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Gardenia jasminoides extract-capped gold nanoparticles reverse hydrogen peroxide-induced premature senescence

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ABSTRACT

This study reports a green approach for synthesis of gold nanoparticles using *Gardenia jasminoides* extract, and specifically, can potentially enhance anti senescence activity. Biological synthesis of gold nanoparticles is ecofriendly and effective for the development of environmentally sustainable nanoparticles compared with existing methods. Here, we developed a simple, fast, efficient, and ecofriendly approach to the synthesis of gold nanoparticles by means of a *Gardenia jasminoides* extract. These *G. jasminoides* extract-capped gold nanoparticles (GJ-GNPs) were characterized by UV–vis, high resolution transmission electron microscopy (HR-TEM), X-ray diffraction (XRD), and Furrier transform infrared spectroscopy (FT-IR). The synthesized GJ-GNPs turned red and showed maximal absorbance at 540 nm. Thus, GJ-GNPs were synthesized successfully. We hypothesized that GJ-GNPs would protect ARPE19 cells from hydrogen peroxide-induced premature senescence. SA-β-gal activity was elevated in hydrogen peroxide-treated cells, however, this effect was attenuated by GJ-GNP treatment. Moreover, compared with the normal control, hydrogen peroxide treatment significantly increased lysosome content of the cells and production of reactive oxygen species (ROS). GJ-GNPs effectively attenuated the increase in lysosome content and ROS production in these senescent cells. According to cell cycle analysis, G2/M arrest was promoted by hydrogen peroxide treatment in ARPE19 cells, however, this change was reversed by GJ-GNPs. Western blot analysis showed that treatment with GJ-GNPs increased the expression of p53, p21, SIRT3, HO-1, and NQO1 in senescent cells. Our findings should advance the understanding of premature senescence and may lead to therapeutic use of GJ-GNPs in retina-related regenerative medicine.

1. Introduction

In recent years, nanotechnology is a rapidly growing field with its applications in various industrial and biomedical. Advance of nanotechnology have caused a wide growth in various fields of science including medicine, energy, electronics and materials [1]. And it is a most promising technic for new applications in biotechnology and nanomedicine. Nanotechnology is a scientific method for manipulation, analysis, and control of materials at the nanoscale. Nanoparticles have a size of 1–100 nm, and easy control of particle size is made possible by such materials as polymers, carbon, and metals. Biological synthesis is clean, nontoxic, nonhazardous, easy to handle, economical, and environmentally friendly, as compared with chemical synthesis such as toxicity and environmental pollution [2]. Among various metals, nanoparticles of gold have unique optical and electrical properties that can be applied to biomedical research and nanomedicine. In addition, they are capable of binding drugs, therapeutic substances, and fluorescent substances for various medical applications. In recent years, because gold nanoparticles can be used for targeted drug delivery, research into molecular imaging, genetic engineering, and tissue engineering has intensified [3–5]. In particular, gold nanoparticles possess high biological stability and low cytotoxicity, and their structure and size can be adjusted with the introduction of a hydrophobic drug, antibody, targeting ligand, or a functional group. Functionalized gold nanoparticles are considered promising tools for nanomedicine, including biosensors, bioimaging, photothermal therapy, and drug delivery and theragnosis [6,7].

Senescence process results in gradual deterioration of cellular functions in an organism and is associated with an increased number of senescent cells with age [8]. Senescence involves changes in molecular and cellular structures and changes in metabolism, eventually leading to death [9]. Cellular senescence was formally described 50 years ago, when Hayflick and colleagues showed that this phenomenon limits the proliferation of healthy cells in culture: all cells gradually lose the ability to divide while cell proliferation declines [10]. Eventually, life span and aging are determined by its cellular life span and cellular

Abbreviations: Cit-GNPs, citric-acid-capped gold nanoparticles; GJ, *Gardenia jasminoides*; GJ-GNP, *Gardenia jasminoides* extract-capped gold nanoparticle; RPE, retinal pigment epithelial; ROS, reactive oxygen species; SA-β-gal, senescence-associated β-galactosidase.

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aging rate [11]. Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly population, and although its pathogenesis is poorly understood, oxidative stress has been suggested as one of the causes of AMD [12]. Aging and oxidative stress are believed to be key mediators of macular degeneration pathogenesis. In particular, senescence of retinal pigment epithelial (RPE) cells is induced by oxidative stress, and genetic factors are also involved in the pathogenesis of macular degeneration [13]. Accordingly, an in vitro model of premature senescence has been established using the ARPE19 cell line as human RPE cells.

G. jasminoides (GJ) has been used in traditional oriental medicine for the treatment of jaundice, fever, hypertension, inflammation, headache, edema, acute conjunctivitis, pyogenic infections and insomnia, and it is known to have hemostatic and diuretic effects [14,15].

We produced ecofriendly nanoparticles through biological methods based on natural materials. In addition, we have studied the development of nanocarriers with antisenescence properties and biocompatibility using a model of premature cellular senescence as well as the relevant mechanisms of the inhibitory effects on senescence caused by oxidative stress. The present study explores the effects of *G. jasminoides* extract-capped gold nanoparticles (GJ-GNPs) on hydrogen peroxide-induced premature senescence of RPE cells and the mechanisms involved therein.

2. Experimental

2.1. GJ-GNP Synthesis

Cit-GNPs (citric acid-capped gold nanoparticles) and GJ-GNPs were prepared by the reduction method using chloroauric acid according to a previously described protocol [16], with slight modifications. To synthesize GJ-GNPs, a mixture of a 1 mM $HAuCl_4$ (Sigma-Aldrich, St. Louis, MO, USA) solution and GJ extract was incubated for 20 min at 200 rpm and 37 °C. Synthesis of GJ-GNPs was confirmed by UV–Vis spectrophotometry using an Ultrospec 6300 Pro (GE Healthcare Life Sciences, Buckinghamshire, UK) at wavelengths in the 300–800 nm range. The size and zeta potential were measured by dynamic light scattering (DLS) using the Data Transfer Assistance (DTA) software and a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK).

2.2. Transmission Electron Microscopy (TEM)

High-resolution TEM (HR-TEM; TALOS F200X, FEI, Oregon, USA) with selected area electron diffraction (SAED) were used to determine the morphological features (shape) and size of the synthesized GJ-GNPs. The SAED pattern of the GJ-GNPs was also consistent with HR-TEM data, which confirmed the crystalline nature of the synthesized GJ-GNPs. The synthesized GJ-GNPs were prepared for analysis by placement on a carbon-coated copper grid. The grids were dried and then the analysis was conducted.

2.3. X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FT-IR)

The structure of GJ-GNPs was analyzed by means of XRD patterns and FT-IR spectra. To confirm the crystalline structure, the GJ-GNPs were freeze-dried and subjected to XRD analysis on an X-ray diffractometer (XRD Empyrean series 2, PANalytical, Almelo, Nederland). The XRD analysis was performed in the scanning range of 30 to 80, at voltage of 40 kV and a current of 30 mA. FT-IR spectra of GJ-GNPs were obtained on an FT-IR spectrophotometer (Spectrum GX, PerkinElmer Inc., Boston, MA, USA) in KBr (potassium bromide) pellets. The FT-IR spectra were recorded in the range of 4000–400 cm^{-1}. FT-IR analysis was carried out to identify various functional groups and to dissect the formation of gold nanoparticles.

2.4. Cell Culture

The human ARPE19 cell line (ATCC® CRL 2302™) was used as RPE cells. ARPE19 cells were grown in the DMEM/F12 medium containing 10% of heat-inactivated fetal bovine serum (FBS) and 1% of penicillin/streptomycin, and incubated at 37 °C and 5% CO_2. DMEM/F12 and FBS were purchased from Gibco BRL (Invitrogen, Carlsbad, CA, USA). The cells were passaged every 2–3 days. The cells between passages 15 and 20 were used for experiments.

2.5. The Cellular Model of Premature Senescence and Treatment with GJ-GNPs

ARPE19 cells (1.5×10^5/dish) were seeded in a 60-mm dish and grown to 70% confluence, then treated with 150 μM hydrogen peroxide (H_2O_2) for 2 h, washed once with PBS, and cultured in the normal growth medium for 20–22 h. This cycle was repeated one more time. The medium was replaced with a fresh medium containing 10% of FBS and 1% of penicillin/streptomycin, and the cells were cultured for another 4 days. In some groups of cells, GJ-GNP (100 μg/mL) treatment was applied 1 h before hydrogen peroxide treatment.

2.6. The Cell Viability Assay

Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were incubated with hydrogen peroxide for 2 h, GJ-GNP treatment was applied at various concentrations 1 h prior to the hydrogen peroxide treatment, and then the cells were incubated for additional 24 h. Next, 20 μL of 5 mg/mL MTT was added to the cells in each well and incubated for 3 h at 37 °C. After removal of the supernatant, 150 μL of DMSO was added to each well, and the absorbance was measured at 570 nm using a microplate reader (Wallac 1420, Boston, MA, USA). Another cell viability assay was carried out by means of CellTrace™ Calcein Green, AM (Molecular Probes Inc., Eugene, OR, USA). The cells were washed with PBS and harvested with trypsin/EDTA, 0.5 μM calcein was added to the cells, with incubation for 15 min in the dark, and the cells were analyzed by flow cytometry (Beckman Coulter, Brea, CA, USA).

2.7. Assessment of Lysosome Content and ROS Production

The cells were washed with PBS and harvested with trypsin/EDTA. Lysosome content of the cells was measured using 50 nM LysoTracker® Green DND-26 (Cell Signaling Technology, Beverly, MA, USA), and ROS production in the cells was quantified by means of 2.5 μM CM-H2DCFDA (Molecular Probes Inc.). The dye was incubated with the cells for 15 min in the dark, and the labeled cells were analyzed by flow cytometry.

2.8. The SA-β-Gal Staining Assay for SA-β-Gal Activity

Staining for senescent cells was performed using the Senescence β-Galactosidase Staining Kit (Cell Signaling Technology). In brief, the cells were washed with PBS and fixed with a 1× fixative solution for 20 min at room temperature. Then, the cells were washed twice with PBS and incubated with the β-galactosidase-staining solution overnight at 37 °C. After that, the cells were examined under a microscope (200× magnification).

SA-β-gal activity in the senescent cells was measured using the Quantitative Cellular Senescence Assay Kit (Cell Biolabs, San Diego, CA, USA). In short, the cells were washed with PBS and harvested with trypsin/EDTA, and a 1× pretreatment solution was added to the cells, which were then incubated at 37 °C for 2 h. The cells were then incubated with a 200× SA-β-gal substrate solution at 37 °C for 4–5 h in the dark, and the labeled cells were analyzed by flow cytometry. Data analyses were conducted in the CXP software 2.0 (Beckman Coulter, Brea, CA, USA).