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Colloidal silver nanoparticles improve anti-leukemic drug efficacy via amplification of oxidative stress

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ABSTRACT

Recently, increased reactive oxygen species (ROS) levels and altered redox status in cancer cells have become a novel therapeutic strategy to improve cancer selectivity over normal cells. It has been known that silver nanoparticles (AgNPs) display anti-leukemic activity via ROS overproduction. Hence, we hypothesized that AgNPs could improve therapeutic efficacy of ROS-generating agents against leukemia cells. In the current study, N-(4-hydroxyphenyl)retinamide (4-HPR), a synthetic retinoid, was used as a drug model of ROS induction to investigate its synergistic effect with AgNPs. The data exhibited that AgNPs with uniform size prepared by an electrochemical method could localize in the lysosomes, mitochondria and cytoplasm of SHI-1 cells. More importantly, AgNPs together with 4-HPR could exhibit more cytotoxicity and apoptosis via overproduction of ROS in comparison with that alone. Taken together, these results reveal that AgNPs combined with ROS-generating drugs could potentially enhance therapeutic efficacy against leukemia cells, thereby providing a novel strategy for AgNPs in leukemia therapy.

1. Introduction

Great advances have been made in the treatment of younger patients with acute myeloid leukemia (AML) in the past thirty years, but prognosis in the elderly patients remains dismal with median survival times of only a few months [1,2]. Although a small percentage of older patients may be cured by standard chemotherapy, it is clear that several aspects of frontline management require improvement and novel strategies and treatment are urgently needed [3,4].

Silver nanoparticles (AgNPs) have been widely used in a variety of biological applications, such as anti-microbial, anti-biofilm, anti-thrombosis, anti-inflammation, and enhancing the wound healing [5,6]. Recently, AgNPs have been proved to have a great potential in the field of cancer treatment because they are selectively involved in disruption of mitochondrial respiratory chain, leading to production of reactive oxygen species (ROS) and interruption of adenosine triphosphate (ATP) synthesis [7–13]. Additionally, the possibility of using AgNPs to treat leukemia cells has been recently explored by our group [14,15]. The fundamental understanding gained in these studies indicated that AgNPs could effectively inhibit the activity of human leukemia cells via generation of ROS and release of silver ions. However, their application in leukemia therapy is greatly restricted due to lack of significant difference of sensitivity upon AgNPs between the clinical isolates from AML patients and cells from healthy donors according to their similar IC50. Interestingly, AgNPs at low concentration but not at high concentrations display stronger AML isolates selectivity over cells from healthy donors, consequently providing a potential way for AgNPs to treat leukemia [14].

Recently, emerging evidence has pointed to oxidative signaling as being a two-edged sword in cancer: moderate increase of ROS may drive cancer, including faster genetic mutations, higher cell proliferation and elevated differentiation rates, whereas higher levels of ROS lead to cell death because cancer cells are more vulnerable to oxidative insults in comparison with normal cells [16,17]. Accordingly, pharmacological agents favoring the generation of ROS are worth exploring in cancer therapy. Indeed, a variety of chemotherapeutic drugs that either generate ROS or inhibit antioxidant enzymes have been developed for cancer treatment [18]. In this regard, the synthetic retinoid fenretinide (N-(4-hydroxyphenyl)retinamide, 4-HPR) has been used clinically

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for some time as an effective chemotherapeutic agent for various cancers by selectively increasing ROS stress [19]. Furthermore, ROS induction has also been shown as a critical mechanism for 4-HPR to eradicate AML cells, including their stem/progenitor cells [20].

Plenty of studies have reported that AgNPs could exhibit the synergistic effect against leukemia cells with chemotherapeutic drugs, such as daunorubicin, cyclophosphamide or busulfan via enhanced the intracellular drug accumulation concentrations [21,22]. However, the role of oxidative stress in this synergistic effect remains unclear. The dual roles of oxidative signaling suggest that it is an effective strategy for cancer therapy to heighten ROS levels and alter redox status [23]. Therefore, we hypothesized that AgNPs in combination with 4-HPR could enhance the cytotoxic effect against AML cells by inducing higher levels of ROS.

In this study, we investigated whether AgNPs could enhance the therapeutic effect of ROS-generating agent, 4-HPR toward AML cells. Both AgNPs and 4-HPR could decrease cell viability and induce apoptosis. Importantly, 4-HPR together with AgNPs displayed higher cytotoxic effect. Meanwhile, their combination could result in higher levels of intracellular ROS in comparison with that alone. The data presented above suggest that the synergistic effect between AgNPs with ROS-generating agents may facilitate the use of AgNPs and chemotherapeutic drugs at lower concentrations, thereby with overall decrease in toxicity to normal cells, offering the possibility of using AgNPs to effectively treat leukemia.

2. Materials and methods

2.1. Preparation of silver nanoparticles

In this study, silver nanoparticles (AgNPs) were prepared by a continuously flow electrochemical method as previously reported [14]. A good biocompatible polymer, polyvinyl pyrrolidone (PVP-K30), was used as a stabilizing agent, and it is of analytical grade and used without further purification. Two silver rods with the diameter of 2 mm and purity of 99.99% were used as electrolytic electrodes. A classical synthesis process is as follows: firstly, the silver electrodes were polished, washed, and fitted on the cover of electrolytic reactor. Secondly, 5 mg/mL PVP aqueous solution was prepared as the electrolyte and stabilizer. Finally, the PVP solution was continuously pumped into the electrolytic reactor by the peristaltic pump under a magnetic stirring condition at 60 °C, and simultaneously a voltage of 10 V was applied to the silver electrodes. After the reactor was filled, the product flowed out from outlet tube of the reactor's cover.

2.2. Nanoparticle characterization

The lyophilized powders of AgNPs were obtained after filtration, centrifugation and lyophilization. The as-synthesized nanoparticles were primarily characterized by UV-vis spectrophotometer (SHIMADZU, Japan) followed by transmission electron microscopy (TEM, JEM-2000EX, JEOL, Japan). TEM samples were prepared by placing a few drops of their aqueous dispersions on carbon-coated copper grids and drying at room temperature. The mean size was calculated from a random field of TEM images that showed the general morphology of nanoparticles. More than 200 particles were counted and measured to determine the mean sizes and size distributions. Fourier transform infrared spectroscopy (FTIR) analysis was performed to identify the possible interfacial groups between capping agent and AgNPs. The final silver concentration in aqueous solution was determined by inductively coupled plasma-mass spectrometry (ICP-MS).

2.3. Cell culture

SHI-1 cells, a human acute myeloid leukemia cell line, were cultured at 37 °C in an incubator with a humidified atmosphere of 5% CO_2 in RPMI 1640 (Hyclone, USA) with 10% fetal bovine serum (FBS, Hyclone) and penicillin/streptomycin mix (100 U/mL and 100 mg/mL, respectively).

2.4. Cell viability assay

Cell viability was measured using Cell Counting Kit-8 (CCK-8) according to the manufacturer's instruction with appropriate controls. Cells were seeded in 96-well microtitre plates (1×10^4 cells/200 μL culture medium/well) with AgNPs and/or 4-HPR. 24 h later, CCK-8 reagent was added to each well and cells were incubated for 4 h, and then the absorbance at two wavelengths (450 nm for soluble dye and 650 nm for viable cells) was detected using a microplate reader (SpectraMax M5, Molecular Devices, Sunnyvale, CA, USA).

2.5. Annexin V/PI double staining assay

For Annexin V–FLUOS/propidium iodide (PI) assays (Roche), cells were stained and analyzed for apoptosis according to the manufacturer's protocol. Briefly, 1×10^5 cells were stained with incubation buffer comprising 2 μL Annexin V–FLOUS and 2 μL PI for 20 min at room temperature in dark. The apoptotic/necrotic cells were analyzed by flow cytometry (Calibur™, Becton-Dickinson).

2.6. Quantification of intracellular ROS

To measure the intracellular generation of ROS, cells were loaded with 10 μM 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCF-DA, Sigma, USA) in RPMI 1640 without phenol red for 30 min, washed with PBS and re-suspended in RPMI 1640 without phenol red. Cells were exposed to AgNPs and/or 4-HPR for 3 h in dark (37 °C, 5% CO_2) and immediately analyzed with flow cytometry. The 488 nm laser was used for excitation and fluorescence was detected in FL-1 by a 525/30 BP filter. For each sample, the mean fluorescence intensity (MFI) was determined and presented its intracellular generation of ROS.

2.7. Intracellular localization of AgNPs

Cells were cultured in 100-mm tissue culture dishes followed by AgNPs treatment. For control experiments, medium without nanoparticles was used. After incubation for 24 h, cell pellets were washed with PBS, and then fixed with 2.5% glutaraldehyde in PBS for 24 h followed by post-fixation in 1% osmium tetroxide (Agar Scientific, Stansted Essex, England, UK) for 1.5 h. Cell pellets were dehydrated through a series of ethanol concentrations (20%, 30%, 40%, 50%, 60%, 70%, and 90%) followed by treatment with 2% uranyl acetate in 95% ethanol (Enblock stain) for 1 h and further dehydration with 100% ethanol for 1 h. Cell pellets were finally treated with propylene oxide (twice for 15 min each) followed by 1:1 propylene oxide: araldite resin overnight, infiltrated with fresh araldite resin (3 changes with a gap of 3–4 h), and then subsequently embedded in araldite resin at 60 °C for 48 h and ultra-thin sections were cut with glass knives in an ultra microtome (LEICA EM UC6, Netherlands). The sections were mounted on copper grids and stained with 1% aqueous uranyl acetate and 0.2% lead citrate. The stained sections were scanned with TEM (JEM-2000EX, JEOL) for ultra structural observations at 80 kV.