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Original Article

Effect of angiotensin-(1-7) on vascular endothelial cell function and correlation with blood pressure variability in patients with secondary hypertension

Haixia Yu[1,2,3], He Huang[1,2,3]

[1]Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; [2]Cardiovascular Research Institute, Wuhan University, Wuhan, Hubei, China; [3]Hubei Key Laboratory of Cardiology, Wuhan, Hubei, China

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Abstract: Objective: The goal of this study was to investigate the effects of angiotensin-(1-7) (Ang-(1-7)) on vascular endothelial cell function and determine the correlation with blood pressure variability in patients with secondary hypertension. Methods: A total of 80 patients with hypertension admitted to the department of cardiovascular medicine of Inner Mongolia People's Hospital from September 2016 to September 2017 (observation group), and 80 healthy subjects in the same period (control group), were compared for the levels of Ang-(1-7), endothelin-1 (ET-1), and inflammatory factors including interleukin-6 (IL-6) and interleukin-8 (IL-8). Results: The observation group presented lower level of serum Ang-(1-7), and higher levels of serum ET-1, IL-6 and IL-8, compared with the control group ($P<0.05$). In addition, the 24-hour measures of blood pressure variability among the hypertensives showed a negative correlation with serum Ang-(1-7) level and positive correlations with the levels of serum ET-1, IL-6, and IL-8. Moreover, univariate logistic regression analysis showed that Ang-(1-7) was a risk factor for blood pressure variability in the hypertensives. Conclusion: Ang-(1-7) can participate in the development and progression of hypertension and blood pressure variability by affecting the function of vascular endothelial cells and mediating inflammatory reactions.

Keywords: Angiotensin-(1-7), vascular endothelial cell function, blood pressure variability, secondary hypertension

Introduction

Hypertension is a common chronic disease as well as a major risk factor for cardiovascular and cerebrovascular diseases such as stroke, myocardial infarction and aortic dissection [1]. More than half of patients died from cardiovascular and cerebrovascular diseases each year have hypertension, and similar proportion of patients died from cerebrovascular accident suffers from hypertension [2]. China presents as a high-incidence area of hypertension with nearly 40 million people who are disabled or have their life span shortened due to hypertension every year, and increasing number of cases as years, reported by epidemiological investigation on hypertension. In addition, epidemiological investigation of hypertension in China shows the number of people suffering from hypertension in the north is higher than that in the south, and the incidence rate of hypertension in Chinese Han population is higher than that in ethnic minorities [3, 4].

With continuous and in-depth study of the causes of hypertension, inflammatory factors have received increasing attention from researchers. Studies have shown that inflammatory reaction can produce excessive reactive oxygen species. The species can damage endothelial cells and thus affect production of nitric oxide synthase, and chronic inflammatory reaction can also reduce the activity of nitric oxide synthase and enhance oxidative stress, thus leading to the formation of hypertension [5-8]. The blood pressure of normal people presents a circadian rhythm with a dynamic fluctuation of two peaks and one valley. Furthermore, the reaction of the body's environment and emotion can also cause changes on blood pressure

in daily life. However, variability appears in the blood pressure values of hypertensives due to vascular lesions and inflammatory factors. Blood pressure variability has been proven to be directly related to target organ damage, and can be used as an independent predictor of cardiovascular disease, but the specific mechanism of blood pressure variability is unclear at present [9].

Studies have confirmed that angiotensin-(1-7) (Ang-(1-7)) can participate in regulating anti-inflammation and vasodilation through ACE2/Ang-(1-7)/Mas axis. ACE2 is a homologue of angiotensin converting enzyme (ACE), which is expressed in endothelial cells and plays an important role in cardiovascular and renal systems. It has ultra-high tissue specificity, and its expression in the body is limited in the heart, kidney, and reproductive systems, with the function of controlling myocardial remodeling and thus indirectly protecting the heart [10]. Ang-(1-7) is a polypeptide, formed from a conversion of Ang-II through cleaving phenylalanine residues within the carboxyl terminus of Ang-II by ACE2, and exerts its effect through Mas receptor. At present, ACE2/Ang-(1-7)/Mas axis has been proven to have the effect of increasing the expression level of anti-inflammatory cytokines, IL-10, and decreasing expression levels of inflammatory factors, IL-6, and IL-8 [6]. Therefore, this study aimed to investigate the effects of Ang-(1-7) on vascular endothelial cell function and potential correlation with blood pressure variability in patients with secondary hypertension.

Subjects

A total of 80 patients with hypertension admitted to the Department of Cardiovascular Medicine of Inner Mongolia People's Hospital from September 2016 to September 2017, and 80 healthy subjects in the same period were analyzed. Although the ACC/AHA hypertension guidelines of the United States lowered the diagnostic blood pressure values of hypertension by 10 mmHg in 2017, China has not yet updated the unified criteria, so the previous diagnostic criteria are still in use. These diagnostic criteria for hypertensives [11] were as follows: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg were measured three times in different days. Inclusion and exclusion criteria: The hypertensives (1) who were first discovered and diagnosed as hypertension followed with treatment, and (2) who agreed to participate in this research were included. While the hypertensives (1) who had secondary hypertension caused by adrenal diseases and cervical spondylosis, or (2) who had heart valve and vascular lesions requiring surgical treatment, or (3) who had any kinds of tissue tumors, or (4) who aged over 80 years old, or (5) who had dilated and hypertrophic cardiomyopathy, or (6) who had major organ dysfunction (liver or kidney), or (7) who had coagulation dysfunction, or (8) who had immune system diseases, or (9) who had no infection in the two weeks before hospitalization, or (10) who had taken immunosuppressants before hospitalization, were excluded. The study was approved by the Medical Ethics Committee of Inner Mongolia People's Hospital, and informed consent was obtained from all the subjects.

Materials and methods

Determination methods of involved factors

Changes of angiotensin-(1-7) (Ang-(1-7)), endothelin-1 (ET-1), inflammatory factors including interleukin-6 (IL-6) and interleukin-8 (IL-8) in the two groups were recorded, and the specific determination methods were as follows: about 6-8 mL peripheral venous blood of each subject of both groups was obtained, and placed in an anticoagulant tube, followed by centrifugation at a speed of 3,000 r/min. Then the supernatant was separated, and placed at -80°C for later determinations. Enzyme-linked immunosorbent assay (enzyme-linked immunosorbent analyzer, Infinity F50, Tecan, Switzerland; kit, Santa, USA) was applied to determine the levels of serum Ang-(1-7), ET-1, IL-6, and IL-8, and specific operations were conducted according to instructions of the kit.

Method for measuring blood pressure

The 24-hour ambulatory blood pressure monitor (Mobil, Germany) was used for monitoring the blood pressure of all subjects. The monitoring time in the daytime was from 6:00 a.m. to 22:00 p.m., and that in the nighttime was from 22:00 p.m. to 6:00 a.m., with a frequency of once in each 30 minutes, and the monitoring time of each subject was at least 23 hours. According to the monitoring results, the coefficient of variation of blood pressure (standard deviation of blood pressure value in a specific time period/average value), 24-hour average

Table 1. Comparison of baseline data between two groups

	Observation group (n=80)	Control group (n=80)	t/χ^2	P
Sex (male/female)	46/34	43/37	0.091	0.763
Age (year)	55.3±5.9	54.8±6.0	0.531	0.596
Diabetes (n)	8	7	1.327	1.000
Body mass index	25.01±0.89	24.97±0.73	0.326	0.745
Smoking history (n)	26	22	0.363	0.547
Systolic blood pressure (mmHg)	157.83±14.70	118.15±15.37	17.502	<0.001
Diastolic blood pressure (mmHg)	101.35±13.53	82.91±13.48	9.057	<0.001

Table 2. Comparison of coefficient of variation of blood pressure between two groups

	Coefficient of variation of systolic blood pressure	Coefficient of variation of diastolic blood pressure
Observation group (n=80)	11.34±3.01	11.02±2.54
Control group (n=80)	8.32±0.89	7.98±2.32
t	9.026	8.290
P	<0.001	<0.001

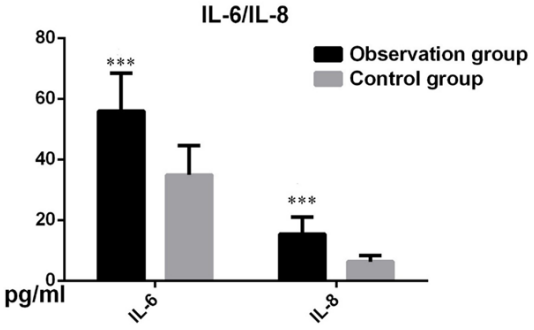

Figure 1. Comparison of serum IL-6 and IL-8 levels between the two groups. For the comparison between the observation group and the control group, ***P<0.001.

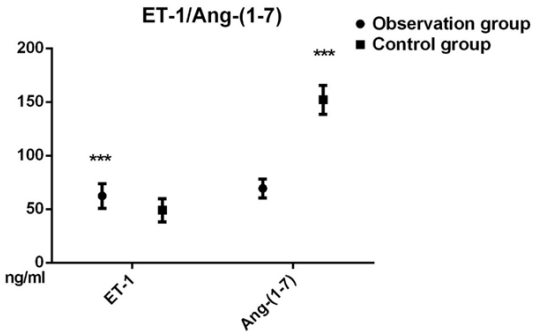

Figure 2. Comparison of serum ET-1 and angiotensin-(1-7) levels between the two groups. For the comparison between the observation group and the control group, ***P<0.001.

systolic pressure and diastolic pressure was statistically calculated.

Statistical analysis

The data obtained in this study was analyzed using the SPSS software version 22.0. The measurement data are expressed as mean ± standard deviation ($\bar{x}$±sd), and inter-group comparison was conducted by independent t-test. The enumeration data were expressed as number of cases (n), and the comparison of the inter-group rates was performed by Chi-square test. Pearson correlation analysis was used to evaluate the correlations between indexes of blood pressure variability and serum factors or between the indexes and Ang-(1-7), and regression analysis was performed with level of serum Ang-(1-7) as dependent variable and the coefficients of variation of systolic and diastolic blood pressure as independent variables. P<0.05 was considered statistically significant.

Results

Comparison of baseline data between two groups

There were no statistical differences in sex, age, diabetes, body mass index, and smoking history between the two groups (all P>0.05), and the two groups were comparable. The values of systolic and diastolic blood pressures of the hypertensives were higher than those of the healthy controls (P<0.001) as shown in **Table 1**.

Comparison of coefficient of variation of 24-hour blood pressure between two groups

There were statistical differences between the coefficients of variation of systolic and diastolic

Table 3. Correlation between serum angiotensin-(1-7) and relevant factors in the observation group

	r	P
Coefficient of variation of systolic blood pressure	0.879	0.030
Coefficient of variation of diastolic blood pressure	0.874	0.014
IL-6	0.923	0.003
IL-8	0.934	0.012
ET-1	0.917	0.018

Note: ET-1: endothelin-1; IL-6: interleukin-6; IL-8: interleukin-8.

Table 4. Correlation between coefficient of variation of blood pressure and IL-6, IL-8, and ET-1 in the observation group

	Coefficient of variation of systolic blood pressure		Coefficient of variation of diastolic blood pressure	
	r	P	r	P
IL-6	0.917	0.011	0.941	0.008
IL-8	0.925	0.039	0.906	0.047
ET-1	0.907	0.019	0.933	0.021

Note: ET-1: endothelin-1; IL-6: interleukin-6; IL-8: interleukin-8.

blood pressure of the hypertensives and those of the healthy controls (all P<0.05), indicating that the blood pressure variability of the hypertensives was higher than that of the normal ones as shown in **Table 2**.

Comparison of serum IL-6 and IL-8 levels between two groups

The levels of serum IL-6 (56.18±11.81 vs 11.38±3.47) and IL-8 (10.09±2.63 vs 3.65±1.03) in the observation group were significantly higher than those in the control group (all P<0.001), indicating that the inflammatory level in the hypertensives was higher than that in the healthy controls, as shown in **Figure 1**.

Comparison of serum Ang-(1-7) and ET-1 levels between two groups

The level of serum ET-1 (62.39±11.48 vs 49.15±10.83) in the observation group was significantly higher than that in the control group (P<0.001), while its level of Ang-(1-7) (69.35±8.76 vs 152.01±13.54) was significantly lower than that in the control group (P<0.001), suggesting that the decrease of Ang-(1-7) and the increase of ET-1 in the hypertensives may be the underlying pathogenesis of hypertension as shown in **Figure 2**.

Correlation between serum Ang-(1-7) and relevant factors in the hypertensives

There was a negative correlation between the coefficient of variation of blood pressure in the hypertensives and Ang-(1-7) (coefficient of variation of systolic blood pressure, r=0.879; coefficient of variation of diastolic blood pressure, r=0.874; all P<0.05), and Ang-(1-7) also presented negative correlations with IL-6, IL-8, and ET-1 (IL-6, r=0.923; IL-8, r=0.934; ET-1, r=0.917, all P<0.05) as shown in **Table 3**.

Correlation between coefficient of variation of blood pressure and IL-6, IL-8, and ET-1 in hypertensives

The coefficient of variation of blood pressure presented positive correlations with IL-6, IL-8 and ET-1 (IL-6, r=0.917; IL-8, r=0.925; ET-1, r=0.907, all P<0.05), as shown in **Table 4**.

Univariate logistic regression analysis of coefficient of variation of blood pressures in hypertensives

In this study, the level of serum Ang-(1-7) was taken as dependent variable, and the coefficients of variation of systolic and diastolic blood pressure as independent variables for regression analysis. Logistic regression analysis showed that the decrease of the level of serum Ang-(1-7) was an independent risk factor for blood pressure variability (the regression coefficients of systolic and diastolic blood pressure were 0.32 and 0.038 respectively, all P<0.001), as shown in **Table 5**.

Discussion

Unceasing alteration of the body's blood pressure is the cause for the variability of blood pressure value [12, 13]. However, it was not until the 1990s that researchers discovered that the variability of blood pressure values was not a random but common phenomenon. Recent studies have certified that blood pressure variability is closely related to cardiovascular events including shock, coronary artery disease, damage of renal function and cardiac pump failure [14, 15]. Therefore, the focus of prevention and treatment of hypertension at

Table 5. Univariate logistic regression analysis of coefficient of variation of blood pressures in the observation group

	Ang-(1-7)			
	Standardized β	OR	95% CI	P
Coefficient of variation of systolic blood pressure	0.326	1.152	1.012-1.435	<0.001
Coefficient of variation of diastolic blood pressure	0.041	1.673	1.157-1.971	<0.001

Note: Ang-(1-7): angiotensin-(1-7); OR: odds ration; CI: confidence interval.

present is gradually shifting to blood pressure variability, and it is of great significance to explore the potential mechanism of variability in hypertensives for reducing the harm of hypertension [16].

At present, many studies have proven that there is a close relationship between the inflammatory response and blood pressure variability [17]. Ang-(1-7), as the latest identified inflammatory regulatory factor, has been proved to play a role in hypertensive chronic nephrosis, and it can partially counteract the inflammatory reaction in the body by combining with its corresponding antagonist [18]. IL-6 and IL-8, as the main representatives of the inflammatory reaction, present their participation by activating the reaction and promoting chemotaxis of inflammatory cells. Animal trials have confirmed that blocking the signaling pathways for Ang-(1-7) significantly increases the levels of inflammatory factors in the body, indicating that Ang-(1-7) serves as a regulator in the inflammatory reactions [19]. This study also proved that the hypertensives in the observation group presented higher levels of IL-6 and IL-8 and lower level of Ang-(1-7) compared with the control group, confirming that Ang-(1-7) participated in the process of hypertension variation by regulating inflammatory factors IL-6 and IL-8, which is consistent with previous studies [20].

Endothelin secreted by vascular endothelial cells can strongly constrict the blood vessels. While Ang-(1-7) can directly act on vascular endothelial cells for decreasing the secretion of endothelin, and fight against hypertension on the basis of promoting the synthesis of nitric oxide. Moreover, endothelin can damage vascular endothelial cells, which leads to transverse and longitudinal shear stress inside the blood vessels, thus resulting in the variability of blood pressure values [21]. Therefore, Ang-(1-7) can decrease variability of blood pressure values by reducing endothelin production. These results showed that the level of serum ET-1 in the hypertensives is higher than that in the healthy controls, indicating that endothelin also plays an important role in the mechanism of blood pressure variability in hypertensives, which is similar to previous studies [22].

This research also analyzed the correlation between those factors. The results showed that the coefficient of variation of hypertensives had a negative correlation with Ang-(1-7), but positive correlations with serum IL-6, IL-8 and ET-1. This study showed that Ang-(1-7) presented negative correlations with IL-6, IL-8, and ET-1, suggesting that Ang-(1-7) participates in blood pressure variability by regulating IL-6, IL-8, and endothelin. Logistic regression analysis also showed that the decrease of the level of serum Ang-(1-7) in hypertensives was an independent risk factor for blood pressure variability, indicating that it may become a potential therapeutic target for blood pressure control. However, this study is a single-center study with a small sample size, which requires further verification by multi-center and large sample studies. In addition, it is quite necessary for us to do further research on ACE2/Ang-(1-7)/Mas axis for further elaborating the mechanism of action of Ang-(1-7).

In summary, the inflammatory response participates in the pathophysiological process of blood pressure variation through either itself or vascular endothelin, and Ang-(1-7), as an important regulatory factor in the body, has the function of anti-inflammation and vasodilation for control blood pressure by regulating IL-6, IL-8, and endothelin.

Disclosure of conflict of interest

None.

Address correspondence to: He Huang, Department of Cardiology, Renmin Hospital of Wuhan University, No.238 Jiefang Road, Wuchang District, Wuhan

430060, Hubei, China. Tel: +86-13986161018; E-mail: huanghe342u@126.com

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