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Platycodon grandiflorum root attenuates vascular endothelial cell injury by oxidized low-density lipoprotein and prevents high-fat diet–induced dyslipidemia in mice by up-regulating antioxidant proteins

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ABSTRACT

We hypothesized that a *Platycodon grandiflorum* root (PG) ethyl acetate extract (PGEA) would help reduce the vascular cell injury caused by oxidized low-density lipoprotein (oxLDL) and prevent high-fat (HF) diet–induced dyslipidemia and oxidative stress by up-regulating antioxidant proteins. We investigated the protective effects of PGEA against vascular endothelial cell injury induced by oxLDL and dyslipidemia induced by an HF diet, and the mechanisms underlying these effects were studied. The protective effects of PGEA were investigated with respect to calf pulmonary arterial endothelial (CPAE) cell viability and the lactate dehydrogenase release during oxLDL treatment. The in vivo effects of PGEA were examined using C57BL/6 mice, which were fed an HF diet for 9 weeks. The HF diet was supplemented with 0, 25, or 75 mg/kg PGEA during the last 4 weeks of the experimental period. Histologic analyses of hepatic lipid accumulation were performed. The changes in antioxidant protein levels induced by PGEA, which protects against HF diet–induced oxidative stress, were measured using a proteomics approach. We found that PGEA exhibited antioxidant activity. In CPAE cells, PGEA inhibited both oxLDL-induced cell death and lactate dehydrogenase release. In the HF diet–induced obese mice that received PGEA, we observed significantly reduced plasma and hepatic lipid levels, demonstrating that PGEA has beneficial effects on hyperlipidemia. In addition, we found that PGEA caused the up-regulation of antioxidant proteins. These findings suggest that the antioxidant effects of PGEA may protect against oxidative stress-related diseases.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CPAE, calf pulmonary arterial endothelial; CAT, catalase; 2-DE, 2-dimensional gel electrophoresis; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GPx, glutathione peroxidase; GST, glutathione-S-transferase; HDL, high-density lipoprotein; HF, high fat; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide; PEDFP, pigment epithelium–derived factor precursor; PG, *Platycodon grandiflorum* root; PGEA, *Platycodon grandiflorum* root ethyl acetate extract; oxLDL, oxidized low-density lipoprotein; Prx, peroxiredoxin; ROS, reactive oxygen species; SOD, superoxide dismutase.

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1. Introduction

During the oxidation of low-density lipoproteins (LDL), reactive oxygen species (ROS) initially target polyunsaturated fatty acids that contain bis-allylic hydrogen atoms [1]. The presence of oxidized LDL (oxLDL) is a major risk factor for atherosclerosis and cardiovascular disease [2]. OxLDL-induced injury to the vascular endothelium [3] is important for the progression of atherosclerosis. Vascular endothelial cell lines have previously been used as experimental models to study atherosclerosis, cardiovascular disease [4], and the inhibitory effects of natural extracts on cardiovascular disease that result from oxLDL-induced damage [4]. Calf pulmonary arterial endothelial (CPAE) cells were used as a vascular endothelial cell model in this in vitro study.

High-fat (HF) diet–induced obesity promotes the production of ROS in mice and humans [5]. The oxidative stress pathway is regulated by balancing ROS production and antioxidant enzyme activity [6]. Reactive oxygen species are normally eliminated through various antioxidant systems, including superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase, catalase (CAT), γ-glutamyl-cysteine synthetase, and metallothionein [7].

Platycodon grandiflorum root (PG), which is commonly known as Doraji in Korea, is popular and commonly used as medicine and food in Korea. It has a wide range of pharmacologic properties, such as reducing adiposity [8] and hyperlipidemia [9], and is known to have antiatherosclerotic effects [10]. However, the mechanisms underlying these effects remain unclear.

In this study, dried PG was extracted with 70% ethanol (crude extract) and partitioned sequentially with hexane, chloroform, ethyl acetate, and butanol. A previous study found that an ethyl acetate extract of PG (PGEA) inhibited the oxidative stress-induced death of human hepatic HepG2 cells and that the levels of SOD, CAT, glucose-6-phosphate dehydrogenase, and metallothionein messenger RNA increased in PGEA-treated HepG2 cells [7]. Given these in vitro effects, we hypothesized that PGEA may function similarly in vivo. We used a mouse model to examine the effects of PGEA on the production of antioxidant proteins during HF diet–induced oxidative stress.

Cell-free systems were used to examine the total phenolic content, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and the reducing power of 6 PG extracts. *P grandiflorum* root ethyl acetate extract was selected for use in both the in vitro and in vivo experiments because it exhibited the highest level of antioxidant activity in each experiment.

We hypothesized that PGEA may help reduce vascular cell injury caused by oxLDL and HF diet–induced dyslipidemia and oxidative stress by up-regulating antioxidant proteins. Because hyperlipidemia and oxidative stress caused by obesity are associated with an increased risk of cardiovascular disease and atherosclerosis [2-5], dyslipidemia may also contribute to atherosclerosis. To test this hypothesis, the protective effects of PGEA on proatherosclerotic stressor oxLDL-induced damage in vascular endothelial cells (CPAE) were examined using mice fed an HF diet.

2. Methods and materials

2.1. Chemicals and reagents

PowerScript reverse transcriptase was obtained from Clontech (Palo Alto, Calif). Oligo(dT)$_{15}$ primers and GoTaq Green Master Mix were obtained from Promega (Madison, Wis). The kits for measuring plasma total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels were purchased from Asan Chemical (Asanpharm, Korea). The alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) assay kits were purchased from Stanbio Laboratory (Stanbio, Tex). The immobilized pH gradient strips were purchased from Bio-Rad (Hercules, Calif). Folin-Ciocalteu reagent, caffeic acid, and all other reagents were purchased from Sigma Chemical Co (St. Louis, Mo). Calf pulmonary arterial endothelial cells were obtained from the Korean Cell Line Bank (Seoul, Korea).

2.2. Preparation of *P grandiflorum* extracts

P grandiflorum root was purchased from Ku-Ryong Pharmaceutical Co, Ltd (Seoul, Korea). The roots were cleaned, dried, and ground to a fine power before being extracted 3 times with 10 volumes of 70% ethanol for 8 hours at –80°C [11].

After filtration, the solvent was removed using a rotary evaporator. The 70% ethanol extract was suspended in water and extracted 3 times with hexane. The ensuing aqueous layer was then extracted sequentially with chloroform, ethyl acetate, and butanol. Each extraction was performed 3 times. The hexane, chloroform, ethyl acetate, and butanol extracts, as well as the final aqueous layer, were concentrated using a vacuum evaporator (Rotary evaporator NE-SERIES; EYELA, Tokyo, Japan) and were freeze-dried in a lyophilizer (Ilshin Freezer, FD5508, Yang Ju Si, Korea). The freeze-dried product was stored at –80°C until use.

2.3. The determination of total phenolic content, DPPH radical-scavenging activity, and reducing power

The concentration of phenolics in the crude, hexane, chloroform, ethyl acetate, and butanol extracts and in the final aqueous layer was determined using the methods described by Folin and Denis [12]. Briefly, 0.5 mL of extract (500 μg/mL) was mixed with 0.05 mL of Foline-Ciocalteau phenol regent, and 0.1 mL of saturated Na_2CO_3 solution was added to the mixture. The absorbance of the mixture was read at 700 nm (VersaMax microplate reader; Molecular Devices, Sunnyvale, Calif). The total phenolic content of the extract was estimated by comparison with a standard curve that was generated by analyzing caffeic acid.

The DPPH radical-scavenging activity and the reducing power of the extracts were determined using the methods described in Chung et al [13]. For the DPPH radical-scavenging activity, the 6 PG extracts or 500 μg/mL of the ascorbic acid standard (180 μL) were each mixed with 120 μL of the DPPH radical solution (1.5×10^{-4} M). The reduction of the DPPH radicals after 30 minutes was measured by a microplate reader at 517 nm (VersaMax microplate reader; Molecular