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Effects of atorvastatin on inflammation and oxidative stress

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Abstract Treatment with inhibitors of 3-hydroxyl-3-methylglutaryl coenzyme A reductase (statins) reduces the incidence of cardiovascular events, but it is unclear whether the beneficial effects are mediated solely by their lipid-lowering properties. We therefore investigated whether atorvastatin reduces inflammation and oxidative stress independently of its lipid-lowering effects. The subjects comprised 71 hyperlipidemic patients (64 ± 9 years old, mean ± SD) who were not receiving medical treatment. Serum lipid and C-reactive protein (CRP) levels, and urine 8-isoprostane level (an index of oxidative stress) were measured before and after 4 weeks of treatment with atorvastatin at 10 mg/day. In 38 patients, these biochemical variables and carotid intima-media thickness (IMT) were also measured after 6 months of treatment with atorvastatin. Atorvastatin markedly reduced CRP (from 0.69 ± 0.36 to 0.42 ± 0.20 and 0.35 ± 0.19 mg/l, median ± median absolute deviation, $P < 0.0001$), 8-isoprostane (from 225 ± 99 to 178 ± 75 and 179 ± 60 ng/g creatinine, $P < 0.05$), and low density-lipoprotein cholesterol (LDLC; from 165 ± 21 to 106 ± 18 and 112 ± 17 mg/dl, $P < 0.0001$) after 4 weeks and 6 months of treatment, respectively. However, the reductions in CRP and 8-isoprostane were not correlated with those of LDLC. After 6 months of treatment, IMT was significantly decreased compared with the baseline value (from 0.94 ± 0.26 to 0.90 ± 0.20 mm, $P < 0.05$), but this was not correlated with the reduction in LDLC. These results suggest that atorvastatin has beneficial effects on inflammation, oxidative stress, and the lipid profile in patients with hyperlipidemia. The extra-lipid effects are not attributable to the lipid-lowering effect of the statin, suggesting that the pleiotropic effects of atorvastatin are independent of its effects on the lipid profile.

Key words Intima-media thickness · Isoprostane · C-reactive protein · Low-density-lipoprotein cholesterol · Statin

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Introduction

Recent clinical trials have demonstrated that treatment with inhibitors of 3-hydroxyl-3-methylglutaryl coenzyme A reductase (statins) reduces the incidence of cardiovascular events in patients with hyperlipidemia.[1] Although the beneficial effects of statins are mediated, at least in part, by their lipid-lowering properties, experimental studies have suggested that statins exhibit a variety of extra-lipid effects including immunomodulation, antiproliferation, and antithrombosis.[2,3] Furthermore, clinical studies using different statins have all demonstrated a favorable reduction in C-reactive protein (CRP),[4–8] a biomarker of systemic inflammation, supporting the concept that statins have pleiotropic effects. Indeed, statins are effective in reducing the risk of stroke,[9] yet epidemiologic studies have not identified low-density-lipoprotein cholesterol (LDLC) as an important risk factor for stroke.

The initiation and amplification of inflammation of the vascular wall by oxidative stress is a central feature of the development of atherosclerosis,[10,11] and CRP is a strong predictor of cardiovascular risk.[6,12,13] Most studies have shown that reductions in CRP are unrelated to the magnitude of LDLC reduction.[5] However, several recent studies have found conflicting results for atorvastatin: there were significant correlations between changes in CRP and LDLC,[14,15] suggesting that the beneficial effects of the statin are mediated by its lipid-lowering effects. Thus, it is not clear whether atorvastatin has extra-lipid, pleiotropic effects.

Table 1. Characteristics of patients and effects of atorvastatin

	Baseline (n = 71)	4 weeks (n = 71)	6 months (n = 38)
Age (years)	64 ± 9		
BMI (kg/m^2)	24.2 ± 3.3		24.2 ± 3.2
SBP (mmHg)	136 ± 20	134 ± 18*	132 ± 18*
DBP (mmHg)	77 ± 10	75 ± 10*	75 ± 9*
TC (mg/dl)	263 ± 24	195 ± 30**	198 ± 23**
TG (mg/dl)	163 ± 51	116 ± 41**	128 ± 41**
HDLC (mg/dl)	54.6 ± 16.7	58.0 ± 14.8*	58.8 ± 15.3*
LDLC (mg/dl)	165 ± 21	106 ± 18**	112 ± 17**
HOMA-R index	2.52 ± 1.13	2.31 ± 0.94	2.29 ± 0.78
CRP (mg/l)	0.69 ± 0.36	0.42 ± 0.20**	0.35 ± 0.19**
ISO/Cr (ng/g Cr)	225 ± 99	178 ± 75*	179 ± 60*
IMT (mm)	0.94 ± 0.26		0.90 ± 0.20*

All data except those of TG, HOMA-R, CRP, and Iso/Cr are expressed as mean ± SD. TG, HOMA-R, CRP, and Iso/Cr are expressed as median ± median absolute deviation and changes in these variables were analyzed after log transformation of these data
BMI, body mass index; SBP and DBP, systolic and diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDLC, high-density-lipoprotein cholesterol; LDLC, low-density-lipoprotein cholesterol; HOMA-R, the homeostasis model assessment of insulin resistance; CRP, C-reactive protein; Iso, 8-isoprostane; Cr, creatinine; IMT, intima-media thickness
* $P < 0.05$, ** $P < 0.0001$ vs baseline

The present study was designed to test the hypothesis that atorvastatin has pleiotropic effects. We investigated the effects of low-does atorvastatin on inflammation, oxidative stress, insulin resistance, LDLC, and carotid intima-media thickness (IMT), and the possible relationships among these effects in patients with mild to moderate hyperlipidemia.

Patients and methods

Subjects and study design

The study protocol was approved by the ethics committee of our hospital, and informed consent was obtained from all subjects prior to the start of the study. The study population comprised 71 hyperlipidemic patients aged 37–80 years, whose baseline characteristics are listed in Table 1. Some of the patients had hypertension or diabetes mellitus, but none had cardiovascular disease. None of the patients were receiving medical treatment. Patients with acute or chronic inflammatory disease, active liver disease, or renal dysfunction were not enrolled. All patients were assigned to receive atorvastatin at 10 mg/day. Baseline measurements include levels of total cholesterol, LDLC, high-density-lipoprotein cholesterol (HDLC), CRP, insulin, fasting plasma glucose, and urine levels of 8-isoprostane (a biomarker of oxidative stress), with repeat measurements at 4 weeks. The homeostasis model assessment of insulin resistance (HOMA-R), an index of insulin resistance, was calculated using the method of Matthews et al.[16] In 38 patients the measurements were also repeated at 6 months, and IMT was measured at baseline and 6 months.

Biochemical measurements

Blood and urine were sampled in the morning following an overnight fast. The blood samples were centrifuged at 1 000 × g for 15 min, and the resulting supernatant was stored at −70°C until use. C-reactive protein was measured using a latex-enhanced immunonephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany). For the measurement of 8-isoprostane, 5 ml of urine was transferred to a tube containing 0.25 mg of indomethacin and centrifuged at 10 000 × g for 10 min, and the resulting supernatant was stored at −70°C until use. The urine concentration of 8-isoprostane was determined using an enzyme immunoassay kit (8-isoprostane EIA Kit; Cayman Chemical, Ann Arbor, MI, USA), whilst correcting for creatinine excretion.

Measurement of IMT

The carotid artery was imaged with an ultrasound system (Philips SONOS 4500 with a 11-3L linear transducer, Philips Medical Systems, Bothell, WA, USA). On a longitudinal, two-dimensional ultrasound image of the common carotid artery, the left common carotid artery was examined 1–2 cm proximal to the carotid bifurcation. The IMT of the posterior wall of the common carotid artery was measured as the distance from the leading edge of the first echogenic line (lumen–intima interface) to the leading edge of the second line (media–adventitia interface).[17] Six measurements were averaged, and this value was thereafter used for further calculations. The examiners of the ultrasound images were unaware of the case status of the subject.

Statistical analysis

Except where stated otherwise, all data values are expressed as mean ± SD. Statistical analysis was performed using StatView software (version 5.0, SAS Institute, Cary, NC, USA). Group differences in continuous variables that had a normal distribution were tested with Student's paired t-test. Since the distributions of CRP, triglyceride, 8-isoprostane, and HOMA-R were skewed toward higher values, median concentrations were computed for these parameters and are expressed as median ± median absolute deviation. Mean concentrations of CRP, triglyceride, 8-isoprostane, and HOMA-R were also computed after log transformation that resulted in normal distributions. Probability values of $P < 0.05$ were considered to indicate statistical significance.

Results

Four weeks of treatment with atorvastatin improved the lipid profile in the hyperlipidemic patients: the levels of LDLC and triglyceride were decreased, and the level of HDLC was increased (Table 1). These effects persisted for 6 months after the start of the treatment.

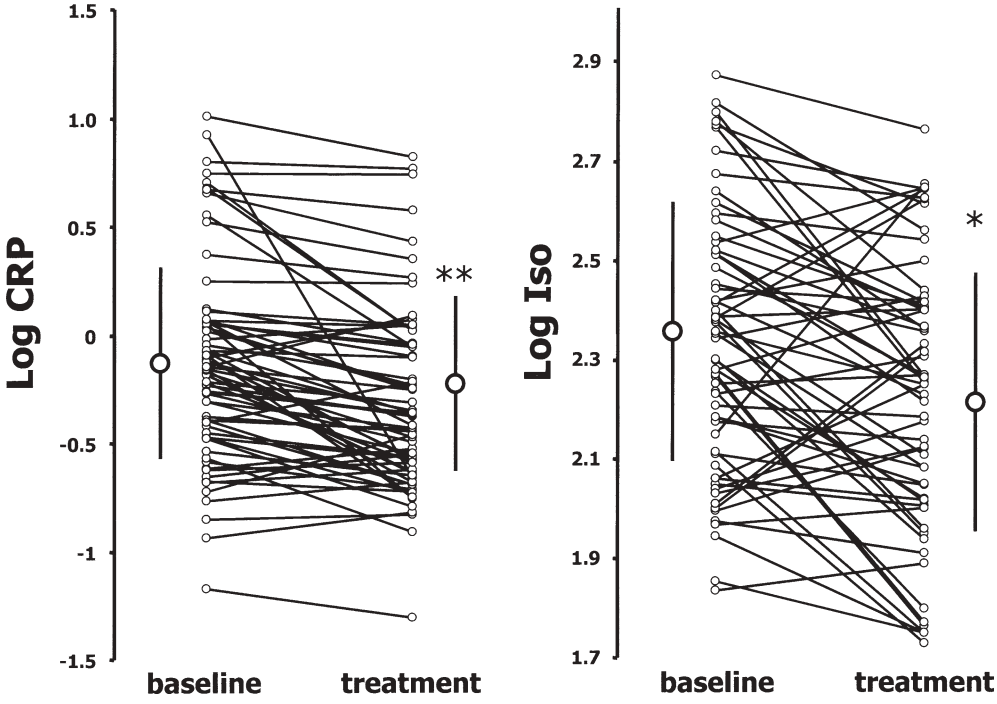

Fig. 1. Changes in serum C-reactive protein (*CRP*; *left panel*) and urine 8-isoprostane levels (corrected by urine excretion of creatinine, *Iso; right panel*) after 4 weeks of treatment with atorvastatin in each patient. *Open circles* and *vertical bars* represent mean and SD, respectively. $*P < 0.05$, $**P < 0.0001$ vs baseline usine paired Student's *t*-test

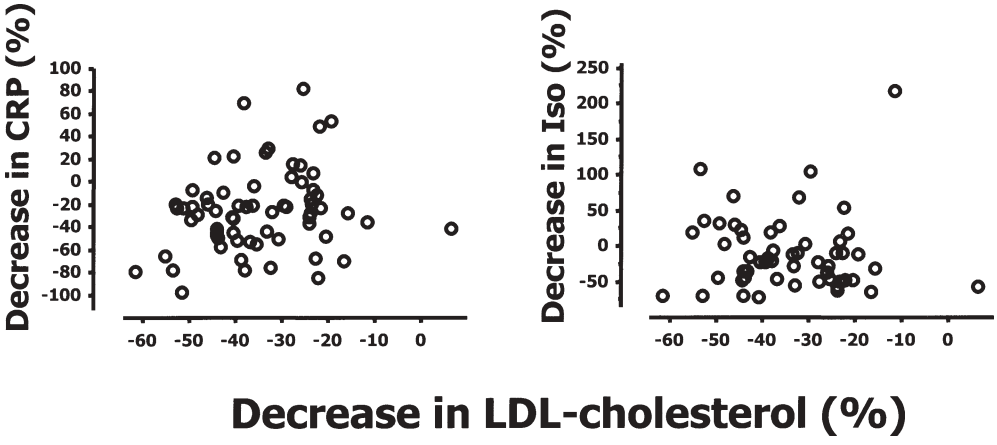

Fig. 2. Relationship between changes in low-density-lipoprotein (*LDL*) cholesterol and C-reactive protein (*CRP*; *left panel*) or urine 8-isoprostane levels (corrected by urine excretion of creatinine, *Iso*; *right panel*) after 4 weeks of treatment with atorvastatin in patients with hyperlipidemia. Reduction in CRP or Iso levels was not correlated with that of LDL cholesterol

Moreover, atorvastatin markedly reduced the serum level of CRP and the urine level of 8-isoprostane after 4 weeks of treatment (Table 1). Most of the 71 patients exhibited reductions in their CRP ($n = 59$, 83.1%) and 8-isoprostane ($n = 48$, 67.6%) levels (Fig. 1), but these reductions were not correlated with that of LDLC ($r = 0.208$, $P = 0.937$ and $r = -0.018$, $P = 0.8988$, respectively; Fig. 2). There was no significant correlation between the percentage reductions in CRP and 8-isoprostane levels ($r = 0.035$, $P = 0.7935$). The levels of CRP and 8-isoprostane remained decreased after 6 months of treatment compared with the baseline data (Table 1).

Other extra-lipid effects of atorvastatin were also observed. Systolic and diastolic blood pressure was decreased after 4 weeks and 6 months of the statin therapy. HOMA-R tended to be decreased by atorvastatin, but this did not reach statistical significance. Strikingly, IMT was significantly decreased after 6 months of treatment with atorvastatin, but this was not correlated with the decrease in LDLC ($r = -0.206$, $P = 0.2692$).

Discussion

The present study demonstrates that treatment with low-dosage atorvastatin has several beneficial effects in hyperlipidemic patients: improvement of inflammation, oxidative stress, blood pressure, and LDLC. The extra-lipid effects of the statin were independent of its effects on LDLC, suggesting that atorvastatin has pleiotropic effects in patients with hyperlipidemia.

Whether the reductions in CRP and LDLC produced by atorvastatin are mutually correlated is unclear.[5,14,15] In the

present study, changes in the CRP levels were not correlated with those of the LDLC levels, suggesting that atorvastatin reduces inflammation through a mechanism unrelated to LDLC reduction. Conflicting results in previous studies[5,14,15] may be attributable to differences in ethnicity, basal levels of CRP and LDLC, and the dose of the statin used. However, it appears clear that atorvastatin reduces inflammation via, at least in part, an LDLC-independent pathway. It is still unclear whether the reduction in CRP by atorvastatin is related to changes in clinical outcomes, but this direct anti-inflammatory effect may contribute to a reduction in cardiovascular events since inflammation is a major factor in atherothrombotic disease.[18] Indeed, atorvastatin reduced IMT in the present study, confirming a previous report.[19] Furthermore, CRP is now recognized to be a mediator of atherothrombotic disease, since it induces complement, regulates the expression of nitric oxide synthase, and upregulates the expression of adhesion molecules.[6,20]

Oxidative stress plays an important role in the pathogenesis of cardiovascular diseases, including atherosclerosis, hypertension, and coronary artery disease.[21,22] Atorvastatin reduced oxidative stress, but this was not related to the changes in LDLC levels in the present study, indicating that the statin has a pleiotropic effect. This finding is consistent with other reports that statins suppress NADH/NADPH oxidase[23] or that a metabolite of atorvastatin has an antioxidant property,[24] which may contribute to the beneficial effects of the statin. Reductions in inflammation and oxidative stress or inhibition of Rho/Rho-kinase activity by atorvastatin may contribute to an improvement in endothelial function,[25,26] which may be related to the reduction in blood pressure observed in the present study.

In conclusion, atorvastatin has beneficial effects on inflammation, oxidative stress, and the lipid profile in patients with hyperlipidemia. The reduction in inflammation and oxidative stress is not attributed to the lipid-lowering effect of the statin, indicating that atorvastatin may have pleiotropic effects independent of its effects on the lipid profile.

References

1. Gresser U, Gathof BS (2004) Atorvastatin: gold standard for prophylaxis of myocardial ischemia and stroke – comparison of the clinical benefit of statins on the basis of randomized controlled endpoint studies. Eur J Med Res 9:1–17
2. Farmer JA (2000) Pleiotropic effects of statins. Curr Atheroscler Rep 2:208–217
3. Takemoto M, Liao JK (2001) Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. Arterioscler Thromb Vasc Biol 21:1712–1719
4. Albert MA, Danielson E, Rifai N, Ridker PM, PRINCE Investigators (2001) Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 286:64–70
5. Rosenson RS, Koenig W (2003) Utility of inflammatory markers in the management of coronary artery disease. Am J Cardiol 92:10i–18i
6. Ridker PM (2003) High-sensitivity C-reactive protein and cardiovascular risk: rationale for screening and primary prevention. Am J Cardiol 92:17K–22K
7. Kinlay S, Schwartz GG, Olsson AG, Rifai N, Leslie SJ, Sasiela WJ, Szarek M, Libby P, Ganz P, Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering Study Investigators (2003) High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study. Circulation 108:1560–1566
8. Nawawi H, Osman NS, Yusoff K, Khalid BA (2003) Reduction in serum levels of adhesion molecules, interleukin-6 and C-reactive protein following short-term low-dose atorvastatin treatment in patients with non-familial hypercholesterolemia. Horm Metab Res 35:479–485
9. Byington RP, Davis BR, Plehn JF, White HD, Baker J, Cobbe SM, Shepherd J (2001) Reduction of stroke events with pravastatin: the Prospective Pravastatin Pooling (PPP) Project. Circulation 103: 387–392
10. Alexander RW (1995) Theodore Cooper Memorial Lecture. Hypertension and the pathogenesis of atherosclerosis. Oxidative stress and the mediation of arterial inflammatory response: a new perspective. Hypertension 25:155–161
11. Willerson JT, Ridker PM (2004) Inflammation as a cardiovascular risk factor. Circulation 109 (Suppl 1):I2–II10
12. Uehara K, Nomura M, Ozaki Y, Fujinaga H, Ikefuji H, Kimura M, Chikamori K, Nakaya Y, Ito S (2003) High-sensitivity C-reactive protein and left ventricular remodeling in patients with acute myocardial infarction. Heart Vessels 18:67–74
13. Tokac M, Ozeren A, Aktan M, Altunkeser BB, Ozdemir K, Duzenli A, Gok H (2003) The role of inflammation markers in triggering acute coronary events. Heart Vessels 18:171–176
14. Kent SM, Flaherty PJ, Coyle LC, Markwood TT, Taylor AJ (2003) Effect of atorvastatin and pravastatin on serum C-reactive protein. Am Heart J 145:e8
15. Ansell BJ, Watson KE, Weiss RE, Fonarow GC (2003) hsCRP and HDL effects of statins trial (CHEST): rapid effect of statin therapy on C-reactive protein and high-density lipoprotein levels. A clinical investigation. Heart Dis 5:2–7
16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
17. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R (1986) Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 74:1399–1406
18. Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105:1135–1143
19. van Wissen S, Smilde TJ, de Groot E, Hutten BA, Kastelein JJ, Stalenhoef AF (2003) The significance of femoral intima-media thickness and plaque scoring in the Atorvastatin versus Simvastatin on Atherosclerosis Progression (ASAP) study. Eur J Cardiovasc Prev Rehabil 10:451–455
20. Yamaoka-Tojo M, Tojo T, Masuda T, Machida Y, Kitano Y, Kurosawa T, Izumi T (2003) C-reactive protein-induced production of interleukin-18 in human endothelial cells: a mechanism of orchestrating cytokine cascade in acute coronary syndrome. Heart Vessels 18:183–187
21. Gryglewski RJ, Palmer RM, Moncada S (1986) Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. Nature 320:454–456
22. Harrison DG (1997) Endothelial function and oxidative stress. Clin Cardiol 20 (Suppl 2):II11–II17
23. Ejiri J, Inoue N, Tsukube T, Munezane T, Hino Y, Kobayashi S, Hirata K, Kawashiwa S, Imajoh-Ohmi S, Hayashi Y, Yokozaki H, Okita Y, Yokoyama M (2003) Oxidative stress in the pathogenesis of thoracic aortic aneurysm: protective role of statin and angiotensin II type 1 receptor blocker. Cardiovasc Res 59:988–996
24. Aviram M, Rosenblat M, Bisgaier CL, Newton RS (1998) Atorvastatin and gemfibrozil metabolites, but not the parent drugs, are potent antioxidants against lipoprotein oxidation. Atherosclerosis 138:271–280
25. Wolfrum S, Jensen KS, Liao JK (2003) Endothelium-dependent effects of statins. Arterioscler Thromb Vasc Biol 23:729–736
26. Wassmann S, Ribaudo N, Faul A, Laufs U, Bohm M, Nickenig G (2004) Effect of atorvastatin 80 mg on endothelial cell function (forearm blood flow) in patients with pretreatment serum low-density lipoprotein cholesterol levels <130 mg/dl. Am J Cardiol 93: 84–88