Impaired endothelium-dependent vasodilation in type 2 diabetes mellitus and the lack of effect of simvastatin

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Abstract

Objective: Although type 2 diabetes is recognized as an independent risk factor for cardiovascular disease and cardiovascular disease is associated with endothelial dysfunction, the influence of type 2 diabetes per se on the endothelial function is controversial. HMG-CoA-reductase inhibitors have been shown to have short-term beneficial effects on endothelial dysfunction among patients with dyslipidemia or cardiovascular disease. The effect of HMG-CoA-reductase inhibitors on the endothelial function in diabetes is largely unknown.

Methods: Seventeen patients with type 2 diabetes, free of cardiovascular disease and no other cardiovascular risk factors, except for dyslipidemia, were studied together with ten healthy volunteers. The effect of 5-hydroxytryptamine, as an endothelium-dependent vasodilator, and sodium nitroprusside, as an endothelium-independent vasodilator, on the forearm blood flow was measured using venous occlusion plethysmography.

Results: 5-Hydroxytryptamine and sodium nitroprusside, infused in the brachial artery, caused a dose-dependent vasodilation. The vasodilator response to 5-hydroxytryptamine was significantly lower among the diabetic patients, 42 and 56%, than among the controls, 73 and 103%, at a dose of 0.3 and 0.9 ng/kg/min, respectively (P<0.05 and P<0.001). Vasodilator responses to sodium nitroprusside were comparable among the diabetic patients and controls. A 6-week treatment with simvastatin 40 mg once daily did not change the vasodilator responses to 5-hydroxytryptamine or sodium nitroprusside among the patients with diabetes.

Conclusions: The results of this study indicate that the endothelial function is impaired in type 2 diabetes and is not restored after a 6-week treatment period with simvastatin 40 mg.

Keywords: Cholesterol; Diabetes; Endothelial function; Serotonin (5HT); Statins

1. Introduction

Cardiovascular disease is an important main long-term complication of type 2 diabetes mellitus (DM 2), causing a two- to four-fold increase in cardiovascular mortality and morbidity. DM 2 is recognized as an independent risk factor for cardiovascular disease, however, most patients have a combination of cardiovascular disease risk factors, such as obesity, hypertension, and dyslipidemia, all contributing to the increased risk for these patients.

The endothelial dysfunction has been proven to be a predictor for future cardiovascular events [1,2]. Acute hyperglycemia and acute hyperinsulinemia tested in clamp studies have been found to impair the endothelial function in healthy subjects [3,4]. In contrast, contradictory results have been reported about whether or not the endothelial function is impaired among patients with chronic hyperglycemia and hyperinsulinemia, i.e. patients with DM 2 [5–10]. Furthermore, the presence of other cardiovascular risk factors and autonomic neuropathy may hamper the interpretation of these studies. Restoration of the impaired
endothelial function has been shown in patients with dyslipidemia after 4–6 weeks of statin treatment [11,12].

To evaluate whether the chronic disturbed glucose metabolism in DM 2 causes endothelial dysfunction and to elucidate the role of dyslipidemia a study was performed on endothelial function in patients with DM 2 and on the lipid levels in the range from normal to the typical diabetic dyslipidemia. Second, to evaluate whether the possible disturbance in the endothelial function was reversed by statin therapy, we treated the DM 2 patients with simvastatin 40 mg daily for 6 weeks.

2. Methods

2.1. Subjects

Seventeen male patients with DM 2 were recruited. Ten healthy middle-aged male volunteers (controls) participated in the study. All patients were free of hypertension, non-smoking, younger than 65 years of age and free of symptoms or signs of cardiovascular disease. DM 2 patients with HbA1c levels above 8.5% were excluded. The presence of autonomic neuropathy was excluded using the methods of cardiac rhythm variability, the Ewing test and the deep breathing test, and the orthostatic blood pressure test [13]. Patients with overt diabetic nephropathy were excluded, in 16 of the diabetic cases the albumin/creatinine ratio was below 2.5 mg/mmol in a morning urine portion while one diabetic patient had microalbuminuria as measured in a timed urine portion; all patients had a plasma creatinine level below the upper limit of normal (<133 µmol/l). Patients using lipid lowering medication were excluded. Female subjects were not included because of possible influences of female hormones on the vascular wall. All patients signed a written informed consent. The Medical Ethics Committee of the Leiden University Medical Center approved the study protocol. This investigation was carried out according to the principles outlined in the Declaration of Helsinki [14].

2.2. Procedures

Blood samples were drawn after 12 h of fasting and measured according to standardized laboratory protocols. A morning urine portion was collected for measurement of the albumin/creatinine ratio.

Forearm blood flow (FBF) was measured by computerized, R-wave triggered, venous occlusion plethysmography, using mercury in silastic strain gauges and a rapid cuff inflator (Hokanson Inc., Bellevue, USA) as described previously [15–17]. Blood glucose was measured 1 min before each new vasoactive compound using a finger stick and a One Touch Pro®le (Johnson & Johnson, Lifescan Benelux). After blood sampling, patients were allowed to eat one slice of white bread. Patients on oral medication did not take any medication until after the examination, patients on insulin received half their morning dose.

2.3. Drugs and solutions

The following compounds were used for intra-arterial infusion: 5-hydroxytryptamine (5HT; ICN Biomedicals, OH, USA), and sodium nitroprusside (SNP; Merck, Darmstadt, Germany). 5HT was dissolved in 0.9% saline, and SNP was dissolved in 5% glucose. 5HT was used because it is a specific stimulator of endothelium-dependent, NO-mediated, vasodilation, in contrast to acetylcholine which has alternative pathways to induce vasodilation, i.e. via stimulating endothelial derived hyperpolarizing factor [18]. On the day of the study the solutions were prepared from sterile stock solutions and ampoules and stored at 4°C until use. Merck Sharp and Dohme (Haarlem, The Netherlands) kindly provided simvastatin tablets of 40 mg.

2.4. Study protocol

2.4.1. Study day 1

A fasting venous blood sample was drawn for measurements of serum lipids. The endothelium-dependent vasodilation in both patients with DM 2 and in the controls was measured using intra-arterial cumulative-dose infusions of 5HT (0.3 and 0.9 ng/kg/min). SNP (30 and 90 ng/kg/min), a direct nitric oxide donor, was infused as an endothelium-independent vasodilator. Doses were chosen in the steep part of the dose response curve, without inducing dilation of the venous vascular bed [19]. The drugs were given in random order and each dose step was administered by means of intra-arterial infusion for 7 min. A washout period of 15 min was applied between the different infusions in order to allow forearm blood flow to return to baseline levels. FBF, blood pressure, and heart rate were measured during the 2 min prior to the start of each intra-arterial infusion, and during the last 2 min of each infusion step. FBF is measured as ml/100 ml forearm tissue/min. Vasodilator responses caused by the infusions of 5HT and SNP are expressed as percentage change from baseline FBF.

2.4.2. Study day 2

The experiments of study day 1 were repeated among the patients with DM 2 after a treatment of 6 weeks with 40 mg of simvastatin administered once daily. Drug compliance was measured by counting the number of tablets returned.

2.5. Statistics

Continuous variables were expressed as mean±S.E.M. Comparisons of continuous variables between baseline characteristics of both groups were made using the Stu-
dent’s t-test. Differences in vasodilator responses were tested for significance using Student’s t-test and ANOVA.

The actual blood glucose, total cholesterol, LDL-cholesterol (LDL-c), HDL-cholesterol (HDL-c) and triglycerides were added as covariates to evaluate their influence on forearm blood flow responses. The effects of simvastatin therapy on lipoprotein profiles were analysed using the Student’s t-test. Statistical significance was accepted at the 5% level. The statistical analyses were performed with SPSS/PC+™ software (SPSS Inc, Chicago, IL, USA).

3. Results

The baseline characteristics of the study groups are listed in Table 1. As expected, fasting glucose was significantly higher in the patients with DM 2, in addition the DM 2 patients were more obese. The patients with DM 2 had a higher mean level of serum triglycerides, while total cholesterol, LDL-c and HDL-c levels showed no difference. According to the inclusion criteria none of the patients showed any evidence of overt nephropathy or autonomic neuropathy. The kind of treatment for glucose control of patients with DM 2 is given in Table 1. None of the patients used aspirin, folic acid or blood pressure lowering medication. No changes in medication or dosage were made throughout the study.

Table 1

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>Controls</th>
<th>DM 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>46±2.2</td>
<td>51±1.7</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.1±0.10</td>
<td>8.7±0.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>–</td>
<td>7.2±0.22</td>
<td></td>
</tr>
<tr>
<td>Time since diagnosis of DM 2 (years)</td>
<td>–</td>
<td>8.4±1.67</td>
<td></td>
</tr>
<tr>
<td>Treatment for glucose control</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Diet only</td>
<td>–</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Oral: SUF</td>
<td>–</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Oral: metformin and SUF</td>
<td>–</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>–</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Insulin and metformin</td>
<td>–</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Insulin and SU and metformin</td>
<td>–</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.2±0.32</td>
<td>5.7±0.28</td>
<td></td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.5±0.29</td>
<td>3.4±0.26</td>
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<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.97±0.064</td>
<td>1.04±0.071</td>
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</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.2±0.089</td>
<td>2.9±0.70</td>
<td>&lt;0.05</td>
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<tr>
<td>Smoking habits</td>
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<tr>
<td>Current</td>
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<tr>
<td>Former (&gt;5 years ago)</td>
<td>2</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>8</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
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<td>0</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>130±3.1</td>
<td>135±4.4</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85±1.6</td>
<td>81±1.67</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4±0.60</td>
<td>26.8±0.70</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Family history for CVD</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Forearm blood flow (ml/100 ml forearm tissue/min)</td>
<td>2.36±0.26</td>
<td>2.84±0.32</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.E.M. CVD, cardiovascular disease; SU, sulphonylurea derivatives.

The local intra-arterial infusions of 5HT and SNP did not induce any significant changes in intra-arterial blood pressure and heart rate. Therefore, the changes in FBF can be interpreted as true local vascular effects of the vasoactive drugs used. No differences were found in baseline FBF of the randomly chosen first and second drug used, nor were differences found between baseline FBF of the two different vasoactive drugs, so the initial 15 min and the 15 min of wash-out were long enough for acclimatisation to the experimental procedure and achieving a stable non-stressed baseline. Even after corrections for actual blood glucose, total cholesterol, LDL-c, HDL-c or triglycerides the outcome of the study did not change. No significant correlation was found between the actual blood glucose levels and the FBF responses. No side effects were reported throughout the study.

3.1. Study day 1

In DM 2 the cumulative intra-arterial infusion of 5HT 0.3 and 0.9 ng/kg/min induced a dose-dependent increase in FBF of 42±6.4 and 56±7.2%, respectively (P=0.01). In the control group this cumulative dose-infusion of 5HT elicited an increase in FBF of 73±12.0 and 103±10.0%, respectively (P=0.033), which is significantly higher compared to the responses in the DM 2 group (P=0.018 for the low dose and P=0.001 for the high dose) (Fig. 1).
The forearm vascular responses to intra-arterially infused SNP 30 and 90 ng/kg/min were similar in both groups, causing a significant dose-dependent increase in FBF of $178\pm 23.1$ and $292\pm 41.0\%$ in the DM 2 group, and of $218\pm 22.4$ and $348\pm 42.1\%$ in the control group ($P<0.001$ for both) (Fig. 1).

3.2. Study day 2

On average $96\%$ (range 83–100\%) of the simvastatin tablets were taken. No changes in fasting glucose were found and no clinical or biochemical side effects were observed. Among the patients with DM 2 the vasodilator responses to 5HT and SNP did not change after 6 weeks of treatment with simvastatin 40 mg compared to the responses before treatment on study day 1, as shown in Fig. 2. During treatment with simvastatin, 5HT 0.3 and 0.9 ng/kg/min increased the FBF with $38\pm 4.2$ and $60\pm 7.5\%$, respectively ($P=0.001$). SNP 30 and 90 ng/kg/min increased the FBF with $163\pm 17.2$ and $269\pm 28.7\%$, respectively ($P<0.001$). The lipid lowering effect of simvastatin 40 mg for 6 weeks in the patients with DM 2 resulted in a significant reduction of the total cholesterol levels from $5.7\pm 0.28$ mmol/l (median 5.5 mmol/l) to $3.9\pm 0.21$ mmol/l (median 1.63 mmol/l), with a $p$ value $<0.001$. The LDL-c was significantly lowered from $3.4\pm 0.26$ mmol/l (median 3.4 mmol/l) to $1.96\pm 0.14$ mmol/l (median 1.80 mmol/l), with a $p$ value $<0.001$. The HDL-c increased significantly from $1.04\pm 0.071$ mmol/l (median 1.00 mmol/l) to $1.10\pm 0.066$ mmol/l (median 1.10 mmol/l), with a $p$ value of 0.01. No significant change of the triglyceride level was found, from $2.9\pm 0.70$ mmol/l (median 1.66 mmol/l) to $2.2\pm 0.39$ mmol/l (median 1.63 mmol/l).

To investigate whether the forearm vascular responses were dependent on lipid levels, the patients with DM 2 were divided in two groups based on lipid levels below and above the median levels in this group on study day 1. The vasodilator responses on study day 1 to both 5HT and SNP were not significantly different in the DM 2 patients with lipid levels above median compared to the DM 2 patients below the median. The vasodilator responses during treatment with simvastatin (study day 2) were not significantly different between the DM 2 patients achieving below median versus above median lipid levels.

4. Discussion

In the present study a significant impairment of the endothelial function was found in patients with DM 2, using the endothelium-dependent vasodilator 5HT as a
pharmacological tool. The endothelial dysfunction was independent of baseline serum lipid levels. Six weeks of treatment with simvastatin 40 mg did not restore this impaired 5HT induced endothelium-dependent vasodilatation, despite significant lowering of lipid levels.

The DM 2 patients in this study had a mild dyslipidemia, which is characteristic for the disease. All patients were free of signs and symptoms of cardiovascular disease and had no additional cardiovascular risk factors. Also autonomic neuropathy was excluded because of the potential disturbing effects on the vascular wall reactivity. It is our assumption that this moderately to well regulated, uncomplicated subset of patients with DM 2, without additional cardiovascular risk factors are suitable to investigate the vascular reactivity, using 5HT and SNP as pharmacological tools. 5HT is an established substance to investigate endothelium-dependent vasodilatation in humans, and SNP is the gold standard to study endothelium-independent vasodilator responses [18,20]. In the control group the vasodilator responses to 5HT and SNP were in the same range as found in earlier forearm studies [18,20–22].

The 5HT-induced forearm vasodilation was significantly impaired among the DM 2 patients, whereas the SNP response was intact, showing a selective impaired endothelium-dependent vasodilatation in DM 2. This impaired endothelium-dependent vasodilatation persisted when only the DM 2 patients with normal serum lipids were analysed, providing evidence that this endothelial dysfunction is not caused by the mild dyslipidemia, but by the long-term disturbance in glucose metabolism. In contrast to the finding in type 1 DM, in whom Clarkson et al. [23] showed LDL-cholesterol dependence of the endothelial function. The present study results are in concordance with the findings in clamp studies in healthy individuals, showing that artificially induced high blood glucose levels or high insulin levels impair endothelial function [3,4]. Also in patients with type 1 diabetes endothelial dysfunction has been associated both with hyperinsulinemia and chronic hyperglycemia [24,25]. High levels of glucose as well as high levels of insulin can be directly toxic for the vascular wall. Thereby, high glucose is accompanied by an increased load of oxygen radicals, interfering with the normal vascular wall function.

After 6 weeks of intensive cholesterol lowering treatment with simvastatin 40 mg, the impaired 5HT induced endothelium-dependent vasodilatation was not restored, despite a significant improvement of the serum lipid levels and was not different in subgroups with or without dyslipidemia. This observation is in sharp contrast with previous studies in both non-diabetic patients with hypercholesterolemia and hypertriglyceridemia where endothelial dysfunction was restored after 4–12 weeks treatment with a HMG-CoA reductase inhibitor, including simvastatin [11,20,26–28]. In two of these studies the same investigational tool was used, i.e. intra-arterial infusions of 5HT in comparable doses, for endothelial function measurement [20,28]. These beneficial effects of the statins on endothelial function are most likely explained by the improvement of the lipid profiles in these dyslipidemic patients [20,28]. However, the DM 2 patients in the present study had only a mild dyslipidemia, which probably explains the fact that treatment with a relatively high dose of simvastatin did not change endothelial function in these patients. This is in agreement with the suggestion that endothelial dysfunction in DM 2 is a consequence of the disturbance in glucose metabolism rather than by dyslipidemia. The present finding corroborate findings of Sheu et al. [10], who did not show improvement of endothelial function, using flow mediated vasodilation in patients with DM 2 after 24 weeks of treatment with a low dose of simvastatin, 10 mg. We are aware of two studies who have recently been completed that also found no improvement of an impaired endothelial function in patients with DM 2 and with multiple other cardiovascular risk factors, treated with high dose atorvastatin (F.V. van Venrooij and R.W. van Etten, personal communications). This rules out the possibility that only simvastatin is unable to restore the endothelial function.

However, it is remarkable that statins are not beneficial for the endothelial function in DM 2. Since the large intervention studies showed remarkable and statistically significant beneficial effects of statins, including simvastatin, on cardiovascular events in the diabetic subgroup [29–32]. While the endothelial function has been shown to be a predictive tool as evaluated among patients with manifest cardiovascular disease [2]. This indicates the further need for evaluation of the endothelial function as a prognostic marker in patients with DM 2. Other factors could play a role in the development of endothelial dysfunction in DM 2, such as the increased oxidative stress. The inactivation of nitric oxide by oxygen derived radicals could explain the persistent impairment of the endothelial function. More proof for this hypothesis is found in studies showing improvement of the endothelial function after the administration of antioxidants like vitamin C and folic acid in DM 2 and hypercholesterolemia [33–35]. An additional study confirms that pretreatment with vitamin C and vitamin E, prevents impairment of the, flow mediated, endothelium-dependent vasodilation after oral glucose loading [36]. However, to date antioxidant interventions have not shown beneficial effects on cardiovascular events [37–39].

From the large intervention studies evaluating the effects of statins it has been suggested that lipid-independent effects of statins explain part of their beneficial effects [40–43]. Among these pleiotropic effects are anti-thrombogenic, anti-inflammatory and anti-proliferative effects. However, the present study showed no evidence for pleiotropic effects, since simvastatin did not result in changes of the endothelial function during 6 weeks of treatment.
5. Conclusion

In the present study we found evidence that type 2 diabetes mellitus causes endothelial dysfunction, which is independent of serum lipid levels and of other cardiovascular risk factors. The finding that treatment with a relatively high dose of simvastatin did not improve the impaired endothelial responses in these patients indicates the further need for evaluation of the endothelial function as a prognostic marker in patients with DM 2.

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