Journal of Steroid Biochemistry & Molecular Biology 155 (2016) 155–165

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb

Diosgenin regulates adipokine expression in perivascular adipose tissue and ameliorates endothelial dysfunction via regulation of AMPK

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ARTICLE INFO

Article history:
Received 20 May 2015
Received in revised form 7 July 2015
Accepted 12 July 2015
Available online 13 August 2015

Keywords:
Diosgenin
Perivascular adipose tissue
AMPK
Inflammation
Endothelial dysfunction

ABSTRACT

Perivascular adipose tissue (PVAT) has been recognized as an active contributor to vascular function due to its paracrine effects on cells contained within vascular wall. The present study was designed to investigate the effect of diosgenin on adipokine expression in PVAT with emphasis on the regulation of endothelial function. Palmitic acid (PA) stimulation induced inflammation and dysregulation of adipokine expression in PVAT. Diosgenin treatment inhibited IKKβ phosphorylation and downregulated mRNA expressions of proinflammatory cytokines/proteins including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein (MCP-1), and inducible nitric oxide synthase (iNOS), while reduced gene expressions for adiponectin, PPARγ, and arginase 1 (Arg-1) were reversed by diosgenin treatment. Diosgenin enhanced AMPK phosphorylation under basal and inflammatory conditions in PVAT, whereas knockdown of AMPK by SiRNA diminished its modulatory effect, indicating that diosgenin inhibited inflammation in an AMPK-dependent manner. We prepared conditioned medium from PA-stimulated PVAT to induce endothelial dysfunction and found that pre-treatment of PVAT with diosgenin effectively restored the loss of ACh-induced vasodilation and increased eNOS phosphorylation in rat aorta. High-fat diet feeding in rats induced inflammation in PVAT and the impairment of endothelium-dependent vasodilation, whereas these alterations were prevented by oral administration of diosgenin at doses of 20 and 40 mg/kg. In conclusion, the obtained data showed that diosgenin ameliorated inflammation-associated adipokine dysregulation, and thereby prevented endothelial dysfunction. Our findings would shed a novel insight into the potential mechanism by which diosgenin protected endothelial function against inflammatory insult.

1. Introduction

Perivascular adipose tissue (PVAT) is the adipose tissue surrounding the vasculature directly, thus allowing for easy access for a large number of mediators that modulates vascular function. Adipocytes and stromal cells contained within PVAT produce adipocytokines such as adiponectin and tumor necrosis factor-α (TNF-α) that act in a paracrine or an endocrine fashion to control metabolic and endothelial functions [1]. It is known that PVAT can protect vessel function by attenuating vasoconstriction through different mechanisms, mainly including secreting adipocyte-derived relaxing factor (ADRF) and stimulating the generation of NO by endothelium [2–4]. Although the identity of ADRF is still unclear, adiponectin has been suggested to be one of the vasodilators [5,6]. In obesity, accumulation of lipids in PVAT stimulates the innate immunity defense leading to the recruitment of monocytes into the adipose tissue and subsequently induces dysregulation of adipocytokine production, leading to endothelial dysfunction [7]. AMP-activated protein kinase (AMPK) is a crucial regulator of energy metabolic homeostasis and emerging evidences demonstrate its anti-inflammatory action in vessel and adipose tissue [8,9]. In a recent work, to be noticed, we also found that pharmacological activation of AMPK beneficially regulated adipocytokine expression in PVAT against inflammatory insult and ameliorated endothelial dysfunction. These findings demonstrate the role of AMPK activation in the regulation of PVAT/endothelial functions [10].

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http://dx.doi.org/10.1016/j.jsbmb.2015.07.005
0960-0760/

Diosgenin, a well-known steroid sapogenin derived from plants, is pivotal in reducing the risks of acquiring cardiovascular disorders. Previous investigations have shown that important biomarkers of the cardiovascular system related to endothelial dysfunction, inflammatory and oxidative stress are affected by diosgenin [11–13]. We previously found that diosgenin ameliorated endothelial dysfunction by increasing insulin-mediated nitric oxide (NO) production through IKKβ/IRS-1 pathway [14]. Moreover, studies support the potential of diosgenin in the management of chronic inflammation in adipose tissues involved in the pathogenesis of obesity-related insulin resistance [15,16]. Based on the potential therapeutic value of diosgenin in obesity-associated cardiovascular disease, it is tempting for us to know the action of diosgenin in the regulation of PVAT function and the potential contribution to the improvement of vascular endothelial homeostasis. In the present paper, we investigated the effect of diosgenin on AMPK activation and adipokine expression in PVAT with emphasis on the regulation of endothelial function and found that diosgenin ameliorated endothelial dysfunction by inhibiting inflammation in PVAT. These findings would provide novel information regarding the potential mechanism of diosgenin in the management of obesity-associated cardiovascular diseases.

2. Materials and methods

2.1. Reagents

Diosgenin (98% in purity) was purchased from Shanxi Huike Plant Co. Ltd (Xian, China) and dissolved in DMSO (0.1% v/v). Resveratrol (98% in purity) was obtained from Nanjing Zelang Medical Technology Co., Ltd. (Nanjing, China). AICA riboside (AICAR) and compound-C were from Sigma (St. Louis, MO, USA). Palmitic acid (PA, Sinopharm Chemical Reagent Co., Ltd. Shanghai, China) was dissolved in ethanol as stock solution and then was diluted with 10% FFA-free BSA. All the primers were obtained from Sangon Biotec (Shanghai, China). The following antibodies were obtained from Cell Signaling Technology, Inc. (Beverly, MA, USA): anti-AMPKα (#2532s), anti-phospho-AMPKα (T172) (#2531s), anti-eNOS (49G3) (#9586) and anti-phospho-eNOS (Ser1177) (#9571). anti-phospho-IKKβ (Y199) (BS4320), and anti-IKKβ (F182) (BS1407), Goat Anti-Rabbit IgG (H+L) HRP (BS13278) and GAPDH (AP0063) were from Bioworld Technology (St. Paul, MN, USA).

2.2. Animals

Sprague-Dawley rats (180–220 g) were supplied by the Laboratory Animal Center of Nanjing Qinglongshan. Animals were kept in a 12-h dark-light cycle and fed standard rodent chow ad libitum in accordance with the Provisions and General Recommendation of Chinese Experimental Animals Administration Legislation. For the high-fat diet feeding, Rats were given the high-fat diet (HFD, 60% kcal fat, 20% kcal carbohydrates, 20% kcal protein) for 8 weeks simultaneously with administration of diosgenin (20, 40 mg/kg) or resveratrol (20 mg/kg) by oral gavage every day. All procedures described were approved by the Animal Ethics Committee of School of Chinese Materia Medica, China Pharmaceutical University.

2.3. Serum analysis in HFD rats

Overnight fasting-rats were anesthetized with diethyl ether and blood was collected from the orbital sinus. Contents of TNF-α and adiponectin in serum were determined with ELISA kits (Jiancheng Bioengineering Institute, Nanjing, China), following the manufacturer's protocols.

2.4. Perivascular adipose tissue-derived conditioned medium (CM) preparation

Rats (200–250 g) were anesthetized with diethylether and killed by cervical dislocation. Perivascular adipose tissues (PVAT) around the aorta were isolated and chopped into small pieces. Equal amount of PVAT was pretreated with diosgenin (0.1, 1 and 10 μM), or resveratrol (50 μM) individually in the presence or absence of AMPK inhibitor compound C (25 μM) and simultaneously stimulated with PA (100 μM) for 2 h. After the treatment, the PVAT was washed with PBS twice to remove the treated agents, and cultured in fresh DMEM for another 22 h. The medium was collected as conditioned medium (CM). For the collection of CM from HFD-fed rats, PVAT were isolated and directly incubated in DMEM for 24 h without any treatment.

2.5. Endothelium-dependent relaxation assessment

Endothelium-dependent relaxation was assayed as we previously described [10]. In brief, the prepared aortic ring was suspended in an organ bath containing 10 mL K-H solution maintained at 37 °C, pH 7.4, and continuously aerated with 95% O_2 and 5% CO_2. After reaching the base tension, the contractive ability of the vessel was examined by contractive response to 60 mM KCl, while the functionality of vascular endothelium was confirmed by relaxation to 10 μM acetylcholine (ACh, Sigma, St. Louis, MO, USA) The aortic ring (relaxation ≥ 80%) was treated with CM for 0.5 h. After washing, the aortic ring was pre-contracted by 1 μM phenylephrine and the endothelium-dependent relaxation was induced by cumulative addition of ACh (0.001–10 μM). The relaxation was expressed as a percentage of the phenylephrine-induced contraction. For the assessment in the aorta from HFD-fed rats, all procedures were as same as described above, but without pretreatment with CM.

2.6. RNA extraction and real-time quantitative PCR

PVAT from normal rats were pretreated as described in section 2.4. The total RNA was obtained by Total RNA Extraction Reagent (SunShineBio, Nanjing, China). RNA was converted into cDNA using the TransScript First-strand cDNA Synthesis Super Mix (TransGen Biotech, Beijing, China). The synthesized cDNA was used for PCR amplification with the primers previously reported [10]. Quantitative PCR was performed using SsoFast™ EvaGree Supermix (Bio-Rad, Hercules, California, USA) with the Bio-Rad iQ5 sequence detection system (Bio-Rad, Hercules, California, USA). Two protocols were used for qPCR of different genes. One protocol: 40 cycles (30 s at 95 °C and 10 s at 54 °C) after an initial activation step for 10 min at 95 °C. The other protocol: 40 cycles (30 s at 95 °C and 10 s at 52 °C) after an initial activation step for 10 min at 95 °C. Primer sequences were shown in Supplementary Table. The mRNA level of individual genes was normalized and presented as a ratio to β-actin and calculated using the $\Delta\Delta$CT method.

2.7. Western blot analysis

Western blot analysis was performed as previously described [17]. For protein analysis, PVAT or aorta was lyzed in RIPA plus PMSF, and then the protein concentration of samples was determined by Bicinchoninic Acid (BCA) Protein Assay kit (Biosky Biotechnology Corporation, Nanjing, China). Equal amount of protein was separated by 10% SDS–polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (0.45 μm, Millipore Co., Ltd.). After blocked with 5% non-fat milk for 2 h at room temperature, the PVDF membrane was incubated with primary antibody overnight at 4 °C. Afterwards, the PVDF membrane was incubated with the secondary antibody at