Effect of vitamin D intervention on the RAAS system activity and cardiovascular remodeling in spontaneously hypertensive rats

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ABSTRACT

Objective: To study the effect of vitamin D intervention on the renin-angiotensin-aldosterone system (RAAS) system activity and cardiovascular remodeling in spontaneously hypertensive rats. Methods: Healthy SD rats were selected as the control group, spontaneously hypertensive rats were randomly divided into hypertension group and VitD group, control group and hypertension group received intraperitoneal injection of propylene glycol, and VitD group received intraperitoneal injection of vitamin D and propylene glycol solution. 6 weeks, 12 weeks and 18 weeks after intervention, the rats were put to death, serum was collected to determine the levels of RAAS system molecules, and the myocardial tissue was collected to determine the levels of cardiovascular remodeling-related molecules. Results: 6 weeks, 12 weeks and 18 weeks after intervention, serum serum renin (PRA), angiotensin II (AngII) and aldosterone (ALD) levels as well as myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group gradually increased and were significantly higher than those of control group (P<0.05); serum PRA, AngII and ALD levels as well as myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of VitD group gradually decreased and were significantly lower than those of hypertension group (P<0.05). Conclusion: Vitamin D intervention for spontaneously hypertensive rats can reduce the RAAS system activity and improve the cardiovascular remodeling.

1. Introduction

Primary hypertension is one of the most common diseases of cardiovascular system, and it is also an independent risk factor that causes coronary heart disease, cerebral infarction, cerebral hemorrhage and other cardiovascular and cerebrovascular diseases. Renin-angiotensin-aldosterone system (RAAS) is the important mechanism in the body that regulates fluid balance and blood pressure stability. Excessive activation of RAAS system can not only lead to vasoconstriction and sodium water retention and cause high blood pressure, but can also lead to cardiac and vascular remodeling as well as affect cardiac function and reduce vascular elasticity[1,2]. At present, the relationship between excessive activation of the RAAS system and essential hypertension has been highly recognized, but the specific mechanism that regulates the RAAS system activity in patients with essential hypertension is still not clear. In recent years, the biological function of vitamin D in the cardiovascular system has received increasing attention, and vitamin D combination with nuclear receptor VDR can exert a variety of biological functions such as regulating vasomotor state, inflammation and oxidative stress reaction[3,4]. Studies have shown that vitamin D deficiency is closely associated with the occurrence of primary hypertension[5,6], but there is no report about the effect of vitamin D supplementation on the RAAS system activity and cardiovascular remodeling in patients with essential hypertension. In the following study, the effect of vitamin D intervention on the RAAS system activity and cardiovascular remodeling in spontaneously hypertensive rats was analyzed.
2. Materials and methods

2.1. Experimental materials

Healthy male SD rats were from Xinjiang Medical Animal Experiment Center, animal license No. was SCXK (Xinjiang) 2011-0004, and spontaneously hypertensive rats were bought from Beijing Yikelihao Biotechnology Co., Ltd.; Vitamin D3 preparations were purchased from Jiangsu Wuzhong Pharmaceutical Group, and rat enzyme-linked immunosorbent kits were purchased from Shanghai Enzyme-linked Detection Technology Co., LTD.

2.2. Experimental methods

2.2.1. Animal grouping and drug intervention methods

A total of 18 healthy male SD rats were selected as control group. Spontaneously hypertensive rats were randomly divided into hypertension group and VitD group, 18 in each group. VitD group received vitamin D for intervention, and the method was as follows: intraperitoneal injection of the solution of 3 μg/kg vitamin D3 preparations and 0.5 mL propylene glycol, 2 times a week, for a total of 18 weeks; control group and hypertension group received propylene glycol intervention, and the method was as follows: intraperitoneal injection of 0.5 mL propylene glycol, 2 times a week, for a total of 18 weeks.

2.2.2. Serum sample collecting and index detecting methods

6 weeks, 12 weeks and 18 weeks after intervention, 6 rats were taken from each group and executed by decapitation, then about 10 mL peripheral blood was collected immediately, let stand at room temperature for 30 min and then centrifuged in the centrifuge for 10 min at a speed of 3 000 r/min to separate the supernatant, and the enzyme-linked immunosorbent assay kits were used to determine serum renin (PRA), angiotensin II (AngII) and aldosterone (ALD) levels.

2.2.3. Myocardial tissue collecting and index detecting methods

6 weeks, 12 weeks and 18 weeks after intervention, 6 rats were taken from each group and executed by decapitation to collect peripheral blood, then they were anatomized to get the heart tissue, the left ventricular myocardial tissue was separated, washed with normal saline for five times to remove the blood, then placed in liquid nitrogen for short freezing and then added in RIPA lysis buffer for splitting, the split tissue suspension was put in the 4 ℃ centrifuge and centrifuged for 20 min at a speed of 12 000 r/min to separate supernatant, and then enzyme-linked immunosorbent assay kits were used to determine Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels.

2.3. Statistical analysis

SPSS21.0 software was used to statistically analyze the experimental data, both measurement data analysis among three groups and the analysis among different time points within group were by variance analysis and P<0.05 indicated statistical significance in differences.

3. Results

3.1. Serum RAAS system activity after vitamin D intervention

6 weeks, 12 weeks and 18 weeks after intervention, analysis of serum RAAS system molecules PRA, AngII and ALD among three groups of rats was as follows: serum PRA, AngII and ALD levels of hypertension group were significantly higher than those of control group, and serum PRA, AngII and ALD levels of VitD group were significantly lower than those of hypertension group. Differences in serum PRA, AngII and ALD levels were statistically significant between control group and VitD (hypertension) group as well as between hypertension group and VitD group 6 weeks, 12 weeks and 18 weeks after intervention (P<0.05); (2) serum PRA, AngII and ALD levels of hypertension group 12 weeks and 18 weeks after intervention were significantly higher than those 6 weeks after intervention, serum PRA, AngII and ALD levels of hypertension group 18 weeks after intervention were significantly higher than those 12 weeks after intervention, and differences in pair-wise comparison of serum PRA, AngII and ALD levels were statistically significant between different time points within hypertension group (P<0.05); (3) serum PRA, AngII and ALD levels of VitD group 12 weeks and 18 weeks after intervention were significantly lower than those 6 weeks after intervention, serum PRA, AngII and ALD levels of VitD group 18 weeks after intervention were significantly lower than those 12 weeks after intervention, and differences in pair-wise comparison of serum PRA, AngII and ALD levels were statistically significant between different time points within VitD group (P<0.05) (Table 1).

3.2. Myocardial remodeling degree after vitamin D intervention

6 weeks, 12 weeks and 18 weeks after intervention, analysis of myocardial remodeling-related molecules Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels among three groups of rats was as follows: myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group were significantly higher than those of control group, and myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of VitD group were...
significantly lower than those of hypertension. Differences in myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between control group and VitD group 6 weeks, 12 weeks and 18 weeks after intervention (P<0.05); (2) myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group 12 weeks and 18 weeks after intervention were significantly higher than those 6 weeks after intervention, myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group 18 weeks after intervention were significantly higher than those 12 weeks after intervention, and differences in pair-wise comparison of myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between different time points within hypertension group (P<0.05); (3) myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of VitD group 12 weeks and 18 weeks after intervention were significantly lower than those 6 weeks after intervention, myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of VitD group 18 weeks after intervention were significantly higher than those 12 weeks after intervention, and differences in pair-wise comparison of myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between different time points within VitD group (P<0.05) (Table 2).

4. Discussion

RAAS system is the important neurohumoral mechanism that regulates water sodium metabolism, vasomotor and blood pressure stability. After renin activates angiotensinogen into angiotensin I, ACE can split angiotensin I into active angiotensin II, which can cause vasoconstriction and lead to high blood pressure. In addition, the angiotensin II can also promote the synthesis and secretion of aldosterone, thus cause the water sodium retention, increase blood circulation and increase blood pressure[7]. Excessive activation of RAAS system is regarded as the important pathological link in the pathogenesis of primary hypertension, and the excessive secretion of PRA, AngII and ALD in the system can raise blood pressure by promoting vasoconstriction and water sodium reabsorption[8]. In the study, the analysis of serum RAAS system molecule levels in spontaneously hypertensive rats and healthy rats showed that serum PRA, AngII and ALD levels of hypertension group gradually increased and were higher than those of control group. This means that there is the pathological situation of excessive RAAS system activation in spontaneously hypertensive rats, and the excessive secretion of PRA, AngII and ALD in RAAS system is related to the generation of hypertension.

In recent years, studies about the primary hypertension have shown that vitamin D deficiency is closely related to high blood pressure. In myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between different time points within hypertension group (P<0.05); (2) myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between control group and VitD group 6 weeks, 12 weeks and 18 weeks after intervention (P<0.05); (3) myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group 12 weeks and 18 weeks after intervention were significantly higher than those 6 weeks after intervention, myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels of hypertension group 18 weeks after intervention were significantly higher than those 12 weeks after intervention, and differences in pair-wise comparison of myocardial tissue Col-I, Col-III, TGF-β1, α-actin and Myom-1 levels were statistically significant between different time points within hypertension group (P<0.05) (Table 2).

Table 1

Comparison of serum RAAS system activity among three groups of rats (n=6, x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time of intervention (weeks)</th>
<th>PRA (ng/mL)</th>
<th>AngII (pg/mL)</th>
<th>ALD (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>0.92±0.11</td>
<td>293.58±33.86</td>
<td>101.35±14.83</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>0.95±0.12</td>
<td>296.12±35.24</td>
<td>102.25±15.02</td>
</tr>
<tr>
<td>Hypertension group</td>
<td>6</td>
<td>1.62±0.20</td>
<td>375.19±42.68</td>
<td>155.62±18.64</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.98±0.25</td>
<td>425.69±51.03</td>
<td>192.21±22.54</td>
</tr>
<tr>
<td>VitD group</td>
<td>6</td>
<td>2.38±0.28</td>
<td>482.9±57.38</td>
<td>232.15±30.35</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.40±0.18</td>
<td>342.19±46.48</td>
<td>140.12±17.93</td>
</tr>
</tbody>
</table>

*: compared with control group at the same time point of intervention, P<0.05; *: compared with same group 6 weeks after intervention, P<0.05; **: compared with hypertension group at the same time point of intervention, P<0.05; ***: compared with hypertension group 12 weeks after intervention, P<0.05.

Table 2

Comparison of serum myocardial remodeling degree among three groups of rats (n=6, ng/mL, x±s).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time of intervention (weeks)</th>
<th>Col-I</th>
<th>Col-III</th>
<th>TGF-β1</th>
<th>α-actin</th>
<th>Myom-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>3.62±0.66</td>
<td>2.05±0.33</td>
<td>4.52±0.63</td>
<td>7.02±0.93</td>
<td>1.90±0.22</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>3.68±0.59</td>
<td>2.11±0.38</td>
<td>4.55±0.69</td>
<td>6.98±0.88</td>
<td>1.94±0.24</td>
</tr>
<tr>
<td>Hypertension group</td>
<td>6</td>
<td>3.54±0.61</td>
<td>2.08±0.31</td>
<td>4.49±0.52</td>
<td>7.08±1.02</td>
<td>1.89±0.21</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5.60±0.72</td>
<td>3.28±0.52</td>
<td>6.98±0.92</td>
<td>9.94±1.17</td>
<td>3.02±0.46</td>
</tr>
<tr>
<td>VitD group</td>
<td>6</td>
<td>7.61±0.82</td>
<td>4.65±0.61</td>
<td>8.41±1.02</td>
<td>12.31±1.52</td>
<td>3.78±0.51</td>
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<td></td>
<td>12</td>
<td>9.24±1.05</td>
<td>6.32±0.95</td>
<td>9.82±1.17</td>
<td>15.28±1.88</td>
<td>4.42±0.59</td>
</tr>
</tbody>
</table>

*: compared with control group at the same time point of intervention, P<0.05; *: compared with hypertension group at the same time point of intervention, P<0.05; **: compared with same group 6 weeks after intervention, P<0.05; ***: compared with hypertension group 12 weeks after intervention, P<0.05.
pressure\textsuperscript{[9,10]}. Vitamin D is a kind of fat-soluble vitamin, and it can act on nuclear receptor VDR and then adjust the expression of multiple genes and produce different biological effects\textsuperscript{[11,12]}. In patients with essential hypertension, vitamin D deficiency is associated with the activation of RAAS system, but it remains uncertain whether vitamin D supplementation can regulate the RAAS system activity in the pathological process of primary hypertension. In the study, in order to define the effect vitamin D intervention on the RAAS system activity in spontaneously hypertensive rats, the serum RAAS system molecule levels were compared after different conditions of intervention, and the results showed that serum PRA, AngII and ALD levels of VitD group gradually decreased and were lower than those of hypertension group. This means that vitamin D can reduce the synthesis and secretion of PRA, AngII and ALD in spontaneously hypertensive rats, and has significant inhibitory effect on the activity of RAAS system.

The activation of RAAS system in patients with essential hypertension is not only involved in the regulation of water sodium metabolism and vasomotor process, but is also involved in the regulation of myocardial tissue and vascular smooth muscle tissue remodeling. The collagen deposition and fibrous tissue proliferation in the extracellular matrix are the important characteristics of myocardial and vascular smooth muscle remodeling, and cardiovascular remodeling can cause myocardial and vascular smooth muscle compliance reduction, which are characterized by decreased myocardial diastolic and systolic function and poor vascular elasticity, and will aggravate the pathological condition of hypertension. TGF-\(\beta\) is the important cytokine that adjusts the collagen deposition and fibroblast proliferation, and it can increase the Col-I and Col-III sedimentation in intercellular substance and promote fibroblast proliferation\textsuperscript{[13,14]}; \(\alpha\)-actin and Myom-1 are the important cytoskeleton proteins, and they show significantly high expression trend in the process of myocardial remodeling\textsuperscript{[15]}. In the study, analysis of remodeling-related molecule levels in myocardial tissue of spontaneously hypertensive rats showed that the myocardial tissue Col-I, Col-III, TGF-\(\beta\)1, \(\alpha\)-actin and Myom-1 levels of hypertension group gradually increased and were higher than those of control group. This means that there is significant cardiovascular remodeling in the pathological process of hypertension. Further analysis of the myocardial remodeling degree in spontaneously hypertensive rats after vitamin D intervention showed that the myocardial tissue Col-I, Col-III, TGF-\(\beta\)1, \(\alpha\)-actin and Myom-1 levels of VitD group gradually decreased and were lower than those of hypertension group. This means that vitamin D could reduce the cardiovascular remodeling degree in spontaneously hypertensive rats.

To sum up, it believed that there are excessive RAAS system activation and cardiovascular remodeling in spontaneously hypertensive rats; vitamin D intervention can significantly reduce the RAAS system activity and improve the cardiovascular remodeling.

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\end{enumerate}