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Preservation of vascular DDAH activity contributes to the protection of captopril against endothelial dysfunction in hyperlipidemic rabbits☆

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ABSTRACT

Endothelial dysfunction plays a pivotal role in the pathogenesis of atherosclerosis. Endogenous inhibitor of nitric oxide synthase (NOS) asymmetric dimethylarginine (ADMA) has been recognized as an independent risk factor of endothelial dysfunction and the biomarker of atherosclerosis. This study was to investigate whether endogenous ADMA and its metabolic enzyme dimethylarginine dimethylaminohydrolase (DDAH) were involved in mechanisms of captopril protection against endothelial dysfunction in high fat diet feeding rabbits. Half of model rabbits were treated with captopril (10 mg/kg/d, i.g.) for 12w. Vascular morphology and serum lipid profiles were detected. Serum ADMA concentration were assayed by high performance liquid chromatography. Recombinant DDAH2 gene adenoviruses were *ex vivo* transferred to thoracic aortas of high fat diet feeding rabbits. Endothelium-dependent relaxation of aortas response to acetylcholine and DDAH activity were measured. Atherosclerosis was confirmed in high fat diet feeding rabbits by increased serum lipid profiles and morphologic changes of vascular wall. Serum ADMA levels were significantly increased in hyperlipidemic rabbits accompanied with impairment of endothelium-dependent relaxation and inhibition of DDAH activity in thoracic aortas. Captopril treatment not only decreased vascular intima thickening and serum ADMA concentration but also preserved vascular DDAH activity and endothelium-dependent relaxation in hyperlipidemic rabbits without influence on serum lipid profiles. Similar beneficial effects on endothelial function and DDAH activity could be achieved by DDAH2 gene transfection. These results indicated that captopril could protect against injuries of vascular morphology and endothelial function in hyperlipidemic rabbits, the mechanisms may be related to the preservation of DDAH activity and decrease of ADMA accumulation in vascular endothelium.

1. Introduction

Atherosclerosis is one of the main causes of cardiovascular diseases. Endothelial dysfunction has been recognized as the early symbol of atherosclerosis and plays a pivotal role in the development of cardiovascular diseases (Jr Gimbrone and Garcia-Cardena, 2016). The characteristic of endothelial dysfunction is the impairment of endothelium-dependent relaxation mediated by nitric oxide (NO). Endothelial-derived NO is synthesized from its precursor L-arginine catalyzed by the endothelial nitric oxide synthase (eNOS) in endothelial cells. In addition to mediating the endothelium-dependent relaxation and regulating vascular tension, endothelial-derived NO (eNO) also inhibit the key steps of atherosclerosis formation such as leukocyte adhesion, platelet aggregation and vascular smooth muscle proliferation (Cooke, 2004). Therefore, eNO is considered as a potent anti-atherosclerotic molecule (Kawashima and Yokoyama, 2004; Cooke and Tsao, 1997).

Asymmetric dimethylarginine (ADMA), a homologue of L-arginine, has been identified as the endogenous NOS inhibitor, which compete with L-arginine for NOS, leading to the reduction of NO generation in endothelial cells (Vallance et al., 1992). It is well known that endogenous ADMA is derived from the degradation of proteins containing methylated arginine residues, and its major metabolic pathway is to be hydrolyzed by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Lin et al., 2002; Cooke, 2010). Accordingly, DDAH is the critical regulator of endogenous ADMA concentration. Elevated endogenous ADMA concentration has been widely found in

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many cardiovascular diseases which are closely associated with endothelial dysfunction. It has been recognized that ADMA is a novel independent risk factor of endothelial dysfunction and has become a biomarker of atherosclerosis (Miyazaki et al., 1999; Boger et al., 1998). In generally, elevated ADMA not only results in endothelial dysfunction, but also results from the suppression of both DDAH activity and protein expression. But in some situations, endothelial dysfunction is only associated with the inhibition of vascular DDAH activity not simultaneously with the suppression of vascular DDAH expression (Lin et al., 2002). It is, hence, of importance to find some medications that can preserve vascular DDAH activity for the prevention of endothelial dysfunction in atherosclerotic cardiovascular diseases.

Angiotensin-converting enzyme inhibitor (ACEI) is the most important kind of medicines for the treatment of cardiovascular diseases in clinical. They have been proved effective in lowering blood pressure of patients with hypertension, inhibiting the progression of atherosclerosis and decreasing mortality of congestive heart failure by blocking the activation of renin-angiotensin system. Sulfhydryl-containing ACEI such as captopril has shown to possess antioxidative or free radical-scavenging properties (Chopra et al., 1992). Furthermore, ACEI has also been demonstrated to improve endothelial dysfunction and reduce endogenous ADMA in cardiovascular diseases (Kawata et al., 2009). However, the mechanism by which catopril protects endothelial function is not completely understood. Therefore, the present study was to determine whether the improvement of endothelial dysfunction by captopril is related to the preservation of DDAH activity, and to further compare the effect of captopril with that of *ex vivo* DDAH2 gene transfection on endothelial dysfunction of high fat diet feeding rabbits, so as to elucidate the underlying mechanisms of captopril protection against endothelial dysfunction.

2. Materials and methods

2.1. Reagents

Phenylephrine, acetylcholine, ADMA, antipyrine and diacetylmonoxime were purchased from Sigma Company (St Louis, MO, USA). Captopril was given by Hunan Xiangya Pharmaceutical Co. Ltd. The commercial kits of total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL-C) were purchased from Zhejiang Dongou bioengineering Co. Ltd (Wenzhou, Zhejiang, P.R.China). The recombinant adenovirus encoding human DDAH2 gene driven by a cytomegalovirus (CMV) promoter were constructed previously by authors' laboratory (Feng et al., 2010), and the adenovirus encoding β-galactosidase gene (Ad5CMVβ-gal) were the products of Biosciences Clontech (Mountain View, CA, USA). The human embryo kidney cell line (HEK 293) was the production of American Type Culture Collection (ATCC, Manassas, USA).

2.2. Experimental animals

All procedures of animal experiments were approved by the Animal Care and Use Committee of Central South University and Guangzhou Medical University. Adult male rabbits (1.5 ± 0.2 kg) were provided by the Animal Services of Central South University (Changsha, P. R. China). After one week of adaptive period, rabbits were randomly divided into control group, hyperlipidemic group and captopril treated hyperlipidemic group; Control rabbits received a standard rabbit chow, hyperlipidemic rabbits received a high fat diet consisting of 1% (w/w) cholesterol, 10% lard (w/w) and 10% yolk (w/w), and captopril treated hyperlipidemic rabbits were given captopril (10 mg/kg/d) by gavage with high fat diet. All animals were caged individually with food and water *ad libitum* for 12 consecutive weeks (12w), maintained on a 12-h day/ night cycle.

2.3. Specimen collection

At the end of the 12w period, rabbits were anaesthetized with sodium pentobarbital (30 mg/kg, i.p.). Blood samples were collected from the carotid artery and then centrifuged at 1000g for 15 min at 4 °C, sera were aliquoted and stored at −70 °C for blood biochemical measurements. After blood taken, the thoracic aorta was immediately isolated and immersed in a pre-cooled (4 °C) Krebs-Henseleit (K-H) buffer containing following composition (in mmol/l): NaCl, 118.3; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; KH_2PO_4, 1.2; $NaHCO_3$, 25.0; and glucose, 11.0, and aerated continuously with 95% O_2 and 5% CO_2. The perivascular connective tissue and fat tissue of thoracic aorta were cleaned with special care to avoid stretching and touching the luminal surface. The aorta was cut into rings of 3–4 mm in length for measurements of histology, vascular function, *ex vivo* gene transferring and DDAH activity as described below.

2.4. Pathomorphologic examination

The vessel segment of 1 cm-long near the aortic arch was cut and the artery lining was observed with naked eyes to identify whether atherosclerotic plaques existed. Then the vessel segment was fixed and immersed in 10% formalin solution, after ethanol dehydration, embedded in paraffin, cut into 6 μm-thick sections and hematoxylin eosin staining. The morphological changes of the artery intima and media were observed under optical microscope.

2.5. Blood biochemical measurement

Serum lipid profiles including TC, TG, HDL-C and LDL-C were assayed spectrophotometrically using the respective commercial kits. Serum level of endogenous NOS inhibitor ADMA was measured by high-performance liquid chromatography (HPLC) method as previously described (Fu et al., 2005). Briefly, Serum (0.1 ml) mixed with 5-sulpfosalicylic acid (2 mg) and stored at 4 °C for 10 min to precipitate protein, which was removed by centrifugation at 2500g for 15 min (4 °C). The supernatant was used for the measurement of ADMA concentration.

2.6. Construction of recombinant adenovirus and ex vivo gene transferring

Previously constructed plasmid pAdeno-hDDAH2 was linearized and transfected into HEK 293 cells (expressing the E1 region of Ad5) through SuperFect® (Hamburg, Germany) to propagate. The primary viral stock was obtained from the cell pellets by three cycles of freezing and thawing. Virus were purified by double cesium chloride (CsCl) step gradient ultracentrifugation and desalted by dialysis (Graham and Prevec, 1995). Viral titer was determined by absorbance at 260 nm ($vp/ml=OD260\times dilution\times 1.1\times 10^{12}$) (Mittereder et al., 1996). The adenovirus encoding β-galactosidase gene (Ad5CMVβ-gal) was used as a control. It was propagated, isolated and quantified as described above. Viral stocks were kept at −80 °C until use (Feng et al., 2010).

Isolated aortic rings from hyperlipidemic and control rabbits were respectively incubated with the suspension of Ad5CMVhDDAH2 (1.5×10^{10} pfu/ml), Ad5CMVβ-gal (1.5×10^{10} pfu/ml) or vehicle (PBS) for 2 h at 37 °C and then kept in DMEM medium (containing 10% fetal calf serum) at 37 °C aerated continuously with 95% O_2 and 5% CO_2 for 24 h after rinsing with PBS. The rings were used for measurements of vascular function and DDAH activity.

2.7. Vascular function testing

The endothelium-dependent relaxation of aortic rings from hyperlipidemic and control rabbits were measured before and after transduction of adenovirus according to the method as previously described