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Effects of atorvastatin and rosuvastatin on thromboxane-dependent platelet activation and oxidative stress in hypercholesterolemia

Luca Puccetti[a,1], Francesca Santilli[b,1], Anna Laura Pasqui[a], Stefano Lattanzio[b], Rossella Liani[b], Federica Ciani[c], Elisabetta Ferrante[b], Giovanni Ciabattoni[b], Francesca Scarpini[a], Anna Ghezzi[a], Alberto Auteri[a], Giovanni Davì[b,*]

[a] Center for Atherosclerosis Research, University of Siena, v.le Bracci, 53100 Siena, Italy
[b] Center of Excellence on Aging, "G. d'Annunzio" University of Chieti, via Colle dell'Ara, 66013 Chieti, Italy
[c] Unit of Neuro-metabolic Diseases, Meyer Children Hospital, v.le Pieraccini, 50139 Florence, Italy

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ABSTRACT

Objectives: We examined the time-dependent effects of atorvastatin and rosuvastatin on *in vivo* oxidative stress and platelet activation, to assess whether these phenomena are related to any pleiotropic effect of any statin or to their LDL-lowering effect. We also asked whether the presence of specific allele frequencies in carriers of the 3′UTR/lectin-like oxidized LDL receptor-1 (LOX-1) polymorphism may influence the effect of either statin.
Methods: We included 60 hypercholesterolemic subjects, previously screened for LOX-1 3′UTR polymorphism, randomized, according to genetic profile (15 T and 15 C carriers for each arm), to atorvastatin 20 mg/day or rosuvastatin 10 mg/day.
Results: After 8 weeks, atorvastatin and rosuvastatin were associated with comparable, significant reductions in LDL cholesterol (40.8% and 43.6%, respectively), plasma hs-CRP (9.5% vs. 13.8%), urinary 11-dehydro-thromboxane (TX) B_2 (38.9% vs. 27.1%) and 8-iso-prostaglandin (PG) $F_{2\alpha}$ (39.4% vs. 19.4%). The impact of rosuvastatin or atorvastatin on CRP, 8-iso-$PGF_{2\alpha}$, and 11-dehydro-TXB_2 did not differ according to the LOX-1 haplotype. On multiple regression analyses, only CRP and LDL were independent predictors of 11-dehydro-TXB_2, and only LDL was a significant predictor of 8-iso-$PGF_{2\alpha}$.
Conclusions: Both atorvastatin and rosuvastatin cause comparable reductions of thromboxane-dependent platelet activation, lipid peroxidation and inflammation. The presence of 3′UTR/LOX-1 polymorphism does not affect the changes induced by either statin.

1. Introduction

Increased incidence of atherothrombotic complications has been associated with hypercholesterolemia [1]. Thromboxane (TX) A_2 biosynthesis, as reflected by urinary 11-dehydro-TXB_2 excretion, is enhanced in the majority of hypercholesterolemic subjects [2]. Moreover, increased lipid peroxidation, as reflected by enhanced urinary excretion of F_2-isoprostanes, has been reported in association with hypercholesterolemia [3,4]. Oxidative stress, in particular oxidized LDL (OxLDL), might play a key role in atherogenic changes in the vascular wall. Lectin-like oxidized LDL receptor-1 (LOX-1), expressed in atherosclerotic lesions, actively incorporates oxLDL and their removal from circulation may exert beneficial effects on atherogenic plaque formation [5].

Several isoprostanes are formed by direct free radical attack on arachidonate in cell membranes, from which they are cleaved by phospholipases. Thus, they are used as markers of free radical generation in biological fluids, such as plasma or urine [6]. Between them, 8-iso-prostaglandin (PG) $F_{2\alpha}$ is an abundant F_2-isoprostane generated *in vivo* in humans, is formed in LDL and endowed with vasoconstrictive and platelet-activating properties [7].

In animal studies, HMG-CoA reductase inhibitors (statins) decrease vascular reactive oxygen species generation independently of cholesterol reduction [8,9]. In humans, statins may exert beneficial effects independently of LDL cholesterol lowering. In fact, in chronic heart failure patients, similar reductions in LDL cholesterol with simvastatin and ezetimibe result in different effects on endothelial function, improved only by simvastatin [10]. However,

* Corresponding author at: Center of Excellence on Aging, "G. d'Annunzio" University Foundation, Via Colle dell'Ara, 66013 Chieti, Italy.
Tel.: +39 0871 541312; fax: +39 0871 541261.
E-mail addresses: puccetti@unisi.it (L. Puccetti), f.santilli@unich.it (F. Santilli), pasquian@unisi.it (A.L. Pasqui), lattanziostefano@yahoo.it (S. Lattanzio), r.liani@unich.it (R. Liani), f.ciani@meyer.it (F. Ciani), e.ferrante@unich.it (E. Ferrante), g.ciabattoni@unich.it (G. Ciabattoni), francy717@inwind.it (F. Scarpini), auteri@unisi.it (A. Auteri), gdavi@unich.it (G. Davì).
[1] These authors equally contributed to this work.

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in patients with coronary artery disease, lipid lowering is more important than pleiotropic effects of statins for improvement in endothelial function [11].

Simvastatin causes significant reduction of both thromboxane biosynthesis and isoprostane generation [12,13]. Thus, we asked whether both phenomena are related to any pleiotropic effect of any statin or to their LDL-lowering effect. Since a dose–response with regard to clinical outcome has been previously reported [14], equivalent doses of statins on LDL lowering should be prescribed in humans with regard to effects on oxidative stress and thromboxane-dependent platelet activation.

Thus, the primary objective of the study was to evaluate the separate effects of two statins on *in vivo* thromboxane-dependent platelet activation. The secondary objective was to assess the potential determinants of enhanced platelet activation in this setting by evaluating the time-dependent effects of either statin on the lipid profile, on systemic inflammation and lipid peroxidation. Finally, to test the hypothesis that specific allele frequencies in carriers of LOX-1 may influence platelet activation changes induced by either statin, as previously described for atorvastatin when other platelet activation markers were employed [15], we randomized patients in the two treatment groups according to the 3′UTR/LOX-1 polymorphism.

2. Materials and methods

2.1. Patients

We included 60 hypercholesterolemic subjects [35 males, 25 females, mean age 51.4 ± 3.07 years] with a baseline serum cholesterol >5.18 mmol/L. To avoid confounding by other determinants of oxidant stress and platelet activation, subjects were excluded if they had a history or evidence of atherothrombotic diseases, diabetes mellitus, cigarette smoking, obesity or arterial hypertension. Patients requiring chronic non-steroidal anti-inflammatory drug therapy or low-dose aspirin were also excluded. Throughout the study the only admitted analgesic was paracetamol (up to 500 mg/day). Exclusion criteria included exposure to cholesterol-modulating drugs within the previous two months, as well as renal and hepatic disease, cancer, and pregnant or lactating women.

The hypercholesterolemic subjects, previously screened for LOX-1 3′UTR/T or C polymorphism, were randomized, according to genetic profile (15 T and 15 C carriers each treatment), to 1 of 2 treatments: atorvastatin (Pfizer, Italy) 20 mg/day, rosuvastatin (AstraZeneca, Italy) 10 mg/day, administered once daily in the evening, to achieve >20% reduction of total cholesterol after 8 weeks.

In each patient, statins were added to diet regimen (AHA step II ineffective after 6 weeks) according to the recommendations of the National Cholesterol Education Program Adult Treatment Panel-III (NCEP ATP-III) [16].

The study protocol was approved by the institutional ethics committee of the recruiting center and the study was carried out in accordance with the principles of the Declaration of Helsinki, as revised in 2004. Patients were informed of the investigational nature of the study and gave written informed consent to participate.

2.2. Study design

This was a prospective, randomized, double-blind study assessing and comparing the effects of two structurally different statins: atorvastatin and rosuvastatin.

The screening visit consisted of a questionnaire, fasting blood work, and an electrocardiogram. Eligible subjects, meeting entry criteria, were asked to return for a pretreatment visit. Seven to 14 days after this, patients returned for randomization in a 1:1 scheme to either receive atorvastatin 20 mg/day, or rosuvastatin 10 mg/day. Study assessment took place after 1, 2 and 8 weeks of therapy. Data obtained at each subsequent visit included fasting blood work, 12-h urine collection, adverse event reporting, and study medication return to assess compliance. Laboratory analyses included lipid analyses and assessment of biomarkers.

For biochemical methods and statistical analysis please see Supplementary material.

3. Results

3.1. Baseline characteristics and study completion

The baseline characteristics of participants are shown in Table 1. There were no significant differences among the 4 groups. There were no drop-outs over the course of the study.

3.2. Control of compliance and lipid-lowering efficacy of study treatment

We monitored plasma lipid levels as a control for the effects of both statins. The effects of study treatments on lipid levels are shown in Fig. 1. Atorvastatin determined a 31% [from 7.46 (7.20–7.51) to 4.92 (4.82–5.05) mmol/L, $P < 0.0001$] reduction of total cholesterol after 8 weeks of treatment. Rosuvastatin determined a 32.6% [from 7.46 (7.20–7.51) to 4.92 (4.82–5.05) mmol/L, $P < 0.0001$] reduction of total cholesterol after 8 weeks of treatment. These reductions were, according to the prespecified aim, >20% in each patient.

The reduction in total cholesterol was due to a 40.8% [from 5.65 (5.31–5.77) to 3.26 (3.14–3.39) mmol/L, $P < 0.0001$] reduction of LDL cholesterol by atorvastatin and to a 43.6% [from 5.77(5.41–5.85) to 3.16 (3.06–3.31) mmol/L, $P < 0.0001$] reduction of LDL cholesterol by rosuvastatin (Fig. 1A), whereas HDL cholesterol was significantly raised by atorvastatin [from 1.27 (1.22–1.32) mmol/L at baseline to 1.29 (1.24–1.35) mmol/L after 8 weeks, $P < 0.0001$] and by rosuvastatin [from 1.24 (1.16–1.35) mmol/L at baseline to 1.29 (1.24–1.45) mmol/L after 8 weeks, $P < 0.0001$].

The median increase at 60 days in HDL-C levels was significantly ($P = 0.023$) higher after rosuvastatin (+8.9%) in comparison to atorvastatin (+6%).

The effect of statins on triglyceride levels did not differ between the 2 treatment arms at any time point. Atorvastatin determined a 4.7% [from 1.08 (0.99–1.17) to 1.02 (0.95–1.08) mmol/L, $P < 0.0001$] reduction of triglyceride levels after 8 weeks of treatment. Rosuvastatin determined a 6.6% [from 1.02 (0.96–1.12) to 0.96 (0.90–1.06) mmol/L, $P < 0.0001$] reduction of triglyceride levels after 8 weeks of treatment.

Changes in these lipid levels were not influenced by LOX-1 3′UTR T or C polymorphism to any significant extent.

No significant differences in Apo A, Apo B, or the Apo B/Apo A ratio, were observed between rosuvastatin and atorvastatin treatment at any time point (Fig. 1B and data not shown). Apo B was significantly reduced vs. baseline as early as after 2 weeks of treatment (15.7% and 19.8% with atorvastatin and rosuvastatin, respectively, $P < 0.0001$), with further reductions thereafter (29.1% and 31.7%, $P < 0.0001$), whereas Apo A significantly increased only after 8 weeks (4.3% and 7.5%, $P < 0.001$). Overall, the median Apo B/Apo A ratio was significantly reduced as early as after 2 weeks of either statin (by 15.7% and 20.4%, respectively, $P < 0.0001$), with deeper reductions after 8 weeks (31.8% and 37.1%, $P < 0.0001$).

Table 1
Clinical characteristics of the patients.

Variable	Rosuvastatin (10 mg/day)		Atorvastatin (20 mg/day)	
	LOX-1 3′UTR/C (n = 15)	LOX-1 3′UTR/T (n = 15)	LOX-1 3′UTR/C (n = 15)	LOX-1 3′UTR/T (n = 15)
Male/female	9/6	8/7	9/6	9/6
Age (yr)	51.5 ± 2.4	50.6 ± 2.7	51.5 ± 3.8	52.1 ± 3.4
BMI (kg/m^2)	24.9 ± 0.9	24.3 ± 1.2	24.9 ± 1.3	24.4 ± 1.4
SBP (mmHg)	130.1 ± 9.1	128.5 ± 9.5	127.9 ± 9.7	128.5 ± 9.2
DBP (mmHg)	76.9 ± 4.8	76.8 ± 5.2	77.9 ± 4.7	76.1 ± 5.1
Glu (mmol/L)	5.07 ± 0.45	5.07 ± 0.49	5.12 ± 0.49	5.04 ± 0.48
Total Chol (mmol/L)	7.36 ± 0.23	7.38 ± 0.26	7.30 ± 0.23	7.30 ± 0.18
LDL-C (mmol/L)	5.65 ± 0.31	5.62 ± 0.28	5.57 ± 0.28	5.52 ± 0.23
HDL-C (mmol/L)	1.24 ± 0.10	1.27 ± 0.10	1.27 ± 0.07	1.27 ± 0.07
TG (mmol/L)	1.05 ± 0.09	1.05 ± 0.10	1.05 ± 0.10	1.11 ± 0.12

Values are mean ± standard deviation. $P > 0.05$ for all between-group comparisons, by χ^2 statistics or Fisher exact test for categorical variables, or one-way ANOVA with the Bonferroni correction for continuous variables.

Consistently, and unlike Apo A, Apo B was significantly higher in C as compared with T carriers both at baseline (median 1.465 g/L vs. 1.33 g/L, $P = 0.001$) and after 1 week of statin treatment, resulting in different Apo B/Apo A ratios at these time points, with C carriers demonstrating a 0.6 median % decrease from baseline in this ratio as compared to a 0.5% increase in T carriers ($P = 0.001$).

3.3. Effects of either statin on urinary levels of 11-dehydro-TXB$_2$ and 8-iso-PGF$_{2\alpha}$

The urinary excretion of 11-dehydro-TXB$_2$ throughout the various phases of the study is shown in Fig. 1C.

At baseline, the rate of excretion of 11-dehydro-TXB$_2$ averaged 1378 ± 684 pg/mg creatinine, consistent with our previous findings of enhanced thromboxane metabolite excretion in hypercholesterolemia [12]. Atorvastatin and rosuvastatin were associated with comparable (P = ns), significant reductions in 11-dehydro-TXB$_2$ at any time point.

Atorvastatin treatment was associated with a significant median reduction vs. baseline in 11-dehydro-TXB$_2$ as early as after 1 week (by 8.9%, $P = 0.03$), which was more pronounced at each subsequent time point, with an overall 38.9% median inhibition [from 1134 (874–1858) to 669 (489–1012) pg/mg creatinine, $P < 0.0001$] after 8 weeks. Rosuvastatin caused a significant median reduction in 11-dehydro-TXB$_2$ after 2 weeks ($P = 0.001$), with a 27.1% median reduction after 8 weeks [from 1370 (743–1950) to 892 (546–1148) pg/mg creatinine, $P < 0.0001$]. Urinary excretion of 8-iso-PGF$_{2\alpha}$ throughout the various phases of the study is shown in Fig. 1D.

At baseline, the rate of excretion of 8-iso-PGF$_{2\alpha}$ averaged 618 ± 278 pg/mg creatinine, consistent with previous findings of enhanced isoprostane excretion in hypercholesterolemia [13]. Atorvastatin and rosuvastatin were associated with compara-

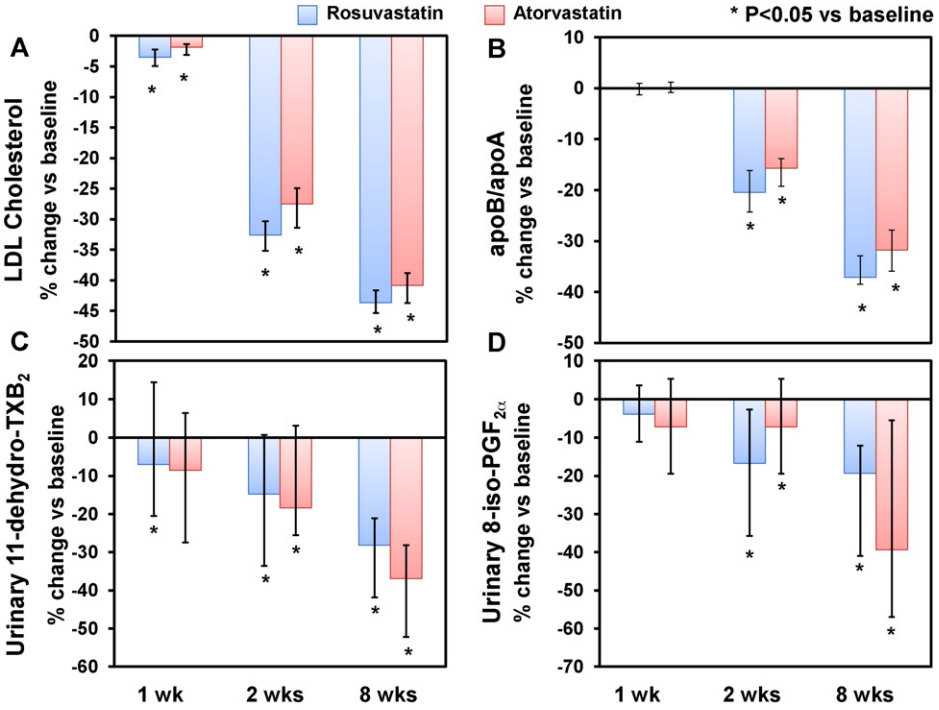

Fig. 1. Changes in LDL cholesterol, Apo B/Apo A, urinary 11-dehydro-TXB$_2$ and 8-iso-PGF$_{2\alpha}$ after rosuvastatin and atorvastatin therapy. Percent changes vs. baseline in LDL cholesterol (panel A), Apo B/Apo A (panel B), urinary 11-dehydro-TXB$_2$ (panel C) and 8-iso-PGF$_{2\alpha}$ (panel D) after 1 week, 2 weeks and 8 weeks of rosuvastatin (blue bars) or atorvastatin (red bars). *$P < 0.05$ vs. baseline. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

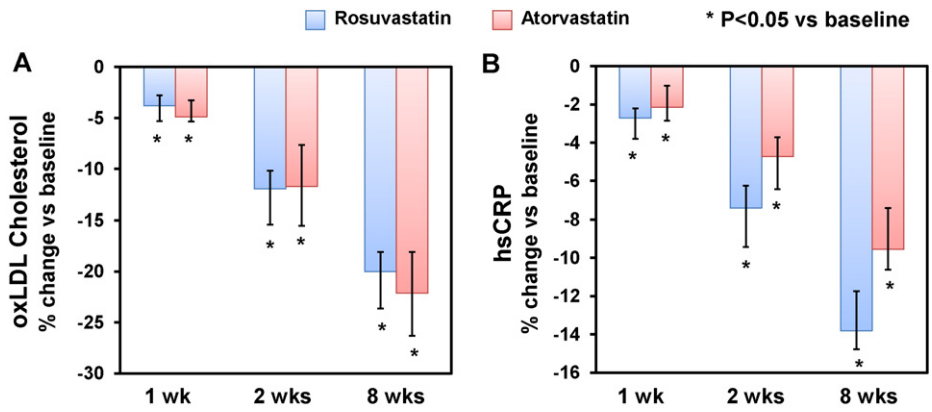

Fig. 2. Changes in oxLDL cholesterol and high-sensitivity (hs)CRP after rosuvastatin and atorvastatin therapy. Percent changes vs. baseline in oxLDL cholesterol (panel A), and hsCRP (panel B), after 1 week, 2 weeks and 8 weeks of rosuvastatin (blue bars) or atorvastatin (red bars). *$P < 0.05$ vs. baseline. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

ble (P = ns), significant reductions in 8-iso-$PGF_{2\alpha}$ at any time point.

Urinary 8-iso-$PGF_{2\alpha}$ excretion was significantly inhibited by either treatment after 2 weeks [median 7.2%, $P < 0.0001$ and 16.7%, $P = 0.001$ with atorvastatin and rosuvastatin, respectively] and 8 weeks [from 600 (344–804) to 338 (278–454), 39.4%, $P < 0.0001$ with atorvastatin and from 594 (404–772) to 369 (315–563), 19.4%, $P < 0.0001$ with rosuvastatin] but not after 1 week with any treatment.

3.4. Contribution of lipids to explaining 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ variability

In the whole group ($n = 60$), when all data throughout the study were pooled ($n = 240$), both 8-iso-$PGF_{2\alpha}$ (Rho = 0.30, $P < 0.0001$) and 11-dehydro-TXB_2 (Rho = 0.27, $P < 0.0001$) levels were directly correlated with LDL-C. Consistently, the % change vs. baseline in LDL cholesterol after 2 and 8 weeks was significantly related to the % change in 11-dehydro-TXB_2 (Rho = 0.35, $P < 0.0001$), 8-iso-$PGF_{2\alpha}$ (Rho = 0.32, $P < 0.0001$) and CRP (Rho = 0.71, $P < 0.0001$) (Table 2). Of note, the significant direct correlations observed between change in HDL or Apo B/Apo A ratio and change in 11-dehydro-TXB_2 in the whole group (Table 2), appear to be attributable to the only group of atorvastatin-treated patients (Rho = −0.45, $P < 0.0001$ and Rho = 0.47, $P < 0.0001$), with no significant correlation in the rosuvastatin group.

A significant direct correlation was also found between LDL and 11-dehydro-TXB_2% change vs. baseline at 1 (Rho = 0.485, $P = 0.007$) and 2 weeks (Rho = 0.405, $P = 0.026$) of rosuvastatin, but not of atorvastatin treatment.

Concerning lipid variables, on multiple linear regression analysis with 11-dehydro-TXB_2 (log transformed) as the dependent variable, only LDL-C was a significant predictor of 11-dehydro-TXB_2 (Beta = 0.22, SEM = 0.32, $P = 0.001$ and Beta = 0.204, SEM = 0.15, $P = 0.002$, $R^2 = 0.11$), independently of treatment, polymorphism, HDL, oxLDL, Apo A and Apo B.

Similarly, urinary 8-iso-$PGF_{2\alpha}$ correlated with LDL-C regardless of the haplotype (Rho = 0.37, $P < 0.0001$ in C carriers and Rho = 0.18, $P = 0.001$ in T carriers). Similarly, apo B/apo A ratio correlated with both 11-dehydro-TXB_2 (Rho = 0.22, $P = 0.013$ and Rho = 0.24, $P = 0.008$ in C and T carriers) and 8-iso-$PGF_{2\alpha}$ (Rho = 0.38, $P < 0.0001$ and Rho = 0.19, $P = 0.038$) regardless of the haplotype.

Moreover, multiple regression analysis with 8-iso-$PGF_{2\alpha}$ as the dependent variable revealed that only LDL (log-transformed) (Beta = 0.286, SEM = 0.13, $P < 0.0001$, $R^2 = 0.12$) was a significant predictor of 8-iso-$PGF_{2\alpha}$, independently of treatment, polymorphism, HDL, oxLDL, CRP, Apo A and Apo B.

3.5. Effects of either statin on other variables (plasma ox-LDL and CRP)

To assess whether a reduction in lipid peroxidation and platelet activation could also be reflected in the oxidation of LDL and chronic, systemic inflammation, we measured oxLDL and hsCRP throughout the various phases of the study (Supplemental table and Fig. 2).

Throughout the study, atorvastatin and rosuvastatin were associated with comparable, significant reductions vs. baseline in CRP and ox-LDL.

Significant reductions vs. baseline were observed as early as after 1 week of treatments for both variables (oxLDL: 4.8% and 3.8%, hsCRP: 2.1% and 2.7% with atorvastatin and rosuvastatin, respectively, $P < 0.0001$) with further reductions thereafter. After 8 weeks, atorvastatin and rosuvastatin caused a median inhibition of oxLDL by 22.1% [from 67 (60–75) to 54 (44.7–62.2) U/L, $P < 0.0001$] and 20% [from 65.5 (59.2–75.2) to 52 (44.7–62.2) U/L, $P < 0.0001$], respectively, and of hsCRP by 9.5% [$P < 0.0001$] and 13.8% [from 0.87 (0.81–0.94) to 0.75 (0.71–0.85) mg/L, $P < 0.0001$], respectively.

Ox-LDL levels were significantly higher at baseline in carriers of the T polymorphism as compared to C carriers [75 (70.7–79) vs. (60 (56.7–61.2) U/L, $P < 0.0001$], as previously reported in a different sample of hypercholesterolemic patients [15].

3.6. Potential influence of LOX-1 3′UTR polymorphism on the response to the randomized statin treatment

To evaluate the potential influence of the LOX-1 polymorphism on the response to either statin, the 60 patients were stratified in 4 groups ($n = 15$ each) according to the assigned treatment and the LOX-1 polymorphism. The impact of rosuvastatin or atorvastatin on 11-dehydro-TXB_2, and other variables such as hsCRP and 8-iso-$PGF_{2\alpha}$, changes did not differ according to the polymorphism. In fact, no differences in these variables were observed among the 4 groups at any time point throughout the treatment period (Supplemental table and Fig. 3). In contrast, the % inhibition in LDL-C after 14 days was more pronounced in T carrier patients treated with rosuvastatin (by 34.1%) than in those treated with atorvastatin (25.7%), whereas the effect of the two treatments did not differ significantly in the C carriers (32.3% vs. 29.1%, respectively, Supplemental figure). In addition, at any time point the % inhibition in oxLDL was significantly deeper in C than T carriers with either

Table 2
Correlation (r^2) between changes over 14 and 60 days of statin therapy in levels of lipoproteins and associated parameters.

	LDL	HDL	OxLDL	hsCRP	8-iso-$PGF_{2\alpha}$	11-dehydro-TXB_2	
Total cholesterol	0.97 ($P<0.0001$)	−0.57 ($P<0.0001$)	0.67 ($P<0.0001$)	0.69 ($P<0.0001$)	0.34 ($P<0.0001$)	0.38 ($P<0.0001$)	0.85 ($P<0.0001$)
LDL	–	−0.63 ($P<0.0001$)	0.68 ($P<0.0001$)	0.71 ($P<0.0001$)	0.32 ($P<0.0001$)	0.35 ($P<0.0001$)	0.86 ($P<0.0001$)
HDL		–	−0.46 ($P<0.0001$)	−0.67 ($P<0.0001$)	0.34 ($P<0.0001$)	−0.16 ($P=0.0.07$)	−0.62 ($P<0.0001$)
OxLDL			–	0.59 ($P<0.0001$)	0.29 ($P<0.0001$)	−0.27 ($P=0.002$)	0.75 ($P<0.0001$)
hsCRP				–	0.12 ($P=0.21$)	0.29 ($P=0.001$)	0.69 ($P<0.0001$)
8-iso-$PGF_{2\alpha}$					–	0.059 ($P=0.55$)	0.27 ($P=0.005$)
11-dehydro-TXB_2						–	0.33 ($P<0.0001$)

treatment, with no difference between rosuvastatin and atorvastatin within carriers of the same LOX-1 haplotype (23.3% vs. 26.2% in C carriers treated with rosuvastatin and atorvastatin, 18.2% vs. 18.2% in T carriers) (Fig. 3). Finally, Apo B was significantly higher at baseline in C than T carriers, regardless of the treatment group, and the difference was maintained after 1 week, with no further difference across groups thereafter (data not shown).

3.7. Associations

In the whole group, pooling all data throughout the study, obtained at baseline and under treatment ($n=240$), 11-dehydro-TXB_2 was significantly correlated with both oxLDL (Rho = 0.14, $P=0.027$) and hs-CRP (Rho = 0.29, $P<0.0001$) (all log transformed) and 8-iso-$PGF_{2\alpha}$ was correlated with oxLDL (Rho = 0.17, $P=0.007$).

Percent changes vs. baseline in hsCRP levels associated with 8 weeks of either treatment were correlated with changes in LDL (Rho = 0.33, $P=0.01$), HDL (Rho = −0.59, $P<0.0001$), Apo B/Apo A ratio (Rho = 0.37, $P=0.003$). The latter was also significantly correlated with percent change in LDL (0.54, $P<0.0001$), HDL (Rho = −0.43, $P<0.0001$), oxLDL (Rho = 0.29, $P=0.02$).

Moreover, in the 30 patients treated with rosuvastatin but not atorvastatin, the % change in oxLDL after 8 weeks correlated with that in both HDL (Rho = −0.52, $P=0.003$) and hsCRP (Rho = 0.40, $P=0.028$). In contrast, atorvastatin treatment experienced parallel reductions after 8 weeks in oxLDL and Apo B/Apo A ratio (Rho = 0.39, $P=0.03$).

Finally, with either treatment, correlations in percent changes vs. baseline over 2 and 8 weeks of treatment were observed among the study variables (Table 2).

3.8. Contribution of other variables to explaining 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ variability

Regardless of the employed statin, only C carriers exhibited a significant direct correlation between hsCRP and both 11-dehydro-TXB_2 (Rho = 0.51, $P<0.0001$) and 8-iso-$PGF_{2\alpha}$ (Rho = 0.23, $P=0.011$).

On multiple linear regression analysis with 11-dehydro-TXB_2 (log transformed) as the dependent variable, only hs-CRP and LDL-C (see Section 3.4) were significant predictors of 11-dehydro-TXB_2 (Beta = 0.22, SEM = 0.32, $P=0.001$ and Beta = 0.204, SEM = 0.15, $P=0.002$, $R^2=0.11$), independently of treatment, polymorphism, HDL, oxLDL, Apo A and Apo B.

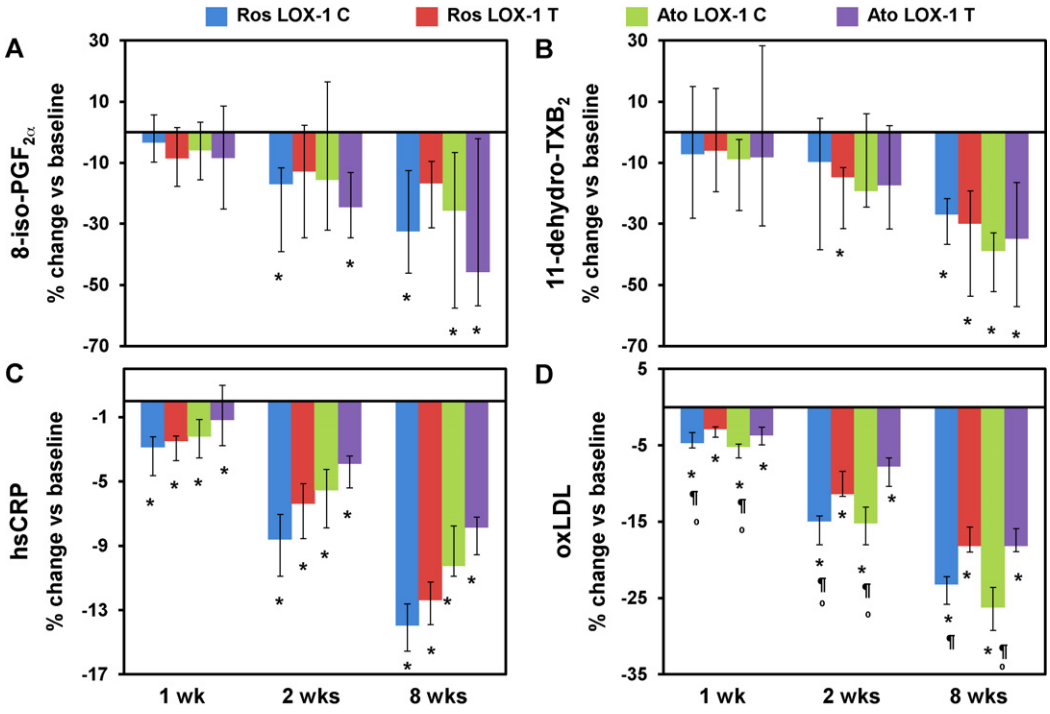

Fig. 3. Impact of rosuvastatin or atorvastatin on 8-iso-$PGF_{2\alpha}$ (panel A), 11-dehydro-TXB_2 (panel B), hsCRP (panel C), oxidized-LDL (panel D) changes according to the 3′UTR lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) polymorphism. Percent changes vs. baseline in urinary 8-iso-$PGF_{2\alpha}$ (panel A), urinary 11-dehydro-TXB_2 (panel B), hsCRP (panel C) and oxLDL cholesterol (panel D) in C and T carriers of LOX-1 polymorphism treated with rosuvastatin (blue and red bars, respectively) or atorvastatin (green and violet bars), after 1 week, 2 weeks and 8 weeks of treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

4. Discussion

Previous studies by our group have provided biochemical evidence of enhanced thromboxane biosynthesis [2] and subsequently demonstrated the efficacy of simvastatin to downregulate this phenomenon [12].

TXA_2 biosynthesis is enhanced in the majority of patients with type IIa hypercholesterolemia; this is, at least in part, a consequence of abnormal cholesterol levels, as suggested by the correlation between the two [2]. *in vivo* formation of the F2-isoprostane 8-iso-$PGF_{2\alpha}$ is enhanced in the vast majority of patients with hypercholesterolemia [3,4]. This provides an aspirin-insensitive mechanism possibly linking lipid peroxidation to amplification of platelet activation in the setting of hypercholesterolemia.

In this study, we confirm the persistent increase in F2-isoprostane formation as well as in thromboxane biosynthesis previously documented in hypercholesterolemia [2–6,12].

A cholesterol-lowering regimen with simvastatin substantially reduces indices of enhanced lipid peroxidation *in vivo* as well as enhanced platelet thromboxane biosynthesis [12,13]. In order to evaluate whether both phenomena are related to any pleiotropic effect of any inhibitor of HMG-CoA reductase (statin) or their LDL-lowering effect, we performed a randomized, double blind study, comparing the effects of two structurally different statins: atorvastatin and rosuvastatin at fully comparable lipid-lowering efficacy dosage.

In our study, both atorvastatin and rosuvastatin cause a comparable, significant reduction of thromboxane-dependent platelet activation and lipid peroxidation mainly related to decreased LDL-C. However these findings are paralleled by directionally similar changes in plasma CRP and oxLDL. Because ox-LDL levels reflect oxidative events within a lipid pool that is highly relevant to atherosclerosis [17], these data complement data derived from peroxidative markers in the total lipid pool (F_2-isoprostanes). Moreover, we also evaluated the Apo B/Apo A ratio, which has been recently shown to be superior to lipoprotein measurement for risk prediction of cardiovascular disorders and proposed as the test of choice to be introduced into routine clinical practice [18,19]. The significant correlation between percent reduction in Apo B/Apo A ratio and in both isoprostane formation and thromboxane biosynthesis further substantiates the importance of modulation of this pathogenetic cascade in preventing atherothrombosis.

Our study as well as previous studies examined with conflicting results the cause-and-effect relation between the lipid-lowering effect of the drug and its alleged anti-platelet or antioxidant effects [10,12]. Lipid-lowering appears to be more important than pleiotropic effects for improvement in endothelial function and inflammatory markers in patients with dysglycemia and coronary artery disease [11]. A rapid effect on endothelium-dependent vasodilation within 1 or 3 days of treatment has been shown for simvastatin and an early decrease (within 3 days) of urinary 8-iso-$PGF_{2\alpha}$ has been described for atorvastatin [20,21], consistent with the concept that statins may also exert pleiotropic effects in humans. However, we did observe significant changes in urinary excretion rates of 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 only after 2 and 8 weeks of either treatment in parallel with a sharp reduction in LDL-cholesterol levels.

A number of findings argue that these changes are a consequence of modifications in the plasma lipid pattern induced by any statin rather than a direct effect of the drug. These include (1) the consistent time-related patterns of lipid-lowering and antiplatelet or antioxidant effects, (2) the highly significant correlation between the two, and (3) the lack of any direct effect of simvastatin or its main biologically active metabolite on platelet thromboxane production *in vitro* [12].

Both statins exert similar and significant reductions vs. baseline in hsCRP levels after 8 weeks, paralleled by significant reductions in lipid profile and significantly related to changes in thromboxane metabolite excretion. Our results are consistent with the recent findings of the Jupiter trial, showing, in apparently healthy subjects with high baseline hsCRP, that concurrent reductions in both LDL and hsCRP with rosuvastatin treatment result in a significantly reduced incidence of major cardiovascular events [22]. Thus, regardless of baseline LDL-cholesterol levels, we can speculate that the magnitude of the benefit associated with statin therapy correlates in part with the achieved high-sensitivity CRP level together with the reduction of *in vivo* lipid peroxidation and thromboxane-dependent platelet activation.

Recently, modifications of platelet-associated ox-LDL were detected in subjects treated with statins and related with decreased platelet expression of the specific ox-LDL receptors CD36 and LOX-1 [17]. LOX-1 may play a role in atherothrombosis mediating platelet–endothelium interactions downregulating endothelial nitric oxide synthase [23,24]. Moreover, a close relationship has been found between coronary artery disease and the 3′UTR (T allele) LOX-1 polymorphism [25]. In our study, we evaluated whether specific allele frequencies in carriers of LOX-1 polymorphism may influence thromboxane-dependent platelet activation changes and *in vivo* lipid peroxidation induced by the two different statins studied. After rosuvastatin or atorvastatin, 11-dehydro-TXB_2, 8-iso-$PGF_{2\alpha}$ and CRP changes did not differ according to the polymorphism. In fact, no differences in these variables were observed among the study groups at any time point throughout the treatment period. This discrepancy with previous observations for atorvastatin when platelet activity was evaluated by P-selectin expression [15] could indicate that a complete platelet deactivation requires a significant LDL-C reduction.

In summary, the results of this study enforce the concept that LDL cholesterol levels are a major correlate and possibly a determinant of enhanced thromboxane biosynthesis and F_2-isoprostane formation as well as systemic inflammation in hypercholesterolemic subjects. Any statin treatment is a powerful means to reduce both enhanced platelet activation and lipid peroxidation in these subjects. These effects may contribute to the overall impact of statin therapy on the thrombotic risk. 3′UTR/T polymorphism did not seem to affect *in vivo* changes in platelet activation and lipid peroxidation induced by atorvastatin or rosuvastatin when evaluated by COX-1 related products.

Finally, measurement of thromboxane metabolite excretion has a remarkable track record of successful characterization of where to use and how to use aspirin for the prevention of atherothrombosis [26].

Because of incomplete inhibition of thromboxane biosynthesis associated with statin therapy, one should further explore the need for concomitant therapy with low-dose aspirin in the subset of hypercholesterolemic patients with persistent thromboxane-dependent platelet activation despite successful LDL-lowering effect obtained by any statin treatment.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.10.006.

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