Plasma urotensin II levels in primary Raynaud's phenomenon and systemic sclerosis

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Background/aim: The pathogenesis of Raynaud's phenomenon (RP) has not yet been fully elucidated. RP is characterized by exaggerated cold-induced vasoconstriction. Urotensin II (UII) is a potent vasoconstrictor. The aim of the present study was to evaluate plasma UII levels in both primary RP and secondary RP associated with systemic sclerosis (SSc).

Materials and methods: Fifteen patients with primary RP, 30 patients with RP secondary to SSc, and 30 healthy controls (HC) were included in the study. Raynaud condition scores (RCS) were determined in the primary RP and SSc groups. Modified Rodnan skin score (MRSS) was determined for the SSc patients. Plasma UII level was analyzed by the ELISA method.

Results: When compared to the HC group, plasma UII level was lower in the secondary RP group, but not in the primary RP group. Plasma UII level was not directly related to RCS in either the primary or secondary RP group. Moreover, it was not correlated with MRSS in the secondary RP group.

Conclusion: The results of the present study suggest that UII is not associated with primary RP. Its level was lower in the secondary RP (SSc) patients. Therefore, it can be concluded that decreased UII level is related to SSc instead of RP.

Key words: Urotensin II, Raynaud's phenomenon, systemic sclerosis

1. Introduction

Raynaud's phenomenon (RP) is an exaggerated vascular response to cold weather and/or emotional stress. It manifests with discoloration of distal parts of the body, i.e. fingers and toes. RP occurring in patients without any underlying disease is called primary (idiopathic) RP, and cases related to systemic lupus erythematosus (SLE), systemic sclerosis (SSc), mixed connective tissue disease, Sjögren's syndrome, or dermatomyositis/polymyositis are called secondary RP. The most marked pathophysiologic mechanism detected in RP is vasoconstriction. However, its underlying mechanism is not fully known (1,2). Increased levels of endothelin-1, which is a potent vasoconstrictor, and decreased activity of nitric oxide (NO), which is a potent vasodilator, have been detected in patients with primary RP (3,4). These changes may explain the mechanism of vasoconstriction in RP (3,4).

Urotensin-2 (UII) is a peptide consisting of 11 amino acids (5). UII was first isolated from the neurosecretory system of a fish. However, nowadays it is known that UII is also secreted from the human central nervous system, spleen, kidneys, small bowel, adrenal glands, thymus, vascular endothelium, heart, white blood cells, and liver. UII receptors have been identified on vascular smooth muscle cells (5). Therefore, UII has been suggested to play a role in cardiovascular diseases and it has been widely studied in cardiovascular diseases (5). Moreover, the relationship between essential hypertension and UII levels has been reported (6). Because UII is one of the most potent vasoconstrictors in the body, its possible role in the pathogenesis of RP has also been studied (7). Buyukhatipoglu et al. (7) have shown that plasma UII level is related to RP in patients with SLE.

Therefore, we aimed to investigate plasma UII levels in primary RP and in RP secondary to SSc.

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2. Materials and methods

2.1. Participants

Fifteen patients with primary RP, 30 patients with RP secondary to SSc, and 30 healthy control volunteers (HC) were enrolled in the study. All participants were female. Primary RP patients fulfilled the established criteria (8), while the secondary RP group was established from patients with SSc who fulfilled the criteria for the American College of Rheumatology classification (9). The HC group consisted of participants without any detected inflammatory disease who came to the outpatient clinics of the Department of Internal Medicine. Participants aged <18 years and >65 years; those with malignant diseases, cardiac disease, or communication problems; pregnant women; and reluctant patients were excluded from the study. The protocol of this study was approved by the institutional ethics committee, and all of the participants gave written informed consent before being enrolled in the study.

2.2. Samples

Blood samples of 5 mL were obtained from the left antecubital vein of each participant in the sitting position. Blood samples were placed in tubes containing aprotinin. The tubes were agitated slowly, placed on ice batteries, and transferred to the biochemistry laboratory immediately. The blood samples were centrifuged at 3500 rpm for 10 min at 4 °C. The obtained plasma samples were stored at −20 °C until the day of analysis.

Plasma UII concentrations were measured using an appropriate commercial kit (catalogue number: yhb3169hu, Shanghai Yehua Biological Technology Co., Shanghai, China) with the ELISA method. The results were expressed as ng/mL (CV [%] = SD / mean × 100; intraassay: CV < 10%; interassay: CV < 12%; sensitivity: 2.23 ng/mL).

Raynaud's condition score (RCS) is used to determine frequency, duration, and severity of RP episodes (10). RCSs of the patients in the primary and secondary RP groups were recorded. In addition, modified Rodnan skin scoring (MRSS) was used to determine the extent of skin involvement in the secondary RP (SSc) group (11).

2.3. Statistical analysis

Data were analyzed using SPSS 21 (IBM Corp., Armonk, NY, USA). Data were expressed as mean ± standard deviation. The normal distribution of the variables was evaluated with the Kolmogorov–Smirnov test. Parametric data were analyzed with analysis of variance (ANOVA) and the post hoc Tukey test, while nonparametric data were analyzed with the Mann–Whitney U test. On the other hand, analysis of covariance (ANCOVA) was also used to adjust variables for age, blood pressure, and the levels of fasting blood glucose. Categorical data were analyzed by the chi-square test. Pearson correlation analysis was used to determine the relationship among the data. P < 0.05 was considered significant.

3. Results

Demographic and clinical characteristics of the participants are shown in the Table. The mean age was higher in the secondary RP group compared to the HC group (P < 0.05). The difference in terms of mean age between primary and secondary RP groups was not statistically significant (P > 0.05).

Plasma UII level was significantly lower in the secondary RP group (Figure) compared to the primary RP and HC groups (P < 0.05 and P < 0.01, respectively). Its level was lower in the primary RP group compared to the HC group, although the difference was not statistically significant (Table).

The mean RCSs were 6.33 ± 1.54 and 4.70 ± 2.38 in the primary RP and secondary RP groups, respectively. There was a significant difference between the 2 groups in terms of mean RCS (P < 0.05). On the other hand, plasma UII levels were not correlated with RCS in either group (P > 0.05). In the secondary RP group, mean MRSS was 13.07 ± 9.16. There was no significant correlation between MRSS and plasma UII level (P > 0.05).

In the control group, plasma UII levels were negatively correlated with diastolic blood pressure (r = −0.377, P = 0.040). However, plasma UII level was not correlated with systolic or diastolic blood pressure in either the primary RP or the secondary RP group. None of the participants had the diagnosis of hypertension in the HC and primary RP groups, although 3 (10%) SSc patients had hypertension (P = 0.096). Moreover, all SSc patient were receiving an antihypertensive drug (24 patients were taking nifedipine and 6 patients were taking losartan) due to RP or hypertension.

Plasma UII levels were again evaluated statistically by ANCOVA test because there were significant differences among the groups in terms of mean age, blood pressure, and fasting blood glucose levels. There was no significant correlation between plasma UII and mean age in all the groups. However, the decrease in plasma UII level in the secondary RP group was not statistically significant after adjusting for the mean age (ANCOVA, P > 0.05).

4. Discussion

The present study evaluated whether plasma UII level is related to RP. For this purpose, plasma UII levels were analyzed in patients with primary RP and secondary RP (SSc). Plasma UII level was similar in healthy subjects and primary RP patients. However, its level was decreased in cases of RP secondary to SSc.

In both in vitro (12) and in vivo (13) studies, potent vasoconstrictor effects of UII, like those of endothelin-1

**Table.** The demographics and laboratory data in the study groups.

	HC (n = 15)	Primary RP (n = 30)	Secondary RP (n = 30)	P
Mean age (years)	27.6 ± 10	30.2 ± 7.9	49.4 ± 13[a]	0.001
Systolic BP (mmHg)	105 ± 20.3	111.1 ± 12.6	119 ± 17.6[a]	0.009
Diastolic BP (mmHg)	67 ± 7.2	76.0 ± 9.2	76 ± 10.2[b]	0.002
BMI (kg/m^2)	25.3 ± 4.9	23.6 ± 4.8	26.4 ± 3.6	0.192
ESR (mm/h)	9.9 ± 8.2	7.1 ± 7.5	18.3 ± 11.1[a,e]	<0.001
CRP (mg/L)	3.9 ± 1.9	4.1 ± 1.8	4.5 ± 3.0[c]	0.007
Glucose (mg/dL)	86 ± 6.8	85.5 ± 8.6	105 ± 19.9[b,d]	0.001
TCL (mg/dL)	168.9 ± 32.5	158.9 ± 30.8	200.6 ± 44.1[b,c]	0.002
LDL (mg/dL)	93.5 ± 20.1	93.5 ± 24.5	124.1 ± 41.0[b,c]	0.003
HDL (mg/dL)	54.3 ± 17.0	54.3 ± 16.5	52.4 ± 16.6	0.889
Triglyceride (mg/dL)	115.7 ± 9.9	101.4 ± 54.5	146.2 ± 108.7	0.203
Urotensin II (ng/mL)	669.1 ± 544.7	533.8 ± 380.2	291.9 ± 268.7[b,c]	0.001

Data are expressed as mean ± SD.
HC, Healthy controls; RP, Raynaud's phenomenon; BP, blood pressure; BMI, body mass index; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; TCL, total cholesterol; LDL, low-density cholesterol; HDL, high-density cholesterol.
When compared to the HC group: [a]$P < 0.05$, [b]$P < 0.01$.
When compared to the primary RP group: [c]$P < 0.05$, [d]$P < 0.01$, [e]$P < 0.001$.
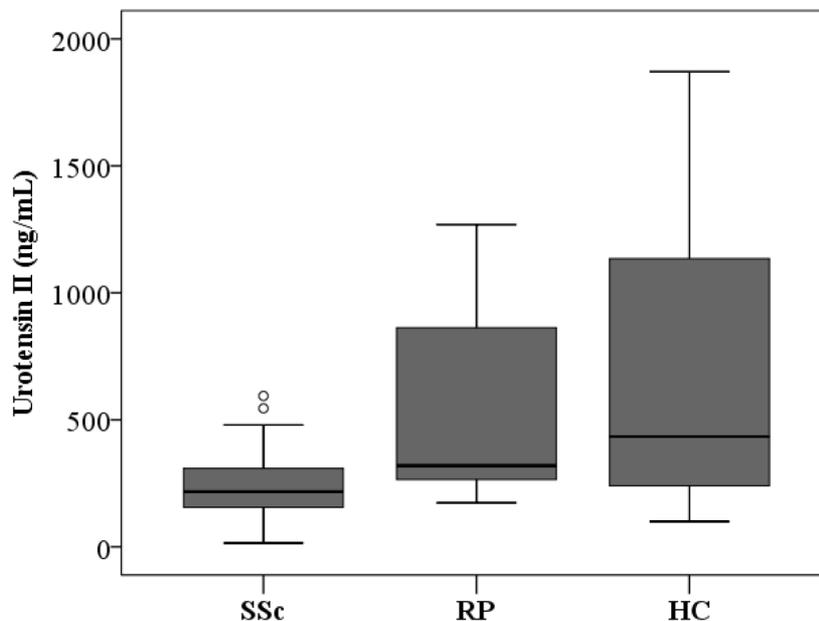
Figure. Plasma urotensin II levels in the study groups.
SSc, Systemic sclerosis; RP, Raynaud's phenomenon; HC, healthy controls.

receptors, have been detected on both arteries and veins. In many studies, the effects of UII on the pancreas (14), pulmonary artery (15), and kidneys (16) have been investigated. However, most studies have concentrated on the cardiovascular system because of the vasoconstrictor effects of UII. In addition to its vasoconstrictor effects, these studies performed on the cardiovascular system have demonstrated that UII can affect the pathogenesis of atherosclerosis (17) and induce fibrosis, cardiac hypertrophy, and coronary vasoconstriction (18). Contrary to these unfavorable data (17,18), Babińska et al. (19) documented the potential cardioprotective effects of UII. In another study (20), it was shown that UII ameliorates reperfusion injury in ischemic heart disease.

In RP, the most predominant and observable pathophysiologic mechanism is vasoconstriction (1,2). In RP, the level of endothelin-1, which is a potent vasoconstrictor, increases (3), while the activity of NO, which is a potent vasodilator, decreases (4). In a study on patients with SLE (7), probable correlation between levels of potent vasoconstrictor UII and RP was asserted. In the aforementioned study (7), the investigators speculated that UII is released from the vascular endothelium and plays a role in the vasoconstriction phase of RP. In our study, plasma UII levels were comparatively lower in the secondary RP group, whereas in the primary RP group, plasma UII levels were similar to those of the healthy control group. In addition, plasma UII level was not correlated with RCS in either the primary or the secondary RP group. These results may suggest that plasma UII levels are not related with RP, in contrast to the previous study (7).

Raynaud's phenomenon progresses with attacks. In our study, none of the patients was experiencing an RP attack during collection of plasma samples. It is difficult to detect patients during RP attacks in order to enroll them in a study. Therefore, the lack of any relation between plasma UII level and RP in our study may be attributed to this issue. It is accepted that UII has a relatively shorter plasma half-life (21). In an in vivo study (21) using an acute liver injury model, tissue UII levels peaked within 30 min, remained at that level for nearly 2 h, and normalized within 6 h. Therefore, it may be claimed that UII levels probably normalize soon after an episode of RP. Thus, it may be speculated that the possible relation between UII and RP could not be observed since patients were not experiencing an RP attack in our study.

In contrast to primary RP, the relatively low plasma UII level in RP secondary to SSc may indicate that plasma UII level decreases due to SSc but not RP. It is widely known that one of the earliest changes in the pathogenesis of SSc is vascular dysfunction (22). The effects of UII on the vascular system have been intensively investigated (17–

20). Increased UII levels were reported in SSc patients (23). Another important clinical finding of SSc is pulmonary hypertension. Previous studies have demonstrated higher UII levels in pulmonary hypertension (24,25). In our study, decreased plasma UII levels were detected in the SSc group.

Plasma UII level was unaltered in the primary RP group, although it decreased in the secondary RP group. Similarly, plasma UII level and RCS were not related in either group. These results suggest that SSc and/or any manifestations of SSc such as fibrosis may contribute to the decrease in UII level, in contrast to RP. However, plasma UII level in the secondary RP (SSc) group was not significantly correlated with MRSS as in RCS. This result may suggest that SSc, or at least skin fibrosis, cannot alter the plasma UII level. It has been shown in an experimental scleroderma model that UII level and skin fibrosis are not correlated (26).

In the SSc group, mean age was relatively higher. Additionally, it is known that the incidence of chronic diseases such as hypertension and diabetes mellitus increases with older age. Previous studies (13–16,18) have shown that UII levels might increase in diseases related to endothelial dysfunction, including essential hypertension, diabetes mellitus, atherosclerosis, ischemic heart disease, and heart failure.

In our study, in the secondary RP group, systolic blood pressure was significantly higher when compared with the control group. Plasma glucose levels in the secondary RP group were significantly higher when compared with both the control and primary RP groups. In our study, we also analyzed the correlation between plasma UII and lipid levels. In all the groups, lipid parameters were within normal limits. However, we determined relatively higher total cholesterol and LDL cholesterol levels in the secondary RP group compared to the healthy control and primary RP groups. In all the groups, there was no significant correlation among the blood lipids and plasma UII levels. However, despite increased levels of relevant pathologies in the secondary RP group, UII levels were not increased, but decreased.

It is widely known that hypertension affects UII level; this subject has been investigated in many studies (6,19). In some studies, increased UII levels have been reported (6). However, some other studies have demonstrated a lack of any significant difference between patients with and without hypertension (19). Mosenkis et al. (27) refuted the presence of a correlation between UII level and blood pressure. Despite all of these reports, the effects of antihypertensive treatments on UII levels have not been investigated so far. In our study, the patients in the secondary RP group were using a vasodilator in order to be able to decrease the frequency of RP episodes. Use

of nifedipine or angiotensin receptor antagonists could probably suppress plasma UII levels.

On the other hand, renal dysfunction is frequently seen in patients with SSc (28). UII can be produced in many parts of the body (5). The kidney is one of the production sites of UII (5). Mosenkis et al. (27) revealed that plasma UII levels decrease in renal failure, and plasma UII level is inversely correlated with creatinine level. Decreased renal function in SSc patients may be another reason for decreased plasma UII levels. This may explain the lack of any correlation between plasma UII level and MRSS.

Our study has some limitations. For example, power analysis was not performed to ascertain the counts of participants in the groups. Second, the patients were not evaluated during RP attacks. Despite these limitations, our study can provide an example for further studies to be performed on this topic.

In conclusion, plasma UII level decreased in RP related to SSc, although it was not altered in primary RP. It may be concluded that UII levels are not related to RP. Its level decreases in secondary RP patients associated with SSc, but not due to RP.

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