PON1 phenotype may be a novel predictive factor for the effectiveness of statin treatment on PON1 activity and serum lipid levels; however, different types of statins may exert different effects on these parameters.

Introduction

The efficacy of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor statins in lowering serum cholesterol levels is well documented [1, 2]. In addition to their lipid-lowering effect, statins and their metabolites may have antioxidant potential [3]. Several clinical studies proved the beneficial effect of statins on high-density lipoprotein (HDL)-associated human paraoxonase-1 (PON1) activity in hyperlipidaemic patients, although the results of these studies are controversial [4, 5]. Statin effect on paraoxonase activity seems to be independent of HDL-cholesterol (HDL-C) elevation [5].

PON1 is a calcium-dependent esterase associated with apolipoprotein A-I and J-containing HDL particles that hydrolyses organophosphates and arylesters [6, 7]. Previous studies have indicated that PON1 can prevent low-density lipoprotein (LDL) oxidation by hydrolysing lipid peroxides in the lipoprotein, therefore it may protect against the development of atherosclerosis [8]. PON1 has recently been shown to catalyse the hydrolysis of a variety of aromatic and aliphatic lactones, and it has been proposed that certain lactones and hydroxy acids might represent the endogenous substrates of PON1 [9].

PON1 activity variations have been mainly related to some common polymorphisms in the coding and promoter regions [10, 11]. Two common coding region polymorphisms are the L55M and the Q192R [12]. Clinically, the most relevant promoter polymorphism is the T-107C [13]. The PON1-192 polymorphism has the most significant impact on enzyme activity [12]. The Q192R polymorphism involves a mutation from glutamine (Q, wild-type) to arginine (R, variant) at amino acid position 192 of the protein sequence. Functional PON1 activity (reflects its antioxidant capacity) can be measured by its ability to hydrolyse exogenous substrates such as paraoxon and phenylacetate, reflecting so-called paraoxonase and arylesterase activity, respectively. Functional differences have been shown in hydrolysis rates of the Q192 vs. R192 alloenzymes using paraoxon as substrate, whereas no difference has been observed regarding their ability to hydrolyse phenylacetate. Therefore, PON1-192 genotypes are distinguishable by the ratio of paraoxonase/arylesterase activity. Using this dual-substrate method to assess phenotype distribution, the occurrence of PON1-192 genotype can be calculated [14].

There are incomplete data on the response of lipid parameters and PON1 activity to statin treatment in patients with different genotypes. Only a few studies have focused on the possible modulatory role of PON1 genotypes in statin treatment. Tomás *et al.* have investigated the effect of 4 months' simvastatin therapy on serum lipid parameters and PON1 activity in familial hypercholesterolaemic patients; however, they could find no differences in the therapeutic response of PON1 activity between various genotype groups [15]. Malin *et al.* found that PON1-192 and -55 genotypes were significant determinants of HDL-C con-

centration change in hypercholesterolaemic male subjects who underwent 6 months' pravastatin treatment [16]. Christidis *et al.* have evaluated the data of >200 hypercholesterolaemic patients treated with low-dose fluvastatin for 6 months. They concluded that PON1-192 and -55 genotypes did not influence the effect of fluvastatin on lipid parameters and PON1 activity, although in the R allele carriers the fluvastatin treatment lowered triglyceride levels more effectively, but the difference was not significant [17].

Recently, Deakin *et al.* have found pharmacogenetic correlation between PON1 gene promoter polymorphism C-107T and simvastatin in HepG2 cells. Hypercholesterolaemic patients homozygous for the C allele showed a significant increase in serum PON1 activity and the mass of paraoxonase during treatment with simvastatin, whereas patients homozygous for the T allele showed no such increase. They concluded that patients with the C allele are likely to derive greater benefit from statin therapy [18]. Some of our unpublished previous observations also suggested that PON1 phenotype estimating the PON1-192 genotype might modulate the effect of statins on PON1 activity.

Our goal was to answer this clinically relevant question: does the PON1 phenotype have an impact on the paraoxonase-activating and lipid-lowering effect of different types of statin.

We hypothesized that PON1 phenotype might influence the effect of simvastatin, atorvastatin and extended-release fluvastatin on serum lipid parameters, especially on HDL-C levels. We also tested if there is a clinically important difference between the effect of the three statins due to their structural and pharmacokinetic differences.

Patients and methods

Study population

Patients were recruited at the First Department of Medicine, University of Debrecen. Subjects between 21 and 70 years old, nonsmokers, with previously untreated type II/b hyperlipidaemia were enrolled. Patients with hepatic disorders, endocrine or renal disorders (serum creatinine level >130 $\mu\text{mol l}^{-1}$), diabetes mellitus, impaired glucose tolerance, alcoholism, drug dependence, gallstones, malignancy, pregnancy or lactation, or patients on anticoagulant or lipid-lowering therapy were excluded. After 6 weeks on the National Cholesterol Education Program step 1 diet, patients were randomly divided into three groups: the first group received 10 mg day^{-1} atorvastatin (ATO), the second 10/20 mg day^{-1} simvastatin (SIM) and the third 80 mg day^{-1} extended-release fluvastatin (FLU) for 3 months. Based on previous data, these statin doses are able to reduce LDL-C levels similarly, by approximately 30–39% [19]. Informed consent was obtained from all patients after explaining the nature and the purpose of the study. The Ethics Committee of the University of Debrecen approved the study.

Blood sampling

After 12 h of fasting, a 10-ml venous blood sample was taken between 07.30 and 08.00 h. Lipid parameters were determined from fresh serum. The sera for paraoxonase activity measurements were kept at -70°C before analysis.

Lipid measurements

Serum cholesterol and triglyceride levels were measured using enzymatic, colorimetric tests (GPO-PAP, Modular P-800 Analyzer; Roche/Hitachi, Basel, Switzerland), whereas HDL-C was assessed by homogenous, enzymatic, colorimetric assay (Roche HDL-C plus 3rd generation). The LDL-C fraction was calculated indirectly using the Friedewald equation [20]. 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

PON1 activity was measured as previously described [5]. Briefly, the following enzymatic reaction was set up using paraoxon (O,O-diethyl-O-p-nitrophenylphosphate; Sigma, St Louis, MO, USA) as substrate and the generation of 4-nitrophenol was followed spectrophotometrically: 50 μl serum was dissolved in 1 ml Tris-HCl buffer (100 mmol l^{-1} , pH 8.0) containing 2 mmol l^{-1} CaCl_2 and 5.5 mmol l^{-1} paraoxon. We measured the absorbance at 412 nm (25°C) using a Hewlett-Packard 8453 UV-visible spectrophotometer. Enzyme activity was calculated using the molar extinction coefficient 17 100 M 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 as previously described [5]. 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 the absorbance was then 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 M cm^{-1} . Arylesterase activity is expressed in U ml^{-1} ; 1 U is defined as 1 μmol phenylacetate hydrolysed per minute.

Paraoxonase phenotyping

The phenotype distribution of PON1 was determined by the dual-substrate method [14]. The genetic polymorphism at codon 192 Q $\rightarrow$ 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.

Statistical methods

SAS for Windows 6.12 (SAS Institute Inc., Cary, NC, USA) computer software was used for 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 $\pm$ SD in case of normal distribution, and medians and 95% confidence intervals for median in case of non-normal distribution. To evaluate the effect of treatment, changes were analysed with repeated-measures ANOVA and with post hoc Newman-Keuls critical ranges test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Demographic data, laboratory parameters and characteristics of the study population are shown in Table 1.

Lipid parameters

The initial cholesterol and LDL-C levels were significantly higher in the FLU group compared with the ATO group (7.61 ± 0.98 vs. 6.98 ± 0.90 mmol l^{-1} , 5.17 ± 0.78 vs. 4.70 ± 0.81 mmol l^{-1} , respectively; $P < 0.05$). We could find no significant differences between the FLU, ATO and SIM groups in the other lipid parameters.

Statin treatment significantly decreased total cholesterol and apolipoprotein B (apoB) levels in the whole study population and in the three statin groups. Significantly decreased triglyceride levels were found in the whole study population and in the ATO group. The LDL-C level significantly decreased in the whole study population and in the ATO and FLU groups. HDL-C levels did not change significantly. A significant increase in the apoA1 levels was found in the SIM group. The LDL/apoB ratio significantly decreased in the whole study population and in the ATO and FLU groups (Table 1).

Paraoxonase and arylesterase activities

Paraoxonase activity significantly increased in the whole study population and in all three statin groups. There was a significant increase in arylesterase activity in the whole study population and in the SIM and FLU groups (Table 1).

Paraoxonase phenotype distribution

The allelic frequencies found in the pooled study population and in the three groups using the phenotypic determination are shown in Table 2. The allelic frequencies were in accordance with the results of our previous studies and the literature, following Hardy-Weinberg equilibrium ($P < 0.05$). PON1 phenotype distribution of the three groups did not differ significantly ($P = 0.1005$).

Characteristics and laboratory parameters of the study population (values are mean $\pm$ SD)

* $P < 0.001$; ** $P < 0.01$; # $P < 0.05$. Students' paired t-test was applied to 'before-' and 'after'-treatment parameters. ATO, atorvastatin; FLU, fluvastatin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; apo, apolipoprotein.

Table 2

Paraoxonase phenotype distribution and allelic frequencies in the whole study population and in the statin groups

	All statins (n = 164)		ATO (n = 61)		SIM (n = 46)		FLU (n = 57)	
	n	%	n	%	n	%	n	%
AA	104	63	38	62	29	63	37	65
AB	50	31	18	30	15	33	17	30
BB	10	6	5	8	2	4	3	5
A	258	78.7	94	77.1	73	79.3	91	79.8
B	70	21.3	28	22.9	19	20.7	23	20.2

ATO, atorvastatin; FLU, fluvastatin; SIM, simvastatin.

Responses of patients to statin treatment according to 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 AB+BB phenotype groups

There was no significant difference between phenotypes in initial lipid levels (data not shown). Statin treatment more effectively decreased the triglyceride levels in the AB+BB group compared with the AA group in the whole study population (24.11 vs. 3.13%) and in the SIM group (17.80 vs. 3.63%). 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. Atorvastatin treatment was significantly more effective on apoB levels in the AB+BB phenotype compared with the AA phenotype group (50.37 vs. 34.83%) (Table 3).

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

In patients with AB+BB phenotype, statin treatment was significantly more effective on paraoxonase activity in the whole study population and in all three statin groups (Figure 1a). Whereas in the AA phenotype group there was no significant change in paraoxonase activity in the whole study population and in the ATO and SIM groups, in the AB+BB phenotype group a significant increase was found in all the studied groups. In the FLU group a significant increase in both phenotype groups was found; however, in the AB+BB group the change was more prominent.

Similar results were found when we examined the changes in arylesterase activity with the exception of atorvastatin (Figure 1b).

Discussion

The putative mechanism of statin effect on PON1 activity has been intensively investigated. Deakin *et al.* examined the influence of simvastatin on PON1 gene expression in HepG2 cells. They found that simvastatin was able to modulate the *in vitro* expression of PON1 regulated by sterol regulatory element-binding protein-2 (SREBP-2) and increased serum PON1 concentration and activity [21]. Data from this study suggest that PON1 gene belongs to the wide range of genes regulated by SREBPs. Ota *et al.* found that pitavastatin significantly increased promoter activity in HEK293 cells after PON1 (587/-6) plasmid transfection. Because PON1 gene promoter activity was also increased by atorvastatin and simvastatin, it was concluded that the transactivation was not specific to pitavastatin, but was rather a general effect of statins. Their results also suggested that the statin effect on PON1 activity may have occurred through the mevalonic acid-derived farnesyl pyrophosphate pathway [22]. Deakin *et al.* have recently proved that patients who were homozygous for the -107C allele showed a significant increase in PON1 activity and concentration after 2 months of simvastatin treatment, whereas in the -107TT homozygotes there was no significant impact of statin treatment [18]. 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 scant.

Previously Christidis *et al.* found that the PON1-192 and -55 genotypes did not influence the change in PON1 activity after 40 mg day⁻¹ fluvastatin treatment [17]. However, they demonstrated that in R allele carriers the increase in paraoxonase activity was larger compared with the QQ homozygotes, although the difference was not significant. Tomás *et al.* have reported similar results with 20 mg day⁻¹ simvastatin treatment [15]. 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 group; nevertheless, PON1 activity presents great interindividual variability [7]. The effect of statins was similar on arylesterase activity, although the difference between the two phenotype groups was not significant in the atorvastatin-treated group. This latter result suggests that there can be differences in the effect of phenotype using different types of statin. Each statin has different pharmacokinetic properties, bioavailability and hydrophilicity. These individual properties, such as affinity to cytochrome P450 isoenzymes, may influence the effect of statins on PON1 activity [23]. Diverse study populations, different types of hyperlipoproteinaemia and various types of statin dosages could contribute to the discrepancies in

Table 3

Relative changes in lipid parameters in the AA and in the AB+BB phenotype groups, expressed as percentage difference between the 'before'- and 'after'-treatment mean values

	All statins		ATO		SIM		FLU	
	AA	AB+BB	AA	AB+BB	AA	AB+BB	AA	AB+BB
Δ chol (%)	-22.63	-24.86	-40.10	-38.79	-1.79	-9.42	-30.34	-26.72
Δ triglyceride (%)	-3.13	-24.11*	-17.07	-33.48	3.63	-17.80*	4.62	-17.56
Δ HDL-C (%)	4.25	9.56	5.73	6.80	2.94	13.11	4.38	7.50
Δ LDL-C (%)	-33.08	-28.89	-43.80	-41.41	-10.15	-11.39	-47.26	-34.20
Δ apoA1 (%)	2.03	5.20	-4.97	1.72	8.57	10.61	-0.49	-2.25
Δ apoB (%)	-18.66	-27.39	-34.83	-50.37*	-2.62	-10.44	-25.98	-28.30

* $P < 0.05$. ATO, atorvastatin; FLU, fluvastatin; SIM, simvastatin; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; apo, apolipoprotein.

the literature. Furthermore, Vincent-Viry *et al.* have reported 7.2% discordance between the results of genotyping and phenotyping in healthy French subjects [24]. It should be noted that Jarvik *et al.* have demonstrated that PON1 phenotype is a better predictor of vascular disease than PON1-55 or PON1-192 genotypes [25].

Malin *et al.* reported first that PON1 genotype could modify statin effect on lipid parameters. They found that pravastatin increased the apoA1 concentration and tended to increase the HDL-C concentration in 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 [16]. On the other hand, Turban *et al.* found that the Q192R variants of PON1 are not associated with plasma lipid levels and response to treatment with 40 mg day⁻¹ fluvastatin [26]. The results of Christidis *et al.* show that in R allele carriers the decrease in triglyceride levels was larger compared with the QQ homozygotes, although the difference was not significant [17]. 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, PON1 paraoxonase activity of the AA phenotype group did not correlate with LPL activity, whereas in the AB+BB phenotype group there was a significantly positive correlation between paraoxonase and LPL activity [27]. It is also known that the activity of LPL inversely correlated with triglyceride concentration. Presumably, the activity of LPL may influence HDL remodelling and, consequently, modify the activity of the HDL-associated PON1. Based on these data, higher paraoxonase activity (AB+BB phenotypes) means higher LPL activity and, consequently, lower triglyceride levels [27].

According to our results there was no significant difference between phenotypes in initial lipid levels. Similarly to

Malin *et al.*, 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 [16]. Tomás *et al.* have shown 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 [15]. We also found that changes in total cholesterol levels tended to be higher in the AB+BB phenotype group.

Interestingly, in the SIM group the changes in cholesterol, LDL-C and HDL-C levels were less than expected compared with previous larger clinical studies [1, 28]. In contrast, we found a remarkable decrease in cholesterol and LDL-C levels in the FLU group. It is known that the efficacy of statin treatment is influenced by the initial lipid levels [29]; initial cholesterol and LDL-C levels were higher in the FLU group, although we could find no significant difference between the FLU and SIM groups. On the other hand, in our study we used low-dose simvastatin and high-dose extended-release fluvastatin therapy.

It must be noted that there are some limitations of this study. The relatively small sample size clearly reduces the power of the study. Presumably, the increase in the size of the study population would result in a significant association between PON1 phenotypes and statin effect on cholesterol and HDL-C levels. Data on PON1 genotypes strengthen the hypothesis that the PON1 phenotype modifies the effect of statins on lipid parameters.

The PON1 phenotype may be a predictive factor for the effectiveness of statin treatment on paraoxonase activity and serum lipid parameters. Different types of statins may exert different effects on these parameters. Therefore, determination of PON1 phenotype can be recommended in future studies on hyperlipidaemic patients before statin treatment. Further studies are needed to clarify the role of PON1 genotype and phenotype in the modification of statin treatment.

Figure 1

Responses of patients to statin treatment in the AA and in the AB+BB phenotype groups. (a) Paraoxonase activity. (b) Arylesterase activity. Results are means and vertical bars denote 0.95 confidence intervals. Repeated measures ANOVA with a post hoc Newman-Keuls test was carried out for comparisons between 'before'- and 'after'-treatment parameters

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