

Low-density lipoprotein reduction by simvastatin is accompanied by angiotensin II type 1 receptor downregulation, reduced oxidative stress, and improved endothelial function in patients with stable coronary artery disease

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Objectives We tested the hypothesis that low-density lipoprotein-cholesterol induces angiotensin II type 1 receptor upregulation that, in turn, accounts for enhanced oxidative stress, and the subsequent endothelial dysfunction in patients with coronary artery disease.

Methods Brachial artery flow-mediated vasodilation, serum 8-*iso*-prostaglandin F_{2α} (8-isoprostane), and angiotensin II type 1 receptor density on platelets were measured in 19 patients with coronary artery disease, at entry and after 12 weeks of simvastatin therapy, 40 mg/day.

Results At entry there was a significant linear correlation between: angiotensin II type 1 receptor density and plasma low-density lipoprotein-cholesterol; plasma 8-isoprostane and angiotensin II type 1 receptor density; and flow-mediated vasodilation and 8-isoprostane. Simvastatin therapy reduced low-density lipoprotein-cholesterol, downregulated angiotensin II type 1 receptor, decreased 8-isoprostane, and improved flow-mediated vasodilation. The slopes of the presimvastatin and the postsimvastatin angiotensin II type 1 receptor/low-density lipoprotein relationships did not significantly differ, indicating that simvastatin caused a downregulation of angiotensin II type 1 receptor that could be predicted by the low-density lipoprotein reduction. In addition, simvastatin-mediated changes in 8-isoprostane could be predicted by angiotensin II type 1 receptor downregulation, and flow-mediated vasodilation improvement by changes in

8-isoprostane. A significant correlation existed between simvastatin-mediated changes in 8-isoprostane and angiotensin II type 1 receptor.

Conclusion The results of this study are consistent with the hypothesis that in coronary artery disease, the impairment of endothelial function is strongly associated with oxidative stress, oxidative stress with cellular angiotensin II type 1 receptor density, and the angiotensin II type 1 receptor density with low-density lipoprotein-cholesterol, suggesting cause-effect relationships between these variables. In support for this notion, these baseline associations were not significantly disturbed by low-density lipoprotein-lowering therapy with simvastatin. *Coron Artery Dis* 18:201–209 © 2007 Lippincott Williams & Wilkins.

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Keywords: angiotensin, coronary artery disease, endothelial dysfunction, hypercholesterolemia, oxidative stress, receptors, statin

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Introduction

Endothelial dysfunction is an essential step in the development of atherosclerosis and coronary artery disease (CAD). Impaired endothelium-dependent and nitric oxide (NO)-dependent vasodilation is a hallmark of endothelial dysfunction [1–3]. In humans, endothelial dysfunction comprises both coronary and peripheral arteries, correlates with risk factor profile, and predicts cardiac events [3,4]. Moreover, interventions improving endothelial function reduce cardiovascular events in patients with CAD and hypercholesterolemia [5–7].

Increased superoxide production contributes to oxidative stress, reduced NO bioavailability, and endothelial dysfunction in animal models of vascular disease [1,3,8]. In humans, the contribution of free radicals to endothelial dysfunction is an independent predictor of adverse cardiovascular risk [9]. In human blood vessels [10], including coronary arteries [11], nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidase is the principal source of superoxide, and is functionally related to clinical risk factors and systemic endothelial dysfunction [12]. Angiotensin II activates

NADPH oxidase, via angiotensin II type 1-receptor (AT1R) stimulation [13]. AT1R blockade [14–16] and NADPH oxidase inhibition by 3-hydroxy-3-methylglutaryl coenzyme A inhibitors (statins) [16–18] have been shown to inhibit vascular superoxide formation and attenuate endothelial dysfunction in animal models. Previous studies have shown that low-density lipoprotein (LDL) mediates AT1R upregulation in isolated vascular smooth muscle cells [19], and that hypercholesterolemic rabbits [20] and men [21] display an enhanced expression of AT1R. In addition, cholesterol-lowering therapy with statins downregulates AT1R in hypercholesterolemic men [21].

These interactions lead to the hypothesis that hypercholesterolemia induces AT1R overexpression that, in turn, increases vascular superoxide production, oxidative stress, and the subsequent endothelial dysfunction [1,22,23]. In humans, the interactions between LDL-cholesterol, AT1R, oxidative stress, and endothelial dysfunction have not been studied in the same group of patients. Therefore, this study examined in CAD patients: (i) whether endothelial dysfunction is related to oxidative stress, AT1R density, and LDL-cholesterol, as predicted by the hypothesis and (ii) whether cholesterol-lowering therapy with statin improves endothelial function by LDL reduction and subsequent downregulation of AT1R, and attenuation of oxidative stress.

Methods

Participants

Nineteen patients with proven CAD (angiography or a history of infarction) were enrolled (Table 1). Exclusion criteria included unstable angina, recent myocardial infarction or coronary revascularization (< 6 months), significant valvular heart disease, heart failure, uncontrolled hypertension, significant endocrine, hepatic, renal or inflammatory disease, history of drug or alcohol abuse, and history of treatment with lipid-lowering drugs for at least 4 weeks before the study. The local ethics committee approved the study, and patients gave written informed consent.

Protocols

The patients were asked to refrain from eating food, drinking alcohol and coffee, smoking, and taking short-acting nitrates for at least 12 h before the study. Calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and long-acting nitrates were withheld for at least 48 h before the study. The patients underwent physical examination, electrocardiography, blood pressure measurement, vascular function measurement, and blood sampling for biochemical determinations, and for the assay of AT1R density on platelets. These determinations were performed at entry and after 12 weeks of simvastatin therapy, 40 mg/day.

Table 1 Characteristics of 19 enrolled patients at entry and after 12 weeks of simvastatin therapy

Variable	Baseline	Simvastatin	P value
Age (year)	62.3 ± 1.6		
Sex (M/F)	15/4		
Body mass index (kg/m ²)	27.9 ± 0.7		
Risk factors (N)			
Hypercholesterolemia	14		
History of hypertension	15		
History of diabetes	1		
Current smoking	1		
History of myocardial infarction	9		
Cardiovascular medication (N)			
Aspirin	19	19	
Beta-blockers	18	18	
ACE-I	12	12	
Diuretics	4	4	
Long-acting nitrates	4	4	
Calcium antagonists	4	4	
Systolic blood pressure (mmHg)	125.7 ± 4.4	124.6 ± 3.9	NS
Diastolic blood pressure (mmHg)	79.4 ± 2.2	78.5 ± 2.0	NS
Heart rate (bpm)	68.0 ± 1.8	66.7 ± 1.5	NS
Lipids (mg/dl)			
Total cholesterol	234 ± 9.5	169 ± 7.4	<0.0001
LDL-cholesterol	151 ± 9.1	90 ± 6.3	<0.0001
HDL-cholesterol	50 ± 2.4	51 ± 2.7	NS
Triglycerides	163 ± 12.2	150 ± 16.3	NS
High-sensitive CRP (mg/l)	3.8 (1.7–8.2)	1.8 (1.0–4.8)	NS
ASPAT (IU)	27.4 ± 2.1	29.1 ± 1.8	NS
ALAT (IU)	31.6 ± 3.3	37.9 ± 3.5	NS
AT1Rs/platelet (B_{max})	15.0 ± 1.1	7.8 ± 0.6	<0.0001
AT1R affinity (K_d) (nmol/l)	2.0 ± 0.5	2.3 ± 0.5	NS
Platelet count ($\times 10^9/l$)	178 ± 45	183 ± 52	NS
Platelet volume (fl)	7.6 ± 0.3	7.6 ± 0.2	NS
8-Isoprostane (pg/ml)	40.2 ± 3.2	27.5 ± 2.5	0.017
Brachial artery diameter (mm)			
Basal	3.70 ± 0.12	3.64 ± 0.12	NS
During reactive hyperemia	4.02 ± 0.14	4.04 ± 0.14	NS
After nitroglycerin administration	4.29 ± 1.4	4.35 ± 0.15	NS
Percentage change during reactive hyperemia	8.9 ± 1.1	11.0 ± 0.9	0.0317
Percentage change after nitroglycerin	17.8 ± 2.7	15.8 ± 2.0	NS

Continuous variables are expressed as mean ± SEM, except for C-reactive protein, which are medians (first and third quartiles). Differences between baseline and postsimvastatin variables were analyzed by paired *t*-test or, as in case of C-reactive protein, by Wilcoxon matched pairs test.

ACE-I, angiotensin-converting enzyme I; ALAT, alanine aminotransferase; ASPAT, aspartate aminotransferase; AT1R, angiotensin II type 1 receptor; CRP, C-reactive protein; F, female; HDL, high-density lipoprotein; LDL, low-density lipoprotein; M, male; NS, non significant

Vascular function

Flow-mediated vasodilation (FMD) was assessed on left brachial artery by ischemia-induced reactive hyperemia [24]. Studies were performed between 7:30 and 9:00 h in a 23°C temperature-controlled room. Patients rested for at least 30 min in the supine position to establish a stable baseline. Brachial artery diameter was measured using a 10 MHz linear phase arrayed ultrasound transducer attached to a GE Vivid FiVe ultrasound machine (General Electric, Horten, Norway). Scans were taken proximal to the bifurcation of the brachial and the ulnar artery, at end-diastole, coincident with R-wave on electrocardiogram (ECG). Vessel diameter was measured at fixed distance from an anatomical marker, over a 1–2-cm segment. Measurements were taken at baseline and

every 15 s between 30 and 120 s after cuff deflation completing suprasystolic compression (240 mmHg) of the left upper arm for 4.5 min. After 15 min of rest and a baseline recording, 0.5 mg nitroglycerin was given sublingually to assess nitrate-mediated (NMD), endothelium-independent vasodilation. Brachial artery diameter was measured every 20 s between 2 and 4.5 min and it was found, as it was established by others [24] and confirmed in our preliminary studies, that peak vasodilation occurred 3–4 min after nitroglycerin administration. Scans were recorded for later analysis. FMD and NMD were defined as the maximum percentage increase in the artery diameter mediated by reactive hyperemia and nitroglycerin, respectively, over the baseline value. Two blinded readers took these measurements, unaware of the patient's clinical characteristics and treatment. Inter-observer and intraobserver coefficients of variation [standard deviation (SD)/mean diameter] for vessel diameter measurements were 2.56 and 2.17%, respectively.

Radioligand binding assay

Blood (20 ml) was drawn into tubes containing anti-coagulant citrate dextrose (ACD) and stored on ice. Platelet-rich supernatant was obtained by centrifugation (4°C for 10 min) at 1100g. The platelet pellet was resuspended and washed twice in ACD, and platelets were counted. Platelets were transferred to a 96-well filter Plate for high-throughput separations (MultiScreen; Millipore, Massachusetts, USA) (100 µl of the suspension containing 2×10^7 to 7×10^7 platelets/well) and incubated with increasing concentrations of ^{125}I -angiotensin II (0.2–2 nmol/l; Amersham, Little Chalfont, UK). Preliminary experiments revealed that in the range of 5×10^6 to 10^8 platelets/well, the final estimate of the AT1R density was not affected by the platelet count. Nonspecific binding was assessed in the presence of 10 µmol/l losartan (a gift of Merck, Whitehouse Station, New Jersey, USA). Incubation was performed at room temperature for 120 min. Reaction was terminated by addition of ice-cold buffer containing 0.5% bovine serum albumin (BSA) and 10 mmol/l Tris-HCl, and subsequent vacuum filtering. The filters were cut out and bound radioactivity was measured.

Assay of 8-isoprostane and C-reactive protein

8-*iso*-Prostaglandin $F_{2\alpha}$ (8-isoprostane) was measured as an index of cellular oxidative stress [25], in duplicate, by ELISA kit (Cayman Chemical, Ann Arbor, Michigan, USA). C-reactive protein (CRP) was determined by turbidimetric immunoassay.

Statistical analysis

Continuous variables were tested for normal distribution with the Shapiro–Wilk test and were expressed as mean \pm SEM if normally distributed or otherwise by median with 25 and 75 percentiles. Differences between

prestatin and poststatin values were analyzed by paired *t*-test or Wilcoxon matched pairs test, respectively. Univariate associations between the study variables were analyzed by calculating Pearson's or Spearman's correlation coefficients, when appropriate.

Poststatin relationships between parameters were compared with prestatin ones by calculating predicted parameters using regression equation ($y = ax + b$) established for baseline relationships and comparing them with authentic poststatin parameters using Dunnett's test. A *P* value less than 0.05 was considered significant.

Results

Baseline characteristics

Table 1 presents demographic and clinical characteristics of 19 enrolled patients. Their total serum cholesterol level ranged from 143 to 320 mg/dl and LDL-cholesterol from 75 to 230 mg/dl. In five individuals, total cholesterol was less than 200 mg/dl and LDL-cholesterol less than 130 mg/dl, and they were considered normocholesterolemic.

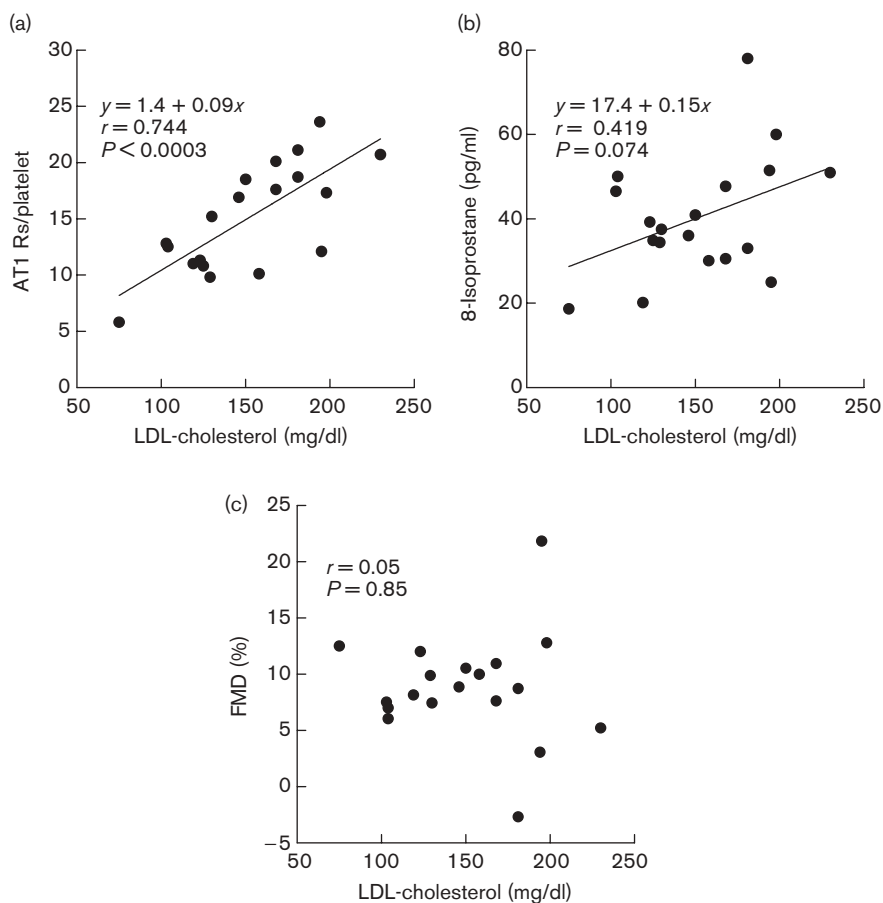
On univariate analysis, AT1R density (B_{max}), measured by radioligand binding assay in isolated platelets, was strongly dependent on concentration of LDL-cholesterol ($r = 0.744$, $P < 0.0003$; Fig. 1a). The ligand affinity (k_d), however, correlated neither with LDL-cholesterol ($r = 0.14$, $P = 0.57$) nor with B_{max} ($r = 0.386$, $P = 0.102$). B_{max} was expressed per single platelet because mean platelet volume, and therefore their size, was similar in all studied individuals, did not correlate with LDL-cholesterol ($r = 0.04$), and was not changed by simvastatin (Table 1).

Although AT1R density strongly correlated with LDL-cholesterol, there was only a trend for the correlation between serum 8-isoprostane and LDL-cholesterol, and no correlation between FMD and LDL-cholesterol (Fig. 1).

Serum 8-isoprostane positively correlated with AT1R density, and there was only a trend for the correlation between FMD and AT1R (Fig. 2). Furthermore, FMD inversely correlated with serum 8-isoprostane (Fig. 3). High-density lipoprotein (HDL)-cholesterol, triglycerides, or CRP were not significantly correlated with LDL-cholesterol, AT1R density, 8-isoprostane, and FMD (not shown).

On multivariate regression analysis, triglycerides, HDL-cholesterol, LDL-cholesterol, AT1R density, and oxidative stress (8-isoprostane) were tested as independent markers of endothelial function. Only oxidative stress significantly predicted FMD ($r = 0.758$, $P < 0.02$; Table 2). Likewise, AT1R density appeared to be the sole predictor of oxidative

Fig. 1



Linear regression analysis of baseline plasma low-density lipoprotein (LDL) concentration and (a) platelet angiotensin II type 1 receptor (AT1R) density ($y = 1.4 + 0.09x$, $r = 0.744$, $P < 0.0003$), (b) plasma concentration of 8-isoprostane ($y = 17.4 + 0.15x$, $r = 0.419$, $P = 0.074$), and (c) flow-mediated vasodilation (FMD) ($r = 0.05$, $P = 0.85$) in 19 patients with coronary artery disease.

stress ($r = 0.685$, $P < 0.003$), and LDL – the sole predictor of AT1R density ($r = 0.731$, $P < 0.001$).

Simvastatin therapy

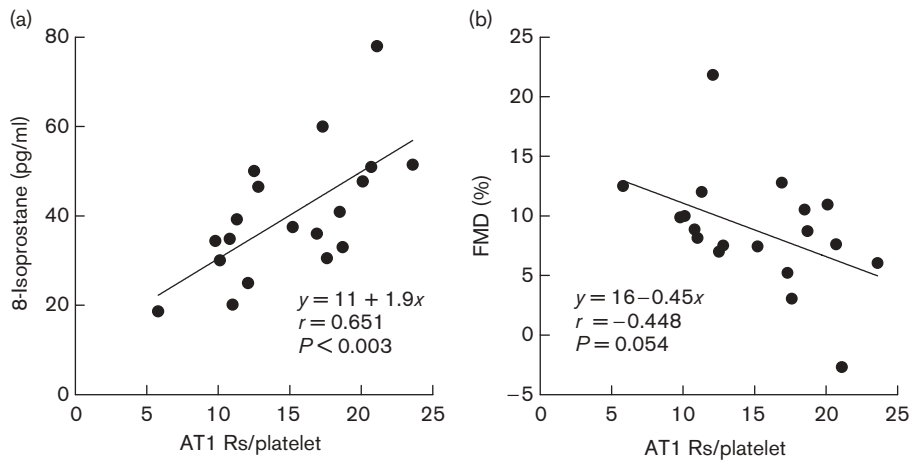
Simvastatin decreased total cholesterol by 28%, LDL-cholesterol by 41%, AT1R density by 48%, and 8-isoprostane by 32% (Table 1). FMD was improved by 24%, and all these changes achieved statistical significance (Table 1 and Fig. 4). Simvastatin reduced LDL-cholesterol (Fig. 4a) and AT1R density (Fig. 4b) consistently in all patients, whereas the changes in other variables were less consistent (Fig. 4). Serum concentrations of HDL, triglyceride, and CRP did not change significantly with simvastatin therapy (Table 1).

Among the simvastatin-induced changes, only those in 8-isoprostane and AT1R density were significantly associated ($r = 0.657$, $P < 0.003$; Fig. 5), and there was no significant correlation between simvastatin-induced changes in (i) AT1R and LDL-cholesterol, (ii) FMD and AT1R, and (iii) FMD and 8-isoprostane (not shown).

After simvastatin therapy, AT1R density was still linearly dependent on LDL-cholesterol ($r = 0.684$, $P < 0.002$; Fig. 6a), and there was no correlation between 8-isoprostane and AT1R (Fig. 6b) and between FMD and 8-isoprostane (Fig. 6c).

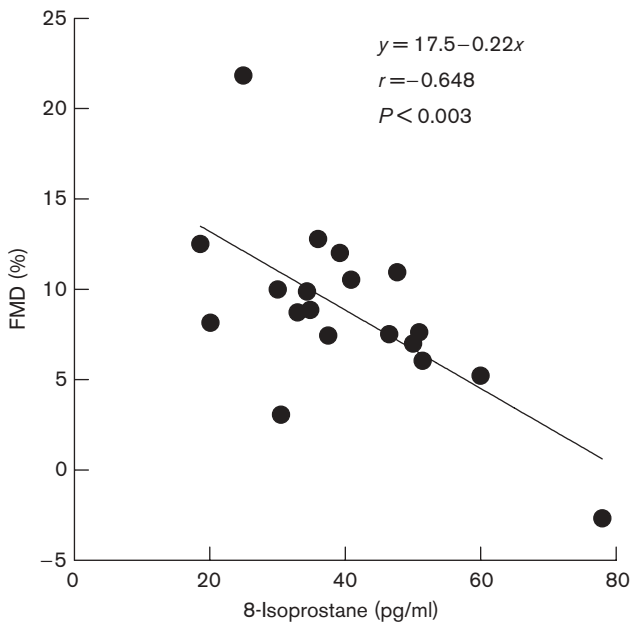
Of note, the slopes of the presimvastatin and the postsimvastatin AT1R/LDL relationships did not significantly differ ($P > 0.05$). Consequently, the mean postsimvastatin AT1R density did not significantly deviate from the regression line describing the baseline AT1R/LDL relationship ($P > 0.05$), indicating that AT1R were downregulated by simvastatin to an extent that could be predicted by the LDL reduction. Likewise, the mean postsimvastatin 8-isoprostane and FMD (Fig. 6) did not deviate ($P > 0.05$) from the regression lines describing the baseline 8-isoprostane/AT1R and FMD/8-isoprostane relationship, respectively. Thus, simvastatin reduced 8-isoprostane and improved FMD to an extent predictable in view of the changes in AT1R and 8-isoprostane, respectively.

Fig. 2



Linear regression analysis of baseline platelet angiotensin II type 1 receptor (AT1R) density and (a) plasma concentration of 8-isoprostane ($y = 11 + 1.9x$, $r = 0.651$, $P < 0.003$) and (b) flow-mediated vasodilation (FMD) ($y = 16 - 0.45x$, $r = -0.448$, $P = 0.054$) in 19 patients with coronary artery disease.

Fig. 3



Linear regression analysis of baseline plasma concentration of 8-isoprostane and flow-mediated vasodilation (FMD) ($y = 17.5 - 0.22x$, $r = -0.648$, $P < 0.003$) in 19 patients with coronary artery disease.

Table 2 Multivariate model for flow-mediated vasodilator response before and after simvastatin therapy

Variable	Before simvastatin		After simvastatin	
	Regression coefficient	P value	Regression coefficient	P value
Triglycerides	0.350	0.244	0.343	0.531
HDL-cholesterol	0.391	0.190	-0.352	0.423
LDL-cholesterol	0.287	0.235	-0.381	0.430
AT1R density	-0.348	0.330	-0.233	0.664
Serum 8-isoprostane	-0.758	0.012	0.303	0.348

Before simvastatin : multiple $r = 0.792$, $F = 3.71$, $P < 0.032$. After simvastatin : multiple $r = 0.354$, $F = 0.34$, $P = 0.876$.

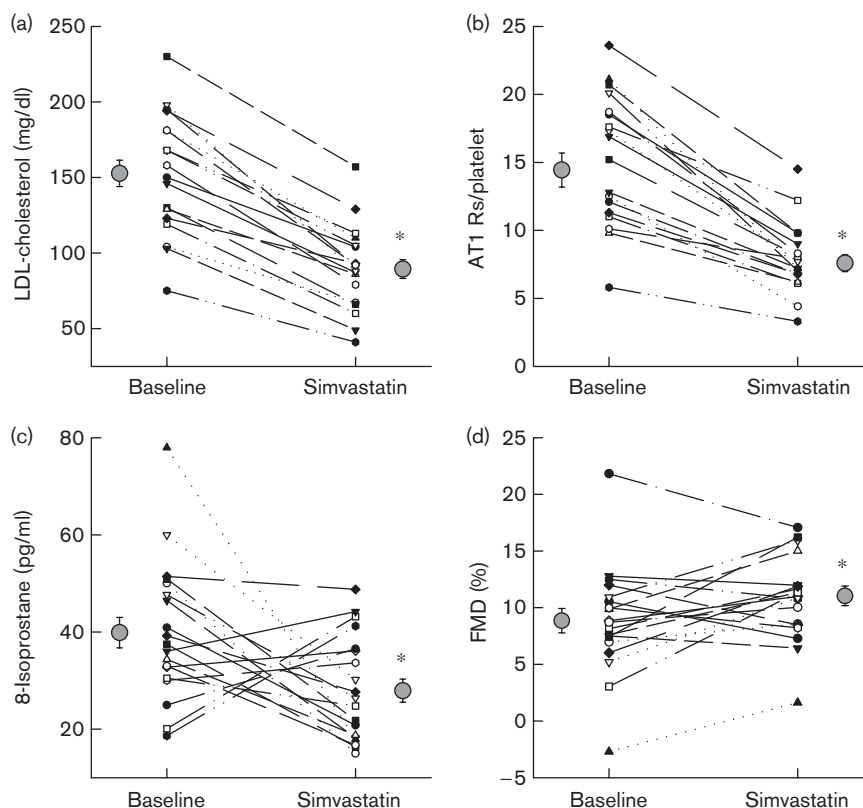
AT1R, angiotensin II type 1 receptor; FMD, flow-mediated dilation; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

We found that in CAD patients, there was a significant linear correlations between (i) cellular AT1R density and LDL-cholesterol, (ii) 8-isoprostane concentration and the AT1R, and (iii) FMD and 8-isoprostane. Thus, in CAD, the impairment of endothelial function is strongly associated with oxidative stress, oxidative stress with cellular AT1R density, and the AT1R density with LDL-cholesterol, suggesting cause-effect relationships between these variables. In line with this hypothesis, statin therapy, which reduced LDL-cholesterol, also reduced AT1R density and oxidative stress, and improved FMD. Furthermore, the slopes of the presimvastatin and the postsimvastatin AT1R/LDL relationships did not significantly differ, indicating that simvastatin downregulated AT1R in a way that could be predicted by the LDL reduction. In addition, simvastatin-mediated 8-isoprostane reductions could be predicted by AT1R downregulation, and FMD improvement by changes in 8-isoprostane.

Discussion

This study tested the hypothesis that in CAD patients, LDL-cholesterol induces AT1R overexpression that, in turn, accounts for enhanced oxidative stress, and the subsequent endothelial dysfunction [1,22,23].

Fig. 4



Simvastatin-mediated changes in (a) low-density lipoprotein (LDL)-cholesterol, (b) angiotensin II type 1 receptor (AT1R) density, (c) serum 8-isoprostane, and (d) flow-mediated vasodilation (FMD) in 19 patients with coronary artery disease. * $P < 0.05$ vs. baseline.

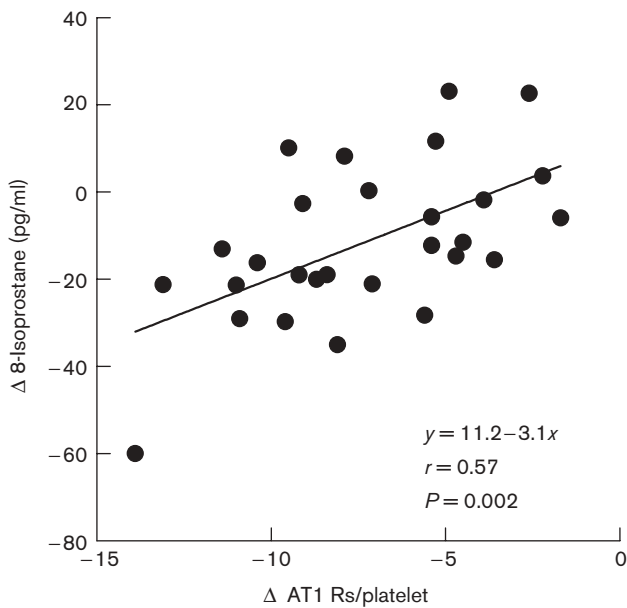
Platelet AT1R density served here as an index of AT1R density in angiotensin II target tissues, including vascular smooth muscle. The rationale for this was two-fold. Human platelets possess angiotensin II receptors whose properties and regulation resemble those characterized in animal smooth muscle and brain [26]. In humans, blood pressure responses to angiotensin II infusion correlate with AT1R density on platelets [21].

This study examined the hypothesis that the mechanism of endothelial dysfunction in CAD involves a cascade of steps with the LDL-cholesterol on the top of the cascade. If this concept is correct, all four steps of the cascade outlined above should correlate with each other, with the strongest correlation expected to occur between any two immediately succeeding steps. In fact, strong associations occurred only between LDL-cholesterol and AT1R, AT1R and 8-isoprostane, and 8-isoprostane and FMD. Neither 8-isoprostane nor FMD, however, correlated with LDL-cholesterol, and there was no correlation between AT1R and FMD. Nevertheless, in keeping with our hypothesis, other authors described, although in larger groups of patients, significant correlations between 8-isoprostane formation and LDL cholesterol [27,28],

and between serum cholesterol and endothelial function [29,30]. One conceivable explanation for these discrepant results would be that various steps of the discussed reaction cascade might be regulated by more than one factor that is expected to weaken the statistical correlations. Indeed, evidence indicates that LDL-cholesterol is not a sole regulator of AT1R expression [31], and angiotensin II is not the only mediator of the vascular oxidative stress [1,3,8]. For instance, oxidized LDL are increased in CAD patients and raise reactive oxygen species formation in endothelium via the lectinlike oxidized LDL receptor-1 (LOX-1 receptor) [32]. This would independently contribute to oxidative stress. We believe, therefore, that the significant correlations may not have been detected in the present study because of insufficient sample size.

In keeping with our hypothesis, simvastatin that reduced LDL-cholesterol simultaneously downregulated AT1R, attenuated oxidative stress, and improved endothelial function. In previous human studies, statin therapy has been already reported to downregulate AT1R [21], attenuate oxidative stress [28,33,34], and improve endothelial function [5–7,34]. Here, these four statin-

Fig. 5



Relationship between simvastatin-induced changes in 8-isoprostane concentration and angiotensin II type 1 receptor (AT1R) density ($y = 11.2 - 3.1x$, $r = 0.57$, $P = 0.002$) in 19 patients with coronary artery disease.

induced effects were studied together, allowing for a closer insight into their mutual interactions. It appeared that among the statin-mediated changes, the only significant correlation was that between changes in 8-isoprostane and AT1R. This, and the significant association between 8-isoprostane and AT1R at baseline, support the view that the oxidative stress in the studied patients was directly mediated by AT1R, and that simvastatin attenuated oxidative stress because it downregulated AT1R and not because it exerted a direct antioxidative effect. Consistent with this, AT1R blockade reduced oxidative stress and improved forearm endothelial function in patients with hypercholesterolemia [35] and hypertension [36].

No correlation was there between simvastatin-mediated changes in AT1R and LDL-cholesterol, and between the changes in FMD and 8-isoprostane, casting doubts as to the nature of the association between these variables. This lack of correlation may simply be because of the insufficient sample size. Alternatively, it may be a reflection of some cholesterol-independent effects of statin therapy.

In this respect, it has been shown [37] that statins directly increase endothelial NO synthase expression and activity. In addition, in some clinical studies [33,38], improvement in endothelial function under statin therapy occurred before significant reduction in cholesterol

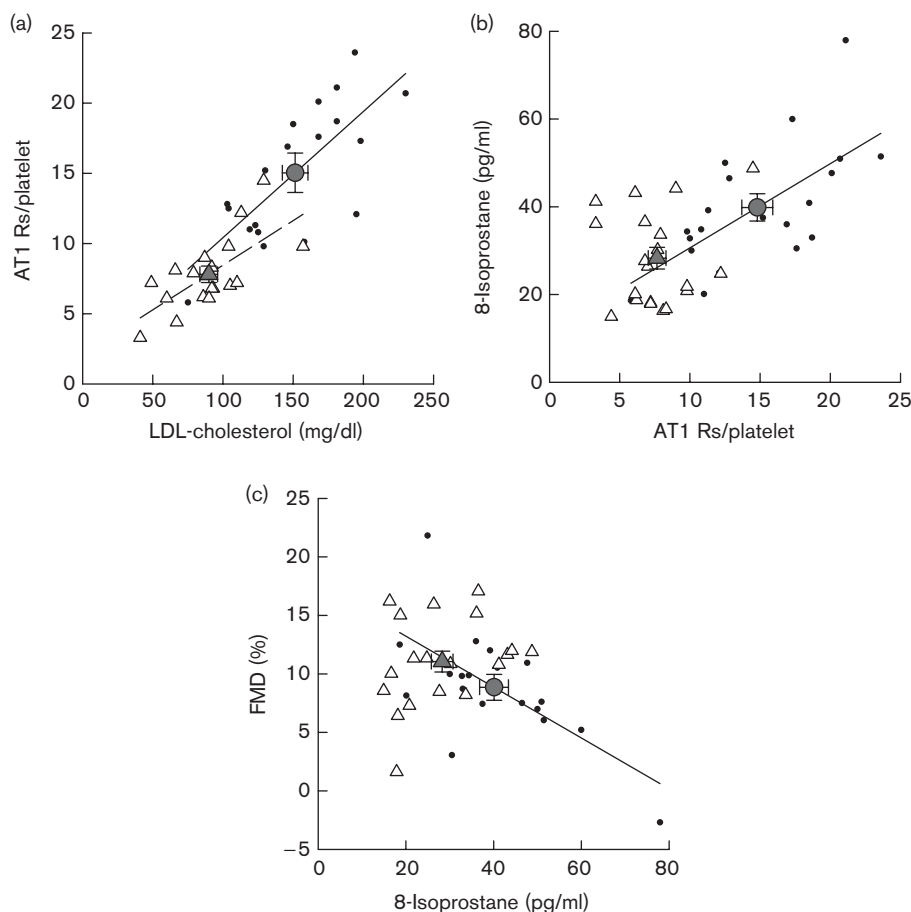
level was evident. Statins were also shown [16,18,39] to exert antioxidant effects in rat vascular smooth muscle by cholesterol-independent depression of NADPH oxidase subunit expression, and by the upregulation of catalase expression. In humans, the reduction in oxidative stress under statin therapy occurs before significant reduction in LDL-cholesterol [33]. Finally, statin therapy was shown [16,18] to directly downregulate AT1R expression in rat vascular smooth muscle. Consistent with its cholesterol-independent activity, 6-week statin therapy in hypercholesterolemic patients exerted greater reduction in AT1R density than could be predicted by the LDL reduction [21].

Unexpectedly, the present study does not provide evidence for cholesterol-independent effects of statin therapy. We demonstrate that statins (i) downregulated AT1R to an extent that could be predicted by the LDL reduction, (ii) decreased 8-isoprostane levels to an extent predictable from reduction in AT1R density, and (iii) improved FMD to an extent predictable from changes in 8-isoprostane. Altogether, these findings are consistent with the notion that the changes in AT1R, oxidative stress, and endothelial function under statin therapy were secondary to their lipid-lowering activity rather than to their putative pleiotropic effects. These effects have been proposed to account for the fact that the clinical benefits observed with statins exceeded the benefits that would be expected from the observed reductions in cholesterol [40].

The reason for these discrepant clinical results is not apparent from this study. We speculate that various doses and/or duration of statin therapy in this and other studies might explain the discrepancies. For instance, the improvement in endothelial function and the reduction in oxidative stress with statins occur within hours to days of the therapy, that is, before the reduction in cholesterol is achieved [33,38]. Thus, early during statin therapy, its cholesterol-independent effects seem to predominate, whereas with longer therapy, the effects secondary to lipid-lowering activity may prevail.

In conclusion, we demonstrate that in CAD patients, the impairment of endothelial function is associated with oxidative stress, oxidative stress with cellular AT1R density, and the AT1R density with LDL-cholesterol, an observation consistent with the hypothesis that these processes are causally related. In further support of the hypothesis: (i) the reduction in LDL-cholesterol with simvastatin was paralleled by AT1R downregulation, attenuation of oxidative stress, and improvement of endothelial function, and (ii) statin therapy downregulated AT1R to an extent predictable by the LDL reduction, decreased oxidative stress in a way predictable from reduction in AT1R density, and improved FMD to

Fig. 6



Relationship between (a) angiotensin II type 1 receptor (AT1R) density and low-density lipoprotein (LDL)-cholesterol concentration, (b) 8-isoprostane concentration and AT1R density, and (c) flow-mediated vasodilation (FMD) and 8-isoprostane concentration in 19 patients with coronary artery disease. Plotted are values obtained at baseline (filled circles), after simvastatin (triangles), and regression lines for the measurements obtained at baseline (solid lines). Dashed line in (a) among the postsimvastatin measurements, the linear correlation could be demonstrated only between AT1R density and LDL-cholesterol ($y = 2.1 + 0.06x$, $r = 0.684$, $P < 0.02$). Crosshatched circles and triangles are mean \pm SEM of baseline and postsimvastatin measurements, respectively. The mean of none of the postsimvastatin measurement deviates significantly from the respective baseline regression line. (●) Baseline and (Δ) simvastatin.

an extent predictable from changes in oxidative stress. The latter suggests that the changes in AT1R, oxidative stress, and endothelial function under statin therapy were mainly related to their lipid-lowering effects.

Acknowledgements

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