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Original Article

Green synthesis of gold nanoparticles using chlorogenic acid and their enhanced performance for inflammation

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Abstract

Here we developed a novel green synthesis method for gold nanoparticles (CGA-AuNPs) using chlorogenic acid (CGA) as reductants without the use of other chemicals and validated the anti-inflammatory efficacy of CGA-AuNPs *in vitro* and *in vivo*. The resulting CGA-AuNPs appeared predominantly spherical in shape with an average diameter of 22.25 ± 4.78 nm. The crystalline nature of the CGA-AuNPs was confirmed by high-resolution X-ray diffraction and by selected-area electron diffraction analyses. High-resolution liquid chromatography/electrospray ionization mass spectrometry revealed that the caffeic acid moiety of CGA forms quinone structure through a two-electron oxidation causing the reduction of Au^{3+} to Au^0. When compared to CGA, CGA-AuNPs exhibited enhanced anti-inflammatory effects on NF-κB-mediated inflammatory network, as well as cell adhesion. Collectively, green synthesis of CGA-AuNPs using bioactive reductants and mechanistic studies based on mass spectrometry may open up new directions in nanomedicine and CGA-AuNPs can be an anti-inflammatory nanomedicine for future applications.

From the Clinical Editor: Gold nanoparticles (Au NPs) have been shown to be very useful in many applications due to their easy functionalization capability. In this article, the authors demonstrated a novel method for the synthesis of gold nanoparticles using chlorogenic acid (CGA) as reductants. In-vitro experiments also confirmed biological activity of the resultant gold nanoparticles. Further in-vivo studies are awaited.
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Key words: Chlorogenic acid; Gold nanoparticles; Green synthesis; Inflammation; NF-κB

With recent advancements in nanotechnology, nanoscale materials have attracted considerable attention because they exhibit unique properties compared to their bulk counterparts. Among metal nanoparticles, gold nanoparticles (AuNPs) have emerged as an important tool for diverse applications.[1-3] The functionalization or bioconjugation of biologically active molecules onto the surface of AuNPs has been widely used to engineer biocompatible AuNPs for the diagnosis and treatment of diseases.[4,5] The most common synthetic method employs a chemical route utilizing chemical reductants to convert Au ions to AuNPs.[6,7]

As an alternative to chemical reductants, green reductants have attracted much attention because of their potential to help realize current sustainability initiatives. Green reductants include diverse types of biological entities.[8-10] The green synthesis of AuNPs offers many advantages, including increased biocompatibility, convenient scale-up, and straightforward reaction procedures. Furthermore, the combination of AuNPs and green reductants may possibly result in synergistic biological activities. Among naturally occurring bioactive compounds, chlorogenic acid (CGA) is a polyphenol compound in plants including green coffee beans. CGA exhibits various functions, such as anti-oxidant, anti-diabetic and anti-tumorigenic effects.[11,12] Previously, we also reported that CGA inhibits endotoxin-induced inflammation in Raw 264.7 macrophages and mouse

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retinal inflammation models through the downregulation of the NF-κB pathway, which activates the transcription of numerous target genes, such as pro-inflammatory cytokine and adhesion molecules.[13] Furthermore, AuNPs (diameter, d = 10-15 nm) synthesized using sodium citrate inhibit nitric oxide (NO) production and inducible nitric oxide synthase (iNOS) expression by blocking the activation of NF-κB and signal transducer and activator of transcription1 (STAT1) in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells,[14] and a single dose of AuNPs (diameter, d = 21 nm) reduces tumor necrosis factors (TNF)-α and interleukin 6 (IL-6) mRNA levels with no measurable organ or cell toxicity in mice.[15] These findings prompted us to study the potential of CGA as a green reductant for producing AuNPs, the characteristics exhibited by the resulting CGA-AuNPs, and the possibility of CGA-AuNPs exhibiting synergistic activity against inflammation.

In the present study, CGA was used as a reductant for the green synthesis of CGA-AuNPs. Spectroscopic and microscopic techniques were used to characterize the CGA-AuNPs, including UV-Visible spectrophotometry, high-resolution transmission electron microscopy (HR-TEM), atomic force microscopy (AFM), field emission scanning electron microscopy (FE-SEM), Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, the reduction mechanism of Au^{3+} to AuNPs was elucidated by characterizing the oxidized form of caffeic acid in CGA using high-resolution liquid chromatography electrospray ionization mass spectrometry (HR-LC-ESI-MS). To verify the activity of CGA-AuNPs, we investigated the anti-inflammatory effects of CGA-AuNPs in LPS-inflamed murine Raw 264.7 macrophage cells and mouse retinal inflammation models. Our findings suggest that CGA-AuNPs may be potential candidates for use in the treatment of many inflammatory diseases, warranting further clinical studies to test whether they can effectively inhibit inflammation.

Methods

Reagents and cells

Raw 264.7 cells, widely used murine macrophages, were obtained from the Korean Cell Line Bank (KCLB) and grown in Dulbecco's Modified Eagle's Medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS; Gibco BRL) and penicillin (100 U/mL)/streptomycin (100 μg/mL) (Gibco BRL). Lipopolysaccharide (LPS), CGA, Griess reagent, caffeic acid, (−)-quinic acid, and hydrochloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Instrument measurements

UV-Visible spectra were recorded on a Shimadzu UV-1800 or UV-2600 (Shimadzu Corporation, Kyoto, Japan). HR-TEM images were obtained on a JEM-3010 model (JEOL Ltd., Tokyo, Japan) operating at 300 kV. The samples were freeze-dried in an FD5505 freeze dryer (Il Shin Bio, Seoul, Korea) for FT-IR and XRD analyses. The freeze-dried CGA-AuNP samples were subjected to FT-IR analysis in a Varian 640 IR in attenuated total reflectance (ATR) mode (Agilent Technologies, Santa Clara, CA, USA). A Bruker D8 Discover high-resolution X-ray diffractometer using CuKα radiation (λ = 0.1541 nm) was used for XRD analysis. AFM images were obtained on a Dimension® Icon® instrument (Bruker Nano, Inc., Santa Barbara, CA, USA) in tapping mode. FE-SEM images were acquired on a JSM-7100 F SEM operated at an accelerating voltage of 15 kV (JEOL, Tokyo, Japan). HR-LC-ESI-MS analyses were performed on a Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap instrument (Thermo Scientific, USA) with an ESI interface in the negative-ion mode. LC separation was conducted on a Dionex Ultimate 3000 RSLCnano HPLC system with an INNO 10 column (ODS, 5-μm particle size, 2.0 mm in i.d. × 100 mm in length, Young Jin Biochrom, Republic of Korea). The injection volume and the flow rate were 10 μL/min and 150 μL/min, respectively. The mobile phase was composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Gradient elution was performed as follows: 0-15 min (5-25% B), 15-17 min (25% B), 17-18 min (25-5% B), and 18-20 min (5% B). For HR-LC-ESI-MS analyses, the sample solution was subjected to ultracentrifugation at 21,130 *g* for 1 h at 18 °C (5424R centrifuge, Eppendorf AG, Hamburg, Germany), and the supernatant was taken for HR-LC-MS analysis. ICP-MS samples were analyzed using an ELAN 6100 (Perkin-Elmer SCIEX, Waltham, MA, USA).

Green synthesis of CGA-AuNPs

For the green synthesis of the CGA-AuNPs, a CGA solution (0.25 mM in deionized water, 800 μL) was placed into a glass vial with a cap and heated to boil on a hot plate (set at 220 °C) with stirring for 2 min. To this solution, a solution of hydrochloroauric acid trihydrate (1.0 mM in deionized water, 200 μL) was added dropwise and stirred on the hot plate for an additional 1 min. Then, the reaction mixture was further incubated in an 85 °C oven for 12 h. Caffeic acid and (−)-quinic acid were used as reductants to synthesize the AuNPs through the same procedure described above for the preparation of CGA-AuNPs. For the preparation of the CA-AuNPs (or (−)-quinic acid-AuNPs), a caffeic acid (or (−)-quinic acid) solution (0.25 mM in deionized water, 800 μL) was placed into a glass vial with a cap and heated to boil on a hot plate (set at 220 °C) with stirring for 2 min. To this solution, a solution of hydrochloroauric acid trihydrate (1.0 mM in deionized water, 200 μL) was added dropwise and stirred on the hot plate for an additional 1 min. Then, the reaction mixture was further incubated in an 85 °C oven for 12 h. For the measurement of anti-inflammatory activities, the CGA-AuNPs were concentrated 25-fold under an N_2 atmosphere.

Nitric oxide (NO) assay

A NO assay was performed as described previously.[13]

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