Effects of Losartan on the Mobilization of Endothelial Progenitor Cells and Improvement of Endothelial Function

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Abstract. Through investigating the effect of the angiotensin receptor blocker (ARB) losartan on the number of endothelial progenitor cells (EPCs) and blood flow-mediated endothelium-dependent function (FMD) in the peripheral blood of patients with coronary heart disease (CHD), we found that FMD was improved and the number of circulating EPCs increased in the ARB treatment group ($P < 0.05$). In addition, the increase in the number of EPCs was positively correlated with the improvement of FMD in the ARB treatment group ($r = 0.52$, $P < 0.01$). These findings suggest that losartan may mobilize EPCs in the peripheral blood, improving endothelial function in CHD.

Key words: Losartan, coronary artery disease (CHD), blood flow-mediated endothelium-dependent function (FMD), endothelial progenitor cell (EPCs).

Introduction

Atherosclerosis (AS) is a systemic arterial disease, with high morbidity and mortality, and it has become one of the major arterial diseases that endanger human health [1, 2]. Endothelial injury is the initiating process of AS, and a variety of risk factors that induce damage to the endothelial cells can initiate or aggravate the pathological process of AS [3]. The balance of arterial damage and repair determines the progress and extent of the AS, whereas protection of endothelial function can reduce or delay the progress of AS and improve prognosis [4]. Celermajer first used high-resolution ultrasound to detect peripheral artery flow-mediated FMD and found that there was a good correlation between FMD and coronary AS. FMD therefore became an important indicator of endothelial function [5].

Endothelial progenitor cells (EPC) are present in the bone marrow, peripheral blood, umbilical cord blood and fetal blood. These cells can proliferate and differentiate into vascular endothelial cells and also secrete a variety of growth factors in promoting neovascularization, promoting angiogenesis in vivo and repairing the damaged blood vessels [6-8]. Reduction in the number and activity of EPCs may be associated with vascular injury in CHD [9]. According to the EPCs-mediated injury and repair hypothesis, intervention or cell transplantation to increase the number and modified function of EPCs could promote angiogenesis and repair of damage. Recent studies have shown that EPCs can quickly repair the damaged endothelium to prevent the development of AS [10, 11].

Losartan belongs to the angiotensin receptor blocker (ARB) class of drugs, inhibiting angiotensin II binding to the receptors and preventing vasoconstriction. It has been reported that the ARB class of drugs modulates EPCs, thereby potentially improving endothelial function, independent of decreased blood pressure [12, 13]. Moreover, in the hypertensive rat model, losartan was discovered to improve the function of EPCs [14].

In this study we explore the effects of losartan on mobilization of EPCs and improvement of endothelial function, providing an experimental and theoretical basis for treatment of CHD by the ARB class of drugs.
Materials and Methods

Clinical data. Sixty-five cases were collected from patients treated in the cardiovascular department of Jinan Military General Hospital from May to October of 2011, including 33 males and 32 females. They were patients with coronary heart disease (CHD) confirmed by coronary angiography, with an average age of 56.8 ± 11.7 years old. All patients chosen for this research are excluded from the following cases: patients with acute myocardial infarction, inflammation, acute bleeding or history of blood transfusion within three months; patients with stroke or history of transient ischemic attack within three months; patients with low blood pressure (≤ 110/70 mmHg); patients with heart failure whose NYHA heart function was classified as IV class; patients with evidence of serious liver or kidney disease; patients who had ever taken the angiotensin converting enzyme inhibitor (ACEI) / ARB class of drugs and patients with contraindications of ACEI / ARB class of drugs. The 65 patients were randomly divided into a standard treatment group containing 33 cases and an ARB treatment group containing the remaining 32 cases. Another group of 31 healthy volunteers without CHD confirmed by coronary angiography constituted the control group. No statistical significance was observed in the gender and age between these groups. All subjects gave informed consent before participating. The study was approved by the local research ethics committee.

EPCs examination. Two milliliters of venous blood were acquired from fasting subjects, placed into vacuum tubes containing EDTA anticoagulant plasma, and examined within 4 h. The samples were labeled with immunofluorescence agents (5 μL Alexa Fluor 647 conjugated anti-human CD309 antibody, 10 μL FITC conjugated anti-human CD34 antibody) for 30 minutes in the dark at room temperature. Then, 2 ml of 1 × hemolytic (BD FACC lysing solution, (Becton Dickinson, Cat No. 349202) were added and incubated for 5-10 minutes at room temperature, protected from light, to fully dissolve the red blood cells. The samples were then centrifuged twice, and the cell suspension containing EPCs was obtained. The samples were subsequently analyzed by flow cytometry, and the proportion of CD34+ / VEGFR-2+ EPCs in whole blood was determined by flow cytometry analysis software.

FMD examination. Brachial artery FMD was determined by the percentage change of blood vessel diameter, according to the principle of Celermajer et al. [5] and methods of Bae et al. [15]. Briefly, the brachial artery in the range of 2 to 15 cm above the elbow stripes was detected and along its longitudinal axis, measurements were performed on the sites where the intima of the anterior and posterior wall was most clearly defined. Three consecutive measurements of brachial artery inside diameter (D0) were conducted within 1 cm, and the mean value was determined. The pneumatic tourniquet was placed upstream of the forearm, inflated to 280 mm Hg and maintained for 5 minutes, followed by a rapid decompression and measurement of the brachial artery inside diameter (D1) of reactive hyperemia in the 60 seconds after decompression were conducted. During the determination process, the probe was always placed in a fixed position, and each measurement was taken in the same location by an experienced ultrasonic technologist without knowledge of these tests. FMD was determined by the following formula: FMD = (D1-D0) / D0 × 100%.

Table 1. Comparison of the number of EPCs in CHD groups before and after treatment with standard therapy or ARB drug (%, X ± S).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>EPCs of baseline (X ± S)</th>
<th>EPCs after treatment (X ± S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard treatment</td>
<td>32</td>
<td>0.040 ± 0.012</td>
<td>0.065 ± 0.014*</td>
</tr>
<tr>
<td>ARB treatment</td>
<td>33</td>
<td>0.040 ± 0.015</td>
<td>0.077 ± 0.021*</td>
</tr>
</tbody>
</table>

Note: Compared with this group before treatment, * P < 0.05; Compared with the standard treatment group, ΔP < 0.05.

Intervention and follow-up. The standard treatment group was treated with conventional drugs, while the ARB treatment group was treated with conventional drugs plus losartan. Conventional oral medications were: aspirin 100 mg, 1 time / day; clopidogrel 75 mg, 1 time / day; isosorbide mononitrate 20 mg, 2 times / day; hydrochloric acid trimetazidine 20 mg, 3 times / day; simvastatin 20 mg 1 time / night. The ARB class of drugs was: losartan (oral) 50 mg once per day. After eight
weeks of treatment, the EPCs and the FMD were examined in the standard and ARB treatment groups. Losartan was purchased from Hangzhou MSD Pharmaceutical Co., Ltd.

**Statistical analysis.** All data were analyzed using the SPSS17.0 statistics software packet, and results were shown as mean ± standard deviation ($\overline{x} \pm s$). Continuous variable differences among the groups were assessed by analysis of variance (ANOVA) while frequency variable differences among the groups were determined by the Nemenyi test. Correlation analysis of the percentage of EPCs and FMD was performed with Spearman. $P \leq 0.05$ was considered statistically significant.

**Results**

**Examination of the number of circulating EPCs.** At baseline level, the number of circulating EPCs in patients with CHD group was (0.040 ± 0.017)%, which was significantly lower than the healthy control group (0.072 ± 0.018)% ($P < 0.05$). After 8 weeks treatment, the number of circulating EPCs in both CHD groups increased in varying degrees compared to those of pre-treatment, and the number of EPCs in peripheral blood of ARB treatment group increased more significantly than the standard treatment group ($P < 0.05$) (Table 1).

**Detection of FMD.** At baseline level, FMD in patients with coronary heart disease group was (5.63 ± 1.92)%, which was significantly lower than the healthy control group (8.68 ± 2.25)% ($P < 0.05$). After eight weeks of treatment, FMD in both CHD groups was increased compared to that of pre-treatment. However, FMD in the ARB group exhibited a more significant increase than the standard treatment group ($P < 0.05$) (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>FMD of baseline</th>
<th>FMD after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard treatment</td>
<td>32</td>
<td>5.74 ± 1.69</td>
<td>7.32 ± 1.47*</td>
</tr>
<tr>
<td>ARB treatment</td>
<td>33</td>
<td>5.53 ± 2.15</td>
<td>9.74 ± 1.46*</td>
</tr>
</tbody>
</table>

Note: Compared with this group before treatment, *$P < 0.05$; Compared with the standard treatment group, $\Delta P < 0.05$.

**The correlation analysis of FMD and EPCs.** At baseline level, FMD in patients with coronary heart disease group has a positive correlation with the number of circulating EPCs ($r = 0.57, P < 0.01$) (Figure 1). After eight weeks of treatment, the increase in FMD and the increased number of circulating EPCs in the peripheral blood of both CHD groups were positively correlated ($r = 0.52, P < 0.01$) (Figure 2).

**Discussion**

Studies have shown that endothelial dysfunction is a key step in the occurrence and development of atherosclerosis and coronary heart disease. EPCs are precursor cells that can directly differentiate into mature endothelial cells. It has recently been found that EPCs are involved in the process of angiogenesis after birth and reendothelialization, playing a critical role in the maintenance of normal vascular function. Decline in the number of EPCs may be responsible for a variety of cardiovascular complications [16]. The clinical research of Hill et al. [17] showed a significantly positive correlation between endothelial function and the number of EPCs in peripheral blood, and they pointed out that the level of circulating EPCs could more efficiently predict the function of endothelial cells than Framingham risk factors. At the same time, studies have shown that EPC cell concentrations in patients with CHD had a negative correlation with myocardial infarction, the rate of hospitalization, revascularization and cardiac death, and are independent of other risk factors for CHD [18]. Thus, the decline in the number or the activity of EPCs has been recognized as a novel biological marker for
Losartan improves endothelial function and cardiovascular risk factors and endothelial dysfunction [19, 20]. These studies describe the important role of EPCs in maintaining endothelial function and integrity.

This study focused on the changes in the level of EPCs in the peripheral blood of patients with CHD, exploring a new target for drug therapy to improve endothelial function and anti-atherosclerosis therapy. In this study, through comparison of coronary heart disease patients and the healthy control group at baseline level, the endothelial function index FMD was found to be significantly lower in patients than in the healthy control group. We also found that FMD had a significantly positive correlation with the number of EPCs, since FMD was improved along with the increase in the number of EPCs. The number of EPCs in peripheral blood was found to be significantly lower in CHD patients than in the healthy control group, indicating that the capacity of EPCs-mediated repair of vascular endothelia after injury was impaired in CHD, potentially contributing to the development of coronary atherosclerosis.

Previous studies have demonstrated that the improvement of endothelial function was not only a result of the reduction in blood pressure, but also from the modified function of EPCs by inhibition of angiotensin II-mediated oxidative stress, inflammation, and insulin resistance [21]. Losartan, as an angiotensin II receptor antagonist, belongs to the class of antihypertensive drugs, and it has been found in animal studies that losartan can improve the function and the number of EPCs in rat peripheral blood [14]. In the present study, through the clinical observation of long-term treatment with losartan, the levels of EPCs in the peripheral blood of patients with coronary artery disease and endothelial function were significantly improved, and the increase of EPCs was positively correlated with the improvement of endothelial function.

In conclusion we speculate that the clinical application of the ARB class of drugs, such as losartan, can also mobilize EPCs, thereby improving endothelial function and providing a new theoretical basis for the ARB class of drugs in the treatment of CHD.

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References