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Short- and Long-Term Changes of Flow-Mediated Vasodilation in Patients under Statin Therapy

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Summary

Background: Flow-mediated vasodilation (FMD) of the brachial artery (BA) has been shown to improve in response to lipid-lowering therapy and other therapeutic interventions, usually within 1 to 2 months. Whether FMD remains improved under therapy in the longer term is unknown.

Hypothesis: The aim of this study was to examine the short- and long-term changes of FMD under statin therapy.

Methods: Flow-mediated vasodilation and nitroglycerin-mediated vasodilation (NMD) of the BA were measured with high-resolution ultrasound (13 MHz) at baseline and at 4 and 10 months in 18 consecutively recruited patients with coronary artery disease (CAD), in whom statin therapy was newly established.

Results: The decrease of total plasma cholesterol levels after 4 and 10 months of statin therapy (243 ± 31 vs. 186 ± 30 vs. 191 ± 40 mg/dl; $p < 0.001$) was accompanied by an increase in FMD from 4.4 ± 3.8% at baseline to 9.6 ± 2.7% at 4 months and to 9.5 ± 2.6% at 10 months ($p < 0.001$). Nitroglycerin-mediated vasodilation showed a trend toward improvement after 4 months (14.6 ± 7.5 vs. 19.1 ± 3.6 vs. 19.4 ± 5.6%; NS). The FMD/NMD ratio also rose significantly after 4 months and remained improved after 10 months of statin therapy (0.31 ± 0.25 vs. 0.52 ± 0.16 vs. 0.50 ± 0.14; $p < 0.01$).

Conclusion: Statin therapy is associated with sustained improvement of endothelial function up to 10 months. These data support the utility of FMD for the assessment of vascular function in response to lipid-lowering therapy or other therapeutic interventions in long-term studies.

Key words: flow-mediated vasodilation, statins, brachial artery, ultrasound

Introduction

Endothelial dysfunction occurs at an early stage of atherosclerosis[1, 2] and is closely associated with most cardiovascular risk factors.[3] Therefore, the clinical assessment of endothelial function has become of major interest in the past years.[4, 5] The measurement of flow-mediated vasodilation (FMD) of the brachial artery (BA) with high-resolution ultrasound is completely noninvasive and has been tested in patients with cardiovascular risk factors[6, 7] and coronary artery disease (CAD).[8, 9] Flow-mediated vasodilation has shown good correlation with coronary endothelial function[10] and with the extent of CAD as assessed by angiography.[9]

However, its value in long-term studies of endothelial function remains unclear. Most studies have reported a short-term (within 1–2 months) improvement of FMD following statin therapy[11–13] or other therapeutic interventions.[14, 15] Whether FMD shows sustained improvement over a longer period of time, which is a prerequisite for the use of FMD as a follow-up parameter, has not been studied.

Therefore, the aim of this study was to examine the course of FMD in patients with newly established statin therapy over 10 months.

Methods

Patients

Written informed consent was obtained from all patients. The study group consisted of 18 consecutive patients (mean age 49 ± 8 years; range 37–67 years) with elevated plasma cholesterol levels, in whom coronary angiography was performed for the evaluation of chest pain. Patients with conges-

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tive heart failure or valvular heart disease were excluded from the study. Coronary artery disease was defined as visually estimated percent diameter stenosis ≥ 30% in one or more major vessels. Statin therapy was begun in accordance with National Cholesterol Education Program (NCEP) guidelines.[16] Nine patients received atorvastatin, five patients pravastatin, and four patients simvastatin. Coronary risk factors were defined as previously reported.[4, 9] Investigations were undertaken in accordance with the Declaration of Helsinki.

Study Protocol

On the day after angiography, patients underwent assessment of FMD by high-resolution ultrasound (13 MHz Sequoia C 256, Acuson [Siemens], Mountain View, Calif.) and plasma lipid levels were measured. Measurements of FMD and plasma lipid were repeated after 4 months in all patients and after 10 months in 16 patients. Two patients withdrew before completion of the study.

Assessment of Vasodilation

Changes of vessel diameter after reactive hyperemia (endothelium-dependent vasodilation) and after sublingual nitroglycerin application (endothelium-independent vasodilation) were measured according to previously described methods.[4, 9] The ultrasound examination was performed between noon and 2 P.M. by an observer blinded to the patient's diagnosis and drug treatment. All vasoactive drugs were withdrawn 18–24 h before examination. Patients were instructed not to smoke and to remain fasting prior to the ultrasound examination.

The right BA was scanned longitudinally above the antecubital fossa. After optimizing the transducer position as well as depth and gain settings, images were stored electrocardiogram (ECG)-triggered to the peak of the T wave on the hard disk. Scans were taken at rest after lying for at least 10 min and the mean of three measurements was defined as resting diameter. After suprasystolic compression at 260 mmHg for 4.5 min of the right upper arm, the cuff was deflated and serial posthyperemia BA images were again stored on the hard disk. The mean of three maximum diameters within 120 s after compression formed the posthyperemia diameter. After sublingual application of 0.8 mg nitroglycerin, the mean of the three maximum diameters within the following 10 min was calculated. In addition, the FMD/NMD ratio was calculated to evaluate the endothelium-dependent dilation (FMD) in relation to the maximum dilation of the BA as assessed by nitroglycerin application.

Assessment of Variability and Repeatability

Interobserver variability was assessed using two methods. First, 136 vessel diameters from 11 patients were measured offline by two observers from images stored on the hard disk. In addition, variability of FMD of these 11 patients was calculated. Second, scans were taken by two observers separately in the same patients (n = 12).

The spontaneous course (repeatability) was assessed by comparing FMD values of two visits (4 months study interval) in 17 patients with CAD or risk factors (Table I), whose medication was left unchanged 3 months before the first visit and during the 4-month interval.

Statistics

Data are expressed as mean ± standard deviation (SD). Normal distribution of variables was tested using the Kolmogorov-Smirnov test with Lilliefors correction. Analysis of variance (ANOVA) followed by the Scheffé-test was used for comparison of variables among the three visits. Interobserver variability was expressed as mean difference of FMD and linear regression analysis was calculated. Differences were considered statistically significant at $p < 0.05$.

Results

Characteristics of patients are shown in Table I. Total and low-density lipoprotein (LDL) cholesterol decreased significantly after 4 months and remained stable after 10 months of statin therapy (Table II). High-density lipoprotein (HDL) cholesterol and triglycerides did not change over the entire study period (Table II).

Flow-mediated vasodilation improved from 4.4 ± 3.8 to 9.5 ± 2.7% after 4 months and to 9.5 ± 2.6% after 10 months of statin therapy ($p < 0.001$) (Fig. 1A), whereas resting diameters did not change (4.3 ± 0.8 vs. 4.1 ± 0.5 vs. 4.1 ± 0.5 mm;

TABLE I Baseline characteristics of patients

	Statin patients	Control patients
Patients	n = 18	n = 17
Age (range)	49.3 ± 7.6 (37–67)	52.1 ± 10.5 (36–68)
Number of risk factors	2.4 ± 0.8 (0–4)	1.2 ± 0.9 (0––2)
CAD (%)	18 (100)	7 (41.2)
Myocardial infarction (%)	7 (38.9)	2 (11.8)
Hypertension (%)	7 (38.9)	9 (52.9)
Smoking (%)	11 (61.1)	2 (11.8)
Hypercholesterolemia (%)	18 (100)	7 (41.2)
Diabetes mellitus (%)	1 (5.6)	1 (5.9)
Positive family history (%)	6 (33.3)	2 (11.8)
Total cholesterol (mg/dl)	243 ± 31 (200–298)	177.6 ± 36.7 (119–233)
LDL cholesterol (mg/dl)	163 ± 22 (120–189)	94.2 ± 29.5 (62–143)
BMI (kg/m^2)	27 ± 3 (20–30)	26.2 ± 3.3 (20.8–31.5)

Abbreviations: CAD = coronary artery disease, BMI = body mass index, LDL = low-density lipoprotein.

TABLE II Changes of plasma lipid levels and body mass index

	Baseline	After 4 months	After 10 months	p Value
Total cholesterol	243 ± 31	186 ± 32	191 ± 40	< 0.001
LDL cholesterol	163 ± 22	105 ± 29	111 ± 35	< 0.001
HDL cholesterol	42 ± 11	45 ± 11	47 ± 15	NS
Triglycerides	242 ± 134	193 ± 78	181 ± 87	NS
BMI (kg/m^2)	27 ± 3	26 ± 3	26 ± 3	NS

Plasma lipid levels are given in mg/dl.
Abbreviations: HDL = high-density lipoprotein, NS = not significant. Other abbreviations as in Table I.

NS). Improvement of FMD > 3% was seen in 10 patients at 4 months follow-up and < 3% in 5 patients. One patient showed a slight decrease. At 10 months follow-up, additional improvement of FMD (> 3%) was seen in three patients, and in two patients FMD decreased by < 3%. Eleven patients remained virtually the same (Fig. 2).

Nitroglycerin-mediated vasodilation increased nonsignificantly from 14.6 ± 7.5 to 19.1 ± 3.6% after 4 months and to 19.4 ± 5.6% after 10 months (p = 0.08) (Fig. 1B). In addition, the FMD/NMD ratio showed an improvement after 4 months (0.52 ± 0.16 vs. 0.31 ± 0.25 at baseline) and remained increased after 10 months of statin therapy (0.50 ± 0.14, p < 0.01).

The mean difference in measurements of the BA diameter from images (n = 136) stored on the hard disk between two observers was 0.04 ± 0.04 mm (r = 0.99; p < 0.001). The interobserver variability of calculated FMD showed a mean difference of 0.5 ± 0.5% (r = 0.99; p < 0.001). In addition, variability of resting diameters in 12 patients, which were measured online consecutively by two observers, was 0.06 ± 0.04 mm (r = 0.98; p < 0.001).

The spontaneous course (repeatability) of FMD in patients whose medication was left unchanged revealed a mean difference of 3.1 ± 2.5% FMD and similar group mean values (8.3 ± 3.7 vs. 8.8 ± 3.2%; NS) between measurements at baseline and at 4 months.

Discussion

In this study we found that FMD showed sustained improvement in patients under statin therapy over the course of 10 months. To the best of our knowledge, this is the first study documenting a long-term improvement in peripheral endothelial function under statin therapy beyond 6 months. In addition, analysis of the spontaneous course (repeatability) revealed that the mean FMD difference in patients with unchanged medication was below the improvement seen in patients under statin therapy.

Previous studies have revealed that statin therapy may improve endothelial function after a very short treatment period. For example, Vogel *et al.* observed an increase of FMD in patients with total cholesterol levels between 175 and 215 mg/dl after 2 weeks of simvastatin therapy.[11] Another study of microvascular endothelial function in the forearm showed an improvement of vascular function under simvastatin therapy after 1 month.[12]

In our study, FMD increased after 4 months of statin therapy and remained improved over a period of 10 months. The demonstration of sustained improvement of FMD under statin therapy is a prerequisite for the use of this noninvasive test as a follow-up parameter in long-term studies, because in most FMD studies the observation period was limited to 1–3 months. Furthermore, spontaneous variations of FMD may result from its susceptibility to current influences such as smoking,[17] high-fat meals,[18] or daytime of examination,[19] all of which are potential limitations for the use of this method as a follow-up test. These influences can be minimized under standardized conditions as shown in this study.

Besides reducing cholesterol levels, Laufs *et al.* have demonstrated another possible mechanism leading to an improvement of endothelial function by statins.[20] They have shown that statins can enhance the stability of endothelial nitric oxide synthase mRNA and thereby increase the generation of nitric oxide (NO).[20] The strong trend toward improvement of NMD seen in our study suggests a possible role of statins also in the modulation of smooth muscle cell function. Indeed,

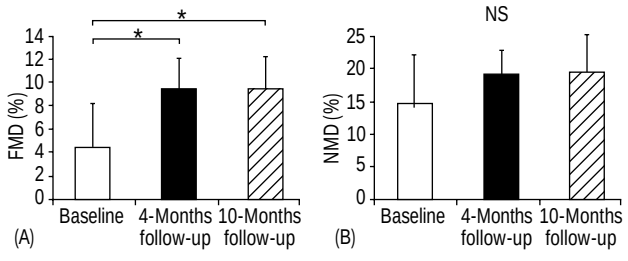

FIG. 1 (A) Long-term course of flow-mediated vasodilation (FMD); FMD increased significantly after 4 months of statin therapy and remained sustained improved after 10 months. (B) Long-term course of nitroglycerin-mediated vasodilation (NMD). Although there was a clear trend toward improvement, the difference did not yield significance. NS = not significant. * p < 0.001.

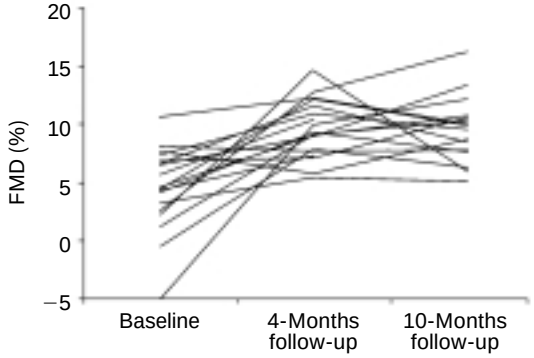

FIG. 2 Line graph showing the long-term course of flow-mediated vasodilation (FMD) in individual patients. Nearly all patients consistently showed an increase in FMD over time.

Dechend *et al.* have recently demonstrated that statins can ameliorate fibrosis in transgenic rats, which could partially explain such an effect.[21]

One major concern with FMD testing has been interindividual variation as well as considerable variability of measurements. Our results show that with higher frequency ultrasound (13 MHz) the interobserver variability of both single-vessel diameter measurements and FMD is very low (0.5%) and compares favorably with an earlier study using 7.5 MHz.[4] The spontaneous course (repeatability) showed a mean difference in FMD of 3%, which is slightly higher than that in previous studies.[4, 9] This is likely related to the longer study interval, to different risk factor profile of patients, and to changes in lifestyle and dietary habits, which have been undertaken during the course of the study.

This study did not include a placebo-control group because of ethical considerations. However, it was our aim to focus on methodological aspects rather than on the efficacy of statin therapy in improving endothelial function in a randomized fashion. The nonsignificant improvement of NMD as well as the additional myogenic and metabolic factors, which may be involved in the stimulus for reactive hyperemia and consequently FMD, may suggest that the statin effect is not specific for endothelial function. Nevertheless, Joannides *et al.* have shown that the NO inhibitor L-NMMA almost completely blocked the posthyperemic large artery vasodilation, suggesting NO to be the most important factor responsible for FMD.[22]

Conclusion

Flow-mediated vasodilation shows sustained improvement under statin therapy over the course of 10 months and a stable spontaneous course in patients with unchanged medication over a 4-month period. These results support the utility of FMD testing in long-term treatment trials.

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