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ANTIOXIDATIVE EFFECTS OF ROSUVASTATIN IN LOW-TO-MODERATE CARDIOVASCULAR RISK SUBJECTS

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ABSTRACT

Background: Although vast clinical evidence supports the oxidative CVD hypothesis, little is known on the effects of statins on LDL/HDL oxidative functionality. Therefore, the aim of this study was to evaluate the antioxidative effects of rosuvastatin by monitoring the susceptibility of LDL to oxidation and the antioxidative HDL potential in low-to-moderate CV risk subjects.

Methods: 40 adult ambulatory patients (aged 53.8±10.9 years, 27 women and 13 men) were included in the study. Data was collected from patients' records, physical examination, and blood sampling. Subjects were prescribed rosuvastatin at 20mg/day. Traditional risk-factors/indicators, lipid parameters, inflammatory/immune markers, LDL susceptibility to oxidation and HDL antioxidative potential were monitored and statistically analyzed with t-test, Chi-square test, one-way ANOVA, Mann-Whitney, and Kruskal-Wallis tests. Multivariate logistic regression analyses were made. Results were considered significant when $p \leq 0.05$.

Results: 67% of the patients showed lower susceptibility of LDL to oxidation after rosuvastatin treatment ($p=0.03$), with no significant effect on baseline LDL oxidation and lag time. All three LDL oxidative indices were seen to be dependent on the subjects' lipid profile, hemoglobin levels and the IL-1α and IL-8 pro-inflammatory marker levels. 53% of the patients showed higher HDL antioxidative capacity after treatment, but without statistical significance ($p=0.07$). Increased antioxidative potential of HDL with rosuvastatin treatment was more likely in males (OR=9.350; $p=0.010$), and subjects achieving lower post-treatment CV relative risk levels (higher CV risk reduction) (OR=0.338; $p=0.027$).

Conclusions: This study suggests the need of a comprehensive approach when investigating oxidative stress and LDL/HDL functions, especially in low-to-moderate CVD risk subjects.

Keywords: CVD risk, rosuvastatin, LDL-oxidation, HDL antioxidative capacity

INTRODUCTION

Oxidative stress indicates an imbalance between the oxidative (such as xanthine oxidase, lipoxygenases, mitochondrial respiratory enzymes, and NADPH oxidases) and antioxidative systems (such as superoxide dismutase, paraoxonase, catalase), which leads to overproduction of reactive oxygen and/or nitrogen entities. [1] Increased ROS levels result in dysfunctional eNOS and

decreased NO bioavailability, thus promoting LDL oxidation, leukocyte adhesion and migration, and endothelial dysfunction. [1] ROS levels are also vastly affected by the transcription of the NF-kB-dependent genes, while ROS levels themselves regulate the NF-kB activity. [2] The oxidative stress-induced NF-kB hyperactivation, dominant in numerous chronic inflammatory conditions such as atherosclerosis, stimulates expression of pro-inflammatory genes, resulting in activation of inflammatory T cells, and differentiation of macrophages into pro-atherogenic M1. [2] Extensive clinical evidence exists on the cross-link of inflammation and oxidative stress in the etiology of CVD. [2]

Friedman et al. (2002) were the first to show that oxidized lipids in ox-LDL are biologically active. [3] Polyunsaturated fatty acids are converted to hydroperoxides, which are broken down to highly reactive molecules, such as 4-hydroxynonenal and malondialdehyde. [3] These reactive aldehydes react with the apolipoprotein-lysine residues, mainly LDLs' ApoB. [3] The oxidatively-modified LDL cannot be recognized by the native LDL-receptors, and is mainly attracted to the macrophage-scavenger receptors. This promotes continuous cholesterol accumulation, and mainly accounts for ox-LDLs' immunogenicity and atherogenicity. [3] Ox-LDL itself stimulates the expression of adhesion molecules and pro-inflammatory cytokines and increases lipid deposition and leukocyte recruitment and endothelial retention, resulting in the formation of foam cells and thrombi. [4] Multiple clinical studies have reported an association between ox-LDL levels and early atherosclerosis, acute coronary syndrome and coronary and peripheral arterial disease, and numerous pathological disorders such as obesity, metabolic syndrome and DM. [4]

On the other hand, in addition to reverse cholesterol transport, HDL neutralizes the atherogenic LDL potential by controlling the membrane cholesterol content, inhibiting the LDL-oxidative modification, and counteracting the LDL pro-inflammatory actions. [5] These beneficial HDL effects can be due to its own actions as a cell-signaling modulator and lipid transporter, or they can be indirect effects of different HDL-linked entities. [5] HDLs are accompanied by multiple antioxidative enzymes, such as paraoxonase, lecithin-cholesterol acyltransferase, thrombocyte-activating factor acetylhydrolase, and reduced glutathione selenoperoxidase. These account for the LDL-oxidation inhibition and establish a role of HDL as a physiologic ox-LDL detoxicator. [6]

Although vast clinical evidence exists that supports the oxidative hypothesis in CVD etiology, and there is an established dependence between ox-LDL levels and cardiovascular risk, [7] few clinical studies have examined the statin effects on ox-LDL. Several studies have shown that simvastatin, atorvastatin, and rosuvastatin reduce circulating ox-LDL levels in atherosclerotic CVD patients, using ELISA quantification methods. [7] However, there is a lack of evidence regarding the effect of rosuvastatin on the intrinsic susceptibility of LDL to oxidation, as well as on the ability of HDL to prevent that oxidation, in dyslipidemic subjects without an established CVD.

Therefore, the aim of this study was to evaluate the antioxidative effects of rosuvastatin, by quantitatively monitoring the susceptibility of LDL to oxidation and the antioxidative HDL potential, before and after treatment, in subjects with low-to-moderate CV risk, without a diagnosed CVD.

MATERIALS AND METHODS

2.1 Subjects

40 adult ambulatory patients (aged 53.8±10.9 years, 27 women and 13 men) from the UKIM-University Clinic of Cardiology in Skopje were enrolled prospectively. Subjects were statin "naïve", with primary hypercholesterolemia or mixed dyslipidemia. Data for the study inclusion were obtained from the patients' physical examination, medical history, and blood analysis on the day of the clinical visit. Standard hematologic, electrolyte, protein indices, and thyroid function measures were used to determine the overall inclusion status of potential subjects. Subjects with known hypersensitivity to rosuvastatin or some of the drug's excipients, diagnosed with thyroid, liver, kidney, and heart diseases, systemic inflammatory diseases, McArdle's disease, cancer history (remission shorter than 5 years), myopathy, rhabdomyolysis or muscle pain of unknown origin, HIV patients,

pregnant and nursing women or women planning to get pregnant, blood-donors (4 wks. before initiation of therapy), subjects with ALT and AST levels 1.5-fold above the upper limit, increased CPK levels 5-fold above the upper limit, and subjects with creatinine clearance below 30 mL/min were not included in the study. Other exclusion criteria included prior treatment with lipid-lowering drugs, drugs known to affect CYP3A4 and OATP1B1 activity, and drugs known to increase LDL-C. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization (ICH) Guidance on Good Clinical Practice (GCP) (CPMP/ICH/135/95). All participants received oral and written information, and gave a written informed consent before entering the study. The final clinical study design was approved by the local Ethics Committee for Human Research (University ss Cyril and Methodius, Faculty of Medicine, 50th Division No.6, 1000, Skopje, RN Macedonia).

2.2 Study design

The study was an open, one-arm longitudinal prospective clinical trial with a 12-week intervention period. Subjects were assigned to rosuvastatin 20 mg/day. It was considered that the patients adhered to the drug treatment when 80% of the dosage units were used. Samples were taken from each subject at baseline, 12 weeks on-treatment, and 4 weeks after drug discontinuation, thus each subject acted as their own control.

2.3 Blood samples

Twelve hour fasting blood samples were collected at study entry, after 12 weeks of rosuvastatin treatment (to evaluate efficacy indicators), and 4 weeks after drug discontinuation (to evaluate safety indicators). 10.0 ml venous blood was collected in two EDTA/K3 vacuum tubes. Plasma was separated by centrifugation at 3000 rpm for 15 min, and stored at -80 °C until analysis.

2.4 Biochemical measurements

Biochemical parameters were determined at the UKIM-University Clinic of Clinical Biochemistry, Skopje. The response to rosuvastatin was evaluated according to the percentage change in total cholesterol (Chol), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), and lipoprotein(a) (Lp(a)). In addition, EURO Score, risk age, and relative risk were also calculated at baseline and after 12 weeks of treatment. As safety profile indicators, aspartate transaminase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), creatine phosphokinase (CPK), myoglobin, glucose, and glycosylated hemoglobin A1c (HbA1c) were determined. Standard laboratory procedures and methods for evaluation of the biochemical parameters were used (COBAS INTEGRA tests), established by the International Federation of Clinical Chemistry (IFCC).

2.5 Inflammatory markers

Inflammatory markers were determined at the Institute of Pathology, UKIM-Faculty of Medicine, Skopje. A panel of 13 inflammatory/immune markers was measured, including interleukins (IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10), cytokines (tumor necrosis factor-α (TNF-α)), interferon-γ (IFN-γ), chemokines (monocyte chemoattractant protein-1 (MCP-1)), growth factors (epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF)), and acute phase proteins (high sensitivity C-reactive protein (hsCRP)). Quantitative determination of the inflammatory markers was done using a commercial Randox cytokine and growth factors array kit, allowing a simultaneous in vitro quantitative detection, based on a sandwich chemiluminescent immune test.

2.6 LDL/HDL preparation and oxidation tests

Plasma samples were transported on dry ice, at -80 °C, to the Cardiovascular ICCC-Program, Research Institute Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, 08025 Barcelona, Spain, in order to isolate the lipoproteins and perform the oxidation tests.

2.6.1 Lipoprotein preparation

LDLs (density range 1.019–1.063 g/mL) and HDLs (density range 1.063–1.210 g/mL) were isolated from plasma samples by sequential ultracentrifugation, according to the method originally described by Havel et al. (1955) [8], modified by De Juan-Franco et al. (2009). [9] In

summary, plasma was adjusted to a density of 1.019 g/mL, with a concentrated KBr solution, and centrifuged at 225,000×g (18 h) in a Beckman L-60 ultracentrifuge with a fixed-angle type 50.4 Ti rotor (Beckman, Brea, CA, USA). After removal of the top layer (VLDL and IDL), the density of the infranatant was adjusted to 1.063 g/mL, and centrifugated at 225,000×g (20h). LDLs were than collected from the top of the tube. The process was repeated with adjustment of the plasma density to 1.210 g/mL, and samples were centrifuged at 225,000×g for 24 h, at 4 °C, to allow HDLs to appear at the tube surface and separate them from the lipoprotein deficient serum.

In addition, LDLs, used in the TRAP test, were isolated from a plasma pool obtained from normolipemic subjects, as described previously, in a Beckman Optima L-100 XP with a fixed-angle type 50.2 Ti (Beckman, Brea, CA, USA).

The obtained LDL and HDL fractions were dialyzed against a phosphate buffer saline 1X (PBS 1X) for 24 h. The protein content of the isolated fractions was determined by the BCA colorimetric assay (Pierce, Thermo Fischer Scientific, Waltham, MA, USA), and samples were adjusted to a protein concentration of 100 μg/mL. Samples were kept protected from light at 4 °C, until analysis.

2.6.2 Conjugated dienes test

Susceptibility of LDL to copper-induced oxidation was assessed by quantitative determination of the formation of conjugated dienes. In summary, freshly prepared LDL samples, previously adjusted to 100 μg/mL, were analyzed after incubation with a copper (II) sulfate ($CuSO_4 \cdot 5H_2O$) solution (final solution concentration was 5 μM). The change of absorbance was determined during 2.5 h at 37 °C, with a SpectraMax 190 Microplate reader (Molecular Devices, Philadelphia, PA, USA), by continuously monitoring the formation of conjugated dienes, a lipid peroxidation product with an absorbance peak at 234 nm. The total amount of conjugated dienes was calculated as previously reported. [10]

2.6.3 TRAP test

The antioxidative HDL potential was assessed by performing the total radical-trapping antioxidative potential (TRAP) test, a method measuring the capability of HDL to prevent LDL oxidation. In summary, LDLs and HDLs were adjusted to a final concentration of 100 μg protein/mL with PBS 1X. LDLs obtained from the control plasma pool were incubated with $CuSO_4 \cdot 5H_2O$ (final solution concentration was 20 μM), in the presence/absence of HDLs isolated from each individual subject, for 4 h at 37 °C. Afterwards, oxidation was stopped with 1 mM EDTA, and the obtained samples were incubated with 10 μM DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate), to measure the oxidation level. Fluorescence intensity was determined with Typhoon FLA9500 (GE Healthcare, Chicago, IL, USA), set at λex = 500 nm and λem = 520 nm. Final fluorescence measurements were expressed as percentage of oxidized LDLs generated in the presence of HDL, relative to the oxidation level of LDLs in the absence of HDL.

2.7 Statistical analysis

Continuous variables are expressed as mean value ± SD, and categorical variables are expressed as absolute numbers. Chi-square test, t-test, and one-way ANOVA were used for the variables that follow the normal distribution, while nonparametric tests, such as Mann-Whitney and Kruskal-Wallis tests, were used to analyze the continuous variables that deviated from the normal distribution. Correlations were computed, and uni- and multivariate logistic regression analysis were made. Results were considered statistically significant when $p \leq 0.05$. Data was analyzed using the IBM SPSS 19.0 statistical software.

RESULTS AND DISCUSSION

3.1 Baseline characteristics of the study population

The distribution of the studied subjects, in relation to traditional CVD risk factors, was as follows: 29 (72.5%) subjects had hypertension, 6 (15.0%) subjects had DM type 2, 3 (7.5%) subjects were smokers, 5 (12.5%) subjects had a family history of CVD. Prediabetes (HbA1c 5.6-6.5%) was present in 22 (55.0%) subjects. 62.5% of the subjects had previously diagnosed untreated hyperlipidemia.

Table 1 shows all clinical characteristics and laboratory measures that were monitored in the patients in the study, before and after rosu-

Table 1. *Characteristics of the studied population, before and after rosuvastatin treatment*

Variable	Before treatment	After treatment	Sig.
Age	53.8±10.9	-	
Gender	40 (100%)		
o **Female**	27 (67.5%)	-	
o **Male**	13 (32.5%)		
EUROScore risk age	58.8±6.8	57.6±8.1	ns
Risk age > biological age	24 (60%)	19 (47%)	ns
EUROSCORE (%)	3.8±3.7	2.9±2.8	0.042
EUROScore relative risk	2.6±1.3	1.8±0.8	0.037
Body mass index, BMI (kg/m^2)	27.5±3.4	27.5±3.4	ns
Systolic blood pressure, SBP (mmHg)	137.1±13.9	130.2±11.7	ns
Total cholesterol, Chol (mmol/L)	6.2±1.4	4.2±1.0	0.000
LDL-C (mmol/L)	3.9±1.4	2.1±0.9	0.000
Non-HDL-C (mmol/L)	4.8±1.3	2.9±1.1	0.000
HDL-C (mmol/L)	1.4±0.4	1.4±0.4	ns
TG (mmol/L)	2.0±1.1	1.6±0.8	0.039
ApoA1 (g/L)	1.6±0.3	1.5±0.3	ns
ApoB (g/L)	1.4±0.4	0.9±0.3	0.002
Lp(a) (mg/dl)	48.2±67.3	56.1±77.6	ns
Creatine kinase, CPK (IU/L)	112.6±65.5	122.9±97.7	ns
Myoglobin (ng/ml)	47.9±18.4	47.7±17,7	ns
AST (IU/L)	22.4±6.7	23.6±7.4	ns
ALT (IU/L)	25.1±10.6	25.5±10.6	ns
GGT (IU/L)	19.4±5.4	20.1±5.6	ns
Glucose (mmol/L)	6.2±3.2	5.6±2.0	ns
HbA1c (%)	6.1±1.0	6.3±0.9	ns
Urea (mmol/L)	4.9±1.5	4.7±1.3	ns
Creatinine (μmol/L)	73.1±15.0	70.6±13.4	ns
Uric acid (μmol/L)	313.5±86.1	288.1±76.4	ns
eGFR (ml/min)	100.7±21.1	104.5±20.7	ns
hsCRP (mg/L)	3.3±4.1	2.1±1.7	ns
IL-2 (ng/ml)	1.7±1.2	2.8±4.5	ns
IL-4 (ng/ml)	1.9±0.7	2.5±1.1	0.012
IL-6 (ng/ml)	5.9±27.0	2.5±3.3	ns
IL-8 (ng/ml)	5.6±6.7	11.9±23.7	ns
IL-10 (ng/ml)	0.8±1.1	1.2±1.6	ns
VEGF (ng/ml)	40.1±37.6	80.9±79.7	0.000
INF-γ (ng/ml)	1.2±5.6	0.6±0.9	ns
TNF-α (ng/ml)	2.4±2.0	3.4±3.8	0.023
IL-1α (ng/ml)	0.3±0.1	0.5±0.9	ns
IL-1β (ng/ml)	1.7±1.5	2.8±6.6	ns
MCP-1 (ng/ml)	99.5±85.9	112.8±81.0	0.000
EGF (ng/ml)	58.1±81.1	111.3±105.6	0.000

vastatin treatment, as well as the statistical significance of the difference in the obtained values, computed with t-test.

As expected, rosuvastatin significantly decreased total cholesterol, non-HDL-C, LDL-C, ApoB and TG, without an effect on HDL-C, ApoA1 and Lp(a). The lipid reduction resulted in a significant reduction of the overall calculated CVD risk (EUROSCORE) and the EUROScore relative risk of the studied population. Taking into account clinical data on statin side effects, rosuvastatin safety was determined by monitoring glycaemia and HbA1c, mean change in liver enzymes, and laboratory measures of muscle toxicity. No significant changes in the safety parameters were seen before and after rosuvastatin treatment. No cases of rhabdomyolysis or liver insufficiency were reported. No changes in the renal function indices were observed.

Vast scientific and clinical evidence has postulated a key role of inflammation in the progress of atherosclerosis, beginning from endothelial dysfunction to plaque formation and rupture, with numerous interleukins, cytokines and cellular entities constituting a chronic inflammatory response. [11] Although statins have been shown to possess lipid-dependent and -independent anti-inflammatory effects, clinical studies evaluating statin actions on different inflammatory/ immune markers, besides hsCRP, are scarce. [11] Rosuvastatin increased the levels of the pro-inflammatory markers IL-4, TNF-α, and MCP-1 in our study population, in accordance with recent clinical findings [12, 13], proposing beneficial immune statin effects only in 'hyper-inflammatory' subjects (with hsCRP >10mg/L). Rosuvastatin significantly increased the levels of the growth factors EGF and VEGF, suggesting a pro-angiogenic effect.

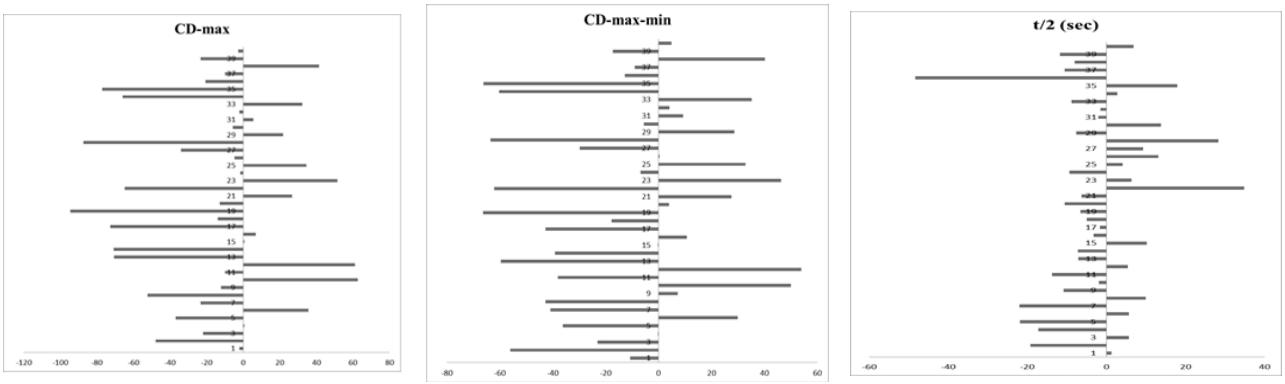
Inflammation and oxidation act in a complex interplay in the CVD pathogenesis. Multiple immune cells are redox-activated, with ROS being the main inflammatory trigger. [2] The mitochondrially produced superoxide/hydrogen peroxide is the main regulator of NAPDH oxidase, which is key in activation, recruitment, and infiltration of monocytes and T cells. [2] Most of the transcription factors involved in inflammatory pathways (such as AP-1 and NF-κB) are redox-regulated. [2]

Even though rosuvastatin inflammatory/ immune effects were not the primary goal of this study, the biomarker levels were included to see if they show an association with the studied rosuvastatin effect on the oxidative LDL/HDL functionality.

3.2 LDL susceptibility to oxidation

LDL susceptibility to oxidation, before and after rosuvastatin treatment, was assessed by the observed quantitative change in the maximal amount of generated conjugated dienes (CD-max), the baseline amount of conjugated dienes (CD-max-min), and the lag time (t/2).

Sixty-seven percent of the patients showed lower susceptibility of LDL to oxidation after rosuvastatin treatment, compared to the paired values obtained before treatment (p=0.03 with the Chi2 test; Figure 1A). Compared to pre-treatment values, fifty-nine percent of the patients showed lower baseline LDL oxidation, and forty percent of the patients showed longer lag time post-treatment, but the observed changes did



(A) (B) (C)

Figure 1. *Effect of rosuvastatin on LDL susceptibility to oxidation. The response to rosuvastatin treatment is given at an individual level for (A) maximal value for generated conjugated dienes, (B) baseline LDL oxidation (baseline value for conjugated dienes), and (C) lag time*

not show a statistical significance (p=0.07 and p=0.10 with the Chi2 test, respectively; Figure 1B and Figure 1C).

3.3 HDL antioxidative capacity

Susceptibility of LDL to oxidation in the presence of HDL was decreased to 33.0 ± 6.1% of the value obtained in the absence of HDL (p<0.01). Fifty-three percent of the patients showed higher HDL antioxidative capacity after treatment, compared to the obtained values before treatment, but without a statistical significance (p=0.07 with the Chi2 test; Figure 2)

3.4 Effect of rosuvastatin on oxidative LDL/HDL functionality

Table 2 shows the obtained values for the examined oxidative measures, before and after rosuvastatin treatment, as well as the statistical significance of the difference computed with t-test.

Much of the interest in LDL oxidation was due to the discovery that native LDL does not lead to endothelial lipid accumulation, i.e. that early atherogenesis is the result of accumulation of a number of modified LDL forms, such as oxidized, glucolyzed, and electronegative LDL. [14]

Although there are studies investigating the effect of statins on circulating ox-LDL levels, the number of studies investigating the effect of statins on the oxidative functionality of LDL and HDL is limited. Lovastatin has been shown to increase paraoxonase-1 activity and prolong LDL lag time with in vitro monitoring of conjugated dien production. [15] Thallinger et al. (2005) compared the effects of simvastatin, pravastatin, and atorvastatin on LDL susceptibility to oxidation in hypercholesterolemic subjects. Pravastatin and atorvastatin reduced CD-max by approximately 9% vs. the basal value (p<0.005), whereas simvastatin resulted

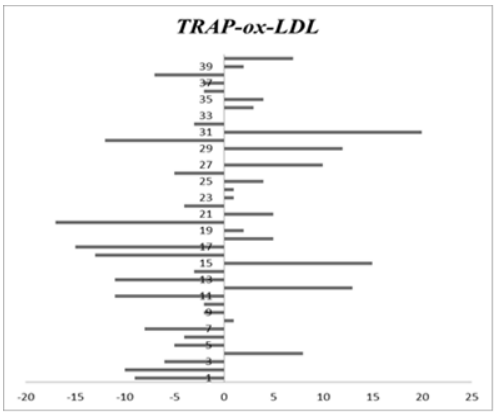


Figure 2. *Effect of rosuvastatin on HDL antioxidative capacity. Functional response of HDL to oxidative stress is given at an individual level.*

Table 2. *Oxidative measures, before and after rosuvastatin treatment*

Variables	*Before treatment*	*After treatment*	*Sig.*
***CD-max** (nmol CD/mg LDL)*	379.7±41.8	365.8±41.8	0.040
***CD-max-min** (nmol CD/mg LDL)*	264.3±33.8	254.3±38.9	0.060
***t/2 (lag time)** (sec)*	61.5±16.2	59.3±14.9	ns
***TRAP-ox-LDL** (%)*	33.0±6.1	32.0±5.8	ns

Table 3. *Multivariate linear regression analysis of CD-max (backward conditional method, mean square 9648.993; p=0.000)*

Model	Standardized Coefficients	t	Sig.
	Beta		
(Constant)		6.988	.000
Total cholesterol	**-.856)**	**-2.338)**	**.026**
non-HDL-C	**1.307**	**3.445**	**.002**
Triglycerides	**-.575)**	**-4.160)**	**.000**
ALT	**-.314)**	**-2.550)**	**.016**
Hemoglobin	**-.256)**	**-2.114)**	**.042**

Table 4. *Multivariate linear regression analysis of CD-max-min (backward conditional method, mean square 6398.880; p=0.000)*

Model	Standardized Coefficients Beta	t	Sig.
(Constant)		6.931	.000
Total cholesterol	**-.780)**	**-2.325)**	**.026**
non-HDL-C	**1.206**	**3.437**	**.002**
Triglycerides	**-.535)**	**-4.047)**	**.000**
Hematocrit	**-.297)**	**-2.633)**	**.013**
IL-1α	**.270**	**2.428**	**.021**

Table 5. *Multivariate linear regression analysis of t/2 (backward conditional method, mean square 1153.717; p=0.000)*

Model	Standardized Coefficients Beta	t	Sig.
(Constant)		6.463	.000
IL-8	**-.381)**	**-2.705)**	**.010**

Table 6. *Multivariate linear regression analysis of TRAP-ox-LDL (backward conditional method, mean square 1420.977; p=0.000)*

Model	Standardized Coefficients Beta	t	Sig.
(Constant)		1.425	.000
female 0/ male 1	**-.368)**	**-2.468)**	**.019**
SCORE relative risk	**.542**	**5.162**	**.000**
Creatinine	**.346**	**2.151**	**.039**
HDL-C	**.311**	**2.671**	**.012**

Table 7. *Univariate analysis in association to the gender*

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	female 0/ male 1(1)	2.235	.866	6.665	1	**.010**	**9.350**	1.713	51.032
	Constant	-.531)	.399	1.773	1	.183	.588		

a. Variable(s) entered on step 1: female 0/ male 1.

Table 8. *Univariate analysis in association to the pre- and post-treatment SCORE relative risk*

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Relative risk (pre-treatment)	-.651)	.343	3.595	1	**.058**	**.521**	.266	1.022
	Constant	1.779	.922	3.720	1	.054	5.921		

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B) Lower	Upper
Step 1[a]	Relative risk (post-treatment)	-1.083)	.491	4.867	1	**.027**	**.338**	.129	.886
	Constant	2.041	.934	4.779	1	.029	7.699		

a. Variable(s) entered on step 1: Relative risk.

in an approximately 5% reduction in CD-max, but without statistical significance. [16] Portal et al. (2003) compared the effects of Fluvastatin and pravastatin on the pro-oxidative potential of LDL in CAD patients. Both statins led to a decrease in CD-max, but did not show an effect on the lag time. [17]. Whether this is the result of the de facto reduction in LDL-C levels, changes in the lipid structure of LDLs, or an indirect result of the effects of statins on ROS production and antioxidative enzymes levels is still a topic of scientific discussion.

Pirillo et al. (2017) showed that pitavastatin increased the antioxidative HDL potential, by stimulating the apoA1-mediated reduction of LDL-phospholipid hydroperoxides. [18] Triolo et al. (2013) showed no effect of simvastatin on the antioxidative effects of HDL. [19]

No studies were found on the effect of rosuvastatin on LDL susceptibility to oxidation, and on the antioxidative HDL potential.

In order to see whether the determined oxidative measures show any associations with the monitored parameters in the study population, correlation analysis, uni- and multivariate linear regression analyses were performed. Only the final models of the multivariate analysis are given, where the oxidative markers were placed as dependent variables. All parameters identified as statistically significant with univariate analysis were placed as independent variables (Tables 3, 4, 5 and 6).

Given the detected independent associations, LDL susceptibility to oxidation seems to be dependent of the lipid profile of the subject, [20] hemoglobin levels, [21, 22] and inflammatory markers levels. [23, 24] Expression and nuclear localization of the pro-inflammatory IL-1α are redox-dependent, [25] whereas, ox-LDL is a major inductor of NF-kB, which in turn stimulates IL-1 production. [26] Given that studies have shown that ox-LDL induces the expression of the NF-kB dependent pro-inflammatory chemokine IL-8, the negative association detected in the present study requires additional research. [27]

The antioxidative HDL capacity seems to be dependent of the gender, the relative CVD risk of the subject, as well as of the basal HDL-C levels. The association of the antioxidative potential of HDL and creatinine levels, in this non-renal impaired patient cohort, was surprising and needs further investigation.

In order to see whether there is a variable that determines the response of the HDL antioxidative capacity to rosuvastatin treatment, given that almost half of the study population did not show an increase, subjects were divided into 2 groups: responders (patients who showed a decrease in TRAP-ox-LDL) and non-responders (patients who did not show a decrease in TRAP-ox-LDL). The new ordinal variable (0/1-non-responders/responders) was tested for associations with the pre- and after-treatment variables with a binary logistic regression analysis. Only the ones that showed statistical significance are presented.

Male gender showed a statistically significant association with category 1 (responders), i.e. males were nearly 10 times more likely to have increased HDL antioxidative capacity in response to rosuvastatin therapy (Exp (B)=OR=9.350) (Table 7).

The SCORE relative risk showed a statistically significant association with category 1 (responders), with a negative B, i.e. those subjects with lower basal relative risk were more likely to show increased antioxidative HDL potential. Additionally, the analysis showed that the likelihood that rosuvastatin will increase the HDL antioxidative potential is nearly sixty-six percent higher in those patients whose CV risk reduction increases with treatment (Table 8). This hypothesis remains to be tested in larger patient cohorts.

This research is part of a national clinical study entitled: Rosuvastatin effects on lipoprotein proteomics in hyperlipidemic patients: new biomarker identification for treatment efficacy monitoring.

Limitations of the study

The small sample size might be a reason for omitting possible statistically significant associations that would appear with a larger sample size.

CONCLUSION

In conclusion, rosuvastatin was associated with beneficial effects on the LDL susceptibility to oxidation, but it did not show an effect on the antioxidative potential of HDL. In the broader sense, this study suggests the need of a comprehensive approach when investigating oxidative

stress and LDL/HDL functions, especially in low-to-medium CVD risk subjects.

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Резиме

АНТИОКСИДАТИВНИТЕ ЕФЕКТИ НА РОСУВАСТАТИНОТ КАЈ СУБЈЕКТИ СО НИЗОК ДО УМЕРЕН КАРДИОВАСКУЛАРЕН РИЗИК

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Заднина: Иако постојат бројни клинички докази што ја поддржуваат оксидативната КВБ-хипотеза, малку е познато за ефектот на статините врз LDL/HDL-оксидативната функционалност. Оттука, целта на оваа студија беше да се евалуираат антиоксидативните ефекти на росувастатинот,преку следење на подложноста на LDL на оксидација и антиоксидативниот HDL потенцијал кај субјекти со низок до умерен КВ-ризик.

Методи: Во студијата беа вклучени 40 возрасни амбулантски пациенти (53,8 ± 10,9 години, 27 жени и 13 мажи). Беа собирани податоци од историите на пациентите, физичкиот преглед и крвните примероци, според дизајнот на студијата. Субјектите беа поставени на терапија со росувастатин 20 мг/ден. Беа следени традиционалните ризик-фактори/индикатори, липидните параметри, инфламаторните/имуните маркери, подложност на LDL на оксидација и HDL-антиоксидативниот потенцијал. Следените обележја беа статистички анализирани со t-тест, Chi-square тест, еднонасочна ANOVA, Mann-Whitney и Kruskal-Wallis тестови. Беа направени мултиваријантни логистички регресиски анализи. Резултатите се сметаа за статистички значајни при $p \leq 0,05$.

Резултати: 67 % од испитаниците покажаа пониска подложност на LDL на оксидација по третманот со росувастатин ($p = 0,03$), без значаен ефект врз базалната LDL-оксидација и lag-времето. Сите три маркери на LDL-оксидацијата покажаа асоцијации со липидниот профил на субјектите, нивоата на хемоглобин и нивоата на IL-1α и IL-8 проинфламаторните маркери. 53 % од испитаниците покажаа повисок антиоксидативен капацитет на HDL по третманот, иако без статистичка значајност ($p = 0,07$). Поголема веројатност за зголемен антиоксидативен HDL-потенцијал по третманот со росувастатин беше забележана кај машкиот пол ($OR = 9,350$; $p = 0,010$) и кај субјектите што постигнаа пониски нивоа на релативен КВ-ризик по третманот (повисока КВ ризик-редукција) ($OR = 0,338$; $p = 0,027$).

Заклучоци: Оваа студија укажува на потребата од паралелно испитување на оксидативниот стрес и LDL/HDL-функционалноста, особено кај субјектите со низок до умерен КВ-ризик.

Клучни зборови: КВ-ризик, росувастатин, LDL-оксидација, антиоксидативен капацитет на HDL