Effects of Xuezhikang (血脂康) and Pravastatin on Circulating Endothelial Progenitor Cells in Patients with Essential Hypertension

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ABSTRACT  Objective: To investigate the impacts of Xuezhikang (血脂康, XZK) or pravastatin combined with antihypertensive drugs on circulating endothelial progenitor cells (CEPCs) in essential hypertensive (EH) patients. Methods: Eighty-eight EH patients were enrolled into the study and randomly assigned to the antihypertensive drug treatment group (ATH group, 29 cases), the pravastatin treatment group (PRA group, 29 cases) and the Xuezhikang treatment group (XZK group, 30 cases). Patients in the 3 groups were treated with routine antihypertensive drugs. In addition, pravastatin and Xuezhikang were given to the patients in the PRA group and XZK group, respectively. After an eight-week treatment, CEPCs were counted using a laser scanning confocal microscope, and their proliferation function was evaluated by the MTT colorimetric assay and the adherent cell number was counted to estimate the adhesion function. Results: After the treatment, CEPCs in the PRA group (116.60 ± 5.70) and XZK group (114.40 ± 6.55) was significantly higher than that in the ATH group (88.00 ± 6.32, P<0.01). CEPCs proliferation capability and the adhesion function in the PRA group (0.406 ± 0.016, 33.60 ± 4.26) and XZK group (0.415 ± 0.018, 34.30 ± 3.77) were obviously superior to those in the ATH group (0.333 ± 0.021, P<0.01; 23.30 ± 3.19, P<0.01). No significant difference was found between the pravastatin group and the XZK group. Conclusions: Combined use of XZK or pravastatin with the anti-hypertensive therapy could increase the CEPCs number and improve their function in EH patients with the blood pressure controlled by antihypertensive drugs, leading to benefits independent of pressure-lowering effects.

KEY WORDS  essential hypertension, endothelial progenitor cell, pravastatin, Xuezhikang

Circulating endothelial progenitor cells (CEPCs), with the proliferation and differentiation potential derived from bone marrow, could differentiate to mature endothelial cells and play an important role in the protection and repair of vascular endothelium. Some studies have shown that the number and function of CEPCs were negatively correlated with cardiovascular disease risk factors, such as smoke, hypercholesterolemia, diabetes mellitus and so on, and they are independent predictive factors for cardiovascular events\(^1\,2\). Essential hypertension (EH) was not only the disorder of circulation and metabolism, but also an independent risk factor for cardiovascular diseases. The injured CEPCs would disrupt the protection and repair of the vascular endothelium and further damage the target organs.

There are in vitro experiments indicating that statins could increase the number of CEPCs and improve the function of CEPCs that accelerate the neovascularization and the re-endothelialization of injured vessels\(^3\,4\). In clinical studies, statins showed protective effects on vascular endothelium independent of the lipid regulating effect when applied to EH patients with hyperlipemia or coronary heart disease. However, there are not many reports on whether statins could benefit the EH patients with normal blood lipids and without target organ damage by regulating the number and function of CEPCs. This study aimed to evaluate the effects of and Xuezhikang (血脂康, XZK, a natural statin derived from Chinese medicine) and pravastatin (a chemosynthesis statin) on CEPCs in EH patients.

METHODS

Inclusion and Exclusion Standard

One hundred and two patients with EH (age...
from 45 to 75 years) were enrolled in the study from December 2006 to February 2008. The diagnosis of EH was based on the standards of the World Health Organization and the International Society of Hypertension. All patients had an EH history of more than six months and regularly took antihypertensive drugs (calcium channel blocker combining β-receptor blocker and/or diuretic as the basic antihypertensive strategy) with their blood pressures under 140/90 mmHg. The patients' body mass index (BMI), blood sugar and serum lipid level were in the normal range (BMI < 24, fasting glucose < 6.1 mmol/L, TG < 1.70 mmol/L, TC < 5.20 mmol/L, LDL < 3.40 mmol/L, HDL > 1.04 mmol/L) and there was no evidence of target organ damage. Patients with secondary hypertension, coronary heart disease, stroke, infectious disease, tumor or other serious systemic diseases were excluded. In addition, patients who had taken angiotensin converting enzyme inhibitor (ACEI) and/or angiotensin receptor blocker (ARB) within two months were excluded. No patient had previously been treated with a statin drug. Informed consent was obtained from all patients and the study protocol was approved by the local Ethics Committee of the First Affiliated Hospital of Chongqing Medical University, China.

Subjects and Grouping

One hundred and two patients were randomly assigned to the antihypertensive drug treatment group (antihypertensive drug group, ATH group), antihypertensive drugs combining pravastatin treatment group (pravastatin group, PRA group) and antihypertensive drugs combining XZK treatment group (XZK group). Among 34 patients in each group, 29 patients completed the study in the ATH and PRA groups, and 30 completed the study in the XZK group. The drop-out reasons were alteration and discontinuation of tested drugs due to uncontrolled high blood pressure or loss of contact. The 29 patients in the ATH group were 13 males and 16 females, with an average age of 61.21 ± 6.95 years old. The 29 patients in the PRA group were 16 males and 13 females, with an average age of 61.66 ± 8.41 years old. The 30 patients in the XZK group were 10 males and 20 females, with an average age of 60.17 ± 7.26. Comparison of the baseline data among the three groups showed no significant difference (P>0.05).

Treatment

All patients were given a calcium channel blocker combined with β-receptor blockers and/or diuretics as an antihypertensive therapy. Additionally, patients in the PRA group and XZK group were given pravastatin 20 mg per day and XZK 0.6 g, twice per day, respectively. The treatment course for the 3 groups was 8 weeks. Before and after the treatment, 20 mL venous blood was collected for the measurement of the CEPCs number, the proliferation capability and adherent function.

CEPCs Culture Assay

Mononuclear cells were isolated by density-gradient centrifugation with Biocoll from 20 mL of peripheral blood. Immediately after isolation, 1 × 10⁶/cm² mononuclear cells were plated on 24-well culture dishes coated with human fibronectin (Santa Cruz, USA), and then 1 mL M199 culture media containing 20% fetal bovine serum (FBS), 10 ng vascular endothelial growth factor (VEGF, PeproTech, UK), 100 U penicillin and 100 μg streptomycin were added into the culture system. Three days later, non-adherent cells were removed by a thorough washing with PBS, and adherent cells went on culture for 4 more days before cytotoxicological analysis.

Identification and Calculation of CEPCs

To detect the uptake of 1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine-labeled acetylated low-density lipoprotein (DiI-acLDL), cells were incubated with DiI-acLDL (2.4 μg/mL; Molecular Probes) at 37 °C for 4 h. Cells were then fixed with 2% paraformaldehyde for 10 min, and lectin staining was performed by incubation with fluorescein isothiocyanate (FITC)-labeled Ulex europaeus agglutinin I (lectin, 10 μg/mL; Sigma, USA) for 1 h. After staining, the samples were observed with a laser scanning confocal microscope (LSCM). Dual-stained cells positive for both FITC-lectin and DiI-acLDL were judged to be CEPCs. Two to three independent investigators evaluated the number of CEPCs per well by counting 15 randomly selected high-power fields (× 200) by an inverted fluorescent microscope.

CEPCs Proliferation Assay with

Methyl thiazolyl tetrazolium (MTT) method was conducted. Isolated CEPCs were detached using 1 mmol/L EDTA in PBS (pH 7.4), harvested by centrifugation, resuspended in 500 μL of M199, counted and placed on 96-well culture dishes. After 20 h in culture, the culture media were changed by
M199 without serum and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added in. After 4 hours, the liquid supernatant was removed and dimethyl sulfoxide was added in. The optical density value was detected under the wavelength of 490 nm. Four repeated wells were set in every experiment, which was repeated three times.

**CEPCs Adherent Function Assay**

Isolated CEPCs were detached using 1 mmol/L EDTA in PBS (pH 7.4), harvested by centrifugation, resuspended in 500 μL of M199, counted, and placed in 96-well culture dishes. After 30-min culture, adherent cells were counted by two to three independent investigators. The experiment was repeated three times.

**Statistical Analysis**

Measurement data were expressed as $x \pm s$. Continuous variables were tested for normal distribution with the Kolmogorov-Smirnov test and compared by One-way ANOVA. Categorical variables were compared using the $\chi^2$ test. In the case of non-normal distribution, nonparametric tests were used (Mann-Whitney U test or Kruskal-Wallis ANOVA on ranks). Differences in CEPC number were examined by repeated ANOVA measurements. Statistical significance was assumed if a null hypothesis could be rejected at $P<0.05$. All statistical analyses were performed with SPSS 13.0 for Windows.

**RESULTS**

**Comparison of Blood Pressure**

There was no significant difference in blood pressure before and after treatment among the three groups (Table 1).

**Comparison of Lipid Levels**

The TG level was significantly lowered by 18.18% after treatment in the XZK group. There was no significant difference in other lipid indices before and after treatment, as well as among the three groups (Table 2).

**Comparison of the CEPCs Number**

CEPCs were isolated and cultivated from peripheral blood and characterized as dual-stained cells positive for Dil-LDL and FITC-lectin (Figure 1). Before treatment, there was no significant difference in the number of CEPCs among the three groups ($P>0.05$). After treatment, the numbers of CEPCs in the XZK group and PRA group were significantly increased compared with the ATH group ($F=191.58, P<0.01$). There was no significant difference between the XZK group and PRA group ($P>0.05$, Table 3, Figure 2).

**Comparison of CEPCs Proliferation**

Before treatment, there was no significant difference in the proliferation capabilities of CEPCs among the three groups ($P>0.05$). After treatment, the proliferation capabilities of CEPCs in the XZK group and PRA group were significantly increased compared with the ATH group ($F=77.77, P<0.01$). There was no significant difference between the XZK group and PRA group ($P>0.05$, Table 3).

**Comparison of CEPCs Adhesion Function**

Before treatment, there was no significant difference in the adhesion function of CEPCs among all groups ($P>0.05$). After treatment, the adhesion function of CEPCs in the XZK group and PRA group was significantly increased compared with the ATH group ($F=191.58, P<0.01$). There was no significant difference between the XZK group and PRA group ($P>0.05$, Table 3).

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**Table 1. Comparison of Blood Pressure Level (mm Hg, $x \pm s$)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Time</th>
<th>SBP  $x \pm s$</th>
<th>DBP  $x \pm s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATH</td>
<td>29</td>
<td>Pre-treatment</td>
<td>122.97 $\pm$ 5.88</td>
<td>77.38 $\pm$ 5.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>122.62 $\pm$ 6.71</td>
<td>76.03 $\pm$ 5.55</td>
</tr>
<tr>
<td>PRA</td>
<td>29</td>
<td>Pre-treatment</td>
<td>122.62 $\pm$ 6.71</td>
<td>77.14 $\pm$ 6.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>122.90 $\pm$ 6.60</td>
<td>75.72 $\pm$ 6.14</td>
</tr>
<tr>
<td>XZK</td>
<td>30</td>
<td>Pre-treatment</td>
<td>123.73 $\pm$ 7.18</td>
<td>75.43 $\pm$ 8.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>122.76 $\pm$ 6.60</td>
<td>75.93 $\pm$ 5.40</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of Blood Lipid Levels (mmol/L, $x \pm s$)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Time</th>
<th>TC $x \pm s$</th>
<th>TG $x \pm s$</th>
<th>LDL-C $x \pm s$</th>
<th>HDL-C $x \pm s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATH</td>
<td>29</td>
<td>Pre-treatment</td>
<td>4.39 $\pm$ 0.68</td>
<td>1.19 $\pm$ 0.35</td>
<td>2.47 $\pm$ 0.56</td>
<td>1.26 $\pm$ 0.28</td>
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<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>4.41 $\pm$ 0.54</td>
<td>1.19 $\pm$ 0.44</td>
<td>2.50 $\pm$ 0.53</td>
<td>1.30 $\pm$ 0.27</td>
</tr>
<tr>
<td>PRA</td>
<td>29</td>
<td>Pre-treatment</td>
<td>4.44 $\pm$ 0.69</td>
<td>1.23 $\pm$ 0.33</td>
<td>2.53 $\pm$ 0.59</td>
<td>1.24 $\pm$ 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>4.59 $\pm$ 0.49</td>
<td>1.17 $\pm$ 0.36</td>
<td>2.56 $\pm$ 0.52</td>
<td>1.27 $\pm$ 0.25</td>
</tr>
<tr>
<td>XZK</td>
<td>30</td>
<td>Pre-treatment</td>
<td>4.46 $\pm$ 0.52</td>
<td>1.32 $\pm$ 0.32</td>
<td>2.55 $\pm$ 0.52</td>
<td>1.21 $\pm$ 0.19</td>
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<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>4.45 $\pm$ 0.58</td>
<td>1.08 $\pm$ 0.32</td>
<td>2.50 $\pm$ 0.49</td>
<td>1.22 $\pm$ 0.20</td>
</tr>
</tbody>
</table>

Note: *$P<0.01$, compared with pre-treatment in the same group
difference in the number of adherent CEPCs among the three groups ($P>0.05$). After treatment, the numbers of adherent CEPCs in the XZK group and PRA group were significantly increased compared with the ATH group ($F=127.74$, $P<0.01$). There was no significant difference between the XZK group and PRA group ($P>0.05$, Table 3, Figure 3).

**DISCUSSION**

Blood pressure control was undoubtedly the

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**Table 3. Comparison of CEPCs Number, Proliferation and Adhesion Function ($\bar{x} \pm s$)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>Time</th>
<th>CEPCs (No. $\times 200$)</th>
<th>Proliferation (OD value)</th>
<th>Adherent CEPCs (No. $\times 200$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATH</td>
<td>29</td>
<td>Pre-treatment</td>
<td>86.70 $\pm$ 5.25</td>
<td>0.333 $\pm$ 0.021</td>
<td>24.00 $\pm$ 3.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>88.00 $\pm$ 6.32</td>
<td>0.333 $\pm$ 0.021</td>
<td>23.30 $\pm$ 3.19</td>
</tr>
<tr>
<td>PRA</td>
<td>29</td>
<td>Pre-treatment</td>
<td>88.30 $\pm$ 5.53</td>
<td>0.335 $\pm$ 0.020</td>
<td>23.60 $\pm$ 3.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>116.60 $\pm$ 5.70$^{*\alpha}$</td>
<td>0.406 $\pm$ 0.016$^{\alpha}$</td>
<td>33.60 $\pm$ 4.26$^{\alpha}$</td>
</tr>
<tr>
<td>XZK</td>
<td>30</td>
<td>Pre-treatment</td>
<td>88.10 $\pm$ 5.79</td>
<td>0.336 $\pm$ 0.020</td>
<td>24.10 $\pm$ 3.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Post-treatment</td>
<td>114.40 $\pm$ 6.55$^{*\alpha}$</td>
<td>0.415 $\pm$ 0.018$^{\alpha}$</td>
<td>34.30 $\pm$ 3.77$^{\alpha}$</td>
</tr>
</tbody>
</table>

Notes: $^*P<0.01$, compared with pre-treatment in the same group; $^{\alpha}P<0.01$, compared with ATH group post-treatment
The primary objective of the treatment for patients with EH. However, as a result of circulatory and metabolic disorder conditions, the vascular endothelium damage and other adverse changes of the cardiovascular system existed for EH. Therefore, blood pressure control was not the only therapeutic target in the treatment of EH. The organism could mobilize CEPCs from the bone marrow to the peripheral blood, homing to the damaged vessel tissues, and differentiating to mature endothelial cells to complete the endothelium repair process under the hypertensive condition\(^8\). Present studies\(^9\) suggested that the damage of CEPCs in patients with EH might weaken the neovascularization and re-endothelialization potentials and was closely related with stroke, ischemic heart disease, renal arterial atherosclerosis and other vascular complications. Moreover, our previous experiment showed that injured CEPCs could not recover completely even when the blood pressure in EH patients was controlled to the target range\(^10\). Because of the important effect of CEPCs on the repair of vascular endothelium, patients with EH might get additional benefits by improving CEPCs.

Statins, including chemical synthetic statins and natural statins from traditional Chinese drugs, are considered to possess cardiovascular protective functions beyond modulating blood lipids. For instance, Rupp S, et al\(^11\) reported that statin therapy could increase CEPCs number and improve their function. Recently, more clinical guidelines have emphasized on the individual use of lipid-modulating drugs and the risk stratification of cardiovascular diseases. People with normal blood lipid levels but high cardiovascular risks were also recommended to undertake statin treatment\(^12\). However, there was no definite result whether EH patients with normal blood lipids but without target organ damage should use statin drugs. On the basis of study evidence mentioned above and the results of our study, we speculated that statins had positive effects on CEPCs in EH patients, even in those with normal blood lipids but without target organ damage.

In this study, the EH patients with normal blood lipids were treated with pravastatin or XZK besides the regular antihypertensive drugs. Related literature\(^13\) demonstrated that there was no significant difference in lowering TC and LDL between XZK 1.2 g per day and pravastatin 10 mg per day, but XZK had stronger effects than pravastatin on lowering TG (36.5% vs 10%-15%). Interestingly, neither XZK capsules nor pravastatin could lower TC and LDL, though the TG level was lower after XZK treatment than that after pravastatin treatment. Thus, the total change tendency of blood lipids in the study suggested statins could not decrease the blood lipid levels any more in EH patients with normal lipid levels. Moreover, changes of the CEPCs number, proliferation function and adhesion function without blood pressure and blood lipid significant fluctuation indicated effects of pravastatin or XZK capsules on CEPCs in our study were independent of the blood pressure and blood lipid levels.

In our study, both pravastatin and the natural Chinese statin drug (XZK) could increase the CEPCs number and improve CEPCs' proliferation and adhesion capacities. Respectively, XZK and pravastatin increased the CEPCs number by 29.9% and 32.1%, enhanced CEPCs' proliferation capacity by 23.5% and 21.2%, and advanced CEPCs' adhesion capacity by 42.3% and 42.4%. There are reports by other researchers indicating that chemical synthetic statins could increase the CEPCs number by 50% to several times in vitro and in patients with coronary heart disease in a dose-dependent manner\(^3,4\), which coincided with the results shown in our study.

The mechanisms of pravastatin on CEPCs may include: improving CEPCs mobilization and differentiation from bone marrow, promoting their migration and differentiation by PI3K/Akt signal conduction pathway, inhibiting CEPCs senescence by modulating cellular cycling related proteins, and up-regulating the integrins expression in CEPCs to enhance their adherent and migratory capacities\(^3,4\). The main components of XZK are natural compound statins, including lovastatin, several unsaturated fatty acids and amino acids\(^15\), which are extracted from red yeast Chinese rice. Our study showed that XZK and pravastatin had similar effects on CEPCs. However, further studies are required to explore the mechanism of XZK.

In summary, our study indicated that EH patients with normal blood lipids would benefit from treatment with statins besides antihypertensive drugs; it also showed that pravastatin 20 mg per day and XZK 1.2 g...
per day had the similar effects on increasing CEPCs number and improving CEPCs function in EH patients.

REFERENCES


