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Original Article

The effect of metformin combined with losartan on VEGF and Ang II in the serum of spontaneously hypertensive rats

Xia Li, Yali Di, Jingjing Qi, Zhaoxiang Wang, Liming Yang, Zheng Ji

Department of Cardiology, Tangshan Gongren Hospital, Tangshan 063000, Hebei, People's Republic of China

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Abstract: Objective: To investigate the effects of metformin combined with losartan on VEGF and Ang II in SHR serum. Methods: Thirty SHR rats were divided into three groups according to the different treatment methods. The SHR group was administered the drugs without any drug intervention. The metformin group was treated with normal saline mixed with metformin tablets. In the stomach, the combination group received losartan potassium tablets in the metformin group. The treatment time was 3 weeks, and we observed the serum expressions of VEGF and Ang II. After the rats were sacrificed, the brain tissues were taken. The VEGF and Ang II in the brain tissue were analyzed using immunohistochemistry and immunoblotting. Results: Before treatment, the blood pressure levels of the rats in the three groups were similar ($P > 0.05$). Compared with before the treatment, the blood pressure levels of the rats in the metformin group and the combined group were decreased ($P < 0.05$). There was no significant change in the blood pressure of the rats in the SHR group. After the treatment, the blood pressure level of the rats in the combined group was lower than it was in the combined group ($P < 0.05$). The expressions of serum vascular endothelial growth factor and Ang II in the three groups were similar before the treatment ($P > 0.05$). Compared with the former, the vascular endothelial growth factor and Ang II levels in the metformin and combination groups decreased ($P < 0.05$). After the treatment, the expressions of vascular endothelial growth factor and Ang II in metformin group were lower than they were in the SHR group. The expression of Ang II in the brain tissue of the metformin group was less than it was in the SHR group ($P < 0.05$), and the Ang II expression in the combined group was less than it was in the metformin group ($P < 0.05$). Conclusion: Joint use in the treatment of spontaneous hypertension has a good antihypertensive effect, and serum and brain tissue VEGF and Ang II levels are significantly decreased, improving the condition.

Keywords: SHR, VEGF, Ang II, metformin, losartan

Introduction

Hypertension is a common clinical disease. At present, more than 10% to 20% of the world population suffers from hypertension, the trend is rising, and it is also the main cause of other heart and brain diseases [1, 2]. Hypertension is a disease caused by many factors, which can be divided into two types: primary and secondary. According to epidemiological surveys, more than 90% of the patients with hypertension are primary. People think that blood vessels are only a simple effector system, but with increased research, it has been found that blood vessels themselves are a complete organ. While transporting blood, blood vessels promote a variety of local factors to exert vascular tension and other functions [3].

Metformin is a common clinical drug and a biguanide. It can reduce blood sugar in diabetes mellitus and has a high utilization rate. However, literature on the treatment of hypertension is relatively rare. Losartan is used to treat spontaneous hypertension clinically. It is an AIIA drug and has the function of inhibiting vasoconstriction [4].

Vascular endothelial growth factor (VEGF) is a specific mitotic agent, and it can be detected in a normal human body and is expressed in many organs, such as the heart, kidneys, and

brain. Given the relationship between vascular endothelial growth factor and various diseases, the relationship between vascular endothelial growth factor and hypertension has been studied continuously [5]. Angiotensin II is an important component of angiotensin. It exists both in adrenal cells and cardiac myocytes. It can bind with angiotensin receptors and produce effects. It can induce thirst in the peripheral blood vessels of patients with hypertension or promote vasoconstriction. However, there are few studies on the effects of these two drugs on the serum levels of vascular endothelial growth factor and Ang II in spontaneously hypertensive rats (SHR). In this study, we observed the serum levels of vascular endothelial growth factor and Ang II by using the above two drugs in spontaneously hypertensive rats (SHR).

Materials and methods

Experimental animals

Thirty SHR male rats, aged 5-6 weeks and weighing about 0.1-0.15 kg, were purchased from Beijing Vitonglihua Company. They were fed a sterile diet at room temperature for 2 weeks in accordance with the Regulations on the Management of Laboratory Animals.

Instruments and reagents

Losartan (Beijing Novartis Pharmaceutical), Metformin Tablets (Mosadong Company), ELISA Kit (Hualianke Biology), Formaldehyde (Taizhou Huayu Company), Goat Anti-Rabbit First Anti-Rabbit (Shanghai Hengyuan Biology).

Grouping and treatment

All the SHR rats were divided into three groups with 10 rats in each group. The SHR group was fed a normal diet without any drug treatment. The metformin group was fed a normal diet. Metformin tablets were intragastrically administered with normal saline at a daily dose of 30 g/kg. The combined group was treated with losartan diluted with normal saline at a daily dose of 30 mg/kg after 12 weeks of treatment.

Blood pressure monitoring

The tail artery blood pressure levels in the three groups of rats were measured before and after treatment.

Quantification of the expressions of serum vascular endothelial growth factor and Ang II in the three groups of rats

Before and after the drug intervention, orbital venous blood was collected from the rats and centrifuged at 2000 r/min. The levels of vascular endothelial growth factor and Ang II in the supernatant were determined using ELISA. Then, the anesthetized rats were subjected to a craniotomy to extract a small amount of brain tissue and euthanized.

Immunohistochemical determination of the vascular endothelial growth factor expression

Brain tissue from the three groups of rats were fixed with medical formaldehyde, then embedded in paraffin with a thickness of 4 microns, dehydrated with 85% alcohol, and sealed with goat serum. Goat anti-rabbit antibody was added, DAB was used for the first staining, and hematoxylin was used for the second staining. The number of positive cells of vascular endothelial growth factor in the brain tissue of the three groups of rats was observed.

Detection of the Ang II protein in the brain tissue using western blot

We extracted brain tissue, added lysate, put it into sterile test tube, and mixed it with trypsin extract at 1:100, then we froze it in a refrigerator for 10 minutes to mix the cells completely and become E solution; we the put myocardial tissue in an EP tube and added 2 ml trypsin extract at 1:100 ratio to mix the cells completely and become F solution; we shook the E and F solutions at an 80:1 volume and mixed them. The working fluid was prepared and put into a 37.5 degree incubator for 20 minutes. GAPDH antibody (1:1000) was added. Then the anti-GAPDH and anti-GAPDH antibodies were hybridized and chemiluminescent reagents were added. The images were processed using LabWorks software.

Statistical processing

The results were analyzed using SPSS22.0 software. The correlation between blood pressure changes, the expression levels of vascular endothelial growth factor and Ang II in the serum and brain tissue before and after the treatment was analyzed. The measurement data were calculated by (X + s). T was used for

Table 1. Comparison of the blood pressure levels in the three groups of rats before and after treatment

Group	n	Before treatment of blood pressure	After treatment of blood pressure
SHR group	10	215.79±5.77	216.32±6.12
Metformin group	10	216.78±5.34	145.57±5.98*,#
Combined group	10	215.8±5.69	124.53±4.94*,#,@
f		0.596	10.51
P		0.556	< 0.001

*Compared with before the treatment, P < 0.05; #Compared with the SHR group, P < 0.05; @Compared with the metformin group, P < 0.05.

the comparisons between groups, and P < 0.05 was used to indicate a statistical difference.

Results

A comparison of the blood pressure levels in the three groups of rats before and after treatment

Before the treatment, the blood pressure levels of the three groups were similar (P > 0.05). Compared with before the treatment, the blood pressure levels of the metformin group and the combined group were decreased (P < 0.05). There was no significant change in the blood pressure of the SHR group. After the treatment, the blood pressure levels in the combined group were lower than the levels in the combined group (P < 0.05). As shown in **Table 1**.

Comparison of the serum vascular endothelial growth factor and Ang II expression in the three groups of rats before and after treatment

The vascular endothelial growth factor and Ang II serum levels in the three groups were similar before treatment (P > 0.05). Compared with the levels before treatment, the levels of vascular endothelial growth factor and Ang II in the metformin group and the combination group decreased (P < 0.05). After treatment, the expressions of vascular endothelial growth factor and Ang II in the metformin group were lower than they were in the SHR group (P < 0.05), and the expressions of these indexes in the combined group were lower than they were in the metformin group (P < 0.05), as shown in **Table 2**.

Immunohistochemical quantification of the expression of vascular endothelial growth factor

The positive expression rate of vascular endothelial growth factor was lower in the metformin group than it was in the SHR group (P < 0.05), and lower in the combination group than in the metformin group (P < 0.05), as shown in **Figures 1**, **2**.

Quantification of the Ang II protein expressions in the brain tissue of the three groups of rats

The expression of Ang II in the brain tissue of the metformin group was less than it was in the SHR group (P < 0.05), and the expression in the combined group was less than it was in the metformin group (P < 0.05), as shown in **Figure 3**.

Discussion

The pathogenesis and mechanism of the spontaneously hypertensive rat model are specific to the pathogenesis of human hypertension. At present, it is an ideal experimental model for clinical research on this kind of disease. The characteristic of this kind of disease is that the cause of the disease is unclear, but the genetic probability is high. At present, the global incidence of the disease is regional. The United States has the highest incidence of the disease. Studies have confirmed that patients with essential hypertension are basically asymptomatic, but they need lifelong treatment. Although they realize that they suffer from the disease, some patients cannot effectively control it and involve other organs. At present, the clinical treatment mainly relies on past treatment experience and involves a variety of antihypertensive drugs [8, 9].

In this study, we found that the levels of vascular endothelial growth factor (VEGF) in the serum and brain tissue of SHR rats increased, but the levels of vascular endothelial growth factor (VEGF) and blood pressure (BP) decreased in the rats treated with metformin. Compared with the metformin group, the expression of vascular endothelial growth factor (VEGF) and the blood pressure in the combined group was lower. Vascular endothelial

Table 2. Comparison of the expressions of serum vascular endothelial growth factor and Ang II in the three groups of rats before and after treatment

Group	n	VEGF		Ang II	
		Before treatment	After treatment	Before treatment	After treatment
SHR group	10	147.49±25.73	149.49±23.69	41.26±4.82	42.76±4.98
Metformin group	10	147.51±25.68	125.89±12.67$^{*,\#}$	40.35±4.87	30.67±3.44$^{*,\#}$
Combined group	10	148.33±24.93	117.56±8.56$^{*,\#,@}$	41.33±4.65	19.85±2.35$^{*,\#,@}$
f		0.089	2.11	1.739	10.06
P		0.929	0.043	0.577	< 0.001

*Compared with before the treatment, P < 0.05; $^{\#}$Compared with the SHR group, P < 0.05; $^{@}$Compared with the metformin group, P < 0.05.

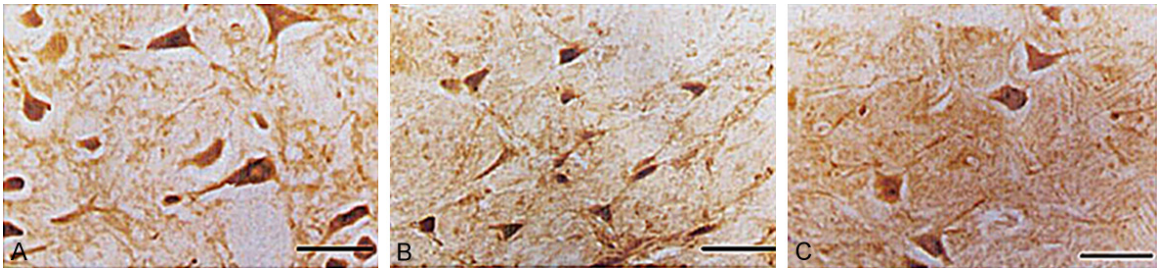

Figure 1. VEGF positive expressions in the brain tissue of three groups of rats (200 ×, (A) is the SHR group, (B) is the metformin group, (C) is the combination group).

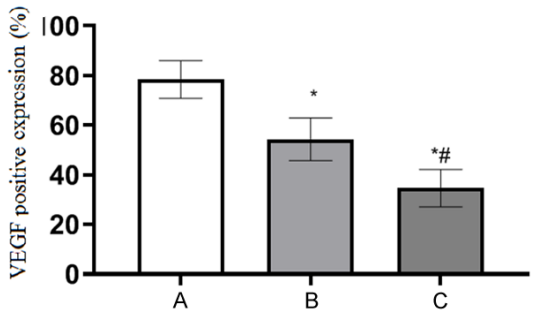

Figure 2. The positive expression rate of VEGF in the brain tissue of the three groups ((A) represents the SHR group, (B) represents the metformin group, (C) represents the combination group; * represents the SHR group, # represents the metformin group, P < 0.05).

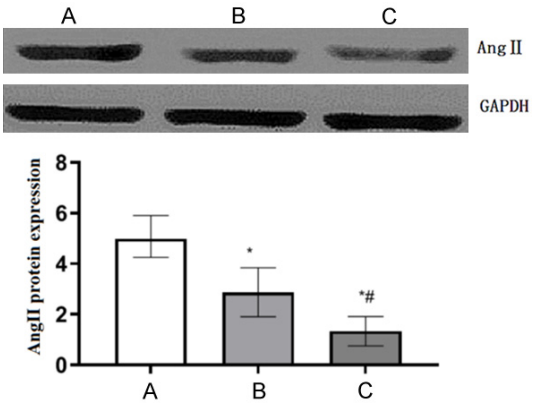

Figure 3. Expressions of the Ang II protein in the three groups ((A) represents the SHR group, (B) represents the metformin group, (C) represents the combination group; * represents the SHR group, # represents the metformin group, P < 0.05).

cell differentiation and potassium capillary regeneration can be accelerated when the expression levels of vascular endothelial cells are within the normal range in healthy people. However, a large number of studies have found that there is an important correlation between vascular endothelial growth factor (VEGF) and elevated blood pressure. When high blood pressure lasts for a short time, the changes in the levels of vascular endothelial growth factor (VEGF) in a patients' serum are small, and there is no pathological change. But when a continuous elevation of blood expression is not effectively controlled, the expression of vascular endothelial growth factor (VEGF) becomes elevated. Then contraction occurs, resulting in a lack of blood oxygen supply to the brain and leading to a variety of kidney and metabolic diseases [11, 12]. Ma Pan's [13] study confirmed that the bodies of patients with essential hyper-

tension have an increased insulin resistance, leading to a variety of metabolic diseases, such as hypertension and diabetes mellitus, but the metformin treatment of patients with this type of insulin sensitivity reduces blood pressure and reduces SHR damage to the heart and brain tissue, improving hemodynamics [14]. Losartan is a drug that can alleviate the progress of blood vessels in patients with hypertension. Zhang Jinjin's [15] study found that the oral absorption of losartan can reduce the level of vascular endothelial growth factor in SHR, reduce the biological activity of blood vessels, and maintain the stability of organs and tissues. Li Xudong's [16] study found that metformin combined with losartan can reduce SHR blood pressure and reduce the expression of vascular endothelial growth factor.

In this study, we found that the expression of Ang II in the serum and tissues of SRR rats was increased significantly, blood pressure was increased, but decreased in the metformin group, while Ang II and blood pressure in the combination group were lower. Ang II has a variety of biological reactions, and they can increase the volume of extracellular fluid, increase vascular resistance, and increase blood pressure. It is mainly divided into two subtypes, AT1 and AT2, which play different roles [17]. The Ang II receptor is distributed in many organs of the body, such as the brain and myocardium. It promotes the increase of smooth muscle by regulating AT1 to exert vasoconstriction and increase the release and absorption of adrenaline. Losartan can be combined with AT1 in SHR, and it blocks the combination of exogenous Ang II and plays a role in lowering blood pressure and cardiovascular and cerebrovascular diseases [19]. Wang Jibo's [20] study showed that metformin combined with losartan in the treatment of SHR blood pressure is more stable, Ang II is decreased, and the drug combination is widely used to treat a variety of cardiovascular and cerebrovascular diseases. To sum up: Metformin combined with losartan has a good effect in the treatment of spontaneous hypertension, and the serum and brain tissue levels of vascular endothelial growth factor and Ang II are significantly decreased, which can improve the patients' conditions. There are some deficiencies in the research process of this experiment. Due to the cost and other problems, the comprehensive physical examination of all the experimental personnel was not carried out, so the impact of other factors cannot be excluded. Also due to the lack of time and other problems, the selected experimental rats were insufficient, so the results may have some deviation. Metformin is expected to be an adjuvant drug in the treatment of malignant tumors.

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Disclosure of conflict of interest

None.

Address correspondence to: Zheng Ji, Department of Cardiology, Tangshan Gongren Hospital, No. 27 Wenhua Road, Tangshan, Hebei Province, People's Republic of China. Tel: +86-0315-2305124; E-mail: bc_45d@163.com

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