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Notoginsenoside Fc attenuates high glucose-induced vascular endothelial cell injury via upregulation of PPAR-γ in diabetic Sprague–Dawley rats

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ABSTRACT

Endothelial injury from high glucose (HG) plays a dominant role in atherosclerosis, diabetes-induced vasculopathy, and vascular remodeling. Notoginsenoside Fc (Fc), a novel saponin isolated from *P. notoginseng*, has been shown to exhibit properties that counteract platelet aggregation. However, the potential roles and molecular mechanisms of Fc in preventing cardiovascular injury have yet to be explored. In this study, we present novel data that show the ability of Fc to prevent early atherosclerosis of diabetic Sprague–Dawley (SD) rats in vivo and to attenuate endothelial cell injury in vitro. Our results indicate that Fc protects rat aortic endothelial cells (RAOECs) from HG-induced injury by inhibiting apoptosis and promoting proliferation as well as by reducing endothelial cell production of pro-inflammatory cytokines: TNF-α, IL-1β, IL-6, ICAM-1. Furthermore, the downregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) in HG-challenged endothelial cells was prevented by Fc. Inhibition of PPAR-γ abrogated the effects of Fc on HG-induced pro-inflammatory cytokine production in RAOECs. These results indicate that Fc has a preventative effect on HG-induced endothelial cell injury partly through a PPARγ-mediated pathway, suggesting that Fc might provide a potential new therapeutic option for the treatment of diabetic vascular complications.

1. Introduction

An aging population and changing lifestyles have both led to an upward trend in the prevalence of diabetes mellitus (DM) in China. Indeed, China is now ranked among the countries with a high global incidence of diabetes [1, 2]. Accumulating evidence indicates that vascular lesions are the most common complication of diabetes [3, 4]. Clinical data also demonstrate that patients with type 2 diabetes with vascular lesions in coronary arteries, cerebral arteries, and lower extremity arteries exhibit the following characteristics: high rate of morbidity, early onset, rapid progression, and poor prognosis. These vascular complications are the main cause of diabetes-related disability and death [5]. Hyperglycemia in DM is thought to play an essential role in the development of atherosclerosis by inducing endothelial cell apoptosis and inhibiting endothelial cell proliferation [6, 7]. Damaged or dysfunctional endothelial cells can synthesize and release pro-inflammatory mediators, leading to an inflammatory response [8]. Taken together, these data indicate that the ability to maintain endothelial cell integrity, number, and function under high glucose (HG) conditions seems to be of pivotal importance in preventing diabetic vascular complications.

The search for more effective therapeutic drugs to prevent vascular endothelial cell injury remains a great challenge [9]. In recent years, studies have shown that traditional Chinese herbal medicine plays an important role in halting the progression of cardiovascular disease due to its advantages of the multi-target treatment and the overall adjustment to disease [10, 11]. The saponins of *Panax notoginseng* have broad pharmacological effects on the cardiovascular system, which include effectively protecting against myocardial ischemia–reperfusion injury, improving myocardial remodeling, inhibiting cardiac hypertrophy, protecting vascular endothelial cells, promoting angiogenesis, and preventing the abnormal activation of platelets [12]. It has previously been demonstrated that notoginsenoside R1 suppressed oxLDL-induced inflammatory cytokine production via inhibiting oxLDL-induced NF-κB and MAPK activation [13]. However, *P. notoginseng* saponins contain a variety of monomer components, such as ginsenoside Rg1, ginsenoside Rb1, ginsenoside Rd, notoginsenoside Ft, and so on [14, 15]. The role of each individual monomer composition in cardiovascular disease still needs to be examined. Among these saponins, Notoginsenoside Fc (Fc), a novel protopanaxadiol-type (PPD-type) saponin isolated from the leaves of *P. notoginseng*, is soluble in saline and has been shown to effectively counteract platelet aggregation and has exhibited various

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pharmacological activities as a folk medicine or diet supplement [16, 17]. However, no previous studies have explored the effect of Fc on cardiovascular disease.

Peroxisome proliferator-activated receptor-γ (PPAR-γ), a member of the nuclear receptor of ligand-activated transcriptional factors, plays a critical role in glucose and lipid metabolism [18, 19]. Its agonists have been used in the treatment of type 2 diabetes [20, 21]. Furthermore, previous studies have demonstrated that PPAR-γ can be effective in the regulation of vascular endothelial function and atherosclerosis [22, 23].

Our aims in this study included exploring the potential protective effect of Fc on vascular endothelial cells in diabetic SD rats in vivo and in vitro as well as examining the possibility that this protective mechanism is mediated by a PPAR-γ pathway. It is hoped that this study will provide an experimental basis for the clinical application of Fc in diabetic vascular disease.

2. Materials and methods

2.1. Drug preparation

Notoginsenoside Fc (chemical structure C58H98O26, molecular weight = 1211.4, purity ≥ 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The molecular structure is shown in Fig. 1.

2.2. Ethics and animals

All animal experiments were approved by the Ethics Committee of the Sixth People's Hospital, which is affiliated with Shanghai Jiaotong University, and were performed in accordance with the Guidelines of the National Institutes of Health for the Care and Use of Animals. The care and handling of rats was approved by the Institutional Animal Care and Use Committee and proceeded in accordance with the Animals (Scientific Procedures) Act, 1986 (UK) (amended 2013). Animals and normal forage were purchased from the Model Animal Research Centre of Nanjing University (Jiangsu, China). All rats were housed in individually ventilated cages (three or four per cage) under specific pathogen-free (SPF) conditions. Housing was temperature-controlled, with a 12-h/12-h light/dark cycle. Eighteen female Sprague–Dawley (SD) rats (100 ± 20 g) were randomly separated into three groups: control group (n = 6), diabetes mellitus (DM) group (n = 6), and DM + Fc group (n = 6).

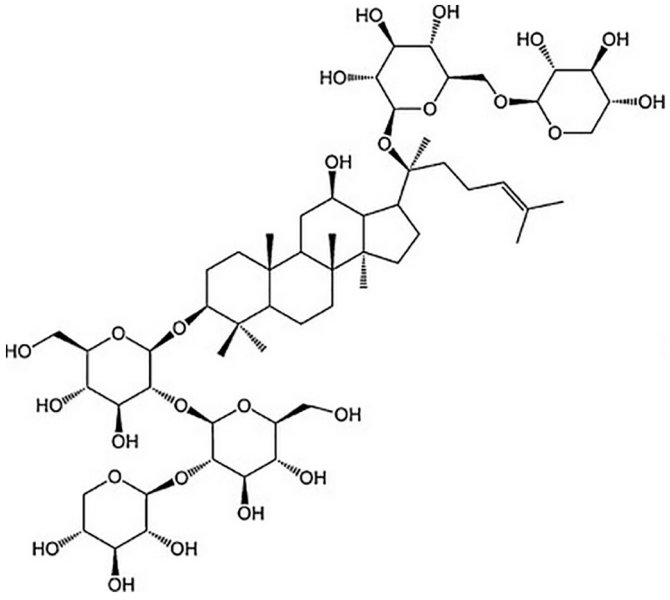

Fig. 1. Molecular structure of Notoginsenoside Fc.

2.3. Animal models and drug treatments

The DM group and the DM + Fc group were fed a high-fat/high-cholesterol (HFHC) diet (Beijing Botai Hongda Biotechnology Co., Ltd.) for 4 weeks. After 12 h of fasting, animals were given an intraperitoneal injection of 40 mg/kg streptozotocin (STZ) and then continued the HFHC diet for 2 weeks. The control group was fed with common feed and injected with citric acid buffer. Fasting blood samples were taken from the tail vein of rats on the 3rd, 7th, and 14th day after STZ injection. A Roche blood glucose meter and Roche test paper were used to measure fasting blood glucose levels. Fasting blood glucose > 16.7 mmol/L represented the successful establishment of a diabetic model of rats. After successful modeling, the DM + FC group began the drug intervention, with Fc 3.5 mg/kg/d gavage, whereas the other two groups were given the same dose of saline. After 8 weeks of gavage, the carotid artery of each rat was obtained for hematoxylin-eosin (HE) staining and immunofluorescence (IF) staining. Every specimen was incubated in 4% paraformaldehyde for 24–48 h and then embedded in paraffin. For HE staining, paraffin sections (3–5 mm thick) were dewaxed, stained, examined microscopically, and photographed. Images were captured using a fluorescence microscope (Jenoptik, Jena, Germany). The general condition of rats was observed during the experiment. Body weight was measured every week, and blood glucose levels were measured every 2 weeks following drug treatment.

2.4. Immunofluorescence staining

3-Nitrotyrosine (3-NT) expression levels were determined using an immunofluorescence assay; 3–5 mm paraffin carotid artery sections were fixed with 4% formaldehyde for 30 min and permeabilized with 0.2% Triton X-100 for 10 min, followed by incubation in 5% skim milk for 1 h at room temperature. The intima were then incubated with primary antibodies: anti-3-NT (1:200, Abcam, Cambridge, UK) overnight at 4 °C. A similar protocol was used for the in vivo experiments; briefly, RAOECs were individually grown on four 35-mm glass bottom dishes for confocal microscopy. The collected cells were fixed and permeabilized and then incubated with anti- PPAR-γ (1:200, Abcam, Cambridge, UK) overnight at 4 °C. After three separate washes with PBS, the intima and cells were incubated with Alexa Fluor 488-labeled goat anti-rabbit IgG (Abcam, Cambridge, UK) in the dark for 1 h. For nuclear staining, the samples were stained with DAPI. After 15 min, the samples were observed and photographed with the confocal microscope (LSM 710 META; Zeiss, Oberkochen, Germany). The fluorescence intensity was averaged from at least six independent visual fields for each group, and the relative fluorescence intensity was assessed as previously described [24].

2.5. Animal biochemical measurements

After 14 weeks, the blood of rats was collected and centrifuged to obtain serum. The TNF-α, IL-1β, and IL-6 levels of each group were quantified by the rat ELISA kit according to manufacturer's instructions (Beyotime Institute of Biotechnology, Shanghai, China).

2.6. Cell culture

Rat aortic endothelial cells (RAOECs) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). According to the manufacturer's instructions, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal bovine serum (FBS) (both from Gibco, Carlsbad, USA). RAOECs were cultivated in a humidified atmosphere at 37 °C with 5% CO_2. The growth medium was replaced every 2–3 days, and passages 4–6 were used for cell experiments.