Original Paper

Low-Dose Statin Therapy Improves Endothelial Function in Type 2 Diabetic Patients With Normal Serum Total Cholesterol: A Randomized Placebo-Controlled Study

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The authors sought to explore the effect of low-dose atorvastatin on endothelial function in normocholesterolemic patients with type 2 diabetes mellitus without evidence of coronary disease. Sixty patients with type 2 diabetes mellitus, normal serum cholesterol, and normal exercise test results were enrolled. Initial assessment was performed by measurement of the brachial artery diameter and flow velocity both at baseline and after induced hyperemia. Patients were randomly assigned to receive either atorvastatin 10 mg daily for 4 weeks (atorvastatin group=30 patients) or matched placebo for the same period (placebo group=30 patients). Follow-up assessment of the brachial artery diameter and flow velocity (both baseline and hyperemic) was performed after 4 weeks. Initially, no significant difference was found between the two groups regarding brachial artery diameter or flow velocity, both at baseline and at peak hyperemia (P>.05 for all). At follow-up, there was a significantly higher flow velocity at baseline (P<.05) and a significantly higher percent increase of brachial artery diameter (from baseline to peak hyperemia) in the atorvastatin group (P<.05). In patients with type 2 diabetes mellitus and normal serum cholesterol without evidence of coronary disease, low-dose atorvastatin improves endothelial function. J Clin Hypertens (Greenwich). *2010;12:820–825.* ©*2010 Wiley Periodicals, Inc.*

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There is a strong body of evidence that type 2 diabetes mellitus (T2DM) is associated with endothelial dysfunction as a result of both decreased production and increased oxidative inactivation of nitric oxide (NO) by free radicals.[1] Several relevant mechanisms may operate in states of insulin resistance and account for the decreased bioavailability of NO and abnormal endothelial function. Insulin resistance is associated with elevated serum markers of inflammation such as C-reactive protein.[2] Increased release of cytokines such as interleukin 6 and tumor necrosis factor α from the adipose tissues stimulates hepatic production of C-reactive protein.[3]

Evidence-based literature indicates that statins may improve the bioavailability of NO by reducing oxidative stress or increasing the activity of NO synthase.[4,5] Recently, it has been proposed that statins may improve endothelial function by decreasing the levels of asymmetric dimethylarginine (ADMA), a known competitive inhibitor of endogenous endothelial NO synthase.[6] The effect

of statins on ADMA levels may be mediated by the decrease in oxidized low-density lipoprotein levels. Similarly, the ability of statins to depress cytokine expression (especially tumor necrosis factor α) might indirectly decrease ADMA levels.[6]

In a prospective, randomized, double-blind, placebo-controlled study, we sought to explore the effect of low-dose atorvastatin on endothelial function, as reflected by flow-mediated dilatation (FMD) of the brachial artery in patients with T2DM and normal serum cholesterol levels without evidence of coronary artery disease.

METHODS

Patient Selection

We enrolled 60 patients with T2DM referred to our diabetes outpatient clinic during the period from October 2007 to July 2008 for routine follow-up. Patients were considered eligible for inclusion if they had normal total serum cholesterol levels (<5.4 mmol/L) and a normal exercise electrocardiographic (ECG) test result. Patients with abnormal serum triglycerides and/or high-density lipoprotein cholesterol levels were also included. We excluded patients with known ischemic heart disease and cerebrovascular or peripheral vascular disease and those with contraindication to statins (eg, history of chronic liver impairment or skeletal myopathy). Before inclusion, informed written consent was obtained from each patient after full explanation of the study protocol, and the study protocol was reviewed and approved by our local institutional human research committee as it conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as revised in 2002.

Definitions

The presence of diabetes mellitus was defined as fasting plasma glucose ≥126 mg/dL and/or 2-hour post-load glucose ≥200 mg/dL or specific antidiabetic drug therapy. Dyslipidemia was defined as serum triglycerides >150 mg/dL and/or high-density lipoprotein cholesterol <40 mg/dL in men and <50 mg/dL in women. Normal exercise ECG test result was defined as attainment of >85% of age-predicted maximal heart rate, without angina or other symptoms suggestive of myocardial ischemia, in the absence of ischemic ST-segment deviation.

Initial Assessment of Endothelium-Dependent FMD

Patients were instructed to discontinue all vasoactive medications for 4 times the half-life and to refrain from cigarette smoking for at least 6 hours before assessment. Assessment was performed in a quiet dark room with a constant temperature (24°C), barometric pressure (760 mb), and humidity (50%). Patients were evaluated after a 12-hour fasting period, in the supine position, with the arm comfortable and supported. Resting blood pressure was measured with a sphygmomanometer cuff. Imaging of the brachial artery in the dominant arm was performed using a General Electric Vivid 7 Pro cardiac ultrasound machine (General Electric, Horten, Norway). After 15 minutes of relaxation, a 7.5-MHz linear array probe was used to obtain good-quality images of the brachial artery in the longitudinal plane, above the antecubital fossa. The baseline brachial artery diameter was measured at rest from media-adventitia interface to media-adventitia interface. The baseline blood flow velocity was measured from the pulsed-wave Doppler signal obtained with the sample volume at mid-artery level. Hyperemic response was induced by inflating the sphygmomanometer cuff (wrapped around the distal forearm) to a pressure 50 mm Hg above the measured systolic pressure for 5 minutes. After release of the cuff, the hyperemic blood flow velocity was measured as before within 15 seconds after cuff release. The hyperemic brachial artery diameter was then measured as before, approximately 60 seconds after cuff release or 45 to 60 seconds after the peak hyperemic flow velocity.[7] FMD was calculated as the percent increase of brachial artery diameter from baseline to peak hyperemic measurement. The percent increase of blood flow velocity from baseline to peak hyperemic measurement was also calculated. Assessment was performed offline by a single experienced operator (Z.A.), blinded to individual group assignment as well as to the chronologic order of assessment (initial or follow-up).

Pharmacologic Intervention

After enrollment and initial assessment of endothelium-dependent FMD, patients were randomly assigned, on an individual basis, to receive either atorvastatin calcium 10 mg daily orally for 4 weeks (atorvastatin group=30 patients) or matched placebo for the same period (placebo group=30 patients). They remained in the same allocation throughout the study period. Standard antidiabetic medications were allowed and remained unchanged during the study period. None of the patients regularly consumed multivitamin/mineral tablets or relevant amounts of food particularly rich in antioxidants.

Table I. Baseline Clinical Characteristics of the Entire Cohort and Both Study Groups

	Cohort (N=60)	Statin Group (n=30)	Placebo Group (n=30)	*P* Value[a]
Age, y	38.3±10.5	38.7±11	37.9±9.9	>.05
Men	30 (50)	14 (46.7)	16 (53.3)	>.05
Duration of diabetes mellitus, y	3.6±8.3	3.0±5.4	3.9±9.5	>.05
Hypertension	48 (80)	25 (83.3)	23 (76.7)	>.05
Smoking	25 (41.7)	13 (43.3)	12 (40)	>.05
Dyslipidemia[b]	20 (33.3)	9 (30)	11 (36.7)	>.05
Total serum cholesterol, mmol/L	4.8±0.3	4.7±0.3	4.9±0.2	>.05

Continuous variables are presented as mean ± standard deviation, while categoric variables are presented as numbers (percentage). [a]Comparison between statin group and placebo group. [b]Abnormal serum triglycerides and/or high-density lipoprotein cholesterol levels.

Follow-Up Assessment of Endothelium-Dependent FMD

Assessment of the brachial artery diameter and blood flow velocity (both baseline and hyperemic) was performed after 4 weeks, as before.

Statistical Analysis

All continuous variables were presented as mean ± standard deviation, if they were normally distributed. Data were tested for normal distribution using the Kolmogorov–Smirnov test. Categoric variables were described with absolute and relative (percentage) frequencies. Comparisons between the two individual groups were performed using the unpaired *t* test for continuous variables and the Pearson chi-square test for categoric variables. Baseline data were compared with follow-up data within the same group using the paired *t* test for continuous variables. All tests were 2-sided and a probability value of $P<.05$ was considered statistically significant. Analyses were performed with SPSS version 12.0 statistical package (SPSS Inc, Chicago, IL).

RESULTS

Baseline Clinical Characteristics

A total of 60 patients with T2DM and normal serum cholesterol levels were enrolled in the current study, which comprised 30 patients randomly assigned to receive atorvastatin (atorvastatin group =30 patients) and 30 others randomly assigned to receive matched placebo (placebo group=30 patients). Table I shows the baseline clinical characteristics of the whole series, as well as the two individual groups. The mean age of the study cohort was 38.3±10.5 years, 30 (50%) being men. The two individual groups were statistically matched regarding age, sex, duration of DM, hypertension, smoking, and dyslipidemia.

Table II. Initial Brachial Artery Measurements in Both Study Groups at Baseline and at Peak Hyperemia

	Statin Group (n=30)	Placebo Group (n=30)	*P* Value
Brachial artery diameter			
Baseline, mm	4.03±0.64	3.89±0.66	.661
Hyperemic, mm	4.43±0.68	4.39±0.73	.902
Percent increase, %	7.7	13.2	.095
Brachial artery flow velocity			
Baseline, cm/s	66.7±23.2	62.8±26.4	.734
Hyperemic, cm/s	71.5±29.2	69.4±27.2	.670
Percent increase, %	12.2	7.5	.367

Continuous variables are presented as mean ± standard deviation.

Initial Brachial Artery Measurements

Table II shows initial brachial artery measurements (both baseline and hyperemic) of the two individual groups. No statistically significant difference was found between the two groups regarding brachial artery diameter or flow velocity, both at baseline and at peak hyperemia ($P>.05$ for all). However, there was a trend toward a higher percent increase of brachial artery diameter (from baseline to peak hyperemia) in the placebo group compared with the atorvastatin group ($P=.095$).

Follow-Up Brachial Artery Measurements

Similarly, Table III shows brachial artery measurements (both baseline and hyperemic) of the two individual groups at 4 weeks of follow-up. There was a significantly higher percent increase of brachial artery diameter (from baseline to peak hyperemia) in the atorvastatin group as compared with the placebo group ($P<.05$). Moreover, there was a significantly higher flow velocity at baseline ($P<.05$) and a trend toward a higher flow velocity

Table III. Follow-Up Brachial Artery Measurements in Both Study Groups at Baseline and at Peak Hyperemia

	Statin Group (n=30)	Placebo Group (n=30)	*P* Value
Brachial artery diameter			
Baseline, mm	3.87±0.74	3.79±0.46	.884
Hyperemic, mm	4.49±0.69	4.33±0.62	.405
Percent increase, %	18.3	13.2	.015[a]
Brachial artery flow velocity			
Baseline, cm/s	70.6±18.2	58.2±20.8	.033[a]
Hyperemic, cm/s	74.5±20.3	63.8±20.7	.091
Percent increase, %	10.9	10.1	.724

Continuous variables are presented as mean ± standard deviation. [a]Significant *P* value.

at peak hyperemia (*P*=.091) in the atorvastatin group as compared with the placebo group. Yet, no statistically significant difference was found between the two groups regarding the brachial artery diameter (both at baseline and at peak hyperemia) or the percent increase of flow velocity (from baseline to peak hyperemia) (*P*>.05 for all).

The drug was well-tolerated by all patients, with no major side effects during the 4-week treatment period. Moreover, no patients reported any clinical events during this period.

DISCUSSION

The current study demonstrated that low-dose atorvastatin (10 mg daily) induced an appreciable improvement of endothelial function in normocholesterolemic patients with T2DM, as reflected by improvement of brachial artery FMD. FMD (the percent increase of brachial artery diameter from baseline to peak hyperemia) has increased in the atorvastatin group, from 7.7% initially to 18.3% at 4-week follow-up (*P*<.05). In comparison, while the FMD was initially higher in the placebo group (as compared with the atorvastatin group), it remained almost the same at 4-week follow-up (*P*>.05). Furthermore, while brachial artery flow velocities (both baseline and hyperemic) were initially similar between the two individual groups (*P*>.05), both values were higher in the atorvastatin group at 4-week follow-up (compared with the placebo group).

Endothelial Dysfunction in T2DM

Hyperglycemia is associated with reduced bioavailability of NO as a result of decreased synthesis by endothelial NO synthase enzyme and increased consumption by oxygen-derived free radicals.[1] A recently proposed pathophysiologic mechanism potentially contributing to endothelial dysfunction in patients with T2DM is mediated by the increase in ADMA levels. By inhibiting endothelial NO synthase, ADMA hampers the regeneration of depleted NO in the vessel wall.[6,8] Moreover, the exposure to hyperglycemia induces a nonenzymatic glycation of proteins and lipids with ensuing formation of advanced glycation end-products, which are prevalent in the vasculature of diabetic patients.[9] Advanced glycation end-products decrease the bioavailability of NO both by reducing endothelial NO synthase activity and by depleting the already present NO.[10]

Effect of Statins on Endothelial Function

Statins likely improve endothelial function by increasing NO synthase enzyme activity, reducing oxidative stress, and decreasing levels of ADMA.[4–6] Supporting the results of the current study, several validated peer-reviewed articles in the literature demonstrated that statins improved endothelial dysfunction in patients with T2DM by a mechanism unrelated to the lipid-lowering effect.[11–13] In contrast, other studies reported no or insignificant improvement of FMD in T2DM patients treated with statins.[14–16] This discrepancy of results among the different studies can be attributed—at least in part—to the divergence in the methodology of measurement of FMD. For instance, defining lumen diameter as the distance between intima-lumen interfaces—rather than media-adventitia interfaces—would decrease reference lumen diameters and augment the calculated FMD. In addition, inducing hyperemia by inflating the sphygmomanometer cuff around the upper arm instead of the forearm would again result in higher FMD. Finally, the timing of measurement of the hyperemic brachial artery diameter would also be critical. Recording measurements every 15 seconds after deflation of the cuff would be more representative than simply taking a single measurement 1 minute later. Inconsistent findings would also reflect the heterogeneous nature of the underlying disease process, the lack of uniformity in patient selection and study protocols among different studies, and the interplay of various genetic and environmental factors related to the diverse ethnical and cultural backgrounds of the studied populations.

Clinical Implications

Two recent mega trials, namely the Collaborative Atorvastatin Diabetes Study (CARDS) and the

Anglo-Scandinavian Cardiac Outcomes Trial–Lipid-Lowering Arm (ASCOT-LLA), reported that treatment with atorvastatin reduced major cardiovascular events in patients with T2DM.[17,18] Importantly, the benefits of atorvastatin use were not related to initial serum cholesterol levels, and these data add further credence to the theory that all patients with type 2 diabetes may benefit from statin therapy. Nevertheless, although these data suggest that treatment with low-dose atorvastatin (10 mg/d) is sufficient to reduce cardiovascular risk in patients with T2DM and normal serum cholesterol levels, this paradigm is not widely acknowledged.[19] We conducted the current study in patients with T2DM and normal serum cholesterol levels, excluding those with known ischemic heart disease and those with equivocal or ischemic response to exercise stress ECG test. The observation, in the current study, that a low-dose atorvastatin regimen improves endothelial dysfunction (as reflected by the improvement of FMD) in normocholesterolemic diabetic patients without evidence of coronary artery disease may provide insight into the underlying mechanism of reduction of cardiovascular risk in this patient category, previously reported in the literature,[17,18] and warrant further reconsideration of such regimens in patients with T2DM. Based on the available body of evidence, statin therapy should now be routinely considered for all diabetic patients at sufficiently high risk for major cardiovascular events, irrespective of their initial cholesterol concentrations.

LIMITATIONS

Our findings are based on a single-center study with a relatively small sample size of the cohort, a fact that makes it difficult to generalize our results to all patients with T2DM and normal serum cholesterol levels. Multicenter studies using the same protocol and examining a larger number of patients are needed. Moreover, the presence of "silent" ischemic heart disease cannot be completely ruled out in this series of diabetic patients. Furthermore, the sensitivity of exercise stress ECG testing might be less than ideal in identifying patients with ischemic heart disease. More sophisticated stress myocardial perfusion imaging studies or stress echocardiography would have better isolated patients with ischemic heart disease.

CONCLUSIONS

In patients with T2DM and normal serum cholesterol levels without evidence of coronary artery disease, low-dose atorvastatin improves endothelial function, as reflected by FMD in the brachial artery.

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