Original Article

Smoking and proteinuria impair vasodilatory response of intrarenal arteries to nitroglycerine in patients with type 2 diabetes mellitus

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Abstract

Background. Few studies have addressed the effect of vasodilatory stimuli on the intrarenal arterial system in type 2 diabetes mellitus (DM), and factors affecting its responsiveness.

Methods. One hundred twenty-four patients with type 2 DM without renal failure were enrolled, and 25 subjects served as controls. Using duplex Doppler sonography, resistive indices (RI) of interlobar arteries were measured before and after sublingual nitroglycerine (NTG) (0.3 mg) spray over a 10-min period.

Results. Per cent changes in RI (%ΔRI) in the DM group were significantly less than in controls (P < 0.05), as was the area over the %ΔRI-time curve (AOC-%ΔRI, total responsiveness to nitroglycerine) (P < 0.05). In the DM group, significant negative correlations were found between AOC-%ΔRI and age (r = -0.492, P < 0.0001). AOC-%ΔRI in DM patients with proteinuria was significantly lower than without it (P < 0.003). AOC-%ΔRI in smokers was also significantly lower than in nonsmokers (P < 0.05).

By multiple regression analysis of the DM group, AOC-%ΔRI was found to be significantly and independently affected by age (β = -0.394), smoking (β = -0.211), and the presence of proteinuria (β = -0.270; R² = 0.354, P < 0.0001).

Conclusions. Diabetic patients with a lower level of responsiveness to NTG. Advanced age, smoking, and proteinuria significantly affect response to NTG in DM patients, suggesting that advanced intrarenal arteriosclerosis may be contributory. Smoking is suggested to be a risk factor for progression of diabetic nephropathy, likely contributing to poor responsiveness of the intrarenal arterial system to vasodilatory stimuli.

Keywords: arteriosclerosis; duplex Doppler sonography; intrarenal haemodynamics; nitroglycerine; smoking; type 2 diabetes mellitus

Introduction

Accelerated arteriosclerotic vascular disease is the principle cause of mortality in patients with type 2 diabetes mellitus (DM). In these patients arteriosclerosis is generally severe and silently progressive, in contrast to non-diabetic patients [1]. It has been reported that arteriosclerosis is one of the risk factors for the progression of diabetic nephropathy [2,3]. The progression of diabetic nephropathy is affected by several factors including high blood glucose concentrations, hypertension, and abnormalities in lipid and lipoprotein metabolism [3]. Recently we reported that intrarenal haemodynamic abnormalities are present in type 2 diabetics with nephropathy [4,5] by demonstrating that the resistive indices (RI) measured by duplex Doppler sonography are significantly increased in type 2 DM patients. In that study the RI values were suggested to be significantly affected by systemic and intrarenal arteriosclerosis [4,5]. Smoking is a major risk factor for arteriosclerosis [1,2,6] and is also reported to be highly associated with advanced progression of diabetic nephropathy [2,6,7].

In diabetic patients, several reports have demonstrated that both endothelial-dependent and -independent vasodilation is impaired [8–10]. Although attenuated vascular smooth muscle dilatation in diabetic patients in response to exogenous nitric oxide using nitroglycerine (NTG) and glyceryl trinitrate has been observed with coronary, brachial, and femoral arteries [8–10], few investigators have studied the effect of exogenous nitric oxide on intrarenal arteries of diabetic patients [11]. The aim of this study is to investigate NTG-induced vasodilatory response of intrarenal arteries in patients with...
type 2 diabetes using duplex Doppler sonography. We analysed the factors affecting the responsiveness to NTG in diabetic patients, particularly focusing on smoking and progression of diabetic nephropathy.

Subjects and methods

Subject and patients characteristics

One hundred twenty-four patients with type 2 DM were evaluated. Patients with renal insufficiency (serum creatinine > 1.5 mg/dl) or low serum albumin concentrations (serum albumin < 3.5 g/dl) were not included in this study. The diagnosis of type 2 DM was established according to the Report of the Expert Committee on Diagnosis and Classification of Diabetes Mellitus [12]. The patients were admitted to Osaka City University Hospital for the treatment of DM including a diabetic education course. Twenty-five individuals served as controls. The age and sex of these controls were comparable to those of the diabetic patients. All subjects gave informed consent to participate in the study. None of the control subjects had proteinuria, renal insufficiency, diabetes, cardiovascular disease, cerebrovascular disease, or peripheral vascular disease. To exclude patients with obstructive kidney disease, patients with a history of nephroureterolithiasis or renal pelvis dilatation on real-time ultrasonography were not enrolled. Patients with severe unilateral and bilateral kidney atrophy were excluded also. Blood pressure was measured with the subject in a supine position for at least 10 min using a standard mercury sphygmomanometer. Smoking habit was obtained represented the average of five complete waveforms. The RI was calculated using the following formula [4,5,11]:

\[ RI = \frac{PSV - EDV}{PSV} \]

Two examiners (MN and EI) blinded to subject characteristics performed all measurements. As previously reported, the coefficient of variance for RI values was 3.8% [4,5].

Response to NTG administration

The subjects were placed in a supine position for at least 15 min before the initial scan. The first RI measurement was defined as RI-0. After sublingual NTG spray (0.3 mg) was administered, RI values of interlobar arteries were measured over a 10-min period: RI values at 1, 3, 5, 7.5, and 10 min were defined as RI-1, RI-3, RI-5, RI-7.5, and RI-10, respectively. Percent changes in RI (%ΔRI) at x-min (%ΔRI-X) were calculated by the following formula:

\[ \%\Delta R1-X\% = \frac{(RI-X - RI-0) \times 100}{RI-0} \]

To evaluate the vasodilatory effects of sublingual NTG administration in this study, we calculated the area under the %ΔRI-time curve from 0 to 10 min by the trapezoidal method and defined it as AOC-%ΔRI.

Statistical analysis

Statistical analysis was performed using the Stat View V system designed for the Macintosh Computer. All data are expressed as mean ± SE. The Student’s t test and the chi-square test were performed to compare control subjects and type 2 DM patients. Multiple regression analysis was performed to assess the combined influence of clinical variables on the AOC-%ΔRI. Correlation and linear regression analyses were performed to examine the relationship between AOC-%ΔRI, age, and estimated Ccr.

Results

Clinical characteristics and basal RI values of the DM patients and control subjects

Clinical and metabolic characteristics of all subjects are summarized in Table 1. In patients with DM, duration of disease was 11.6 ± 9.0 years, HbA1C values before taking anti-hypertensive did not significantly differ from those 3–4 h after taking them. Images were obtained using a duplex Doppler imaging system (Aloka SSD 2000; Aloka, Tokyo, Japan) employing a 5-MHz convex array probe in both real-time/colour-coded Doppler and pulsed Doppler modes. The ultrasound probe was positioned gently on the flank in an oblique projection, and the kidney was visualized in the longitudinal axis. Real-time/colour-coded Doppler mode images, in which intrarenal arterial and venous flows are shown in different colours, were acquired to examine the interlobar arteries. The pulsed Doppler mode was used to obtain quantitative measurements of velocity by placing the cursor along the course of the interlobar arteries. Sample volumes were obtained by positioning the cursor of the pulsed Doppler at the midportion of the interlobar arteries. The sample volume was adjusted to a pulse length of 1.0 mm.

The ultrasound apparatus automatically calculated peak systolic flow velocity (PSV) and end-diastolic flow velocity (EDV). Flow velocities were determined from signals that were stable for at least five pulse beats, and the measurements obtained represented the average of five complete waveforms. The RI was calculated using the following formula [4,5,11]:

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values at 10 min were still lower than at baseline peak decrease, RI values increased gradually, although in most controls occurred at 3 min. After the values in patients with DM was seen from 3 to 5 min after sublingual administration of 0.3 mg NTG, RI arteries

Effect of nitroglycerine on RI values of interlobar arteries

After sublingual administration of 0.3 mg NTG, RI values were diminished. The greatest decrease in RI values in patients with DM was seen from 3 to 5 min and in most controls occurred at 3 min. After the peak decrease, RI values increased gradually, although values at 10 min were still lower than at baseline (RI-0).

The time course of changes in %ΔRI values is shown in Figure 1. Decrease in RI values was less in DM patients compared with control subjects over the 10-min period. Percent decrease in RI values at 3, 7.5, and 10 min in DM patients were significantly lower than in control subjects (P < 0.05; Figure 1).

Because the time of the peak effect of NTG administration on RI values was different in controls and patients with DM, the total effect of NTG on the changes in %ΔRI was examined by calculating the area over the %ΔRI-time curve (AOC-%ΔRI). AOC-%ΔRI values in DM patients were significantly lower than in control subjects (29.4 ± 3.2 vs 49.8 ± 10.5, P < 0.05).

Relationship between AOC-%ΔRI, age, estimated Ccr, smoking, and nephropathy stage in DM patients

Clinical factors associated with the renal vascular response to NTG were analysed in DM patients. There was a significant negative correlation between age and AOC-%ΔRI (r = −0.492, P < 0.001; Figure 2). There was a significant positive correlation between estimated Ccr and AOC-%ΔRI (r = 0.441, P < 0.001). There was a significant but weak correlation between systolic blood pressure and AOC-%ΔRI (r = −0.216, P < 0.05). There were no significant correlations between AOC-%ΔRI and other clinical factors, such as triglyceride, HDL cholesterol concentrations, non-HDL cholesterol concentrations, duration of DM, body mass index, serum albumin concentration, HbA1C, or diastolic blood pressure. In the stages of diabetic nephropathy characterized by more severe proteinuria, AOC-%ΔRI decreased. AOC-%ΔRI values in patients with overt proteinuria were significantly less than in patients without overt proteinuria (15.0 ± 3.4 vs 35.8 ± 4.2%·min, P < 0.003, Figure 3 left), although AOC-%ΔRI values in patients with microalbuminuria (30.8 ± 6.8) were not significantly different from controls

<table>
<thead>
<tr>
<th>Table 1. Clinical characteristics of the study subjects</th>
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<tbody>
<tr>
<td>Control subjects</td>
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<tr>
<td>Male/female</td>
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<tr>
<td>Age (years)</td>
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<tr>
<td>Body mass index (kg·m⁻²)</td>
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<tr>
<td>Serum creatinine (mg·dl⁻¹)</td>
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<td>Serum albumin (g·dl⁻¹)</td>
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<tr>
<td>Duration of type 2 DM (years)</td>
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<tr>
<td>Haemoglobin A₁C (%)</td>
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<tr>
<td>Triglycerides (mg·dl⁻¹)</td>
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<tr>
<td>HDL cholesterol (mg·dl⁻¹)</td>
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<tr>
<td>Non-HDL cholesterol (mg·dl⁻¹)</td>
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<tr>
<td>Systolic blood pressure (mmHg)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
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<tr>
<td>Smoking (yes/no)</td>
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<td>Baseline resistive index (RI-0)</td>
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*P < 0.0001 (Student’s t test), (mean ± SE).

9.0 ± 0.2%, triglyceride concentration 125 ± 7 mg·dl⁻¹, HDL cholesterol concentration 52 ± 2 mg·dl⁻¹, and non-HDL cholesterol concentration 155 ± 4 mg·dl⁻¹. There was no significant difference in the prevalence of smoking and hypertension between controls and individuals with DM.

Baseline resistive index (RI-0) of the DM patients was 0.722 ± 0.007, being significantly higher than that of the control (0.644 ± 0.012). This result was consistent with our previous study [4].

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Fig. 1. Time course of percent changes in resistive index (%ΔRI) after sublingual nitroglycerine administration in all subjects. After sublingual administration of 0.3 mg nitroglycerin, %ΔRI values were decreased. The peak responses in %ΔRI values were seen from 3 to 5 min, being different from patients to patients with DM (●) although the peak decrease was seen at 3 min in most of control subjects (○) (*P < 0.05 vs controls).

Fig. 2. Relationship between AOC-%ΔRI in DM patients. There was a significant negative correlation between AOC-%ΔRI and age in DM patients (r = −0.492, P < 0.0001).
different from those in individuals with normal albumin excretion \((37.9 \pm 5.1)\). AOC-%\(\Delta\)RI values in patients with a smoking history were significantly less than without smoking \((23.3 \pm 4.4 \text{ vs } 40.0 \pm 4.6\% \cdot \text{min}, P < 0.05; \text{Figure 3 right})\).

**Multiple regression analysis of clinical variables affecting AOC-%\(\Delta\)RI in DM patients**

Multiple regression analysis was performed to examine the combined influence of clinical variables on AOC-%\(\Delta\)RI in DM patients, using a model incorporating age, HDL cholesterol concentration, non-HDL cholesterol concentration, HbA1c, duration of DM, presence of smoking, systolic blood pressure, presence of overt proteinuria, and estimated Ccr as independent variables. In the model, a binary system was used to assess the effect of smoking (absence = 0, presence = 1), and overt proteinuria (absence = 0, presence = 1). For all variables, the decrease in AOC-%\(\Delta\)RI values was significantly and independently affected by age \((\beta = -0.394)\), the presence of smoking \((\beta = -0.211)\), and the presence of overt proteinuria \((\beta = -0.270; R^2 = 0.354, P < 0.0001; \text{Table 2})\).

**Discussion**

Recently several authors have examined vasodilatory responses by endothelium-dependent and-independent methods [8–10,14,15]. Although these studies have examined the vasodilatory response in the brachial, coronary, carotid, and femoral arteries, no author has examined the vasodilatory response of intrarenal arteries, since these small vessels cannot be directly visualized by currently available methods such as ultrasonography. Recently Frauchiger et al. [11] reported changes in RI values measured by Doppler sonography after sublingual NTG administration. We also examined haemodynamic changes in intrarenal arteries after administration of sublingual NTG, which has an endothelial-independent vasodilatory effect. In response to sublingual NTG administration, RI values decreased, indicating that NTG-induced intrarenal vasodilatation decreases RI values. Thus, we consider that the vasodilatory response of intrarenal arteries could be assessed by measuring RI and by calculating the changes in RI values (\(\Delta\)RI values), and that this method is clinically efficacious in determining the intrarenal vascular response to vasoactive stimuli.

In examining 800 asymptomatic adults at risk for atherosclerosis, Adams et al. [15] demonstrated that the vasodilatory response of the brachial artery to exogenous NO (sublingual NTG administration) is impaired, suggesting that smooth muscle dysfunction is present in these patients. They found DM to be an independent factor significantly associated with a reduced vasodilatory response [15]. The endothelial-independent vasodilatory response of several arteries (such as radial, carotid, femoral, and coronary arteries) in DM patients has been reported to be

![Fig. 3. Nitroglycerine responsiveness (AOC-%\(\Delta\)RI) in DM patients with or without proteinuria (left), and with or without smoking (right). AOC-%\(\Delta\)RI values with proteinuria were significantly lower than those without proteinuria (*\(P < 0.003\)). AOC-%\(\Delta\)RI values in DM patients with smoking were significantly lower than in those patients without smoking (**\(P < 0.05\)).](image-url)

### Table 2. Clinical factors affecting AOC-%\(\Delta\)RI in patients with type 2 diabetes mellitus

<table>
<thead>
<tr>
<th>Variables</th>
<th>(\beta)</th>
<th>(P)</th>
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<tbody>
<tr>
<td>AUC-%(\Delta)RI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>-0.394</td>
<td>0.0013</td>
</tr>
<tr>
<td>Smoking</td>
<td>-0.211</td>
<td>0.0225</td>
</tr>
<tr>
<td>Presence of proteinuria</td>
<td>-0.270</td>
<td>0.0234</td>
</tr>
<tr>
<td>Estimated creatinine clearance</td>
<td>0.177</td>
<td>0.1408</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.132</td>
<td>0.2305</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.100</td>
<td>0.2689</td>
</tr>
<tr>
<td>Duration of type 2 diabetes mellitus</td>
<td>-0.106</td>
<td>0.2834</td>
</tr>
<tr>
<td>Non HDL cholesterol</td>
<td>0.091</td>
<td>0.3226</td>
</tr>
<tr>
<td>Haemoglobin A1c</td>
<td>-0.041</td>
<td>0.6462</td>
</tr>
</tbody>
</table>

\(R^2 = 0.354, P < 0.0001\). AOC-%\(\Delta\)RI, area over the %\(\Delta\)RI-time curve of the renal interlobar arteries. \(\beta\), the standard regression coefficient. \(R^2\), the multiple coefficient of determination.
impaired [8–10]. The vasodilatory response in patients with microalbuminuria is reported to be more markedly impaired than in those with normoalbuminuria [9]. As for intrarenal arteries, Frauchiger et al. [11] demonstrated that the vasodilatory response (represented by changes in RI values) in a relatively small number (n = 20) of DM patients with mild to moderate nephropathy is significantly lower than in non-diabetic control subjects. In the present study examining a larger number of DM patients (n = 124), we clearly demonstrated that the vasodilatory response was significantly decreased in DM patients compared to healthy controls, whose age and sex were comparable to the diabetic patients. Thus, in patients with DM, vasodilatory function of vascular smooth muscle was impaired in intrarenal arteries in addition to other arteries including coronary, brachial, and carotid arteries as previously reported. The present study determined which factors are responsible for decreased responsiveness to NTG. We found that lower responsiveness is associated with older age, although age could not be a single predictor of decreased responsiveness to NTG. In older DM patients, arteriosclerosis usually is more advanced, and the arteries are stiffer [1]. Thus, the higher resistance of arteriosclerotic arteries in older DM patients may have contributed to the attenuated response to NTG in the present study.

Smoking is widely known to be an important risk factor for advanced arteriosclerosis, particularly in patients with DM [1]. Smoking also has been shown to hasten the progression of nephropathy in diabetic patients [16]. Adams et al. [15] demonstrated that cigarette smoking reduces the vasodilatory response of the brachial artery to NTG. Halimi et al. [17] reported that nicotine is associated with renal vasoconstriction, possibly through an alteration in a cyclic-GMP-dependent vasoactive mechanism. Gambaro et al. [18] found that renal plasma flow of chronic smokers is reduced. In the present study the vasodilatory response of intrarenal arteries to NTG stimulation was impaired in patients with current smoking. These reports and the results of the present study could imply impaired vasodilatory capacity of the renal vasculature in cigarette smokers. Intrarenal arteries of patients with a smoking history may not be able to generate the appropriate vasodilatory response in such a condition as renal blood flow is required to increase, leading to relative ischaemia, loss of perfusion, and eventually progression of diabetic nephropathy.

Patients with proteinuria have an increased risk of cardiovascular disease and generalized atherosclerosis [19,20]. Particularly in patients with type 2 DM, gross proteinuria is associated with a significant increase in mortality due to atherosclerotic cardiovascular disease and stroke, compared to patients with microalbuminuria or normal albumin excretion [19,20]. Stroes et al. [14] showed that patients with nephrotic-range proteinuria have a defect in vasodilation. In the present study, patients with proteinuria showed significantly lower responsiveness to NTG.

Taken together, these data show that vascular function in patients with advanced diabetic nephropathy who have overt proteinuria is impaired probably as result of both functional and structural abnormalities of extra- and intrarenal arteries.

In conclusion, our study demonstrates that intrarenal vasodilatory responsiveness in diabetic patients is significantly impaired. Decreased responsiveness in diabetic patients is associated with advanced age, cigarette smoking, and advanced nephropathy. Advanced arteriosclerosis is a common factor in the conditions. Impaired vasodilatory responsiveness in a diabetic kidney may be one of several mechanisms that contribute to the progression of diabetic nephropathy. Furthermore, smoking appears to be a risk factor for the progression of diabetic nephropathy because it further compromises the already decreased ability of the intrarenal arteries to vasodilate.

References


