Near-IR sensitive Au-Au$_2$S nanoparticles with biocompatibility for drug delivery

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Abstract

The near-infrared (NIR) sensitive Au-Au$_2$S nanoparticles (NPs) have shown many advantages as potential drug delivery systems. To further investigate biological safety of Au-Au$_2$S NPs, cytotoxicity was estimated by calcein AM/EthD-1 fluorescence staining and the lactate dehydrogenase (LDH) release. The effects of NPs on apoptosis of CHL cells were determined by flow cytometry with Annexin V-FITC/PI double staining. It is evident that the Au-Au$_2$S NPs are non-cytotoxic below IC$_{50}$ dosage.

Introduction

Au-Au$_2$S nanoparticles (NPs) are composites of amorphous Au$_2$S and Au crystallites having distinctive near-IR sensitive properties that depend on their size and composition, being tailored by varying concentration ratio of precursors [1]. Since NIR light (650-1000 nm) with deep penetration in living tissues, the unique optical property of Au-Au$_2$S NPs might open up their fascinating applications in biomedical imaging, photoablation and photodynamic therapy. In our early previous studies [2], cisplatin, one of the most widely used and effective cytotoxic agents in treatment of malignancy of lung, head, neck, testes, and ovary [3], was loaded onto Au–Au$_2$S NPs through an 11-mercaptoundecanoic acid (MUA) layer to develop a targeted drug delivery system. Moreover, irradiation of pulsed NIR laser light (Nd:YAG pulse laser at $\lambda = 1064$ nm) could effectively induce the release of cisplatin from the NPs [2]. However, a crucial issue, which needs to be addressed before NIR sensitive Au-Au$_2$S NPs are commercially available for drug carriers, is toxicity of these materials. The present study was thus undertaken in order to further investigate the cytotoxicity and cell apoptosis inducing by NIR sensitive cisplatin-loaded Au-Au$_2$S NPs for potential cancer therapy.

Materials and methods

Au-Au$_2$S NPs and cisplatin-loaded Au-Au$_2$S NPs were prepared as previously described [2]. The reaction was monitored by using a UV-visible spectrophotometer (DUTM800, Beckman coulter) at a range of 400 - 1100 nm. The morphological examination of the as-synthesized NPs was performed by transmission electron microscope (TEM, JEM 2100) at 120 kV. Cisplatin was loaded onto Au-Au$_2$S...
NPs via a MUA layer.

For cell culture, NPs were filtered by 0.22 µm membranes to be sterilized before use. Cells were cultured in DMEM with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin at 37°C and 5% CO₂. For cell viability assay, Hela cells at a density of 10⁵ cells/well were respectively exposed to cisplatin (100 µg/mL) and cisplatin-loaded Au-Au₂S NPs (100 µg/mL) for 24 h, and then transferred to a glass microscope slide, and incubated with LIVE/DEAD Viability/Cytotoxicity Kit (Sigma) following the protocol. A confocal fluorescence microscope (Olympus FluoView FV300, Japan) supplied with a filter set (EX 450-490 nm, EM 520 nm) was used in order to carry out the observations. For LDH assay, CHL cells at a density of 5×10⁴ cells/well were respectively co-cultured with Au-Au₂S NPs (500 µg/mL) or cisplatin-loaded Au-Au₂S NPs (200 µg/mL) for 24 h, the cell culture medium was then collected for LDH determination, utilizing a commercial available kit following the protocol. The data is presented as mean and standard deviation (SD) calculated over three data points at least. Statistical significance of differences was evaluated by a two tailed unpaired Student t-test. A value of \( P < 0.05 \) was considered significant.

Cell apoptosis induced by NPs was assayed by annexin V-FITC apoptosis detection kit. The kit comprised of two individual stains: the binding of annexin V-FITC to phosphatidylserine present in the outer membrane of cells that are starting the apoptotic process and propidium iodide (PI) to assess cell viability. The cells at a density of 5 x 10⁴/well were co-cultured with NPs for 24 h, and subsequently mixed with binding buffer (HEPES buffered PBS supplemented with 2.5 mM CaCl₂), 2 µL of annexin V-FITC, and 5 µL of PI at 25°C for 15 min in the dark, following the protocol. Fluorescence-activated cell sorting (FACS) was finally used to determine the apoptotic or necrotic cells. Viable, apoptotic, and necrotic cells are designed as annexin-/PI-, annexin+/PI-, and annexin+/PI+, respectively.

Results and discussion

Au–Au₂S NPs were obtained by reduction of tetrachloroauric acid (HAuCl₄) using sodium sulfide (Na₂S) [2]. As illustrated in Fig. 1, the polygonal particles with ~ 40 nm in size exhibited two absorption bands: band I at 527 nm was assigned to the surface plasmon resonance of the Au NPs, whereas band II at 766 nm (NIR region) was attributed to multiply-twinned Au-rich particles containing S [1]. Moreover, we also loaded 30 mg cisplatin onto per mg Au–Au₂S NPs via a MUA layer [2].

To detect the cytotoxicity, calcein AM/EthD-1 fluorescence staining assay was carried out. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, which converts the virtually nonfluorescent cell-permeant calcein AM to the intensely fluorescent calcein, producing an intense uniform green fluorescence in live cells [4]. Dead cells usually are characterized by EthD-1 could enter dead cells, due to the damaged membranes, thereby producing a bright red fluorescence in dead cells; whereas EthD-1 is excluded by the intact plasma membrane of live cells [4]. As shown in Fig.2, it is apparent that exposure to 100 µg/mL cisplatin caused significant cell death compared to the control (unexposed); whereas viable
cells was observed after co-culture with 100 µg/mL cisplatin loaded Au-Au$_2$S NPs, though with an altered shape.

According to the MTT assay [5], 500 µg/mL of Au-Au$_2$S NPs and 200 µg/mL of cisplatin loaded Au–Au$_2$S NPs inhibited cell growth by 50% (IC$_{50}$), respectively. For further evaluating the cytotoxicity at the dosage of IC$_{50}$, LDH release assay was carried out. As shown in Fig. 3, though the LDH level of the cell culture media respectively increased 4.95% and 10.68% for Au-Au$_2$S NPs or cisplatin-loaded Au-Au$_2$S NPs groups, there was no significant difference comparing with the control group. It is thus suggested that at IC$_{50}$ level, those NIR sensitive nanoparticles did not cause cell death.

To further understand whether the those NIR sensitive nanoparticles might cause cell damage through apoptosis in cells, the annexin V- FITC/PI apoptosis assay was...
employed, in which apoptotic cells were probed by green annexin V- FITC dye and the necrotic cells were stained by red dye propidium iodide. Compared to the control group (Fig. 4A), the population of health cells in the Au-Au$_2$S NPs and cisplatin-loaded Au-Au$_2$S NPs groups, decreased by 21.3% (Fig. 4B III) and 22.4% (Fig. 4C III), respectively. Meanwhile, early apoptosis cells showed an increase of 14.7% (Fig. 4B I), 17.6% (Fig. 4C I), and late necrotic cells increased 5.86% (Fig. 4B II), 4.42% (Fig. 4C II), respectively. Nevertheless, 500 $\mu$g/mL Au-Au$_2$S NPs and 200 $\mu$g/mL cisplatin-loaded Au-Au$_2$S NPs could only induce slight necrosis (Fig. 4B, C IV). Combination the earlier data of MTT assay, two-stage cell transformation assay in vitro, hemolysis assay, pathological examine [5-7], it is thus suggested that those NIR sensitive nanoparticles are non-toxic below the maximum recommended dosage.

Summary

The calcein AM/EthD-1 fluorescence staining assay, LDH release assay, and annexin V-FITC/PI apoptosis assay revealed that below IC$_{50}$ dosage, Au-Au$_2$S NPs and cisplatin-loaded Au-Au$_2$S NPs could only induce slight necrosis, but not cell death. Considering the earlier reported data [1, 5-7], it is possible to employ NIR sensitive cisplatin-loaded Au-Au$_2$S NPs for cancer therapy in the future.

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