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Analysis on the relationship and mechanism of high blood pressure and vascular aging on the condition that the gender and age matches

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Abstract. – OBJECTIVE: The relationship between hypertension and the mechanism of the expression of T-lymphocyte Kv1.3 channels in vascular aging has been analyzed in this study based on the gender and age matches' principle.

PATIENTS AND METHODS: Thirty patients have been consecutively chosen with vascular aging caused by hypertension (group A), while 30 cases of high blood pressure not merged with vascular aging (group B) were chosen, and 30 cases of healthy volunteers (group C), conforming to gender and age 1:1 and the closest matching principle, were studied. The aim of this study was to separate the peripheral blood mononuclear cells and give intervention of 0.2 nmol/L ANGII to CD4 + T-lymphocytes, and store them in the incubator 48 h. The difference of Kv1.3 channel current of CD4 + T-lymphocyte, mRNA, angiotensin receptor (AT1R) protein mRNA, and IFN-γ density has also been compared.

RESULTS: The membrane capacitance, peak current, and current density of group A, are higher than those of the other two groups, and the differences have statistical significance ($p<0.05$). After adding ANGII intervention to group A, the expression levels of T-lymphocyte Kv1.3 potassium channels mRNA, AT1R mRNA, and IFN-γ are significantly increased, so that the difference has statistical significance $p<0.05$, while the other two groups have no significant change ($p>0.05$). The levels of Kv1.3 potassium channels, AT1R mRNA, and IFN-γ of group A before and after the intervention are significantly higher than those of the other two groups, and the differences are statistically significant ($p<0.05$).

CONCLUSIONS: Vascular aging caused by hypertension may be linked to the increase of Kv1.3 potassium channel activity of T-lymphocyte, while ANGII can improve the high expression of Kv1.3 potassium channel and AT1R, to stimulate lymph cells to secrete IFN-γ.

Key Words
T-lymphocyte, Kv1.3 channel, High blood pressure, Vascular aging, ANGII, Angiotensin receptor.

Introduction

Kv1.3 potassium channels are the most critical potassium channels subtypes on the human T-cell membrane. With the voltage dependence, they can maintain the membrane potential and act on the T-lymphocyte activation, cytokine secretion and proliferation[1]; what is more significant is that they can effectively regulate the immune function of human body[2]. Studies[3] have shown that, through the increase of expression, the T-lymphocyte Kv1.3 channels have a close relation with autoimmunity and a variety of autoimmune and chronic inflammatory diseases; they are the targets of ease and adjust the immune function of this kind of disease. Kv1.3 channels play a major role in the pathogenesis of many diseases of heart head blood vessels such as atherosclerosis, myocarditis, and hypertension[4,5]. The study further explores the expression differences in vascular aging diseases and possible internal mechanism of T-lymphocyte Kv1.3 channels.

Patients and Methods

Patients

Thirty patients have been consecutively chosen with hypertension combined with vascular aging diagnosed in our hospital from January 2015 to January 2016 (group A). Also, 30 cases of high blood pressure not merged with vascular aging (group B) were chosen, and 30 cases of healthy volunteers (group C), conforming to gender and age 1:1 and the closest matching principle, were studied. Hypertension target-organ damages, such as hypertensive nephropathy, ocular fundus retinopathy, fundus retinopathy, hypertensive heart disease, hypertensive cerebral ischemia or cerebral hemorrhage, high blood pressure difficult to

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control, diabetes mellitus, autoimmune diseases, malignant tumors, and poor compliance were excluded from the study. The study has obtained the informed consent of the Hospital Ethics Committee and patients, as well as their family members. In the group A, there were 16 cases of male and 14 cases of female; their age was between 48 and 72, with an average age of 63.1 ± 13.5. The average systolic blood pressure was 168.7 ± 13.5 mmHg, average diastolic blood pressure 85.4 ± 6.6 mmHg, the average duration was 3.3 ± 1.0 years. In the group B, there were 15 cases of male and 15 cases of female, their age was between 45 and 70, their age was between 44 and 73, with an average age of 62.8 ± 14.7 years. The average systolic blood pressure was 166.9 ± 15.2 mmHg, the average diastolic blood pressure was 85.5 ± 6.8 mmHg, and the average duration was 3.5 ± 1.2 years. In the group C, there were 15 cases of male and 15 cases of female; their age was between 44 and 73, with an average age of 63.0-14.5.

Methods

Vascular aging is diagnosed by using ultrasonic Doppler (SonoScape, Shenzhen, China) to check the pulse wave velocity (PWV) of carotid artery pulse. Ten milliliters of peripheral venous blood were collected and stored in the anticoagulant tube.

Isolating and Culturing CD4 + T-cells

Mononuclear cells were isolated using density-gradient centrifugation method and CD4 + T-cells were separated using magnetic beads immune method. Cells counting and cell viability were performed by trypan blue staining. The amount of CD4 + T-cells was about 1-2 × 10^6/mL and cell activity was more than 98%. After separation, the CD4 + T-cells into no dosing and dosing were grouped; angiotensin II (ANGII) (Yangzijiang, Taizhou, China) in dosing group was added, with a final concentration of 2 × 10^6 mol/L. After centrifugation, the supernatant was discarded and corresponding medium was added, followed by incubation for 2 d.

Records of Kv1.3 Potassium Channel Current of CD4 + T-cells

Culture medium was removed and CD4 + T cells were resuspended using electrode fluid (KaiGen, Nanjing, China). Electrode fluid mainly contains NaCl160, CaCl22, MgCl21, Glucose 10, etc. The electric current of Kv1.3 CD4 + T-cells in each group was measured by EPC-9 amplifier (HEKA Company, Berlin, Germany).

Rt-PCR Detecting Kv1.3 Potassium Channels of T-cells and the Expression of AT1R

After incubation for 48 h, the medium containing lymphocytes was transferred to sterile EP tubes (KaiGen, Nanjing, China), followed by centrifugation (3000 r/min) for 15 min. After that, the supernatant was discarded. The lymphocytes were washed with sterile PBS, followed by centrifugation for 15 min and the supernatant was discarded. After that, 1 mL Trizol (Yi Fei Xue Bio TECh, Nanjing, China) was added and mixed with the cells. Then, 250 μl chloroform was added and mixed by oscillation for 15 s. After 5 min at room temperature, the mixture was centrifuged and obtain RNA pellet. The concentration and purity of RNA samples were tested using ultraviolet spectrophotometer. RNA was dissolved in 12 μl RNase-free H_2O, following by denaturing at 65°C for 5 min. The reverse transcription reaction was performed using the following conditions: 42°C for 25 min and 85°C for 5 min.

Statistical Analysis

Quantitative data were expressed in terms of mean ± standard deviation using SPSS19.0 software (SPSS Inc., Chicago, IL, USA) for data analysis; comparisons between groups were done using single-factor ANOVA analysis; comparisons in groups have been done using paired *t*-test; count data were expressed with cases number or (%), using χ^2-inspection. When $p<0.05$, the differences were considered to be statistically significant.

Results

The Expression and Comparison of Kv1.3 Potassium Current of T-cell

The membrane capacitance, peak current, and current density of Group A were significantly higher than those of the other groups; the differences were statistically significant ($p<0.05$) (Table I).

The Comparison Between Groups Before and After the Intervention of Kv1.3 Potassium Channels of T-lymphocyte Cells and AT1R mRNA Expression

After ANGII intervention, the expression levels of Kv1.3 potassium channels mRNA and AT1R mRNA in group A were significantly increased ($p<0.05$), while no significant changes were observed in other two groups. The expression levels of Kv1.3 potassium channels mRNA

Table I. The expression and comparison of Kv1.3 potassium current of T-cell.

Groups	Membrane capacitance (pF)	Peak current (pA)	Current density (pA/pF)
Group A	1.3 ± 0.4	312.5 ± 45.8	264.8 ± 54.8
Group B	0.6 ± 0.2	142.3 ± 32.6	213.2 ± 43.5
Group C	0.3 ± 0.1	100.5 ± 28.4	165.6 ± 47.8
F	8.627	7.524	9.633
p	< 0.001	< 0.001	< 0.001

Note: Group A, vascular aging caused by high blood pressure; Group B, high blood pressure not merged vascular aging; Group C, healthy volunteers.

and AT1R mRNA in group A were significantly higher than in other two groups before and after the intervention ($p<0.05$) (Table II).

Discussion

There have been many kinds of research about voltage dependence Kv1.3 channels' role in lymphocyte activation[6,7], the outflow of lymphocyte cell Kv1.3 channels can effectively maintain the flow dynamics in the Ca^{2+} to act on lymphocytes activation. Researches have confirmed that[8] vascular aging has close relationship with the disease development of high blood pressure and heart cerebrovascular, but there are fewer researches about the vascular aging patients with T-cell Kv1.3 channel. This study showed that T-cell membrane capacitance, peak current, and current density of patients with vascular aging are significantly elevated. After adding ANGII intervention, the expression levels of Kv1.3 potassium channels mRNA of T-lymphocyte, AT1R mRNA and IFN-γ are significantly increased, whereas the other two groups have no obvious change. Before and after the intervention, the levels of Kv1.3 potassium channels, AT1R mRNA, and IFN-γ of group A are significantly higher than those of the other two groups, the differences are statistically significant. The research of Dörffel et al[9] shows that after the ANGII intervention, single mononuclear cell in patients with vascular aging secretes more IL-1β than that of the healthy group, hinting that mononuclear cells in patient with vascular aging have pre-active function. The results have confirmed that the increased expression of Kv1.3 channel of T-lymphocyte is associated with vascular aging occurring and the inflammatory response. Vascular aging is the occurrence of reduced function of vessels, increased arterial stiffness and the increased transmission rate of pulse wave along with aging. The fact that Kv1.3 channels' role mechanism in T-cell activation, T-lymphocytes and macrophages are the immune cells in atherosclerosis, Kv1.3 can become important targets for the prevention and treatment of atherosclerosis[11], Kv1.3 can provide the limited expression for the lymphatic and nervous system, which confirms that the important role of immune mechanism in atherosclerosis[12]. Moreover, some cytokines IFN-γ protein expressions of artery atherosclerosis lesions are more obvious than the control group, in accordance with the literature reported that IL-2 and IFN-γ can act on the sclerosis of arterial congee[13]. The formation and development of T-lymphocytes integrated into atherosclerosis are mostly secreted by the Thl/Th2, for example, cytokines of IL-2 or IFN-γ to effect[14], while Kv1.3 channels open is the focus of the activation of T-lymphocytes and its factor[15], so that we can speculate in atherosclerosis, Kv1.3 channel has great influence.

Table II. Che comparison between groups before and after the intervention of Kv1.3 potassium channels of T-lymphocyte cells and AT1R mRNA expression.

Groups	Kv1.3 mRNA		AT1R mRNA		IFN-γ (pg/mL)	
	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
Group A	0.7568 ± 0.0234	0.9245 ± 0.0625	0.6254 ± 0.0326	0.8427 ± 0.0374	72.5 ± 10.3	86.4 ± 12.5
Group B	0.3256 ± 0.0125	0.3421 ± 0.0145	0.3314 ± 0.0265	0.3469 ± 0.0264	32.4 ± 12.3	36.2 ± 15.4
Group C	0.1257 ± 0.0302	0.1326 ± 0.0369	0.1027 ± 0.0524	0.1125 ± 0.0597	21.5 ± 10.5	23.9 ± 12.5
F	10.325	15.427	9.754	13.628	8.354	14.205
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Conclusions

To sum up, vascular aging caused by high blood pressure, may be related to increased Kv1.3 potassium channel activity of T-lymphocyte, while ANGII can improve the high expression of Kv1.3 potassium channel and AT1R, to stimulate lymph cells to secrete IFN-γ.

Conflict of Interests:

The Authors declare that they have no conflict of interests.

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