RAPID COMMUNICATION

Increased plasma urotensin II levels in patients with diabetes mellitus

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ABSTRACT

Urotensin II (UII) is the most potent vasoconstrictor peptide, whereas it acts as a vasodilator on some arteries. We studied plasma levels of UII in diabetic patients with normal serum creatinine levels (<90 μmol/l) and the expression of UII and its receptor in cultured human vascular endothelial cells. Plasma UII levels were significantly elevated by 1.8-fold in diabetic patients without proteinuria (7.8 ± 0.6 fmol/ml; P < 0.0001) and 1.7-fold in those with overt proteinuria (7.3 ± 0.9 fmol/ml; P = 0.0018) when compared with healthy subjects (4.4 ± 0.2 fmol/ml). No significant correlation was obtained between plasma UII levels and fasting blood sugar (P = 0.631 and P = 0.853 in non-proteinuric and proteinuric diabetic patients respectively), glycated haemoglobin levels (P = 0.376 and P = 0.888 respectively) or serum creatinine levels (P = 0.301 and P = 0.568 respectively). Reverse transcriptase-PCR analysis showed the expression of mRNAs encoding UII precursor and UII-receptor precursors in cultured human coronary artery endothelial cells and umbilical vein endothelial cells, suggesting that vascular endothelial cells are one of the sources of UII in blood. These findings suggest that elevation of plasma UII levels may be an important background factor in diabetic cardiovascular and organ complications in diabetic subjects without renal failure.

INTRODUCTION

Angiopathy is a major complication in diabetes mellitus [1]. Various vasoactive substances secreted from the vascular endothelium, such as endothelin-1, may be involved in the pathogenesis of diabetic vascular complications [2,3]. Urotensin II (UII) is the most potent vasoconstrictor peptide, which was initially isolated from the caudal neurosecretory system of teleost fish [4,5]. The potency of UII vasoconstriction is an order of magnitude greater than that of endothelin-1 [4,6]. UII also has a vasodilatory effect on the small arteries of rats [7] and on the resistance arteries of humans [8] through the release of endothelium-derived hyperpolarizing factor and nitric oxide. Infusion of UII into the brachial artery caused vasoconstriction in human volunteers [9], although a similar treatment did not alter local or systemic haemodynamics in another study [10]. This peptide has a positive inotropic action [11] and stimulates proliferation of vascular smooth muscle cells [12,13]. Furthermore, it inhibited insulin release from the perfused rat pancreas [14].

We have reported recently [15] that plasma immunoreactive UII levels are elevated in patients with chronic renal failure. UII and UII-receptor mRNAs are expressed in various peripheral organs, including heart, kidney and vascular tissues [4,15], as well as various tumour cells [16]. It is therefore likely that UII acts as a circulating vasoactive hormone and may be involved in the pathogenesis of diabetic vascular complications. We therefore studied plasma UII levels in patients with diabetes mellitus. Furthermore, we postulated that vascular endothelial cells might be one of the sources of UII in blood and thus we studied the expression of UII and its receptor in cultured human vascular endothelial cells.

Key words: diabetes, plasma, reverse transcriptase-PCR, RIA, urotensin II, vascular endothelial cell.

Abbreviations: BMI, body mass index; HbA1c, glycated haemoglobin; RT, reverse transcriptase; UII, urotensin II.

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MATERIALS AND METHODS

Subjects
Plasma samples were obtained from 22 healthy controls (12 males and 10 females, aged 15–63 years old) and 16 diabetic patients (7 males and 9 females, aged 20–78 years old) undergoing insulin therapy. The diabetic patients were divided into two groups by the presence or absence of proteinuria. Presence of proteinuria was examined by a routine urine test with the detection limit of 100 mg of albumin/g of creatinine per day. The clinical characteristics of these subjects are summarized in Table 1. The diabetic patients consisted of one with Type I diabetes and 15 with Type II diabetes. None of these diabetic patients had renal failure (serum creatinine was 44–88 μmol/l) or other critical diseases. On the other hand, when compared with the controls, the non-proteinuric and proteinuric diabetic patients had significantly higher levels of blood urea nitrogen (P = 0.0003 and P = 0.0059 in non-proteinuric and proteinuric diabetic patients respectively) and serum creatinine concentrations (P = 0.0436 and P = 0.0011 respectively). The diabetic patients had significantly higher arterial systolic blood pressure than the controls (P = 0.0287 and P = 0.0018 respectively), whereas there was no significant difference in diastolic blood pressure among three groups.

There were no significant differences between non-proteinuric and proteinuric diabetic groups in blood pressure, blood urea nitrogen levels, serum creatinine levels, body mass index (BMI), fasting blood sugar or glycated haemoglobin (HbA1C) levels. Creatinine clearance, which was calculated with Horio’s formula for Japanese people [17], was slightly lower in the proteinuric diabetic group, but this was not statistically significant.

Informed consent was obtained from each subject. The study was performed in accordance with the principles expressed in the Declaration of Helsinki, and was approved by the Ethics Committee of Tohoku University School of Medicine. Blood samples were obtained from a subcutaneous vein in the forearm after overnight fasting, collected into tubes containing aprotonin (500 kallikrein inhibitor units/ml of blood; Bayer, Leverkusen, Germany) and EDTA (1 mg/ml of blood), and centrifuged at 1800 g for 15 min at 4 °C. The plasma samples were separated and stored at −20 °C until extracted.

Plasma extraction and RIA
Plasma samples were extracted and assayed as described previously [15,16]. Briefly, plasma (3 ml) was acidified with 3 ml of 0.75 mol/l acetic acid, and loaded on to a Sep-Pak C18 cartridge (Waters, Milford, MA, U.S.A.), which was pretreated with 10 ml of acetonitrile, 10 ml of methanol and then 10 ml of 0.75 mol/l acetic acid. After the cartridge was washed with 10 ml of 0.75 mol/l acetic acid, the adsorbed peptide was eluted with 2 ml of 60% (v/v) acetonitrile in 0.1% trifluoroacetic acid. The eluate was air-dried, reconstituted with assay buffer [0.1 mol/l sodium phosphate buffer (pH 7.5) containing 0.1% (w/v) BSA, 0.2% (v/v) Triton X-100 and 0.1% (w/v) sodium azide] and assayed. The recovery of peptide following this extraction procedure was determined by adding synthetic human UII to plasma (50 and 200 fmol UII/ml of plasma) and yielded > 95% recovery (n = 6).

Human UII (Peptide Research Institute, Osaka, Japan) was used as a standard, and 125I-labelled UII (Amer sham Biosciences, Tokyo, Japan) was used as a radioligand in the RIA. The sample (200 μl) or standard peptide (200 μl) was incubated at 4 °C for 48 h with a polyclonal anti-(human UII) antibody (1:6000 final dilution; lot 992-500601; Peptide Research Institute). 125I-labelled UII (4000 c.p.m./100 μl) was then added to each assay tube, and incubated at 4 °C for a further

| Table 1 Clinical characteristics of the control subjects and the diabetic patients with (+) or without (−) proteinuria
| Results are expressed as the means ± S.E.M. n.e., not examined. |

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Proteinuria (−)</th>
<th>Proteinuria (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>22</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>55</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>Age (years)</td>
<td>15–63</td>
<td>20–78</td>
<td>46–77</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>112 ± 1</td>
<td>129 ± 6</td>
<td>142 ± 13</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71 ± 2</td>
<td>80 ± 4</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Blood urea nitrogen (mmol/l)</td>
<td>4.3 ± 0.2</td>
<td>6.0 ± 0.5</td>
<td>7.1 ± 0.3</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>49 ± 3</td>
<td>62 ± 4</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>n.e.</td>
<td>90 ± 8</td>
<td>69 ± 5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>n.e.</td>
<td>26.0 ± 1.4</td>
<td>20.9 ± 1.6</td>
</tr>
<tr>
<td>Fasting blood sugar (mmol/l)</td>
<td>n.e.</td>
<td>9.4 ± 1.1</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>n.e.</td>
<td>7.1 ± 0.6</td>
<td>7.5 ± 0.6</td>
</tr>
</tbody>
</table>
Urotensin II and diabetes

Figure 1 Plasma concentrations of UII in healthy volunteers and diabetic patients with (+) or without (−) proteinuria

Closed triangle represents the patient with Type I diabetes mellitus. The means (○)±S.E.M. (bars) are shown. n.s., not significant.

RESULTS

Plasma UII levels were significantly elevated by approximate 1.8-fold in 10 non-proteinuric diabetic patients (7.8 ± 0.6 fmol/ml; P < 0.0001) and by approximate 1.7-fold in six proteinuric diabetic patients (7.3 ± 0.9 fmol/ml; P = 0.0018) compared with normal volunteers (4.4 ± 0.301 fmol/ml) (Figure 1). There was no significant difference in plasma UII levels between the two diabetic groups.

In either non-proteinuric or proteinuric diabetic patients, plasma UII concentrations showed no significant correlation with serum creatinine concentrations (P = 0.301 and P = 0.568 in non-proteinuric and proteinuric diabetic patients respectively), creatinine clearance (P = 0.675 and P = 0.716 respectively), and blood urea nitrogen concentrations (P = 0.092 and P = 0.475 respectively). There was also no significant relationship found between plasma UII levels and fasting blood glucose (P = 0.631 and P = 0.853 respectively), HbA1c levels (P = 0.376 and P = 0.888 respectively), BMI (P = 0.054 and P = 0.181 respectively), systolic blood pressure (P = 0.481 and P = 0.104 respectively), diastolic blood pressure (P = 0.786 and P = 0.220 respectively) or age (P = 0.055 and P = 0.571 respectively).

RT-PCR analysis showed the expression of UII and UII-receptor mRNAs in two types of vascular endothelial cells as well as the spinal cord (Figure 2). The...
intensity of the bands of UII mRNA in cultured human coronary artery endothelial cells and human umbilical vein endothelial cells was not as strong as that in spinal cord, but was comparable with that in human heart, liver and kidney. UII-receptor mRNA was expressed in all of the samples examined, although the band in the liver was very weak.

**DISCUSSION**

The present study has shown for the first time the elevation of plasma UII levels in diabetic patients, and the expression of both UII and UII-receptor mRNAs in two types of cultured human endothelial cells. It was unlikely that hyperglycaemia stimulated the production and/or secretion of UII, because plasma UII levels had no significant correlation with fasting blood sugar or HbA1c levels. Elevated plasma UII concentrations were found not only in diabetic patients with overt proteinuria, but also in diabetic patients without proteinuria. Plasma UII levels had no significant correlation with serum creatinine concentrations or with creatinine clearance. The elevated plasma UII levels, therefore, do not appear to be secondary to the renal dysfunction in diabetic patients. We could not discount the possibility, however, that early-stage diabetic nephropathy may contribute to the elevated plasma UII levels in these diabetic patients, because some patients with microalbuminuria of less than 100 mg/g of creatinine per day might be included in the non-proteinuric group.

It has been reported [3,21] that plasma concentrations of endothelium-derived peptides, such as endothelin-1 and adrenomedullin, were elevated in patients with diabetes mellitus, possibly due to accompanying vascular endothelial cell damage. We have therefore hypothesized that vascular endothelial cells are one of the important sources of UII in blood, and vascular endothelial cell damage may elevate plasma UII concentrations in diabetic patients. Interestingly, Richards et al. [22] have reported recently that plasma UII levels are elevated in patients with heart failure and correlated significantly with plasma endothelin-1 and adrenomedullin levels. In the present study, we have shown the expression of UII mRNA in two types of vascular endothelial cells. Since the vascular bed occupies a considerable part of the human body and endothelial cells may be able to secrete UII directly into the circulation, endothelial cells may be one of the major sources of UII in plasma. The expression levels of UII mRNA in vascular endothelial cells, however, did not appear to be as high as in spinal cord and were comparable with that in human heart, liver and kidney. We therefore could not discount the presence of other sources of UII in blood.

The vasoconstrictor action of UII is still controversial in humans [9,10] and in experimental animals [4,6–8]. We are therefore not certain whether elevated UII in plasma is related to vasoconstriction in diabetic patients. Another possible pathophysiological role of UII in diabetes may be its mitogenic action on vascular smooth muscle cells [12,13]. Furthermore, the expression of UII-receptor mRNA in vascular endothelial cells may support an autocrine role of UII in these cells, such as for release of NO [7]. The RT-PCR method for UII and UII-receptor used in the present study is, however, not quantitative. Moreover, mRNA expression does not always equate the secretion of UII peptide with the expression of the functioning receptor. Further studies are therefore required to clarify whether UII secretion from the vascular endothelial cells or other types of cells is enhanced in patients with diabetes mellitus and whether UII-receptor activity is altered or not in these patients.
Recent studies [23,24] have shown that vasoconstrictor peptides with mitogenic actions, such as angiotensin II and endothelin-1, are involved in the pathogenesis and the progression of various cardiovascular and renal diseases, such as diabetic nephropathy. Angiotensin-converting-enzyme inhibitors and angiotensin-II-receptor antagonists have been shown to be effective drugs for diabetic nephropathy [25]. Endothelin antagonists are expected to be of benefit to diabetic patients with vascular complications. The present study has suggested that increased plasma UII is another important background factor in diabetic cardiovascular complications in patients with diabetes mellitus. Development of clinically useful UII antagonists may open new strategies for the treatment of diabetic vascular complications. On the other hand, the present study studied only a limited number of patients with diabetes mellitus. Future studies in a larger number of diabetic patients and comparison of UII with other parameters, such as endothelin-1, may further reveal roles of UII and UII-receptor in the pathogenesis of diabetic vascular complications.

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