

Clinical Research

Association of urotensin II with angiographic severity of coronary artery disease

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Objective The goal of this study was to examine the association between urotensin II (U II) concentration and the severity of coronary artery disease (CAD). **Methods** We studied U II concentrations in 100 patients with known or suspected CAD referred for cardiac catheterization. Based on coronary angiograms, subjects were classified as having no or mild CAD (stenosis $<50\%$) and significant CAD (stenosis $\geq 50\%$). Micheal score system was used to estimate the severity of CAD. **Result** U II concentration in the significant CAD group had no difference compared with the no or mild CAD group ($1.95 \pm 1.18 \text{ pmol/L}$ vs $2.04 \pm 1.47 \text{ pmol/L}$, $P > 0.05$), but higher in the severe group (score ≥ 9) than in the normal or nearly normal group (score < 3) ($2.50 \pm 1.62 \text{ pmol/L}$ vs $1.61 \pm 1.05 \text{ pmol/L}$, $P = 0.03$). U II concentration had no relationship with other known risk factors, but it correlated with CAD severity ($r = 0.213$, $P = 0.034$). In multiple regression analysis, U II is one of the determinants of the severity of CAD, other than age, abnormal glucose, hypertension and gender. **Conclusions** U II is elevated in severe CAD and there is a significant relationship between U II concentration and CAD severity. (*J Geriatr Cardiol* 2007;4:229-232.)

Key Words arteriosclerosis; urotensin II ; coronary artery disease

Introduction

Urotensin, the most potent endogenous vasoconstrictor peptide identified to date, can induce endothelial cells and vascular smooth muscle cells (VSMCs) proliferation,¹ inhibits endothelial cells apoptosis via extracellular signal-regulated protein kinase (ERK),² accelerates the formation of macrophage-derived foam cells,³ up-regulates the expression of collagen I and decreases the expression and activity of matrix metalloproteinases-1 (MMP-1).⁴ Further the levels of U II and UT receptor are up-regulated within the atherosclerotic plaque⁵ and injured vascular wall.⁶ These data support that U II may be involved in the pathogenesis of atherosclerosis and vascular remodeling after injury.

The present study was therefore undertaken to investigate, in a cohort of patients with known or suspected coronary artery disease (CAD), the association between U II concentration and severity of CAD, based on coronary angiograms.

Methods

Study population

Patients who underwent coronary angiography for evaluation of known or suspected CAD at the China-Japan

Friendship Hospital were recruited in this study. The first patient was enrolled on Nov 2005. The exclusion criteria included patients with: 1) a history of coronary revascularization within 6 months; 2) acute myocardial infarction within 3 months; and 3) clinically evident heart failure, uncontrolled hypertension (systolic blood pressure $> 180 \text{ mmHg}$ and/or diastolic blood pressure $> 100 \text{ mmHg}$), renal insufficiency (serum creatinine $> 1.5 \text{ mg/dl}$).

A total of 100 patients were enrolled.

Interpretation and scoring of coronary angiograms

Coronary angiography was performed by the Judkins technique. Multiple projections of the right and left coronary arteries were recorded and cineangiograms were saved on disk. Subsequently, all cineangiograms were reviewed by two investigators. Scoring of severity of CAD was performed with a modification of the coronary atherosclerosis scoring system described previously.⁷ For analysis, the coronary circulation was divided into eight proximal segments. Disease in the distal segments was not considered because of difficulty in quantifying the severity of lesions in these areas. The eight proximal segments included the left main coronary artery, the left anterior descending artery (LAD) up to the junction of the middle and distal third of the vessel, the proximal third of the major septal branch of the LAD, the proximal third of the major diagonal branch of the LAD, the circumflex coronary artery (LCX) up to the junction of the middle and distal thirds of the vessel, the proximal third of the major obtuse marginal branch of

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the LCX, the right coronary artery (RCA) up to and including the origin of the posterior descending coronary artery (PDA), and the proximal third of the PDA. In cases in which the PDA was supplied by the LCX vessel (LCX dominance), lesions in the LCX up to the origin of the PDA were included, as were lesions of the RCA up to the origin of the middle and distal thirds of the vessel. The PDA was scored identically for RCA and LCX-dominant circulations. The percentage by which each lesion in the proximal coronary circulation narrowed the artery was assessed according to the maximal narrowing of the diameter of the artery in all projections. The extent and severity of the proximal coronary disease was assessed by assigning points to each lesion as follows: less than 50% stenosis of the luminal diameter, 1 point; 50% to 74% stenosis, 2 points; 75% to 99% stenosis, 3 points; total obstruction, 4 points. The points for each lesion in the proximal coronary circulation were added and a score for severity of coronary atherosclerosis was obtained.

U ϕ concentration measurement

Blood samples for U ϕ measures were collected into the VACUETTE tubes (Greiner, Austria) which contain EDTA just before coronary angiography, separated by centrifugation and stored at -80°C until analyzed. Urotensin enzyme immunoassay kit (Phoenix Pharmaceutical Inc, USA), was pre-coated with secondary antibody and the nonspecific binding sites were blocked. Intra-assay coefficients of variation were $<5\%$ and inter-assay variance $<10\%$.

Definition of risk factors

Risk factors were defined as before.⁹ Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared (kg/m^2). Obesity was defined as $\text{BMI} \geq 30$. Creatinine clearance rate (Ccr) was calculated by the Cockcroft-Gault formula.⁸ Renal insufficiency was defined as $\text{Ccr} < 80 \text{ ml/minute}$. Hypertension was defined as current use of antihypertensive drugs or diagnosed after admission. Smoking meant smoking until being studied, and no smoking included those who had quit. Abnormal glucose was defined as a history of known diabetes or a fasting plasma glucose $\geq 6.1 \text{ mmol/L}$. Risk factors score was calculated as in the EUROPA study.¹⁰

Statistical analysis

All statistical analyses were performed using SPSS for Windows 10.0. Data are presented for frequencies or percentages for categorical variables and means (SD) for continuous variables. Correlations were calculated by univariate Spearman correlation coefficients. Between-group differences were assessed by *t*-test or One-Way ANOVA. A stepwise multiple regression analysis was performed to determine independent predictors of severity of CAD.

Result

Baseline patient characteristics

The baseline characteristics of the 100 study subjects, including 25 patients with normal angiography and 75 patients with angiographic CAD were shown in Table 1.

Relationship between U ϕ and the severity of CAD

We found higher values of U ϕ concentration without statistical significance in the group with CAD compared with the group with no or mild CAD ($2.04 \pm 1.47 \text{ ng/ml}$ vs $1.95 \pm 1.18 \text{ ng/ml}$, $P > 0.05$). Furthermore we set 4 groups according to the Michael score, and saw a stepwise increase in U ϕ

Table 1 Demographic and clinical characteristics of the study population ($n=100$)

Male/female	65/35
Age (yrs)	59.99 ± 10.50
Obesity	25%(25)
Hypertension	73%(73)
Dyslipidemia	56%(56)
Abnormal glucose	33%(33)
Renal impairment	38%(38)
Previous cerebrovascular disease	8%(8)
PVD	3%(3)
Positive family history	17%(17)
Smoker	37%(37)
Crea(mol/L)	72.68 ± 15.79
Systolic blood pressure (mmHg)	133.23 ± 15.9
Diastolic blood pressure (mmHg)	79.48 ± 11.5
Cholesterol(mmol/L)	4.56 ± 1.07
LDL-C(mmol/L)	2.65 ± 0.86
Fasting plasma glucose(mmol/L)	5.60 ± 1.53
LVEF(%)	66.61 ± 6.4
Risk score	7.10 ± 3.0
Michael score	5.68 ± 4.15
U ϕ (ng/ml)	2.01 ± 1.40

Note: PVD, peripheral vascular disease; LDL-C, low-density lipoprotein cholesterol; LVEF, left ventricular ejection fraction; U ϕ urotensin ϕ

Table 2 Concentration of U ϕ in different severity groups

Michael score	n	U ϕ ng/ml
<3	32	1.61 ± 1.05
3-6	30	1.98 ± 1.17
6-9	20	2.27 ± 1.83
≥ 9	18	$*2.50 \pm 1.62$

Note: *compared with group 1, $P < 0.05$.

Table 3 Relationship between U ϕ and other risk factors

	Gender	Age	Smoker	BMI	Renal impairment	Hypertension	Abnormal glucose	Dyslipidemia	Risk score
<i>r</i>	-0.036	0.041	-0.061	0.023	-0.021	0.147	0.096	0.083	0.069
<i>P</i>	0.722	0.686	0.547	0.819	0.835	0.143	0.343	0.413	0.498

Table 4 Multivariate analysis of predictors affecting CAD stenosis

	Unstandardized coefficient		Standardized coefficient Beta	<i>t</i>	<i>p</i>
	B	Std. Error			
(Constant)	-0.900	1.140		0.789	0.432
Age	2.750	0.841	0.284	3.269	0.002
Abnormal glucose	2.421	0.768	0.275	3.152	0.002
Hypertension	2.085	0.816	0.224	2.556	0.012
U ϕ	0.559	0.261	0.188	2.148	0.034
Gender	1.603	0.747	0.185	2.145	0.035

Note: A Dependent Variable: SCORE
 $R^2=0.313$, $F=8.562$, $P<0.001$.

Especially there was a significant increase in group 4 (score=9) compared with group 1 (score<3). (Table 2) U ϕ concentration was significantly associated with the Michael score ($r=0.213$, $P=0.034$), but it had no relationship with other risk factors, as outlined in Table 3.

Multivariate regression analysis

To establish independent determinants of CAD via the Michael score, we performed a linear regression analysis controlled for age, gender, hypertension, abnormal glucose, dyslipidemia, smoking, abuse, renal impairment, and U ϕ concentration. The most important determinants were age, abnormal glucose, hypertension, U ϕ concentration and gender. (Table 4)

U ϕ levels in patients with hypertension or abnormal glucose versus control

In our study there was no difference in the hypertension or abnormal glucose group compared with control. (Table 5)

Discussion

Table 5 Concentration of U ϕ in patients with or without hypertension or abnormal glucose

Patients	<i>n</i>	U ϕ (ng/ml)
with abnormal glucose	33	2.14 \pm 1.59
with normal glucose	67	1.93 \pm 1.31
with hypertension	73	2.13 \pm 1.59
with normal blood tension	27	1.8 \pm 0.99

$P>0.05$

There have been a few recent studies about the effect of various vasoactive agents in both hypertension and atherogenesis. In particular, human U ϕ was suspected in playing a key role in the pathogenesis of atherosclerosis, not only through its hemodynamic effects but through direct cellular and molecular actions on the vessel wall. Some important processes of atherogenesis are known to be enhanced by U ϕ . In this research protocol, we evaluated the relationship between U ϕ and Michael score which represent the severity and extent of CAD.

Heringlake et al.¹¹ showed an elevated urotensin U ϕ plasma immunoreactivity in CAD (1511 \pm 886pg/L vs 1015 \pm 650 pg/L, $P<0.01$), while Hu et al.¹² found lower UII level in CAD than in control (1.61 \pm 1.02pg/ml vs 3.70 \pm 1.30pg/ml, $P=0.0001$). These inconsistent results may be due to the proportion of patients with acute coronary syndrome (ACS) in the study population. Both Joy et al.¹³ and Hu et al found a fall of U ϕ concentration in patients with ACS, a fact which might be explained by a reduced release of U ϕ from the injured endothelial cell.

In our study, we found a significant higher U ϕ in the severe CAD group (score =9) than in the normal or nearly normal group (score<3), (2.50 \pm 1.62pmol/L vs 1.61 \pm 1.05pmol/L, $P=0.03$). In a multiple regression analysis, U ϕ concentration was found to be one of the determinants for severity of CAD, other than age, abnormal glucose, hypertension and gender. However U ϕ concentration in the total CAD group was not different when comparing with patient without CAD or only mild CAD (1.95 \pm 1.18pmol/L vs 2.04 \pm 1.47pmol/L, $P>0.05$); perhaps U ϕ is a predictor for systemic atherosclerosis, as seen in patients with severe CAD or those with large atheroma burdens while the coronary angiogram may be normal. Suguro et al.¹⁴ demonstrated a

correlation between plasma U ϕ level and progression of carotid atherosclerosis in hypertensive patients. In their study the intima-media thickness (IMT) and plaque score in the carotid artery, blood pressure (BP), plasma levels of U ϕ and atherosclerotic parameters were determined in 50 hypertensive patients and 31 normotensive controls. In all subjects, plasma U ϕ level showed significant positive correlations with systolic BP, maximum IMT, plaque score, and homeostasis model assessment for insulin resistance (HOMA-IR). Multiple logistic regression analysis indicated that the contribution of plasma U ϕ levels to carotid plaque formation was significantly still greater with a 60% increase than those of established risk factors, such as age, systolic BP, high-sensitive CRP, small dense LDL, and HOMA-IR. Both of our results suggest that increased levels of U ϕ play a crucial role in the development of atherosclerosis.

Studies have demonstrated increased U ϕ in hypertensive or type 2 diabetic patients,^{15,16} however both of those patients may have a renal dysfunction, which may have a higher U ϕ concentration (10-100 fold than normal),¹⁷ so in our study those with renal insufficiency (serum creatinine > 1.5mg/dl) had been excluded. Thus in our study there was no difference in the hypertension or abnormal glucose group compared with control. Further analysis showed U ϕ had no relationship with hypertension or abnormal glucose, as they are independent predictors for CAD in multivariate analysis.

In the study by Heringlake et al, a positive relationship was found between U ϕ and proANP, proBNP, and mean right ventricular pressure (RVPM) which reflect the heart function. This is why in our study, subjects with heart failure were excluded.

It is unclear at the present, whether the predominant action of U ϕ in human disease will be protective or deleterious. SB-611812 was found to be the effective UT receptor antagonist. In a study by Ewa Rakowski et al,¹⁸ who examined the change and treatment in rat carotid arteries before and after balloon angioplasty, treatment with SB-611812 resulted in a significant 60% reduction in intima-to-media area ratio when compared to vehicle treatment ($P < 0.005$). The authors also demonstrated upregulation of U ϕ following balloon angioplasty, and a significant reduction in intimal lesion in response to UT receptor blockade. This study suggests an important role for U ϕ in the pathogenesis of restenosis following balloon angioplasty.

In summary our study showed a significant high U ϕ level in severe CAD. U ϕ could be considered as a determinant of severity for CAD, other than age, abnormal glucose, hypertension and gender. Further studies using several selective UT receptor antagonists or U ϕ converting enzyme inhibitors, and knockout or transgenic animals are required to investigate the real role of U ϕ on treating atherosclerosis.

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