

Increased plasma urotensin-II levels are associated with diabetic retinopathy and carotid atherosclerosis in Type 2 diabetes

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A B S T R A C T

Human U-II (urotensin-II), the most potent vasoconstrictor peptide identified to date, is associated with cardiovascular disease. A single nucleotide polymorphism (S89N) in the gene encoding U-II (*UTS2*) is associated with the onset of Type 2 diabetes and insulin resistance in the Japanese population. In the present study, we have demonstrated a relationship between plasma U-II levels and the progression of diabetic retinopathy and vascular complications in patients with Type 2 diabetes. Eye fundus, IMT (intima-media thickness) and plaque score in the carotid artery, BP (blood pressure), FPG (fasting plasma glucose), HbA_{1c} (glycated haemoglobin), U-II, angiogenesis-stimulating factors, such as VEGF (vascular endothelial growth factor) and heregulin- β_1 , and lipid profiles were determined in 64 patients with Type 2 diabetes and 24 non-diabetic controls. FPG, HbA_{1c} and VEGF levels were significantly higher in patients with Type 2 diabetes than in non-diabetic controls. Diabetes duration, insufficient glycaemic and BP control, plasma U-II levels, IMT, plaque score and nephropathy grade increased significantly across the subjects as follows: non-diabetic controls, patients with Type 2 diabetes without retinopathy (group N), patients with Type 2 diabetes with simple (background) retinopathy (group A) and patients with Type 2 diabetes with pre-proliferative and proliferative retinopathy (group B). The prevalence of obesity and smoking, age, low-density lipoprotein, triacylglycerols (triglycerides) and heregulin- β_1 were not significantly different among the four groups. In all subjects, U-II levels were significantly positively correlated with IMT, FPG, and systolic and diastolic BP. Multiple logistic regression analysis revealed that, of the above parameters, U-II levels alone had a significantly independent association with diabetic retinopathy. In conclusion, the results of the present study provide the first evidence that increased plasma U-II levels may be associated with the progression of diabetic retinopathy and carotid atherosclerosis in patients with Type 2 diabetes.

INTRODUCTION

Human U-II (urotensin-II), a cyclic peptide of 11 amino acids (Glu-Thr-Pro-Asp-cyclo[Cys-Phe-Trp-Lys-Tyr-

Cys]-Val) with a molecular mass of approx. 1388 Da, is the most potent vasoconstrictor hormone identified to date [1]. U-II acts through its G-protein-coupled receptor UT [1]. Intracerebroventricular administration of U-II

Key words: carotid atherosclerosis, diabetic retinopathy, Type 2 diabetes, urotensin-II, vascular endothelial growth factor (VEGF).
Abbreviations: ABI, ankle-brachial index; ACE, angiotensin-converting enzyme; ARB, angiotensin-II type 1 receptor blocker; BP, blood pressure; BMI, body mass index; BUN, blood urea nitrogen; CAD, coronary artery disease; CI, confidence interval; DBP, diastolic BP; EC, endothelial cell; FPG, fasting plasma glucose; group A, patients with Type 2 diabetes with simple retinopathy; group B, patients with Type 2 diabetes with pre-proliferative and proliferative retinopathy; group N, patients with Type 2 diabetes without retinopathy; HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein; OR, odds ratio; PKC, protein kinase C; PWV, pulse wave velocity; ROS, reactive oxygen species; SBP, systolic BP; U-II, urotensin-II; UT, U-II receptor; VEGF, vascular endothelial growth factor; VSMC, vascular smooth muscle cell.

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into sheep caused hypertension and hyperglycaemia [2]. In perfused rat pancreas models, U-II inhibited insulin release in response to glucose [3]. U-II circulates in human plasma and its main sources are vascular wall cells [4], the heart, liver and kidneys [5]. Plasma levels of U-II are increased in vascular-endothelial-dysfunction-related diseases, such as essential hypertension, ischaemic heart disease, congestive heart failure, renal failure, Type 2 diabetes and portal hypertension caused by liver cirrhosis [6–8]. Moreover, certain polymorphisms in the *UTS2* gene (the gene encoding U-II) are associated with Type 2 diabetes and insulin resistance in the Japanese population [9]. Palosuran, the first UT antagonist to be tested in humans, decreased microalbuminuria after approx. 2 weeks in 18 patients with hypertension and Type 2 diabetic nephropathy [10].

Both U-II and UT are found at high levels in renal tubular epithelial cells in patients with diabetic nephropathy [11], in VSMCs (vascular smooth muscle cells), ECs (endothelial cells) and within the regions of monocyte/macrophage infiltration in atherosclerotic plaque from human coronary and carotid arteries [4,12]. We have demonstrated that U-II accelerates human macrophage foam cell formation [13] and VSMC proliferation [14], and that the mitogenic effect of U-II on VSMCs is potentiated by oxidized LDL (low-density lipoprotein), ROS (reactive oxygen species) and serotonin [14,15]. U-II also stimulates EC proliferation and permeability [16,17], VSMC migration [18] and extracellular matrix production [19]. These observations suggest that U-II may contribute to the development of atherosclerosis and angiogenesis [6,20].

Diabetes is a worldwide medical problem and is a significant cause of morbidity and mortality. Diabetes has major complications such as neuropathy, nephropathy, retinopathy and angiopathy. As diabetic retinopathy leads to blindness and visual impairment, it is becoming not only one of the most important complications of diabetes, but also a social problem. However, it remains unclear whether U-II can induce the progression of diabetic retinopathy in patients with diabetes. In the present study, we investigated the significance of U-II compared with angiogenesis-stimulating factors, such as VEGF (vascular endothelial growth factor) and heregulin- β_1 [21,22], as a risk factor for micro- and macro-angiopathy in patients with Type 2 diabetes.

MATERIALS AND METHODS

Subjects

A total of 64 consecutive patients referred to our outpatient hospital for diabetes and 24 gender- and age-matched non-diabetic control subjects (17 healthy volunteers and seven patients with mild hyperlipidaemia and/or mild hypertension) without antidiabetic therapy

or a history of diabetes mellitus were enrolled in the study. All patients with diabetes fulfilled the World Health Organization criteria for Type 2 diabetes. Patients with angiographically proven CAD (coronary artery disease), heart failure, liver cirrhosis and severe renal failure were excluded from the study because plasma U-II levels are known to be increased in patients with these diseases [6]. All participants co-operated to stop smoking at least by the evening prior to blood sampling, because smoking within 10 min increases plasma U-II levels [23].

The study was conducted according to the principles of the Declaration of Helsinki and approved by the local institutional review committee. Informed consent for participation in this investigation was obtained from all subjects.

Ophthalmologic examination

Fundoscopy, retinal photograph and/or fluorescein angiography after pupillary dilation were performed in all of the subjects. The diagnosis of diabetic retinopathy was performed by experienced ophthalmologists based on the presence of one or more of the following clinical features in the fundus: haemorrhages, hard or soft exudates, venous beading, intra-retinal microvascular abnormalities, cotton-wool spots, pre-retinal new vessels, fibrous proliferation and photo-coagulation scars. Diabetic retinopathy was graded on the basis of the Davis classification as follows: no retinopathy, simple (background) retinopathy, and pre-proliferative and proliferative retinopathy [24].

Carotid ultrasonography

Carotid atherosclerosis was evaluated by high-resolution B-mode ultrasonography using a 7.5-MHz linear-array transducer (SSA-770A; Toshiba). The IMT (intima-media thickness) and plaque score, as indices of carotid atherosclerosis, were determined by trained operators blinded to the subjects' clinical records. All ultrasound images were obtained with the subject in the supine position with the neck mildly extended and rotated to the contralateral side, and measurement of IMT and plaque score was performed on the frozen frame, perpendicular to the vascular walls, by scanning bilateral common and internal carotid arteries at the time of examination. In each subject, the maximum and mean IMT values were obtained by IMT measurements in six sites of the far walls in bilateral carotid arteries excluding the plaque. The upper normal limit of IMT was 1.0 mm, and the lesions with a focal IMT ≥ 1.1 mm were defined as plaques [7]. The plaque score was calculated by summing all plaque thickness measurements in both carotid arteries. The severity of plaque score was graded as follows: none, 0; mild, 1.1–5; moderate, 5.1–10; and severe, ≥ 10.1 [7].

PWV (pulse wave velocity) and ABI (ankle-brachial index) measurements

Along with ultrasonographically measured carotid atherosclerosis, ankle-brachial PWV and ABI were used to assess the degree of atherosclerosis. PWV and ABI were measured in all patients with Type 2 diabetes using a volume plethysmographic apparatus (from PWV/ABI version 112; Colin Co.). Briefly, after an overnight fast, the subjects were examined in the supine position, with ECG electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The characteristic points of waveforms were determined automatically, and the results were printed out. All procedures took approx. 5 min. The mean PWV and ABI values measured on either side of each patient were used for the analysis.

Assays

Blood samples were collected in the morning after overnight fasting and in a non-smoking state. Plasma glucose levels and serum levels of LDL-cholesterol, triacylglycerols (triglycerides), creatinine and BUN (blood urea nitrogen) were analysed by enzymatic methods (SRL Inc.). Serum levels of HDL (high-density lipoprotein)-cholesterol were determined by the precipitation method. HbA_{1c} (glycated haemoglobin) was determined using latex agglutination. Plasma levels of VEGF and heregulin- β_1 were measured by ELISA kits (Apollo Cytokine Research and R&D Systems respectively). Plasma U-II measurements were carried out with commercial ELISA kits (Phoenix Pharmaceuticals) [7]. The kit has a detection limit of 0.05 ng/ml and 100% cross-reactivity against human U-II (15.7% for human U-II-related peptide, 0% for human prepro-U-II, and 15 and 22% for human U-II₄₋₁₁ and U-II₅₋₁₁ fragments respectively). Intra- and inter-assay coefficients of variation were 4 and 9% respectively.

Nephropathy grading

Spot urine samples were collected on at least three different days and the average albumin/creatinine index in the urine was calculated. Urine specimens contaminated with bacteria, white blood cells or red blood cells were excluded. The urinary albumin concentration was measured by the latex turbidimetric immunoassay method using a commercially available kit (LA-system; AIC) and the patients with Type 2 diabetes were classified as follows: normoalbuminuria (albumin/creatinine index < 30 mg/g; grade 1) microalbuminuria (albumin/creatinine = 30–300 mg/g; grade 2) and overt diabetic nephropathy (albumin/creatinine index > 300 mg/g; grade 3) [25].

Diabetes duration, glycaemic and BP (blood pressure) control, and obesity

The duration of diabetes was estimated from the time of the occurrence of the first symptoms attributable to

the disease or, if symptoms were absent, from the time of the first detection of glycosuria. Insufficient control of glucose was defined as HbA_{1c} \geq 6.5% according to the guidelines of the Japan Diabetes Society. Insufficient control of BP was defined as BP \geq 130/85 mmHg for patients with Type 2 diabetes and BP \geq 140/90 mmHg for non-diabetic controls according to the guidelines of the Japanese Society of Hypertension. Obesity was defined as a BMI (body mass index) \geq 25 kg/m² according to the criterion of the Japanese Society for the Study of Obesity.

Past history of smoking and alcohol intake

Information on cigarette smoking and alcohol intake was obtained from the subjects including the age that the individual started smoking, total number of years spent smoking, amount of cigarettes consumed and the usual weekly intake of alcoholic beverages over the previous several months. Alcohol intake was converted into a daily equivalent in terms of the number of 'go', a traditional Japanese unit of volume for rice wine sake (1 go = 180 ml and contains 22.7 g of ethanol). One go corresponds to one bottle (633 ml) of beer, two single shots (75 ml) of whisky or two glasses (180 ml) of wine. In the present study, men who reported consuming > 1 go/week were regarded as drinkers, and a smoker was defined by current smoking of > 10 cigarettes/day for more than a year [7].

Statistical analysis

Results are expressed as means \pm S.E.M. for continuous variables, and as frequencies for categorical variables. The data were compared using a two-tailed unpaired Student's *t* test between two groups and by one-way ANOVA, followed by Bonferroni's post hoc test, when more than two groups were involved. Pearson's correlation coefficient was used to analyse the relationships between plasma U-II levels and other continuous variables. Multiple logistic regression analysis was performed to assess independent risk factors for diabetic retinopathy; associations were calculated as ORs (odds ratios) with the corresponding 95% CIs (confidence intervals). *P* < 0.05 was taken to indicate statistical significance.

RESULTS

Table 1 summarizes the clinical characteristics of the non-diabetic controls and patients with Type 2 diabetes divided according to their degree of retinopathy (group N, patients without retinopathy; group A, patients with simple retinopathy; and, group B, patients with pre-proliferative and proliferative retinopathy). The mean age, gender, BMI, incidence of smoking habit, alcohol consumption, cataract surgery, hypertension and hyperlipidaemia, and the prevalence of therapy using insulin,

Table 1 Clinical characteristics of patients with Type 2 diabetes and control subjects

Values are means \pm S.E.M. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.0001$ compared with the control group; † $P < 0.05$ and †† $P < 0.01$ compared with patients with Type 2 diabetes in group N.

Characteristic	Control group	Patients with Type 2 diabetes		
		Group N	Group A	Group B
<i>n</i>	24	36	16	12
Age (years)	58 \pm 3	58 \pm 2	59 \pm 3	63 \pm 3
Male (%)	63	67	56	50
BMI (kg/m ²)	22 \pm 1	25 \pm 1	23 \pm 1	26 \pm 2
Obesity (%)	17	39	19	42
Smoking habit (%)	42	36	50	42
Alcohol consumption (%)	29	24	29	30
Cataract surgery (%)	8	17	6	17
Hypertension (%)	25	40	38	33
Hyperlipidaemia (%)	17	34	38	25
Insulin therapy (%)	0	20	25	25
Sulfonylureas (%)	0	16	25	58
Antihypertensive drugs (%)	13	20	31	33
Statins (%)	13	28	25	25
Insufficient BP control (%)	17	61**	81***	92***†
Insufficient glycaemic control (%)	0	78***	81***	92***
Diabetes duration (years)	0 \pm 0	6.9 \pm 1.2	8.2 \pm 1.2	12.3 \pm 2.5††
SBP (mmHg)	128 \pm 2	135 \pm 3	140 \pm 3	142 \pm 4
DBP (mmHg)	74 \pm 2	78 \pm 2	81 \pm 2	81 \pm 3
FPG (mg/dl)	92 \pm 3	118 \pm 6***	118 \pm 12***	121 \pm 7***
HbA _{1c} (%)	5.1 \pm 0.1	8.9 \pm 0.4*	8.8 \pm 0.6*	9.0 \pm 0.6*
Triacylglycerols (mg/dl)	117 \pm 17	167 \pm 17	136 \pm 24	173 \pm 33
LDL-cholesterol (mg/dl)	100 \pm 6	118 \pm 6	118 \pm 12	121 \pm 7
HDL-cholesterol (mg/dl)	46 \pm 3	53 \pm 3	51 \pm 4	58 \pm 6
VEGF (pg/ml)	214 \pm 44	812 \pm 113*	303 \pm 141	173 \pm 86
Heregulin- β_1 (ng/ml)	10.1 \pm 4.0	13.2 \pm 3.8	15.6 \pm 9.2	4.9 \pm 3.2
Creatinine (mg/dl)	0.69 \pm 0.02	0.70 \pm 0.03	0.75 \pm 0.04	0.81 \pm 0.04*
BUN (mg/dl)	13.7 \pm 0.7	13.73 \pm 0.7	14.1 \pm 1.2	16.8 \pm 1.4*

sulfonylureas, antihypertensive drugs or statins did not differ significantly among the four groups. In addition, neither SBP (systolic BP) nor DBP (diastolic BP) was significantly different among the four groups. However, the prevalence of insufficient BP and glycaemic control, FPG and HbA_{1c} were significantly greater in all of the patients with Type 2 diabetes compared with the control group (Table 1). Among the patients with Type 2 diabetes, the prevalence of insufficient BP control and diabetes duration were significantly greater in group B compared with group N, but there was no significant difference in the prevalence of insufficient glycaemic control.

As shown in Figure 1(A), plasma U-II levels increased significantly across the groups as follows: control group, group N, group A and group B. The maximum IMT (Figure 1B) and mean IMT (Figure 1C) were significantly greater in the patients with Type 2 diabetes compared with the control group, and tended to increase across the groups as follows: group N, group A and group B.

Carotid plaque score (Figure 1D), nephropathy grade (Figure 1E), and serum levels of creatinine and BUN (Table 1) were significantly greater in group B compared with the control group. Plasma VEGF levels were significantly higher in patients with Type 2 diabetes compared with the control group (565 \pm 105 compared with 214 \pm 44 pg/ml; $P < 0.05$), with the significantly highest levels of VEGF observed in group N (Table 1). However, the plasma heregulin- β_1 level and serum levels of triacylglycerols, LDL-cholesterol and HDL-cholesterol were not significantly different among the four groups (Table 1). In groups N, A and B, PWV (1452 \pm 90, 1655 \pm 87 and 1822 \pm 99 cm/s respectively) and ABI (1.05 \pm 0.02, 1.05 \pm 0.03 and 1.16 \pm 0.04 respectively) increased with the severity of retinopathy, but there were no statistically significant differences between the groups.

In all of the subjects, plasma U-II levels increased as the carotid plaque score increased, with a significantly

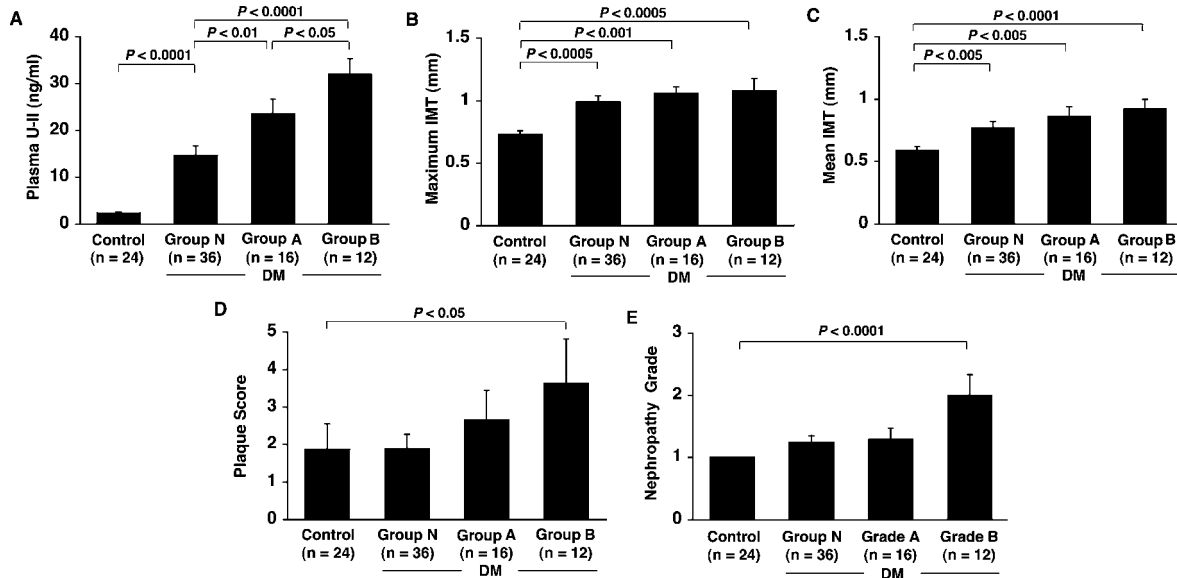


Figure 1 Comparisons of plasma U-II levels (A), maximum IMT (B), mean IMT (C), carotid plaque score (D) and nephropathy grade (E) in non-diabetic controls and patients with Type 2 diabetes in groups N, A and B. Values are means \pm S.E.M. DM, Type 2 diabetes.

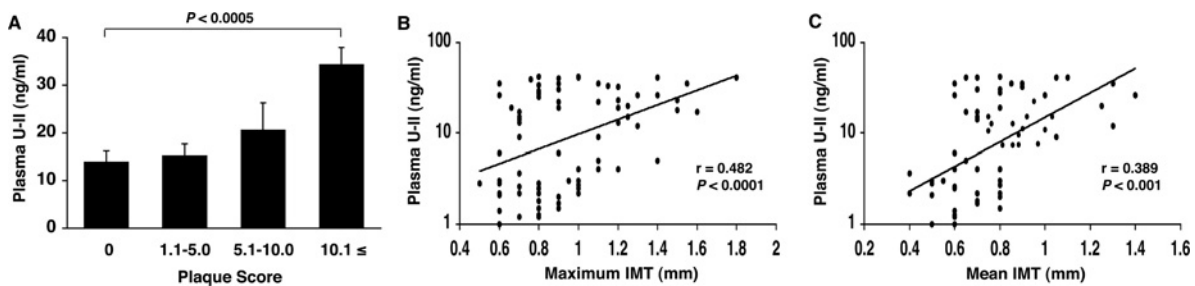


Figure 2 Relationship between plasma U-II levels and carotid plaque score (A), maximum IMT (B) and mean IMT (C) in all subjects

In (A), results are means \pm S.E.M.

greater level of U-II observed when the most severe was compared with the least severe carotid plaque (Figure 2A). Plasma U-II levels were significantly positively correlated with SBP ($r = 0.386$, $P = 0.0003$), DBP ($r = 0.357$, $P = 0.0009$), FPG level ($r = 0.382$, $P = 0.0004$), maximum IMT (Figure 2B) and mean IMT (Figure 2C). Multiple logistic regression analysis was performed in all subjects to evaluate the association between diabetic retinopathy (groups A and B) and the regarded risk factors. It revealed that, of the risk factors investigated, plasma U-II levels alone had a significantly independent association with diabetic retinopathy (Table 2).

DISCUSSION

To our knowledge, this is the first study to demonstrate a significant relationship between increased plasma levels

Table 2 Multiple logistic regression analysis of risk factors for diabetic retinopathy (groups A and B) in all subjects

Risk factor	OR (95% CI)	P value
Insufficient BP control	1.15 (0.19–6.97)	0.8833
Insufficient glycaemic control	2.42 (0.41–14.1)	0.3275
Diabetes duration (years)	1.04 (0.93–1.17)	0.4737
Cataract surgery	2.62 (0.31–22.4)	0.3797
Carotid plaque score	1.07 (0.82–1.39)	0.6266
Nephropathy grade	2.36 (0.67–8.25)	0.1807
Obesity	0.70 (0.14–3.44)	0.6602
Smoking habit	1.84 (0.42–8.06)	0.4183
LDL-cholesterol (mg/dl)	1.01 (0.99–1.03)	0.4757
U-II (ng/ml)	1.21 (1.04–1.37)	0.0008
VEGF (pg/ml)	1.00 (1.00–1.00)	0.6875
Heregulin- β_1 (ng/ml)	1.00 (1.00–1.00)	0.6816

of U-II and severe diabetic retinopathy and carotid atherosclerosis in patients with Type 2 diabetes. The present study shows that plasma U-II levels have a significantly independent association with diabetic retinopathy when compared with VEGF, which is regarded as a risk factor [26]. Previous studies have shown that plasma VEGF levels were significantly increased in patients with diabetes compared with non-diabetic controls [21,26]. In addition, the observation of retinal VEGF expression early in diabetic retinopathy and the finding in non-diabetic animals that exogenous intraocular VEGF administration can elicit retinal abnormalities resembling diabetic retinopathy suggest that VEGF may play a role in mediating the development of the earliest stages of retinopathy [26,27], although VEGF does not induce proliferative retinal changes [27]. VEGF is essential for early-stage vascular development [28], but mature vessels no longer require VEGF [29]. Heregulin- β_1 is regarded as a stimulator of angiogenesis and atherosclerosis [30–32]; however, a recent study has shown that heregulin- β_1 attenuates neointimal formation following vascular injury and inhibits VSMC proliferation [33]. In the present study, there was no significant association between plasma heregulin- β_1 levels and diabetic retinopathy in patients with Type 2 diabetes. Therefore, rather than VEGF and heregulin- β_1 in plasma, U-II may be a more suitable biomarker for reflecting the presence and severity of diabetic retinopathy. However, it is not exactly known whether changes in plasma U-II levels may contribute to, or result from, diabetic retinopathy and carotid atherosclerosis. Further studies are required to investigate this.

In general, diabetic micro- and macro-angiopathy have different mechanisms of pathogenesis, but some similar pathways are used, such as increased production of AGEs (advanced glycation end-products) and stimulation of the polyol pathway by hyperglycaemia [34]. In addition, EC dysfunction, PKC (protein kinase C) activation, ROS generation, altered gene expression of growth factors and cytokines, and macrophage activation are involved in diabetic vascular complications, which are closely linked with U-II. U-II plays a key role in the development of atherosclerosis [6] or angiogenesis [20] by inducing EC permeability and proliferation [16,17], macrophage foam cell formation [13], VSMC migration and proliferation [14,15,18], and extracellular matrix production [19]. U-II also stimulates collagen-1 expression in ECs [35], and the expression of PAI-1 (plasminogen activator inhibitor-1) and ROS production via the activation of NADPH oxidase in VSMCs [36]. U-II activates PKC when inducing VSMC contraction and proliferation [37,38]. We have shown that U-II exerts a synergistic effect with oxidized LDL, lysophosphatidylcholine, ROS or serotonin in inducing VSMC proliferation [14,15].

Plasma and urinary levels of U-II increase with the severity of diabetic nephropathy [39]. In addition, plasma U-II levels are significantly higher in patients with three-

vessel CAD than healthy volunteers or patients with one- or two-vessel CAD [40]. The expression of U-II is at high levels in renal tubular epithelial cells in patients with diabetic nephropathy [11], and U-II is expressed at higher levels in ECs, foam cells and VSMCs within atherosclerotic lesions in human coronary arteries compared with those in normal coronary arteries [41]. Boussette et al. [12] reported that lymphocytes are by far the largest producers of U-II, whereas monocytes/macrophages are the major cell types that express UT, with relatively little expression in lymphocytes or platelets. The levels of U-II and UT expression are up-regulated by lipopolysaccharide and inflammatory cytokines, such as IL (interleukin)-6 and -1 β , IFN- γ (interferon- γ) and TNF- α (tumour necrosis factor- α) [42,43]. The expression of U-II in the vascular wall is also increased by hypoxia [44] and mechanical stimuli, such as balloon injury [45], but not by either pressure load or shear stress [46].

UT antagonists have been developed over the last decade as potential drugs for the treatment of hypertension, congestive heart failure, ischaemic heart disease and stroke [47]. Our recent results have shown that 4-aminoquinoline, a UT antagonist, inhibited the development of atherosclerotic lesions both in the presence and absence of exogenous U-II administration in apoE (apolipoprotein E)-knockout mice [48]. Rakowski et al. [45] have shown that SB-611812, a specific UT antagonist, decreases neointimal thickness and increases lumen diameter in a rat restenosis model of carotid artery balloon injury. Recently, palosuran was the first UT antagonist to be tested in humans and has been shown to decrease microalbuminuria after approx. 2 weeks in 18 subjects with hypertension and Type 2 diabetic nephropathy, probably by increasing renal blood flow [10].

There are several potential limitations of the present study. First, there is some controversy with regard to the immunoassays used for measuring U-II levels. Disparity was found between results from the different assays, such as RIAs and ELISAs from different commercial or in-house sources. A reason for the discrepancy in these findings between studies employing different immunoreactive methodologies might be attributed to the differing selectivity of the antibody used for mature human U-II over human prepro-U-II, human U-II-related peptide and human U-II fragments [23]. Douglas [49] stated that establishment of selective assays for immunoreactive and bioactive human U-II may help explain the wide variation in results obtained from ELISAs; however, such sensitive assays are not yet available. Secondly, the influence of statins and antihypertensive drugs on retinopathy and carotid atherosclerosis must be considered, as a number of the patients with Type 2 diabetes in the present study took these drugs. In addition to their lipid-lowering effects [50], statins have anti-atherosclerotic effects, such as anti-inflammatory effects and effects on atherosclerotic plaque stabilization. However, in the present study, there

was no difference in the prevalence of statin use among the patients with Type 2 diabetes. Thus the influence of these drugs on our results was considered negligible. Among the antihypertensive drugs, ARBs (angiotensin-II type 1 receptor blockers) and ACE (angiotensin-converting enzyme) inhibitors have been reported to decrease carotid IMT in patients with hypertension [51,52]. However, in the present study, most of the patients taking ARBs and ACE inhibitors had an increased IMT rather than decreased one. So far, there is no evidence that any of the drugs taken by the subjects in the present study affect plasma U-II levels. Another limitation is that the number of study subjects was relatively small, and the results of the present study should be interpreted with caution. Therefore large population studies are necessary to confirm the role of U-II as a risk factor for diabetic retinopathy.

In conclusion, the results of the present study suggest that increased levels of U-II may be associated with the development and progression of diabetic retinopathy and carotid atherosclerosis in patients with Type 2 diabetes. Therefore blockade of the U-II/UT system may become a promising therapeutic strategy for diabetic retinopathy and vascular complications in patients with Type 2 diabetes.

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