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Icariin regulates PRMT/ADMA/DDAH pathway to improve endothelial function

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ABSTRACT

Background: Oxidative stress may affect PRMT/ADMA/DDAH (protein arginine methyltransferases/asymmetric dimethylarginine/dimethylarginine dimethylaminohydrolase) pathway to impair endothelial dysfunction. The present study was carried out to test the effect of icariin on endothelial function and the mechanisms responsible for this.

Methods: Eighty mice at 12 weeks of age were separated randomly into four groups ($n = 20$): C57BL/6J control, untreated apolipoprotein E-deficient (ApoE$^{-/-}$), two groups of icariin-treated (10 or 30 mg/kg body wt/day, intragastrically) ApoE$^{-/-}$. Primary human umbilical vein endothelial cells (HUVECs) were randomly divided into 7 groups: control group, vehicle of icariin (10 μmol/L) group, icariin (10 μmol/L) group, lysophosphatidylcholine (LPC) (10 μg/mL) group, LPC plus icariin (1 μmol/L) group, LPC plus icariin (3 μmol/L) group, and LPC plus icariin (10 μmol/L) group.

Results: In ApoE$^{-/-}$ mice and primary HUVECs, icariin treatment decreased reactive oxygen species production, PRMT I expression, ADMA level, half-maximum effective concentration of ApoE$^{-/-}$ mice aortic rings. Icariin increased DDAH II expression, DDAH activity, maximal relaxation value and endothelium-dependent vasorelaxation in aortic rings from ApoE$^{-/-}$ mice ($p < 0.05$ or $p < 0.01$).

Conclusions: The present results suggest that icariin regulates PRMT/ADMA/DDAH pathway to improve endothelial function.

Introduction

Endothelial dysfunction is a key trait of atherosclerosis [1]. Asymmetric dimethylarginine (ADMA) has been regarded as marker of it [2]. Dimethylarginine dimethylaminohydrolase (DDAH) catalyzes ADMA to produce dimethylamine and L-citrulline. The decreased DDAH activity and expression play a key role in the accumulation of ADMA [3]. Protein arginine methyltransferase (PRMT) is an enzyme catalyzing the production of ADMA [4]. Therefore, the PRMT/ADMA/DDAH pathway may be a new pharmacologic target for treatment of endothelial dysfunction.

Previous investigations show there is a close relation between the accumulation of ADMA and the aggravation of oxidant stress [5]. Gained from several kinds of Epimedium plants, icariin is the prenyl acetylation of kaempferide 3, 7-O-diglucoside. It has been shown to treat sexual dysfunction and improve impotence. It also has nootropic, antidepressant and antioxidant characteristics [6]. There is evidence to suggest that icariin can ameliorate the function of endothelium and the manufacture of bioactive NO [7], but the mechanisms involved are not yet outright described. As said before, PRMT/ADMA/DDAH pathway may be a new pathway related to endothelial dysfunction. Previous investigations have shown that icariin has powerful antioxidant activity [8]. Due to its antioxidant properties, we postulated that icariin could regulate PRMT/ADMA/DDAH pathway to improve endothelial function by inhibiting oxidative stress.

In the present study, therefore, we examined whether icariin could regulate the PRMT/ADMA/DDAH pathway to improve the endothelial function by restraining oxidative stress in Apolipoprotein E deficient (ApoE$^{-/-}$) mouse and human umbilical vein endothelial cells (HUVECs).

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Materials and methods

Reagents

ADMA standard, LPC, and acetylcholine (Ach) were bought from Sigma. Antibody was obtained from Santa Cruz Biotechnology (USA). Icariin (purity: 98.0%) (Fig. 1) was obtained from Yingxuan Chempharm Co., Ltd (Shanghai, China). Other chemical agents were purchased from Sinopharm Chemical Reagent (Shanghai, China).

Experimental animals

ApoE$^{-/-}$ mice and C57BL/6J mice were respectively bought from Beijing University (Beijing, China) and Hunan Agricultural University (Changsha, China). Exposed to 12 h light:12 h dark cycle, mice were retained at a constant temperature of 23 ± 1 °C and provided with high-fat chow and water *ad libitum*. In accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 80-23, revised 1996), all animals received humane care.

Experimental protocols

In our methods section, research was approved and granted by Hunan Agricultural University ethics review board. In the first series of experiments, eighty mice at 12 weeks of age were separated randomly into four groups ($n = 20$): C57BL/6J control, untreated ApoE$^{-/-}$, two groups of icariin-treated (10 or 30 mg/kg body wt/day, intragastrically) ApoE$^{-/-}$. Icariin was melted in Dimethyl sulfoxide (DMSO) before use as described previously [9]. In untreated ApoE$^{-/-}$ mice group and the control group, vehicle of icariin (30 mg/kg per day) was given. The experiment lasted 6 weeks, at which time all mice were fasted nightlong before euthanasia. Anesthetized (pentobarbital 80 μg/kg *ip*) as reported previously [10], samples of plasma and aorta were obtained from the mice.

In the second series of experiments, primary HUVEC were isolated, grown, and identified as described previously [11]. Primary HUVECs were cultured in Dulbecco's modified Eagle's medium containing 100 U/ml penicillin, 10% (v/v) fetal bovine serum, and 100 U/ml streptomycin. HUVECs were randomly divided into 7 groups: control group, vehicle of icariin (10 μmol/L) group, icariin (10 μmol/L) group, lysophosphatidylcholine (LPC) (10 μg/mL) group, LPC plus icariin (1 μmol/L) group, LPC plus icariin (3 μmol/L) group, and LPC plus icariin (10 μmol/L) group. Cell injury was induced by LPC (10 μg/ml) for 24 h. For Icariin, HUVECs were exposed to icariin (1, 3 or 10 μM) for 1 h and then exposed to LPC for 24 h in the presence of icariin. Icariin was dissolved in DMSO. Reactive oxygen species (ROS) production, DDAH II expression, DDAH activity, PRMT I expression, and ADMA concentration were analyzed.

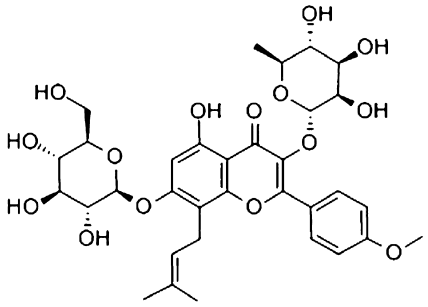

Fig. 1. Chemical structure of icariin.

Determination of ROS

In the absence or presence of icariin (1, 3 or 10 μM), cells in 6-well culture dishes were incubated with 10 μg/ml LPC or control media for 24 h. Using a fluorospectro-photometer to determine the oxidative conversion of cell-permeable 2′, 7′-dichlorofluorescein diacetate to fluorescent dichlorofluorescein, concentrations of intracellular ROS were measured as described previously [12]. After mice were treated with icariin for 6 weeks, isolated aorta was cut into 2-mm fragments. It was placed into 1 mL of Kreb's bicarbonate buffer which was aerated with 5% CO_2/95% O_2 and incubated at 37 °C for 2 h in advance. With the nonfluorescent probe 2′, 7′-dichlorodihydrofluorescein diacetate, ROS produced by aortic ring segments was measured. The average fluorescence from two rings per mouse was determined and showed as the average arbitrary units (AU) per mg protein according to a previously method [13].

Analysis of mRNA expressions of PRMT I and DDAH II

Real-time PCR was performed to quantify DDAH II and PRMT I mRNA. Briefly, Total RNA was isolated from aorta using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and reverse-transcribed. The following primer pairs were used: human DDAH II (5′-GATCTGGCCAAAGCTCAAAG-3′ forward and 5′-CAACCCAGGAC-GAAGAAAGA-3′ reverse) [14]; mouse DDAH II (5′-GGTTGATG-GAGTG CGTAAAGC-3′ forward and 5′-TCCACAATTCGGAGTCCCAA-3′ reverse) [15]; human GAPDH (5′-CTGCTCCTCCTGTTCGACAGT-3′ forward and 5′-CCGTTGACTCCGACCTTCAC-3′ reverse) [14]; mouse GAPDH (5′-GAGAATG GGAAGCTTGTCATC-3′ forward and 5′-GTCCACCACCCTGTTGCTGTA-3′ reverse) [16]; human PRMT I (5′-GATGCTGAAGGACGAGGTGC-3′ forward and 5′-ACTCGATCCCGAT-GACCTTGCG-3′ reverse) [17]; mouse PRMT I (5′-AATGGGAT-GAGCCTCCAGC-3′ forward and 5′-TGCTTGGCCACAGGAAACTT-3′ reverse) [18]. The relative abundance of PRMT I/GAPDH or DDAH II/GAPDH in control group was expressed as 100% for comparative purposes.

Measurement of aortic protein expressions of PRMT I and DDAH II

The segregated aorta was homogenized in ice-cold Dulbecco's phosphate-buffered saline. Cells were lysed in SDS sample buffer. Equal concentrations of protein were separated on a 12% SDS-PAGE and the separated proteins transferred to PVDF membranes. The Western Blotting of PRMT I and DDAH II expressions were carried out as described previously [17–20].

Assay of DDAH activity

Aortic DDAH enzyme activity was measured in aorta homogenates. According to the amount that catalyzed formation of 1 μmol/l L-citrulline from ADMA per minute at 37 °C, one unit of the enzyme was defined as described previously [21]. According to the amount of ADMA metabolized by DDAH, its activity in HUVECs was determined. Its activity of the control group was defined as 100% for every experiment. Compared with the control, DDAH activity in the other group was expressed as a percentage of the ADMA metabolized as previously reported [22].

Measurement of ADMA concentration

Using high-performance liquid chromatography (HPLC) as described previously [23], the proteins in plasma and the conditioned medium were eliminated utilizing 5-sulfo-salicylic acid. And then the supernatant was utilized for determination of ADMA. HPLC was performed with a Shimadzu LC-6A liquid chromatograph using Shimadzu SIC-6A autosampler and Shmadzu