Atorvastatin normalizes endothelial function in healthy smokers

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ABSTRACT

Endothelial function is known to predict cardiovascular disease. The aim of the present study was to examine whether endothelial function in smokers with normal cholesterol levels could be normalized by treatment with 80 mg of atorvastatin in comparison with a control group. Healthy smokers (n = 20) and non-smokers (n = 20) were randomized to receive 80 mg of atorvastatin or placebo in an open randomized cross-over study, followed by measurement of endothelial functional [FMD (flow-mediated dilation)]. At baseline, smokers had a lower FMD compared with the non-smoking group (2.2 ± 0.5 and 4.5 ± 0.8 % respectively; P < 0.05). In the smoking group, FMD increased significantly (P < 0.05) to 4.0 ± 0.8 % during treatment with atorvastatin and returned to basal levels during placebo (2.3 ± 0.6 %). In the non-smoking group, FMD was unaffected by both atorvastatin and placebo. The net change of total cholesterol or LDL (low-density lipoprotein)-cholesterol was not associated with the net change in FMD when the study group was considered as a whole or the smoking group was considered separately. In conclusion, improved endothelial function was observed in a group of smokers when they were received 80 mg of atorvastatin, whereas atorvastatin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the decrease in LDL-cholesterol during atorvastatin treatment. The results show that poor endothelial function in smokers with normal lipid levels can be improved by a statin treatment.

INTRODUCTION

Both primary and secondary prevention studies have demonstrated that statin treatment reduces cardiovascular end points [1–3]. There are several ultrasound and angiographic studies showing that lipid-lowering treatment is associated with a reduction in IMT (intima-media thickness) or improvement in the lumen area compared with placebo [4–6]. However, the clinical benefit of the drugs used in clinical studies [3] was manifest early in the course of lipid-lowering therapy before regression of atherosclerosis could occur. Therefore mechanisms other than cholesterol-lowering may be responsible for the positive outcome after treatment with statins.

Modifications of endothelial function, inflammatory responses and plaque stability have been suggested as possible mechanisms.

Impaired endothelium-dependent relaxation has been observed in subjects with atherosclerosis [7]. Moreover, studies have demonstrated that the presence of cardiovascular risk factors, such as cigarette smoking [8] and hypercholesterolaemia [9–11], is associated with endothelial dysfunction. Furthermore, cholesterol-lowering has been shown to improve the endothelial function [9–11]. In one study [10], an improvement in endothelial function after 1 month on simvastatin treatment was demonstrated in hypercholesterolaemic subjects, whereas most other studies have reported an improvement after...
However, there are also studies failing to show a positive effect on endothelial function with statin treatment in certain populations [12,13]. The underlying pathophysiological explanation behind the improved endothelial function after treatment with statins in subjects with hypercholesterolaemia is not known. However, it has been observed that statins activate endothelial NOS [NO (nitric oxide) synthase] independent of its cholesterol-lowering actions and this has been suggested to be a possible explanation for the positive outcome after treatment with statins [14,15]. It is unclear if different cardiovascular risk factors mediate endothelial dysfunction via the same pathophysiological pathway. Recently, it has been shown that pretreatment with vitamin C attenuates endothelial dysfunction after acute cigarette smoking [16]. Cigarette smoking is also associated with increased monocyte–endothelial cell adhesion, and a study has shown that l-arginine (the physiological substrate for NO), but not antioxidants, reversed that abnormality [17]. Thus there is a possibility that an agent, such as a statin, which activates NO formation, may improve endothelial function in smokers, independent of a cholesterol-lowering effect.

The aim of the present study was to examine whether endothelial function in smokers with normal cholesterol levels could be normalized by treatment with 80 mg of atorvastatin compared with a group of non-smokers.

**MATERIALS AND METHODS**

**Subjects**

Healthy smokers (smoking more than ten cigarettes per day) under the age of 40 years were recruited from the Department of Cardiology and by advertisement (n = 20). An aged-matched non-smoking group (n = 20) was recruited in the same way. Exclusion criteria included signs of cardiovascular disease, pharmacological treatment, diabetes mellitus and pregnancy. Contraception was necessary for all women included in the study. Snuff and nicotine-replacement treatment were not allowed. All subjects gave written informed consent, and the Ethics Committee of the Karolinska Institute at Huddinge University Hospital approved the study.

**Design**

The study was an open randomized cross-over design (Figure 1). After an initial physical examination, FMD (flow-mediated dilation) was determined and the subjects were randomized to received either active treatment with 80 mg of atorvastatin daily or no treatment (placebo). The non-smoking group underwent the same procedure. After 4 weeks, each subject returned for a second determination of FMD. There was a cross-over between the treatment arms, as outlined in Figure 1, and FMD was re-examined in the subjects after another 4 weeks.

**Measurements**

Total cholesterol, HDL (high-density lipoprotein)-cholesterol, LDL (low-density lipoprotein)-cholesterol, triacylglycerols (triglycerides) and plasma glucose, haemoglobin, CK (creatine kinase), ASAT (aspartate aminotransferase) and ALAT (alanine aminotransferase) were controlled at baseline, and at 4 and 8 weeks. Resting BP (blood pressure) was measured in the right arm after 10 min of supine rest. Smoking was assessed by a questionnaire. No subject experienced any side effects of the drug or elevated CK, ASAT or ALAT levels.

**FMD and IMT of the brachial artery**

The ultrasound procedures for assessing endothelium-dependent FMD were performed as described previously [18,19]. The subjects were examined in the morning after an overnight fast since midnight. A high-resolution ultrasound scanner (System Five; GE Vingmed) with a 10.0-MHz linear array transducer was used. After a 10 min equilibration period at rest in the recumbent position, a single dedicated ultrasonographer performed measurements of the left brachial artery FMD. Scans of the brachial artery were taken proximal to the antecubital fossa and saved on videotape. The transducer was fixed with a stand during the FMD examinations. Baseline diameter recordings were obtained after which arterial
**Table 1** Baseline demographics of the two groups of subjects

Values are means ± S.D., or means ± S.E.M. for ultrasound measurements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Smokers (n = 20)</th>
<th>Non-smokers (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30 ± 6</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>119/71 ± 15/9</td>
<td>116/72 ± 12/7</td>
</tr>
<tr>
<td>Lipid levels (mmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>4.2 ± 0.4</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>2.4 ± 0.5</td>
<td>2.4 ± 0.6</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.4*</td>
</tr>
<tr>
<td>Triacylglycerols</td>
<td>1.1 ± 0.6</td>
<td>1.0 ± 0.5</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.9 ± 0.3</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Ultrasound measures</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen (mm)</td>
<td>3.6 ± 0.09</td>
<td>3.5 ± 0.13</td>
</tr>
<tr>
<td>IMT (mm)</td>
<td>0.24 ± 0.004</td>
<td>0.25 ± 0.007</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>2.2 ± 0.5</td>
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occlusion was performed by inflating a forearm BP cuff (12.5 cm wide) to 250 mmHg for 4.5 min. After cuff release, diameter recordings were repeated during the post-occlusive increase in brachial artery blood flow. Arterial flow velocity recordings were obtained using pulsed wave Doppler at 60° to the vessel with the sample volume (1.5 mm) in the centre of the artery. The complete experimental sequence was performed twice at 30 min intervals.

Images were acquired digitally from the videotape and measured in random order by a single observer blinded to the conditions under which the ultrasonic images were obtained. Measurement of the brachial artery diameter was defined as a distance from the leading edge of the near-wall intima-lumen echo to the leading edge of the far wall lumen-intima echo along a line perpendicular to the artery's long axis. A computer system [20] with automated tracing of echo interfaces and measurements of distances between the wall echoes within a 5-mm-long section of the brachial artery was used. Brachial artery diameter was calculated in diastolic frames taken coincidentally with the R wave on the ECG twice at rest and then at 45, 60 and 75 s after cuff deflation. A mean of the diameters after 45, 60 and 75 s was calculated.

Diameter changes were expressed as the percentage change relative to the mean baseline value. The mean of two FMD examinations was used.

IMT was defined as the distance between the leading edge of the lumen-intima echo and the leading edge of the media-adventitia echo.

### Statistical analysis

Descriptive measurements are reported as means ± S.D. Experimental measures are reported as means ± S.E.M. Changes within a study group were compared with a paired Student t test, whereas comparisons between the study groups were made using an unpaired Student t test. Pearson’s correlation coefficients were calculated. In a clinical trial using this technique, a mean improvement in FMD of at least 2 % would usually be required to detect a treatment benefit. In a cross-over trial design, with 80 % power and 95 % confidence interval, a less than 1.5 % change in FMD will be detected with 20 subjects [21]. In our hands, the coefficient of variation is 2 % and a significant enhancement of 1.7 % induced by a treatment was detected in a cross-over design in 12 subjects [19].

### RESULTS

Baseline characteristics of all individuals enrolled are shown in Table 1. The only difference observed between the study groups was a significantly (P < 0.05) higher HDL-cholesterol level in non-smoking subjects compared with the smokers. The lumen and IMT of the brachial artery did not differ between the study groups (Table 1), although smokers had a significantly (P < 0.05) lower FMD compared with non-smokers (Table 1).

The effect of 80 mg of atorvastatin on total and LDL-cholesterol levels is shown in Table 2. Total cholesterol and LDL-cholesterol decreased significantly (P < 0.05) compared with baseline in both the smoking and non-smoking groups during treatment with 80 mg of atorvastatin. Triacylglycerol levels decreased significantly (P < 0.05) compared with baseline during atorvastatin

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treatment in the non-smoking group, but not in the smoking group. No effect on HDL-cholesterol levels was observed with 80 mg of atorvastatin compared with baseline in either of the study groups (Table 2).

During placebo administration, all lipid levels returned to basal levels in both study groups, and none of the levels were significantly different from those at baseline (Table 2).

FMD measurements are shown in Table 2. In the smoking group, FMD increased significantly ($P < 0.05$) during treatment with atorvastatin and returned to basal levels during placebo. In the non-smoking group, FMD was unaffected by either atorvastatin or placebo (Table 2).

The net change in total cholesterol or LDL-cholesterol was not correlated with the net change in FMD when the groups were considered as a whole or when the smoking group was considered separately.

**DISCUSSION**

In the present study, we confirm that cigarette smokers have a worse FMD compared with an age-matched non-smoking group. In the smoking group, endothelial function was improved to the same level as in the non-smoking group when they received 80 mg of atorvastatin, whereas atorvastatin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the decrease in LDL-cholesterol during atorvastatin treatment.

Despite a clearly low FMD, IMT of the brachial artery was not enlarged in the smoking group. It is more common to use IMT of the common carotid artery as a surrogate measure of atherosclerosis; however, we have reported previously [18] that brachial artery IMT is significantly associated with the artery IMT in the common carotid artery. In a recent autopsy study by Sorensen et al. [22], the grade of lesion severity in the brachial and the left anterior descending coronary arteries was significantly correlated and so was the severity in the brachial and carotid arteries. Thus the finding in that autopsy study supports the notion that IMT of the brachial artery may also serve as a surrogate variable when studying atherosclerosis. However, it is important to realise that it is not possible to differentiate between intimal and medial thickening with ultrasound techniques. Wiesmann et al. [23] found, using high-resolution MRI (magnetic resonance imaging), a poor central vascular distensibility in both the common carotid arteries and at multiple sites in the aorta in a group of smokers compared with non-smokers. The discordance between the present study and the study by Wiesmann et al. [23] is probably explained by different assessment methods and examination of different vessel beds.

The underlying mechanism of why cigarette smoking causes a deterioration in endothelial function is not completely known. FMD gives an indirect measure of NO bioavailability in the brachial artery. Since snuff and nicotine spray [24,25] also decrease FMD, nicotine has been suggested to be responsible for this action. However, because there are numerous known and unknown components of cigarette smoke whose metabolic fate in the human body is unknown, an appropriate *in vitro* model of cigarette smoking exposure remains to be established. Previous reports suggest that statins activate endothelial NOS [14,15], and our present observation of an improved FMD in the group of smokers might be explained by deactivated NOS among smokers, which returns to a normal activation level during statin treatment, whereas NOS among non-smokers is already activated and therefore statin treatment has no effect. In the present study, we found no relationship between the improvement in FMD in the smoking group and the improved lipid profile. This observation supports the hypothesis that statins activate endothelial NOS independent of the cholesterol-lowering actions.

Other studies [26] have suggested damage of NO by free radicals as the potential mechanisms through which cigarette smoking impairs endothelial function. This theory was supported by an observation by Papamichael et al. [27], who found that simultaneous consumption of red wine when smoking partly decreased the harmful acute effect of smoking via its effect on FMD. Furthermore, treatment with vitamin C attenuates endothelial dysfunction after acute cigarette smoking [16].

We found a lower HDL-cholesterol level in the smoking group, which is a phenomenon recognized previously [28]. The mechanisms responsible are not clearly elucidated, and the role of dietary differences between smokers and non-smokers is unknown. As expected, we observed a dramatic decrease in total cholesterol and LDL-cholesterol levels in both study groups following statin treatment, but the improved FMD level in the smoking group was independent of these lipid level changes.

In the present study, FMD was used as a surrogate variable and one can question the relevance of this selection. However, we know from numerous studies that FMD is associated with cardiovascular risk factors, including cigarette smoking [8–11], and that FMD predicts a poor outcome in different patient groups [29,30]. Our results are supported by a previous study [31]. The results may suggest that the cardiovascular risk in smokers with normal lipid levels can be reduced by a statin treatment; however, to confirm this, a large study with robust end points is needed.

In summary, we have observed improved endothelial function in a group of smokers when treated with 80 mg of atorvastatin, whereas this statin had no effect on endothelial function in the non-smoking group. The improved FMD among smokers was independent of the decrease in LDL-cholesterol levels during atorvastatin treatment.
ACKNOWLEDGMENTS

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REFERENCES


