

# Changes in Flow-Mediated Dilatation, Cytokines and Carotid Arterial Stenosis During Aggressive Atorvastatin Treatment in Normocholesterolemic Patients

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**Background:** Aggressive statin therapy to reduce low-density lipoprotein (LDL) cholesterol in patients with normal LDL-cholesterol levels reduces the incidence of future cardiovascular events and enhances atherosclerotic regression in the common carotid artery. We tried to quantify changes in flow-mediated dilatation (FMD), inflammatory cytokines, and the severity of carotid arterial stenosis, after aggressive statin administration to patients with normal LDL-cholesterol levels, and stroke or transient ischemic attack ipsilateral to carotid arterial stenosis.

**Methods:** Twenty patients with at least a 50% reduction in the diameter of the carotid artery were studied. Atorvastatin 10 mg daily was prescribed for 4 months. Serial changes in the severity of carotid arterial stenosis, and brachial-artery FMD were evaluated by high-resolution ultrasound. Tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1beta (IL-1 $\beta$ ), interleukin-6 (IL-6), vascular cell adhesion molecule (VCAM), intracellular adhesion molecule (ICAM), soluble endothelin-1 (ET-1), and high-sensitivity C-reactive protein (hs-CRP), were measured before, and after 2 and 4 months of, atorvastatin treatment.

**Results:** Atorvastatin significantly decreased plasma levels of total cholesterol and LDL-cholesterol after 1, 2 and 4 months of treatment ( $p < 0.01$ ). FMD showed significant improvement after only 4 months (10.0% vs 6.8% at baseline;  $p = 0.019$ ). The overall severity of carotid arterial lesions was not significantly reduced by atorvastatin. Changes in TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and ET-1 showed trends towards a progressive decline after atorvastatin, but none of the cytokines was reduced significantly. FMD did not correlate with the severity of carotid arterial stenosis, lipid profile, or cytokine levels.

**Conclusion:** Atorvastatin effectively reduced plasma concentrations of total cholesterol and LDL-cholesterol, and had beneficial effects on endothelial function, in Chinese patients with carotid arterial stenosis and normal LDL-cholesterol levels. [*J Chin Med Assoc* 2005;68(2):53–58]

**Key Words:** carotid arterial stenosis, cytokines, duplex Doppler ultrasound, hydroxymethylglutaryl-CoA reductase inhibitors, vascular endothelium

## Introduction

Endothelial dysfunction has been demonstrated in adults with established atherosclerosis, and is also an important early event in atherogenesis.<sup>1</sup> Dysfunction

of the endothelium is a critical factor in the pathogenesis of several vascular diseases and thrombus formation.<sup>2,3</sup> Noninvasive evaluation of brachial-artery reactivity (endothelium-dependent vasodilatation; flow-mediated dilatation [FMD]) using B-mode

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ultrasound is a useful surrogate for detecting systemic endothelial dysfunction in several diseases.<sup>4-7</sup> Our previous study found that patients with symptomatic carotid arterial stenosis had impaired FMD compared with controls and patients with asymptomatic carotid arterial stenosis.<sup>8</sup> Thus, endothelial dysfunction contributed to cerebral ischemic events and stenotic lesions of the carotid artery. These findings suggested that aggressive treatment to improve endothelial function might prevent future cerebrovascular events. Preliminary reports in stroke patients demonstrated that statins might shorten hospital stay and help to control refractory transient ischemic attacks.<sup>9,10</sup>

The National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) recommends an optimal low-density lipoprotein (LDL) cholesterol level of less than 100 mg/dL for patients at high cardiovascular risk. Further LDL-cholesterol reductions, to levels far below 100 mg/dL, are of unknown benefit, although some reports have suggested that treatment to "ultra-low" values may provide superior outcomes.<sup>11-13</sup> However, the mechanisms of statin benefit in patients with normal LDL-cholesterol levels are not clear. Besides their lipid-lowering effects, statins have other therapeutic effects, including endothelial protection, and antioxidant, anti-inflammatory and antiplatelet effects. Clinically, statin therapy has shown improvements in FMD, and reduced plasma concentrations of inflammatory cytokines. A recent, randomized trial demonstrated superior atherosclerotic regression in the common carotid artery in patients treated to lower LDL-cholesterol levels.<sup>14</sup>

Whether aggressive lipid-lowering therapy might accelerate regression of atherosclerotic lesions that cause carotid arterial stenosis is also unclear. We proposed that the beneficial effects of statin therapy in patients with normal plasma cholesterol levels might result from improved endothelial function, or from anti-inflammatory effects. We thus conducted the present study to evaluate changes in carotid-plaque severity, FMD, and inflammatory cytokines, after atorvastatin administration to patients with carotid arterial stenosis and normal plasma cholesterol levels.

## Methods

### *Patient selection*

From February 1 to July 31, 2001, a total of 1,152 consecutive patients who received high-resolution duplex ultrasound examination of the carotid arteries were prospectively evaluated. Ultrasound was carried out with a computed, high-frequency ultrasound sys-

tem (Hewlett Packard, Sonos 5500, Andover, MA, USA), with a linear multifrequency 7.5 MHz probe for B-mode imaging and 3.6 MHz probe for pulsed-wave Doppler velocity measurement. Pulsed-wave Doppler velocities were determined at the center of the narrowest carotid arterial lesion. Sixty-two patients who satisfied the study criteria for significant carotid arterial stenosis were screened: (1) more than 50% focal diameter reduction on B-mode images; and (2) peak systolic velocity at the stenotic site > 120 cm/sec, or more than twice the peak systolic velocity at a nearby non-stenotic segment. Patients were excluded for the following reasons: plasma LDL-cholesterol concentration above 130 mg/dL ( $n = 12$ ); currently receiving lipid-lowering therapy ( $n = 16$ ); unstable vital signs or heart failure ( $n = 6$ ); severe disabling stroke ( $n = 7$ ); or severe peripheral vascular insufficiency in the upper limbs ( $n = 1$ ). Thus, 20 patients (aged 62–80 years; 15 males) with carotid arterial stenosis and normal LDL-cholesterol concentrations were recruited. All participants signed informed consent before entering the study, which was approved by the local Institutional Review Board.

### *Study protocol*

All recruited patients underwent ultrasound examinations in the morning after an overnight fast. All vasoactive medication was withheld at least 12 hours before testing. Fasting blood samples were obtained from all patients. After centrifugation, all plasma samples were frozen and stored at  $-20^{\circ}\text{C}$  until assay. A detailed medical history was taken and a neurologic examination performed during the first visit. Then, treatment was started with atorvastatin 10 mg daily for 4 months. Patients were asked to return every month, and any reported clinical or adverse events were recorded. FMD was measured, and blood samples collected, after 1, 2 and 4 months of treatment. All patients underwent ultrasound examination after 2 and 4 months of treatment.

As described previously,<sup>4,5,8</sup> patients were assessed in a temperature-controlled room ( $25^{\circ}\text{C}$ ) with the left arm in the recumbent position. High-frequency ultrasound was used to measure changes in brachial-artery diameter in response to reactive hyperemia (leading to FMD), and in response to 0.4 mg sublingual nitroglycerin (leading to nitroglycerin-induced, endothelium-independent dilatation). Using 7.5 MHz linear-array ultrasound, the brachial artery was longitudinally imaged approximately 2–10 cm proximal to the antecubital crease, after at least 10 minutes' rest in the supine position. Hyperemia was induced by inflating a pneumatic cuff placed around the arm,

above the scanned part of the artery, to 200 mmHg for 5 minutes, and then deflating the cuff. The artery was scanned before cuff inflation and immediately, 1, 3 and 5 minutes after deflation. After another 10 minutes' rest, a further control scan was recorded. A single dose of nitroglycerin 0.4 mg was administered sublingually and brachial-artery diameter was recorded after 3 and 5 minutes. Great care was taken to maintain the transducer in a fixed position relative to the subject's arm. Sonographic images were recorded on super-VHS videotape for subsequent analysis. All vasodilatation measurements were made at end diastole (concurrent with the onset of the QRS complex on electrocardiogram). For the calculation of FMD, the change in vessel diameter 1 minute after cuff deflation was divided by the average control diameter. Endothelium-independent dilatation was calculated as the change in vessel diameter, 3 minutes after nitroglycerin, divided by the average control diameter.

An ultrasound assessment of the cervical carotid artery was carried out with each subject lying in the supine position. Using 7.5 MHz linear-array ultrasound, the cervical carotid arteries were first scanned horizontally and then longitudinally. Great care was taken to maintain the transducer perpendicular to the artery during all measurements. The intima-media layer on the far wall was to be clearly seen to ensure the imaging artery was in axis during measurement. The maximal thickness of plaque over the carotid artery was recorded. Scan images were also recorded on super-VHS videotapes for later analysis.

Plasma glucose concentrations were determined using the glucose oxidase method. Plasma cholesterol and triglyceride concentrations were measured after precipitation of apolipoprotein B-containing lipoproteins by phosphotungstic acid and magnesium chloride reagent.<sup>15</sup> LDL-cholesterol concentration was calculated by the Friedewald equation. Plasma concentrations of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), soluble vascular cell adhesion molecule (VCAM), soluble

intracellular adhesion molecule (ICAM), and soluble endothelin-1 (ET-1), were measured by a commercial enzyme-linked high sensitivity immunoassay (R&D Systems, Minneapolis, MN, USA). The assay for high-sensitivity plasma C-reactive protein (hs-CRP) was performed by latex-enhanced immunoturbimetry (Good Biotech Corp, Taichung, Taiwan); the assay has inter- and intra-assay coefficients of variation of 1.42% and 1.40%, respectively.

### Statistical analysis

All values are expressed as mean  $\pm$  standard deviation. Comparisons of serial changes in FMD, inflammatory cytokine concentrations, and maximal diameter reduction of the carotid artery, were analyzed compared with baseline by non-parametric Wilcoxon's signed-rank test. Correlations between measured parameters were assessed according to Spearman's method. All statistical calculations were performed using a commercially available statistical package (Statistical Package for the Social Sciences, version 9.0 for Windows, Chicago, IL, USA). A *p* value of less than 0.05 was considered statistically significant.

## Results

Atorvastatin significantly decreased plasma levels of total cholesterol and LDL-cholesterol after 1, 2 and 4 months of treatment (*p* < 0.001) (Table 1). The mean LDL-cholesterol level was lowered to less than 70 mg/dL. No patient developed any adverse event during atorvastatin therapy.

FMD improved only after 4 months' atorvastatin treatment (*p* = 0.019). The severity of carotid arterial lesions was not significantly reduced by atorvastatin (Table 2). Changes in inflammatory cytokine levels after atorvastatin are also listed in Table 2. hs-CRP decreased significantly after 2 months (*p* = 0.04), but demonstrated no significant change after 4 months (*p* = 0.852). Atorvastatin tended to produce a pro-

**Table 1.** Changes in lipid profile during atorvastatin therapy

	Baseline	Atorvastatin therapy					
		After 1 month		After 2 months		After 4 months	
			<i>p</i>		<i>p</i>		<i>p</i>
Total cholesterol (mg/dL)	163.4 $\pm$ 37.0	136.1 $\pm$ 31.3	0.002	136.4 $\pm$ 32.0	0.004	137.7 $\pm$ 28.1	0.005
HDL-cholesterol (mg/dL)	44.6 $\pm$ 12.9	48.6 $\pm$ 11.3	0.136	48.2 $\pm$ 11.8	0.116	47.1 $\pm$ 11.5	0.278
LDL-cholesterol (mg/dL)	88.3 $\pm$ 21.9	60.7 $\pm$ 15.6	0.000	62.6 $\pm$ 21.4	0.001	63.0 $\pm$ 15.5	0.001
Triglyceride (mg/dL)	121.3 $\pm$ 60.0	104.1 $\pm$ 46.4	0.240	98.2 $\pm$ 30.8	0.235	107.8 $\pm$ 46.7	0.955

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

**Table 2.** Changes in flow-mediated dilatation (FMD), nitroglycerin-induced dilatation (NID), carotid arterial stenosis, and cytokines, during atorvastatin therapy

	Baseline	Atorvastatin therapy			
		After 2 months	<i>p</i>	After 4 months	<i>p</i>
FMD (%)	6.8 ± 2.8	7.6 ± 4.5	0.204	10.0 ± 4.6	0.019
NID (%)	8.8 ± 7.3	11.3 ± 5.8	0.247	10.5 ± 6.8	0.313
Carotid arterial stenosis (%)*	62.2 ± 17.8	58.2 ± 17.1	0.332	52.0 ± 18.1	0.063
hs-CRP	0.23 ± 0.14	0.20 ± 0.16	0.040	0.27 ± 0.29	0.852
TNF- $\alpha$	7.13 ± 8.11	6.61 ± 6.35	0.287	5.30 ± 3.23	0.985
IL-1 $\beta$	1.31 ± 2.67	0.84 ± 1.23	0.654	0.54 ± 0.54	0.123
IL-6	3.47 ± 6.41	3.27 ± 5.58	0.778	2.79 ± 4.91	0.406
ICAM	269.4 ± 83.1	260.0 ± 93.1	0.370	270.7 ± 92.7	0.765
VCAM	902.1 ± 439.1	827.8 ± 319.1	0.575	825.7 ± 324.9	0.232
Endothelin-1	1.36 ± 1.37	1.20 ± 0.73	0.964	0.97 ± 0.40	0.432

\*Carotid arterial stenosis = maximal percentage diameter reduction in carotid arteries. hs-CRP = high-sensitivity C-reactive protein; TNF = tumor necrosis factor; IL = interleukin; ICAM = intracellular adhesion molecule; VCAM = vascular cell adhesion molecule.

gressive decline in the levels of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and ET-1. However, none of these cytokines were reduced significantly after 4 months. Although the overall severity of carotid arterial stenosis did not decrease significantly, atorvastatin caused regression of plaque thickness to less than 30% diameter reduction in 2 patients, whose LDL cholesterol levels after 4 months were 34.8 mg/dL and 79.4 mg/dL, respectively. Atorvastatin did not significantly alter endothelium-independent dilatation induced by sublingual nitroglycerin.

FMD did not correlate with LDL-cholesterol, high-density lipoprotein cholesterol, severity of stenosis, or any of the inflammatory cytokines studied. Plasma concentrations of TNF- $\alpha$  significantly correlated with those of hs-CRP ( $r = 0.282$ ;  $p = 0.028$ ), IL-1 $\beta$  ( $r = 0.425$ ;  $p = 0.001$ ), IL-6 ( $r = 0.455$ ;  $p < 0.001$ ), VCAM ( $r = 0.414$ ;  $p = 0.001$ ) and ICAM ( $r = 0.304$ ;  $p = 0.018$ ). IL-6 further correlated with IL-1 $\beta$  ( $r = 0.353$ ,  $p = 0.006$ ) and hs-CRP ( $r = 0.454$ ;  $p < 0.001$ ).

## Discussion

In this study, atorvastatin caused about a 30% decrease in plasma LDL-cholesterol concentration in Chinese patients with normal pretreatment LDL-cholesterol. The lipid-lowering effect was comparable to that in the Anglo-Scandinavian Cardiac Outcomes Trial—Lipid-Lowering Arm (ASCOT—LLA), which demonstrated a 35% decrease in LDL-cholesterol with atorvastatin 10 mg daily.<sup>16</sup> The recent Heart Protection Study<sup>12</sup>

demonstrated that simvastatin reduced major vascular events by 25% in patients with baseline LDL-cholesterol less than 3 mmol/L (115 mg/dL). Observational studies indicate a continuous positive relationship between coronary disease risk and blood LDL-cholesterol concentration, with no definite threshold below which a reduced concentration fails to be associated with reduced risk.<sup>17,18</sup> Our study also showed that atorvastatin improved endothelial function in patients without hypercholesterolemia when LDL cholesterol was reduced to less than 70 mg/dL. Sheu et al showed that FMD improved in diabetic patients only when LDL-cholesterol was less than 80 mg/dL.<sup>19</sup> A subgroup analysis of the ASCOT study also found improved FMD in patients with normal LDL-cholesterol.<sup>20</sup> As endothelial dysfunction is a critical factor in the pathogenesis of several vascular diseases and thrombus formation,<sup>2,3</sup> improved endothelial function seems to be a reasonable mechanism for reduced cardiovascular events during statin therapy in patients with normal LDL-cholesterol levels.

Although atorvastatin did not produce significant, overall regression of carotid arterial lesions in our study, it markedly reduced the thickness of carotid arterial stenotic lesions in 2 patients. More interestingly, the effects on plaque regression became evident within 4 months. Our results also suggested that aggressive statin treatment in selected patients with normal plasma cholesterol levels probably helped to accelerate regression of carotid arterial plaques. We could not find a target LDL-cholesterol level, or any other parameter, that ensured regression of carotid plaques, because multiple factors, besides reduced

cholesterol, are likely to be involved in the regression of arterial stenotic lesions. Kent and Taylor showed that patients with higher-than-average, on-therapy CRP levels are most likely to benefit from LDL-cholesterol reductions beyond the traditional goal of less than 100 mg/dL.<sup>21</sup> In our study, the cytokines and vasodilatory responses were not significantly different between patients with and those without regression of carotid arterial lesions, probably because the response rate was too low and the follow-up period too short. Prospective studies recruiting more patients are mandatory to understand the mechanisms of atherosclerotic plaque regression. Accumulating data strongly suggest that inflammation plays an important role in the process of atherosclerosis.<sup>22,23</sup> Such accentuated inflammatory responses may contribute to the high level of mortality from coronary heart disease in these subjects. Inflammatory cytokines, particularly TNF- $\alpha$  and IL-1 $\beta$ , stimulate a complex cascade of events.<sup>24,25</sup> Secondary responses include the release of chemotactic IL-6 and IL-8, upregulation of leukocyte adhesion molecule, and decreased fibrinolytic activity. Changes in inflammatory cytokines after atorvastatin administration have been variable.<sup>26,27</sup> In this study, plasma concentrations of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and ET-1 showed a trend towards progressive reduction after atorvastatin therapy. However, none of these cytokines were reduced significantly or correlated with FMD in our small trial. TNF- $\alpha$  concentrations correlated with hs-CRP, IL-1 $\beta$ , IL-6, VCAM and ICAM concentrations. Thus, TNF- $\alpha$ , as an initiator of the inflammatory cascade, may be useful as a marker of various inflammatory cytokines in future clinical studies. Previous studies showed that statins, including pravastatin, cerivastatin, lovastatin, simvastatin and atorvastatin, reduced circulating levels of hs-CRP.<sup>9,28-31</sup> However, it is unclear if statin therapy also reduces the levels of hs-CRP or inflammatory cytokines in patients with carotid stenosis. Our study showed that atorvastatin significantly reduced hs-CRP after only 2 months' treatment. Conversely, the mean hs-CRP level after 4 months was higher than the baseline level (not significant). These inconsistent changes suggest that elevated hs-CRP is a non-specific marker of inflammation and may be biased in individual cases or small-scale trials.

There are some limitations to this study. Firstly, there may have been confounding effects from medications on vasoreactivity, as we were unable to withdraw medication from chronically ill patients. Indeed, in all patients, atorvastatin was an "add-on" drug, and previous medication was unaltered. The effects of atorvastatin could, nonetheless, be assessed by com-

paring serial changes in the same individual. Secondly, the number of patients was small, and the follow-up period was relatively short. In addition, there are certain racial differences to consider regarding carotid arterial lesions. Further large-scale studies in different ethnic groups are necessary to provide more robust evidence for the management of patients with carotid arterial stenosis and normal LDL-cholesterol levels.

In conclusion, 4 months' atorvastatin therapy improved flow-mediated FMD in patients with carotid arterial stenosis and normal LDL-cholesterol levels, but did not cause significant regression of carotid arterial lesions. The endothelial protective effect of atorvastatin contributed to the drug's therapeutic benefit in patients with normal LDL-cholesterol levels.

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