Original Article

DOI: 10.5582/bst.2015.01087

Protective effects on vascular endothelial cell in *N'*-nitro-*L*-arginine (*L*-NNA)-induced hypertensive rats from the combination of effective components of *Uncaria rhynchophylla* and *Semen Raphani*

Yunlun Li[1,*,], Wenqing Yang[2,*], Qingjun Zhu[2], Jinguo Yang[3], Zhen Wang[2]**

[1] *Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Ji'nan, Shandong, China;*
[2] *Shandong University of Traditional Chinese Medicine, Ji'nan, Shandong, China;*
[3] *Shenzhen Longgang District Hospital of Traditional Chinese Medicine, Shenzhen, Guangdong, China.*

Summary **Endothelial dysfunction is closely associated with hypertension. Protection of vascular endothelial cell is the key to prevention and treatment of hypertension. *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid, isolated from traditional Chinese medicine Uncaria rbyncbopbylla and *Semen Raphani* respectively, exhibit properties of anti-hypertension and protection of blood vessels. In the present study, we observed the protective effect of the combined use of *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid to the vascular endothelial cell in *N'*-nitro-*L*-arginine-induced hypertensive rats and investigate the preliminary mechanism. Blood pressure was detected by non-invasive rats tail method to observe the anti-hypertension effect of drugs. Scanning electron microscopy was used to observe the integrity or shedding state of vascular endothelial cell. The amount of circulating endothelial cells and CD54 and CD62P expression on circulating endothelial cells were tested to evaluate the endothelium function. In this study, we found that the *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility can effectively lower the blood pressure, improve the structural integrity of vascular endothelium, and significantly reduce the number of circulating endothelial cells. Furthermore, the mean fluorescence intensity of CD54 and CD62P expressed showed decrease after the intervention of *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility. In conclusion, the combination of effective components of the *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid demonstrated good antihypertension effect and vascular endothelium protective effect. The preliminary mechanism of the protective effect may attribute to relieve the overall low-grade inflammation.**

Keywords: *Uncaria rhynchophylla* total alkaloids, *Semen Raphani* soluble alkaloid, endothelial dysfunction, inflammation

1. Introduction

Hypertension is the most common cardiovascular risk factor and a major public health problem in the world. Development of hypertension was closely related to the vascular endothelial dysfunction. Endothelial dysfunction may contribute to increased systemic vascular resistance and lead to the development of hypertension. Endothelial dysfunction is commonly manifested as impaired endothelium dependent vasodilation due to an imbalance between vasoconstrictors and vasodilators (*1*). Thus, improving vascular endothelial dysfunction plays an important role in hypertension treatment.

In recent years, hypertension was considered to be a low-grade inflammatory disease (*2*), and vascular endothelial is the key part. When vascular endothelial damage, the activation of endothelial cells can secrete proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumor necrosis factor

*These authors contributed equally to this works.
***Address correspondence to:*
Dr. Yunlun Li, Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Ji'nan 250011, Shandong, China.
E-mail: yunlun.lee@hotmail.com

alpha (TNF-α) and also express the immunoglobulin superfamily cell adhesion molecules and selection family. These cytokines facilitate the adhesion of neutrophils and monocytes to endothelium affecting production of endothelium-derived nitric oxide (NO), impairing endothelial dependent vasodilation function, inducing inflammatory damage of vascular walls (*3*). Therefore, it is a key factor to lower blood pressure that secretion of inflammatory substances was regulate.

Although drugs and changing life styles have been widely promoted to control the hypertension, unfortunately, the prevention of the progression of vascular damage in hypertension patients remains pessimistic. Traditional Chinese medicine (TCM) provides a potential option to the treatment of hypertension. From the 1950s, Chinese physicians have been concentrating on effective prevention and cure for hypertension using TCM, and considerable progress has been achieved (*4*). Many TCMs and their active components have been reported to have anti-hypertension effects.

Syndrome differentiation is a diagnostic and treatment method used in TCM. It plays an important role in the therapeutic process and affects the therapeutic result of hypertension. Some scholars have found that yang hyperactivity was the most common excessive syndrome elements of hypertension (*5*). Hyperactivity of liver yang is characterized by vertigo, tinnitus, headache, flushing, red eyes, irritability, insomnia, lassitude in lion and legs, bitter mouth, red tongue, and wiry pulse. Our previous studies showed that hyperactivity of liver yang might be related to the fifteen compounds of the structure and metabolic pathways mainly including amino acids, free fatty acids, and sphingosine by high performance liquid chromatography coupled with time of flight mass spectrometry (HPLC-TOFMS) (*6*). Usually, calming the liver wind and liver Yang is an important treatment method.

Uncaria rhynchophylla Miq Jacks (Gouteng in Chinese) belongs to the family of Rubiaceae. Currently, *Uncaria rhynchophylla* is generally used to treat ailments in the cardiovascular and central nervous systems, such as lightheadedness, convulsions, numbness, and hypertension, *etc.* (*7,8*). The effective component of *Uncaria rhynchophylla* is *Uncaria rhynchophylla* total alkaloids. Our previous study had proved that *Uncaria rhynchophylla* total alkaloids had the pharmacological effects of lowering blood pressure, protecting vascular endothelium, inhibiting cell aging and improving the thoracic aorta wall reconstruction (*9-11*). *Semen Raphani* (Laifuzi in Chinese) belongs to the family of Brassicaceae and *Semen Raphani* soluble alkaloid is the main effective ingredient. Several researches indicated that *Semen Raphani* soluble alkaloid can had prominent function to lower the hypotension, improve the process of cardiovascular remodeling and protect target *via* antioxidation (*12,13*).

Uncaria rhynchophylla combined with *Semen Raphani* is widely used in clinics for hyperactivity of liver yang in hypertensive treatment in the Hospital of Shandong University of Traditional Chinese Medicine. The classical prescription can effectively reduce blood pressure in hypertensive patients. We speculate that *Uncaria rhynchophylla* combined with Semen Raphan should exhibit protective effects of vascular endothelial cells. Thus, in the present study, we investigated the protective effects of *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid against vascular endothelial dysfunction in hypertensive rats induced by *N'*-nitro-*L*-arginine (*L*-NNA). Furthermore, the underlying mechanisms for *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid-induced protective effects were investigated. Valsartan was purchased from Beijing Novartis Pharmaceutical Co., Ltd. (Beijing, China).

2. Materials and Methods

2.1. *Materials*

L-NNA was purchased from sigma (St. Louis, MO, USA). Anti-Rat CD54 PerCP-eFluor 710 and Anti-Rat CD3PE were purchased from eBioscience (San Diego, California, USA). CD31 FITC and rabbit monoclonal antibody to CD146 were purchased from Abcam (London, UK). CD62P PerCP was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). *Uncaria rhynchophylla* and *Semen Raphani* were purchased from Jianlian Traditional Chinese medicine Store (Jinan, China).

2.2. *Preparation of drugs*

Acid dyes staining method was utilized to determine the content of *Uncaria rhynchophylla* total alkaloids, and high performance liquid chromatography (HPLC) method was adopted to establish the fingerprint. The HPLC method was also applied to ensure that the contents of rhynchophylline accounted for more than 5.5% in *Uncaria rhynchophylla* total alkaloids and of sinapine cyanide sulfonate accounted for more than 40% in *Semen Raphani* soluble alkaloid. *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid were provided by professor Honglei Zhou (Shandong University of Traditional Chinese Medicine). According to the result of multiple regression analysis and partial least-squares regression, we had proved that the optimal ratio of the two components in lowering blood pressure was 5/6 (*14*).

Then the mixture was dissolved in physiological saline and prepared into three suspensions, which contained the *Uncaria rhynchophylla* total alkaloids with concentration of 3.853mg/mL + the *Semen*

Raphani soluble alkaloid with concentration of 4.623 mg/mL, the *Uncaria rhynchophylla* total alkaloids with concentration of 3.853mg/mL and the *Semen Raphani* soluble alkaloid with concentration of 4.623 mg/mL. Valsartan dissolved in physiological saline to a concentration of 1.335 mg/mL and kept at 4°C. The suspensions were stored at 4°C before use.

2.3. *Modeling and grouping*

One hundred and twenty male Wistar Kyoto rats (WKY), SPF level, 5-week-old, 165-212 g, were purchased from Shandong Lukang Pharmaceutical Co., Ltd. (certificate: SCXL (Lu) 20080002). The animals were taken care under standard conditions (12 h light/dark cycle, ventilated, fixed temperature and humidity room). Rats were provided standard rat pellet food for nourishment and tap water as drinking water. All animal experiments were performed in accordance with the guidelines of Shandong University of Traditional Chinese Medicine for the care and use of laboratory animals and approved by the Animal Ethics Review Committee of Shandong University of Traditional Chinese Medicine.

One hundred and twenty rats were randomly divided into 2 groups, namely, the normal control group (n = 20), model group (n = 100). The modeling started after 7-day adaptive feeding. The model group was induced by intraperitoneal injection of *L*-NNA at a dose of 7.625 mg/kg·d, while the normal control group was injected the same volume of physiological saline. Blood pressure began to increase in the first modeling week. The injection lasted 2 weeks and formed stability hypertension, indicating successful modeling.

Rats proven to be hypertension (n = 100) were randomly assigned to five groups with 20 rats in each group: model group, valsartan group, *Uncaria rhynchophylla* total alkaloids group (U group), *Semen Raphani* soluble alkaloids (R group) and *Uncaria rhynchophylla* total alkaloids + *Semen Raphani* soluble alkaloid group (U-R group). And 20 Wistar Kyoto rats were recruited as the normal control group. Intragastric administration was performed once a day for each treatment group. The dosages of suspensions were calculated according to the body surface area of human and rat. The Valsartan was intragastrically administered at a dose of 2.67 mg/200g (prescription/body weight). The doses of *Uncaria rhynchophylla* total alkaloids, *Semen Raphani* soluble alkaloids and *Uncaria rhynchophylla* total alkaloids + *Semen Raphani* soluble alkaloid groups were 7.705 mg/200 g, 9.246 mg/200 g and (7.705 mg + 9.246 mg)/200 g (prescription/body weight) respectively. The normal control group and model group were intragastrically administrated by physiological saline at the equivalent dose. Every group was given suspensions for 6 days per week and 5 weeks in a row.

2.4. *Detection of blood pressure of tail artery*

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) were detected by a tail-cuff sphygmomanometer with an automated system photoelectric sensor (ALC-Non-Invasive Blood Pressure System, Shanghai Alcott Biotech Co., Ltd., Shanghai, China). Rats were heated to dilate rat-tail artery for blood pressure measurements. All rats were measured 3 times in parallel, and data was collected as a mean.

2.5. *Observation of rat vascular endothelial morphological*

Following 24 h fasting, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg). After drawing blood, the thoracic aorta, renal artery and mesenteric artery were gently separated as fast as possible, cleaned of connective tissue and flushed by saline. Samples were fixed in the 2.5% glutaraldehyde solution for 24 h. Scanning electron microscopy was used to observe the integrity or shedding state of vascular endothelial cell.

2.6. *Measurement of the number of circulating endothelial cells*

Peripheral blood was sampled at different time points: before modeling, the 1st, the 3rd and the 5th weekend after administration. The number of circulating endothelial cells (CECs) was measured by indirect FACS-fluorescence labeled antibody by flow cytometry (Shanghai, Chain). The percentage of mononuclear cells in white blood cells (WBC) (M1) was detected by flow cytometry. And then detected by flow cytometry the percentage of $CD3^-CD31^+CD62P^+$ cells in the mononuclear cells (M2). The results of M1 multiplied M2 were the circulating endothelial cells accounted leukocyte percentage (M). The absolute value of WBC was counted with hemocytometer under microscopy (N1). The results of M multiplied N1 were the circulating endothelial cell number ($N = M \times N1$).

2.7. *Detection of circulating endothelial cells CD54 and CD62P expression*

FACS combined with fluorescently labeled antibodies indirect detection method was used to detect the mean fluorescence intensity of CD54 and CD62P on $CD3^-CD31^+$cell.

2.8. *Statistical analysis*

All the data was processed with SPSS 15.0 software (SPSS Inc., USA). The results were expressed as means ± SEM. The multi-group comparisons used one-way

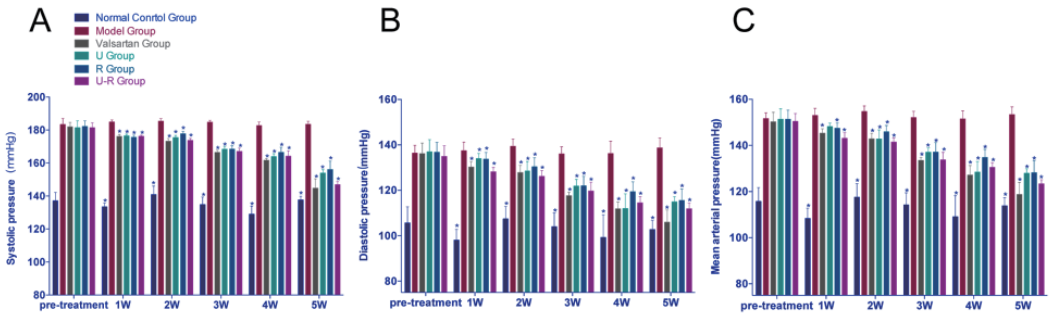

Figure 1. The changes of blood pressure in different groups. Systolic pressure **(A)**, diastolic pressure **(B)** and mean arterial pressure **(C)** of rats kept decreasing gradually during the 5-week-study in Valsartan group, *Uncaria rhynchophylla* total alkaloids group (U group), *Semen Raphani* soluble alkaloids group (R group), and *Uncaria rhynchophylla* total alkaloids + *Semen Raphani* soluble alkaloid group (U-R group), while model group have no significant changes. Significant difference ($p < 0.05$) due to comparing with model group is denoted by asterisk (*).

analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant.

3. Results

3.1. *The changes of blood pressure in different groups*

Baseline SBP, DBP and MAP were similar in the six experimental groups ($p > 0.05$). The blood pressure of rats in the Valsartan group, U group, R group and U-R group were decreased significantly compared to the model group($p < 0.05$) (Figure 1A). DBP and MAP decreased in different extent after administration, but the effect was not as obvious as SBP (Figures 1B and 1C). From the perspective of lowering the blood pressure, the antihypertensive effect of *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility was similar with that of valsartan ($p < 0.05$). When treated with *Uncaria rhynchophylla* total alkaloids, *Semen Raphani* soluble alkaloid and combination of the two, respectively, the blood pressure decreased at different degrees, however, the combination of the two demonstrated the most powerful effect on decreasing the blood pressure. This indicates the *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility have an additive effect.

3.2. *Improve the morphology of vascular endothelial cells*

Endothelial morphology of thoracic aorta, renal artery and mesenteric artery were observed with scanning electron microscopy. The endothelial cells of rats in normal control group were showed neat cord blood vessels, completely connecting and mucosal smoothing endothelial cells, without significant fiber plaques adhesion on cell surface. In contrast, the model group had significantly shedding endothelial cells that gathered into a group. The disordered cable, loss of connections between cells that resulted in voids or

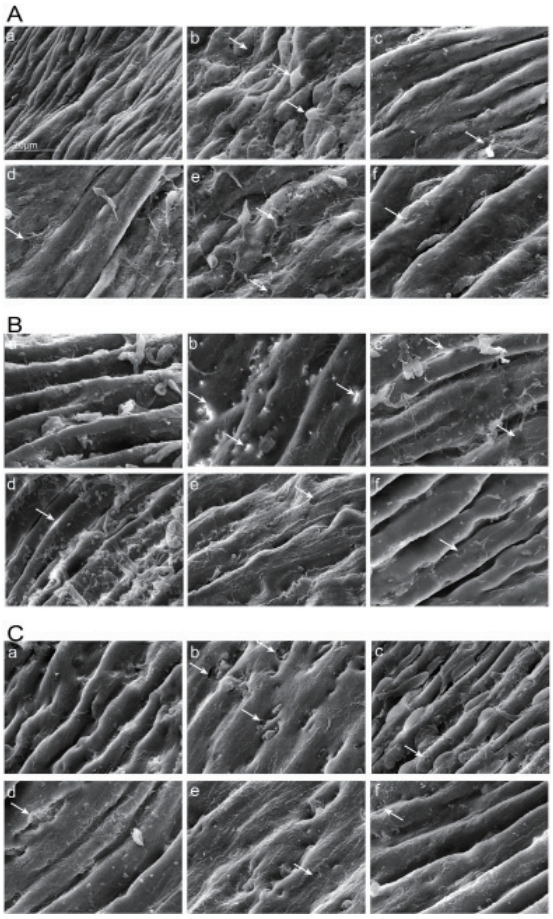

Figure 2. Endothelial morphology of thoracic aorta (A), renal artery (B) and mesenteric artery (C) were observed by scanning electron microscope after 5 weeks of drug treatment. a: normal control group; **b:** model group; **c:** valsartan group; **d:** U group; **e:** R group; **f:** U-R group. The arrows point to the endothelial damage and the change of endothelial morphology.

"honeycomb" shape endometrium was also observed by electron microscopy. Endometrial attachments were increased simultaneously. Injury of thoracic aorta was most, while mesenteric artery injury the degree lightest. After treatment, the rats endometrial integrity and shedding state of endothelial cells were significantly

improved which corresponded to the flow cytometry results. The improved effects of *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility were better than single active fraction (Figure 2).

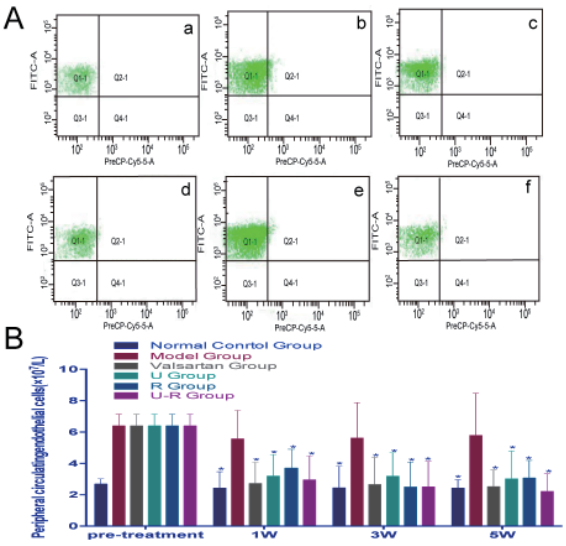

Figure 3. The number of CECs. $CD3^{-}CD31^{+}CD62P^{+}$ identifies the shedding number of circulating endothelial cells by flow cytometry. Figure 3A shows the CECs flow diagram of each group after 5 weeks of administration. Figure 3B shows the changes of CECs number in each group. Significant difference ($p < 0.05$) due to comparing with model group is denoted by asterisk (*). **a:** normal control group; **b:** model group; **c:** valsartan group; **d:** U group; **e:** R group; **f:** U-R group.

3.3. *The amount of CECs*

After treatment for 1, 3, 5 weeks, the number of CECs were detected. As shown in Figure 3, the number of CECs in model group was significantly increased compared to the control group ($p < 0.05$). While compared with the model group, the number of CECs in Valsartan group, U group, R group and U-R group have different extents of reduction in the administration of 1, 3, 5 weeks ($p < 0.05$). The number of CECs in U-R group was declined in early intervention and dropped 62% after administration for 5 weeks, which is better than U group and R group (decreased by 48% and 47% at 5 weeks end).

3.4. *The expression levels of CECs CD54 and CD62P*

Similar with the relationship of CECs number and blood pressure, the trend of mean fluorescence intensity of CD54 and CD62P was positively correlated with blood pressure. As shown in Figure 4, compared with the normal group, the mean fluorescence intensity of CD54 and CD62P in model group was significantly increased ($p < 0.05$). After treatment, with the reduction in blood pressure, the treatment group rats mean fluorescence intensity of CD54 and CD62P expressed with different degree of reduction ($p < 0.05$). We have observed CD62P expression on CECs continued to fall in U-R group (decreased by 30% at 5 week end), while the efficacy of U group and R group was not stable. Treated with drugs, CD54 expression on CECs has improved different degrees over the 5 weeks. U-R group showed

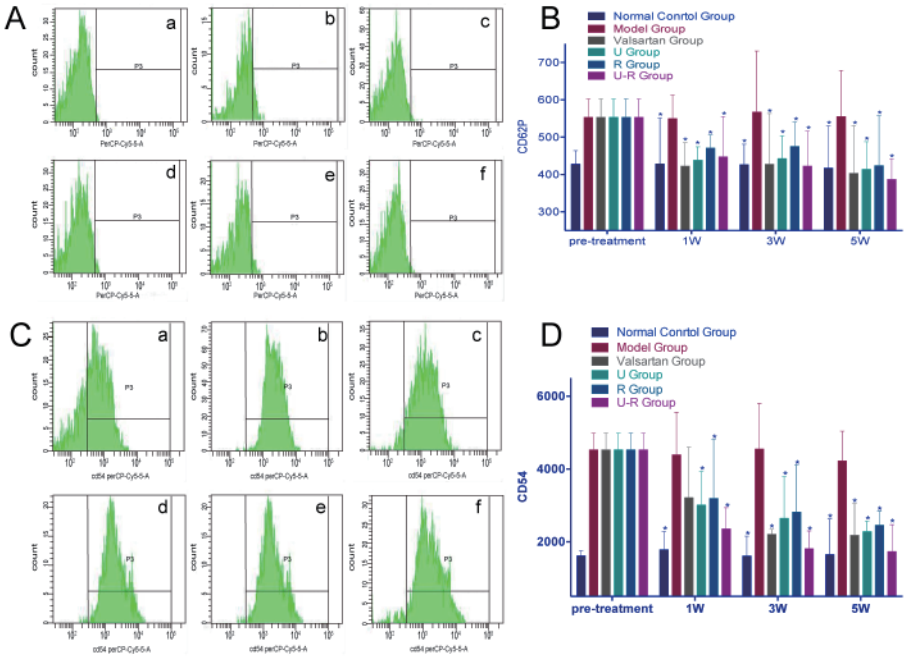

Figure 4. The expression levels of CECs CD54 and CD62P. 4A and **4C** show the CD54 and CD62P flow diagram of each group after 5 weeks of administration. **4B** and **4D** demonstrate the changes of the mean fluorescence intensity of CD54 and CD62P in each group. Significant difference ($p < 0.05$) due to comparing with model group is denoted by asterisk (*). **a:** normal control group; **b:** model group; **c:** valsartan group; **d:** U group; **e:** R group; **f:** U-R group.

the best efficacy, because of the decreased by 59% at the end 5 week.

4. Discussion

In this study, we have found that *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility has efficacy on antihypertensive function and protection on vascular endothelial cells. Specifically, we have demonstrated protection vascular endothelial in three ways. First, the compatibility can significantly improve the endometrial integrity and shedding state of endothelial cells. Second, *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility can reduce the number of CECs. Finally, the compatibility can decrease the expression of CD54 and CD62P on CECs.

The combination of *Uncaria rhynchophylla* and *Semen Raphani* contains complex chemical compositions and has the characteristics of multi-component, multi-level, multi-target and multi-metabolic pathways, resulting in difficulty to clarify the mechanism of action. In order to solve this problem, we use technology of composition compatibilities of traditional Chinese medicinals that persist syndrome differentiation and have the advantage of controllable compositions, clear targets and explicit mechanism. So, we extracted the effective components of *Uncaria rhynchophylla* and *Semen Raphani* (*Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid). *Uncaria rhynchophylla* total alkaloids contain multiple alkaloids, such as rhynchophyline, isorhychophyline and corynantheine *etc*. The rhynchophyline and isorhychophyline are the main active ingredient. The pharmacological studies indicated that the rhynchophyline and isorhychophyline showed antihypertensive activity, antiarrhythmic, inhibition of platelet aggregation and antithrombotic (*15*). The major active compounds in *Semen Raphani* are alkaloids, glucosinolates, brassinosteroids, flavonoids and so on. *Semen Raphani* soluble alkaloid demonstrated to have antihypertensive effects. Sinapine is the major bioactive alkaloid, existing in the form of sinapine thiocyanate in *Semen Raphani* (*16*). The pharmacological functions of sinapine include antihypertensive effect, antioxidative, and neuroprotective, *etc*. (*17*).

Vascular endothelial cells are the major regulator of vascular homeostasis. The endothelium is a single cell layer that lines the luminal surface of blood vessels and is involved in regulation of vascular tone and structure. They adjust the cardiovascular system by secreting a variety of active substances. In hypertensive pathological conditions due to blood flow shear stress and flow fluctuation is too strong, the structure and function of vascular endothelial cells are changed. This became one of the initial factors of endothelial cells dysfunction (*18*). On the other hand, injury of vascular endothelial cells undermined the self-regulating system balance, namely, increasing the synthesis of ET and decreasing the synthesis and release of NO, leading to vascular tension adjustment disorder and structural changes in the vascular wall, and ultimately triggering increased blood pressure (*19*).

CECs are a novel means of assessing endothelial dysfunction, meaning that mature cells are detached from the vascular intimal layer due to a great deal of insults (*20*). CECs are a stable and sensitive marker for endothelia damage, and may serve as a clinical marker in diagnosis, monitor and curative effect evaluation. Circulating levels of CECs are increased in hypertension associated with endothelial dysfunction (*21,22*). Research suggested significantly higher CECs counts among seven patients with hypertension, when compared with 22 matched, healthy control subjects (*23*). However, the precise surface antigen(s) used to identify CECs has not been established; CD31, CD54, CD106, CD141, CD146, *etc*. have all been used to identify cells of endothelial origin (*22*). These markers were not specific to vascular endothelial cells, and actually there is no such a standard yet. CD31 is a constitutive marker expressed on endothelial cells. CD62P can be used as a molecular marker of endothelial dysfunction (*24*). Referring to the most commonly used CD molecules and considering two positive markers are highly recommended, we chosed, in this experiment, CD3-PE, CD31-FITC and CD62P-PerCP to mark CECs.

In our study, the amount of CECs of model group was much higher than that of normal group. During the 5-week study, the CECs count of each treatment group kept decreasing, especially in valsartan and U-R groups. This showed that *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility could protect the vascular endothelial cells effectively and prevent vascular endothelial cells falling off.

Vascular wall inflammation reaction exists in hypertension patients, and inflammation is involved in the pathophysiological process (*25*). In the hypertensive state, vascular endothelial cells could induce secretion of a variety of inflammation-related substances, such as the immunoglobulin superfamily cell adhesion molecule-1 (ICAM-1; CD54), vascular cell adhesion molecule-1 (VCAM-1) and selectin family of P-selectin (CD62P). CD54 is the important adhesion molecule and plays an important role in both innate and adaptive immune responses. It is involved in the trans-endothelial migration of leukocytes to sites of inflammation (*26*). CD62P is also an important adhesion molecule and is stored in the α granules of platelets and the Weibel-Palade bodies of endothelial cells. CD62P mainly mediated leukocytes adhesion and activated endothelial cells, which was a direct result of adhesion between cells and matrix. Inflammation-related substances would further damage and activate endothelial cells.

The vicious cycle is created, which is prompting the development of hypertension. Therefore, improving endothelial function and inhibiting the expression of inflammation-related substances in the wall is not only the basic strategy of vascular endothelial protection, but also an important goal of antihypertensive.

In our experiment, we observed that the model group rats increased expression of CD54 and CD62P. The changes above verified elevated blood pressure could indeed increase various inflammatory substances secretion and expression. After treatment, the number of vascular endothelial cell adhesion molecule expression was significantly decreased. And *Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid compatibility exhibited the best efficiency, which suggested that these two components can protect vascular endothelial cells by reducing adhesion molecule expression, inhibiting leukocyte and endothelial cell adhesion and suppressing vascular wall inflammation.

5. Conclusion

The effective components of the combination of *Uncaria rhynchophylla* and *Semen Raphani* (*Uncaria rhynchophylla* total alkaloids and *Semen Raphani* soluble alkaloid) demonstrated good anti-hypertension effect and vascular endothelium protective effect. The preliminary mechanism may attribute to relieving the overall low-grade inflammation. The results also proved that composition compatibilities of traditional Chinese medicinals show potential in analysis of multi-target and multi-pathway mechanism of Chinese herbs.

Acknowledgements

This work was funded by Natural Science Foundation of China # 81072794.

References

1. Chrissobolis S, Faraci FM. The role of oxidative stress and NADPH oxidase in cerebrovascular disease. Trends Mol Med. 2008; 14:495-502.
2. Varpula M, Pulkki K, Karlsson S, Ruokonen E, Pettilä V; FINNSEPSIS Study Group. Predictive value of N-terminal pro-brain natriuretic peptide in severe sepsis and septic shock. Critical Care Medicine. 2007; 35:1277-1283.
3. Polovina MM, Potpara TS. Endothelial dysfunction in metabolic and vascular disorders. Postgrad Med. 2014; 126:38-53.
4. Xiong X, Yang X, Liu W, Chu F, Wang P, Wang J. Trends in the treatment of hypertension from the perspective of traditional chinese medicine. Evid Based Complement Alternat Med. 2013;2013:275279.
5. Wang J, Xiong XJ, Liu W. Traditional Chinese Medicine syndromes for essential hypertension: a literature analysis of 13,272 patients. Evid Based Complement Alternat Med. 2014;2014:418206.
6. Jiang HQ, Li YL, Xie J. Urine metabonomic study on hypertension patients of ascendant hyperactivity of gan yang syndrome by high performance liquid chromatography coupled with time of flight mass spectrometry. Zhongguo Zhong Xi Yi Jie He Za Zhi. 2012; 32:333-337. (in Chinese)
7. Lee JS, Kim J, Kim BY, Lee HS, Ahn JS, Chang YS. Inhibition of phospholipase cgamma1 and cancer cell proliferation by triterpene esters from *Uncaria rhynchophylla*. J Nat Prod. 2000; 63:753-756.
8. Shi JS, Yu JX, Chen XP, Xu RX. Pharmacological actions of Uncaria alkaloids, rhynchophylline and isorhynchophylline. Acta Pharmacol Sin. 2003; 24:97-101.
9. Zhang YH, Li YL, Zhao J, Hou Q. The Effect of Total Alkaloids in Rhynchophylla on β-galactosidase and Telomerase in Vascular Endothelial Cell in Spontaneous Hypertensive Rat. Shan Xi Zhong Yi. 2011; 27:44-50. (in Chinese)
10. Jiang YH, Li YL, Zhao J, Hou Q. Uncaria Alkaloids-Intervention on the Aged Endothelial Cell Induced by D Galactose. Zhongguo Dong Mai Ying Hua Za Zhi. 2011; 19:474-478. (in Chinese)
11. Sun JC, Qi DM, Zhou HL, Li YL. The influence of gouteng alkaloid on SHR aorta smooth muscle cell apoptosis and proliferation. Zhongguo Yao Li Xue Tong Bao. 2011; 27:925-928. (in Chinese)
12. Li BG, Li TY, Zhang HX, *et al*. Experimental Study of Hypotensive Effect of Soluble *Semen Raphani* Alkaloids in Spontaneous Hypertensive Rats. Changchun Zhong Yi Yao Da Xue Xue Bao. 2007; 23:7-8. (in Chinese)
13. Li TY, Li TG, Zhang GX, *et al*. Experimental Study of Hypotensive Effect of Soluble *Semen Raphani*. Shi Jie Zhong Xi Yi Jie He Za Zhi. 2007; 2:25-28. (in Chinese)
14. Jiang HQ, Nie L, Zhou HL, Li, YL. Optimization for compatibility of RamulusUncariae cum Uncie total alkaloids and *Semen Raphani* total alkaloids based on partial least-squares regression analysis. Zhong Cao Yao. 2013; 44:2531-2536. (in Chinese)
15. Ndagijimana A, Wang X, Pan G, Zhang F, Feng H, Olaleye O. A review on indole alkaloids isolated from *Uncaria rhynchophylla* and their pharmacological studies. Fitoterapia. 2013; 86:35-47.
16. Xing J, Yuan YX, Feng BM. Extraction and high performance liquid chromatographic determination of sinapine in five cruciferous plants. Journal Home of Analytical Science. 2012; 28:523-526.
17. Sham TT, Yuen ACY, Ng YF, Chan CO. Mok DKW, Chan SW. A review of the phytochemistry and pharmacological activities of raphani semen. Evid Based Complement Alternat Med. 2013;2013:636194.
18. Ercan E, Tengiz I, Ercan HE, Nalbantgil I. Left ventricular hypertrophy and endothelial functions in patients with essential hypertension. Coron Artery Dis. 2003 Dec;14:541-544.
19. Wallace SM, Yasmin, McEniery CM, Mäki-Petäjä KM, Booth AD, Cockcroft JR, Wilkinson IB. Isolated systolic hypertension is characterized by increased aortic stiffness and endothelial dysfunction. Hypertension. 2007; 50:228-233.
20. Karthikeyan VJ, Lip GY. Endothelial damage/dysfunction and hypertension in pregnancy. Frontiers in Front Biosci (Elite Ed). 2011; 3:1100-1108.
21. Boos CJ, Lane DA, Karpha M, Beevers DG, Haynes R,

Lip GY. Circulating endothelial cells, arterial stiffness, and cardiovascular risk stratification in hypertension. Chest. 2007; 132:1540-1547.
22. Burger D, Touyz RM. Cellular biomarkers of endothelial health: microparticles, endothelial progenitor cells, and circulating endothelial cells. J Am Soc Hypertens. 2012; 6:85-99.
23. Koc M, Bihorac A, Segal MS. Circulating endothelial cells as potential markers of the state of the endothelium in hemodialysis patients. Am J Kidney Dis. 2003; 42:704-712.
24. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. Circ Res. 2001; 89:763-771.
25. Vardas P, Marketou M. CRP in non-dippers: new perspectives and old queries. J Hum Hypertens. 2008; 22:447-449.
26. Lawson C, Wolf S. ICAM-1 signaling in endothelial cells. PPharmacol Rep. 2009; 61:22-32.

(Received June 22, 2015; Revised August 23, 2015; Accepted August 24, 2015)