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Captopril inhibits maturation of dendritic cells and maintains their tolerogenic property in atherosclerotic rats

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ABSTRACT

Atherosclerosis (AS) is a systemic disease of the immune system featuring hyperactive dendritic cells (DCs) in atherosclerotic plaques and organs. Captopril, a representative medicine of angiotensin-converting enzyme inhibitors, has been demonstrated to be effective in treating AS. However, captopril's anti-atherosclerotic mechanism is still poorly understood. Therefore, this study was primarily performed to investigate the effects of captopril on the function of DCs in vivo. AS in rats was induced by feeding them with atherogenic diets, and it was evaluated by the levels of plasma lipids and aortic cholesterol. DCs' activity was appraised by endocytic activity, mixed lymphocyte reactions and cytokine secretion. The markers of DCs (CD103, CD80, CD86 and MHC-II) and Treg ($CD4^+$, $CD25^+$ and $Foxp3^+$) were assayed by western blotting analysis and flow cytometry. Cytokine level was measured by an enzyme-linked immunosorbent assay. The results showed that captopril treatment (10, 20 mg/kg/d) obviously improved dyslipidemia and reduced the levels of aortic cholesterol. Captopril significantly reduced CD103, CD80, CD86 and MHC-II protein expression while increasing that of Foxp3 in aortic tissue. Further study indicated oral administration of captopril up-regulated endocytic activity and reduced the immunostimulatory function of splenic DCs. Captopril treatment also promoted IL-10 & TGF-β production while decreasing that of IL-6 & IL-12 in splenic DCs. Finally, the results of flow cytometry indicated that captopril obviously inhibited DC maturation and promoted Treg polarization. Captopril treatment was able to inhibit DC maturation and maintain their tolerogenic property, which is closely associated with DC anti-atherosclerosis activity.

1. Introduction

Atherosclerosis (AS)—the underlying cause of myocardial infarction, stroke and thrombus—is a chronic vascular disease that occurs within the artery wall [1]. Traditional viewpoints have proposed that the pathogenesis of AS is composed of a disordered lipid metabolism and cholesterol precipitation in the walls. However, increasing evidence indicates that inflammatory response could be another vital factor contributing to the formation and progression of AS. Indeed, inflammatory diseases [2–4], such as rheumatoid arthritis, systemic lupus erythematosus and systemic sclerosis, are usually associated with AS. Conversely, AS patients also present significantly high levels of inflammatory mediators. It has been previously confirmed that hyperactivity and expanded inflammatory cells, such as macrophages, DCs and T lymphocytes, were presented in atherosclerotic plaques [5–7]. Meanwhile, these cells were also found to be dysfunctional in spleens, peripheral blood and peripheral lymph nodes [8,9]. Therefore, inflammatory status and immunological dissonance are important factors for AS progression.

It is well-known that DCs are members of antigen presenting cells (APC) and specialize in capturing, processing and presenting antigens to T lymphocytes, which induce and modulate the immune response [10]. DCs are divided into mature and immature phenotypes. Mature DCs stimulate T cell proliferation, activation and differentiation into effector T cells (Th1 and Th17) and thereby amplify local inflammatory response. Immature types exhibit tolerogenic properties through induction of regulatory T cells (Tregs) and suppression of naïve T cell activation [11,12]. Recently, several studies have demonstrated that DCs are directly involved in the process of cholesterol homeostasis and formation of atherosclerotic plaque. Mature DCs [12,13] have been shown to increase in number in advanced plaques and are arrayed in clusters with T cells that are primarily located at the exposed shoulder and rupture-prone regions of plaques. However, increased immature types [14] located in the plaques are also closely linked with the reduction of atherosclerotic lesions. Consequently, it has been suggested that overactive DCs promote plaque formation.

Captopril, as a representative medication of an angiotensin converting enzyme inhibitor [15], is widely used in treating angiocardiopathy, including hypertension and congestive heart failure. Recently, captopril has been confirmed to be effective in treating AS, as well by attenuating aortic lipid lesions and reducing the thickness of the carotid artery

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intima-media [16]. In addition, the combination of an antilipemic agent with captopril has presented dramatically synergistic clinical effects [17]. However, captopril's anti-atherosclerotic mechanism remains poorly understood. It has previously been demonstrated that the inflammatory response (such as arthritis, uveitis and encephalomyelitis) in murine models could be improved after treatment with captopril [18–20]. Other published papers further noted that captopril caused significant reduction in inflammatory cytokines production in active human DCs, while no direct effect was presented on antigen-specific T cell responsiveness in *vitro* [21,22]. Therefore, this study was primarily performed to investigate the effects of captopril on the functions of DCs in murine models of AS.

2. Materials and methods

2.1. Animals

The current study was approved by the Ethical Committee on Animal Research of Anhui Provincial Hospital. Sprague Dawley (SD) rats (male, 160 ± 20 g, Grade II, Certificate No. 2012-0012) were purchased from the Animal Department of Anhui Medical University (Hefei, Anhui Province, China). All animals were housed under standard laboratory conditions at a temperature of 25 ± 1 °C. The water and commercially available food was provided ad libitum. The lighting in the feeding room lasted from 7:30 am to 7:30 pm.

2.2. Drug and reagents

Captopril (purity beyond 98%) and simvastatin (purity beyond 97%) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The measurement kits for serum total cholesterol (TC), total triglyceride (TG), low density lipoprotein (LDL) and high density lipoprotein (HDL) were from Zhongsheng Beikong Biotech Company (Beijing, China). The primary antibodies of CD103, the major histocompatibility complex-II (MHC-II) and Foxp3 were from Abcam plc (Cambridge, UK), while those of CD80 and CD86 were from Santa Cruz Biotechnology, Inc. (CA, USA). Lipopolysaccharide (LPS) was from Sigma Chemical Co. (St. Louis, MO, USA). Fluorescein isothiocyanate (FITC)-labeled monoclonal antibody to CD80, CD86 and APC-conjugated anti-MHC-II antibody were from Biolegend (San Diego, CA, USA). FITC-labelled-CD4, PE-CD25 and PEcy5-Foxp3^{+} were from eBioscience, Inc. (San Diego, CA, USA).

2.3. Induction of AS model

AS in rats was induced by feeding them atherogenic diets according to a published method [23]. In brief, rats were intraperitoneally injected with a single dose of 600,000 IU/kg vitamin D_3 and fed with a diet containing 3% cholesterol, 0.5% sodium cholate, 0.2% propyl-thyracil, 5% refined sugar, 10% lard, and 81.3% base feed (15 g/d) with free access to drinking water. The control rats received an intraperitoneal injection with isovolumic saline and were maintained on normal rat chow and drinking water ad libitum. Eight weeks later, several rats were killed, and their aortic lipids were assayed. Subsequently, the remaining rats were provided with atherogenic diets and were divided into five groups as follows: the atherosclerotic model, captopril at different dosages (5, 10, and 20 mg/kg/d), and simvastatin (4 mg/kg/d). AS rats were orally treated with captopril and simvastatin from days 56 to 84, respectively; meanwhile, those rats of the normal and model groups were administered isovolumic saline in the same way.

2.4. Determination of plasma lipid

Rats were anesthetized at eight and twelve weeks. Blood was collected from the angular vein, stored at room temperature for 30 min, and then centrifuged at 3000 rpm for 15 min to separate the serum. Serum TC, TG, HDL and LDL were measured enzymatically using an autoanalyzer (Hitachi High-Technologies, Tokyo, Japan).

2.5. Determination of aortic lipid [24]

Aortic tissues (0.05 g) were removed, and then minced, grinded and extracted twice with chloroform/methanol (2:1, *v*/*v*) at 4 °C (first for 4 h and then for 16 h). Subsequently, lipid extracts were removed by filtration, and then the filtrate was mixed with 3 ml of 0.05% vitriol to the separated organic phase (final volume was 10 ml). Shortly thereafter, 200 μl of the organic phase was removed and evaporated to dryness. Finally, residue in the tube was resolved by 200 μl ethyl alcohol, and the cholesterol contents were assayed according to the manufacturer's instructions (Roche Diagnostics Corp, Indianapolis, IN, USA).

2.6. Western blotting analysis

Thoracic and abdominal aorta was lysed in lysate followed by homogenization at 4 °C for 1 h. Subsequently, the homogenate was centrifuged (3000 rpm, 4 °C) for 20 min. Finally, the homogenate was diluted with 5 mg protein/ml and kept frozen at −80 °C before use. The expression of CD103, CD80, CD86, MHC-II and Foxp3 was examined using a routing method [25]. The total protein concentrations in homogenates were determined by the BCA Protein Assay Kit (Thermo Scientific, Waltham, MA, USA). Identical amounts of protein extracts were separated with 10% SDS-polyacrylamide gels and immobilized on polyvinylidene difluoride (PVDF) membranes. A SDS–PAGE protein standard (Institute of Shanghai Biochemistry, Shanghai, China) and a molecular weight marker were used to check transfer efficiency. PVDF membranes were incubated with blocking buffer (0.05% Tween 20-PBS with 5% nonfat milk) for 2 h. Primary antibodies to CD103, CD80, CD86, MHC-II and Foxp3 were used at a final dilution of 1:1000 overnight at 4 °C, respectively. After incubation, membranes were washed and further incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000) at 37 °C for 1 h. Following another three washes, immunodetection was performed using an enhanced chemiluminescence reagent according to the manufacturer's instructions (Pierce Biotechnology, Inc.). Equivalent protein loading and transfer efficiency were verified by staining for β-actin.

2.7. Detection of endocytic activity [26,27]

The splenic DCs were prepared according to a previously published method. Briefly, the spleen was minced in sterile conditions. Then, the cells were suspended in RPMI (containing 0.04% EDTA) and mixed with Optiprep™ (3:1 *v*/*v*) to form a solution. Subsequently, the mixtures were centrifuged for 15 min (2000 rpm, 4 °C). The fraction at the interface of the top two layers was collected. Finally, splenic DCs were positively selected by anti-rat OX62 microbeads (Miltenyi Biotec, Germany) using an AutoMACS separator (Miltenyi Biotec) according to the manufacturer's instructions. The endocytic activity of the DCs was assayed by the extent of FITC dextran uptake. Briefly, DCs were cultured for 7 days and were counted; next, the density was adjusted to 1×10^8 cells/L with RPMI-1640 (Sigma-Aldrich, St Louis, MO) medium containing 10% fetal calf serum (FCS). Subsequently, 5×10^4 cells were mixed with 0.5 g/L FITC-dextran solution followed by incubation at 4 °C and 37 °C for 2 h, respectively. After twice washing in cold PBS, cells were resuspended with 1% paraformaldehyde and detected by flow cytometry. The mean fluorescence intensity (MFI) represented the cells' uptake ability. In each group, cells were consistently incubated at 4 °C as a control, and the fluorescence density was subtracted to avoid the non-active uptake.