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Doxorubicin-conjugated pH-responsive gold nanorods for combined photothermal therapy and chemotherapy of cancer

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ABSTRACT

Cancer chemotherapy can be hindered by drug resistance which leads to lower drug efficiency. Here, we have developed a drug delivery system that tethers doxorubicin to the surface of gold nanorods via a pH-sensitive linkage (AuNRs@DOX), for a combined photothermal and chemical therapy for cancer. First, AuNRs@DOX is ingested by HepG2 liver cancer cells. After endocytosis, the acidic pH triggers the release of doxorubicin, which leads to chemotherapeutic effects. The gold nanorods are not only carriers of DOX, but also photothermal conversion agents. In the presence of an 808 nm near-infrared laser, AuNRs@DOX significantly enhance the cytotoxicity of doxorubicin via the photothermal effect, which induces elevated apoptosis of hepG2 cancer cells, leading to better therapeutic effects in vitro and in vivo.

1. Introduction

The development of drug resistance is a major obstacle to the success of cancer chemotherapy [1–6]. Developing drug delivery systems using nanotechnology can potentially address this challenge. One possible approach is to inhibit or bypass the P-glycoprotein (P-gp) channel, a membrane-bound active efflux pump which is often overexpressed in the plasma membrane of drug resistant cancer cells. This pump can efflux a broad range of anticancer drugs, so such a strategy might achieve better therapeutic effect [7,8]. For example, it has been shown that certain polymeric carriers can sensitize drug resistant cells to a group of cytotoxic drugs by inhibiting the P-gp drug efflux system [9–11]. Another possible approach is to deliver short interfering RNA (siRNA) that targets the gene encoding P-gp to cancer cells. This would down-regulate P-gp expression and thereby restore intracellular drug levels to the concentrations required for induction of cytotoxicity [12,13].

Combinational therapy has also been acknowledged as a promising strategy to improve therapeutic efficiency. The combination of different drugs and therapeutic modalities may cooperatively suppress cancer development through synergistic effects and reversal of drug resistance [14–22]. Photothermal therapy (PTT) is a rapidly developing cancer phototherapy modality that combines a photosensitizer and a light source. It can effectively induce toxicity to malignant and diseased cells. Near infrared (NIR) laser mediated photothermal therapy in particular has attracted increasing attention for cancer therapy due to its deep tissue penetration and minimal skin/blood absorbance [23–26]. One potential cancer that can be treated with phototherapy is melanoma, a cancer of the skin. Other tumors inside the body require additional equipment, such as optic fiber to guide light into body. Among various NIR photothermal transducers reported thus far, gold nanorods (AuNRs) have been extensively investigated due to their excellent biocompatibility and tunable surface plasmon resonance (SPR) property to convert NIR light into local heat [27,28]. Therefore, we expect advanced anti-cancer efficiency can be achieved by

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combining AuNRs-based PTT and simultaneous application of chemotherapeutic drugs.

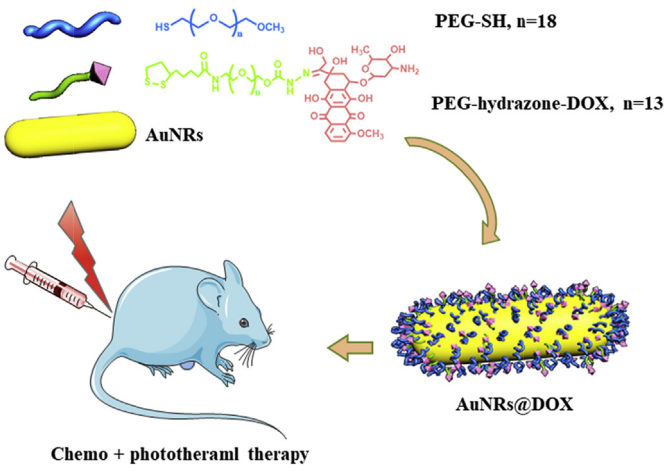
There have been several previous investigations of using spherical gold nanoparticles to deliver small chemotherapeutic molecules and large biomacromolecules, including doxorubicin (DOX), small interfering RNA (siRNA), and proteins [19–22]. However, the drugs are usually stably conjugated to the gold nanoparticles, leading to slow release and subsequently reduced toxicity which is unfavorable for the combinational therapy. It would be useful to develop a responsive drug delivery system based on AuNRs with controlled drug release in certain organelles. Wang et al. successfully conjugated DOX to the surface of spherical gold nanoparticles via an acid-labile linkage to overcome multidrug resistance in cancer cells. This represented a combination of highly efficient cellular entry and a responsive intracellular release of DOX from the gold nanoparticles in acidic organelles [29–31]. The photothermal effect may also lead to much faster drug release in tumor cells/tissues, leading to a better tumor therapy effect [16].

Therefore in this study, we conjugated DOX onto the surface of AuNRs with a poly(ethylene glycol) spacer via a pH-responsive linkage (Scheme 1) [29]. This arrangement is favorable for chemotherapy because of its combination of enhanced DOX cellular entry and rapid drug release in acidic organelles. In addition, photothermal therapy was achieved in the presence of 808 nm NIR light to completely ablate cancer cells in vitro and in vivo.

2. Materials and methods

Materials. Doxorubicin hydrochloride was purchased from Zhejiang Hisun Pharmaceutical Co. Ltd., China. α-lipoyl-ω-doxorubicinyl poly(ethylene glycol) with a hydrazone linker (LA-PEG-Hyd-DOX) was synthesized according to literature (Fig. S1) [29]. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), thiol functionalized mPEG (Mn ~800 D), propidium iodide (PI) and Annexin V-FITC apoptosis detection kit were purchased from Sigma-Aldrich. Milli-Q water was used throughout the experiments. Other common chemicals were of analytical grade and used as received if without specific description.

Preparation of DOX and PEG modified AuNRs. Cetyltrimethylammonium bromide (CTAB) coated AuNRs were prepared according to previous established methods [32]. About 10 mg AuNRs were centrifuged twice at 10,000× g for 15 min to remove free CTAB ligands, and then were incubated in 5 mg/mL thiol PEG or LA-PEG-Hyd-DOX solutions in the presence of 1 mM $NaBH_4$, followed by gentle shaking for 2 days. AuNRs@PEG and AuNRs@DOX were collected by centrifugation (10,000× g for 15 min) and washed five times against water to remove free polymers. The production yields for AuNRs@PEG and AuNRs@DOX from CTAB capped AuNRs are 63.7%wt and 57.6%wt respectively. The loss of AuNRs is mainly happened during centrifugation process after ligand exchange.



Scheme 1. Schematic illustration to show synthesis of α-lipoyl-ω-doxorubicinyl poly(ethylene glycol) with a hydrazone linker and its application to cap gold nanorods to prepare doxorubicin (DOX) conjugated pH responsive gold nanorods (AuNRs) for cancer therapy.

General characterizations. The AuNRs were observed under transmission electron microscopy (TEM, JEM-1230EX). Hydrodynamic size and zeta potential of the AuNRs were measured by dynamic light scattering (DLS, Zetasizer Nano, Malvern), using a 633 nm He-Ne laser at room temperature. The content of gold was determined using an ICP-MS (Xseries II, Thermo Elemental Corporation, USA).

Release of DOX from DOX-conjugated AuNRs. AuNRs@DOX were incubated in phosphate buffer (PBS, 0.02 M, pH 7.2) or acetate buffer (0.02 M, pH 4.5). The fluorescence emission spectra were recorded at various time intervals. To quantitatively determine the release of DOX, AuNRs@DOX (100 μg/mL) were suspended in PBS or acetate buffer in a dialysis membrane tube (M_W cutoff = 14,000 D, Spectrum Laboratories, USA), and the tube was immersed in 15 mL of PBS or acetate buffer, in a shaking water bath at 37 °C. At predetermined time points, the external buffer was collected and replaced with an equal volume of corresponding buffer. The collected release medium was freeze-dried and dissolved in acetonitrile/water (50/50, v/v), and the concentration of DOX was analyzed by HPLC.

Cell uptake. Human liver carcinoma cell (HepG2) was purchased from Typical Culture Collection of Chinese Academy of Science (Shanghai, China). HepG2 cells were cultured with Dulbecco's modified Eagle medium (DMEM, Gibco), supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. In order to quantify the amount of ingested AuNRs, cells were seeded in a 6-well plate and cultivated to over 80% confluence. The medium was replaced with fresh one containing different AuNRs. After specific time intervals, the cells were carefully washed with PBS to remove the free AuNRs in the medium and the loosely adsorbed ones on the cell surface, and then the cells were harvested by trypsinization [33,34]. The number of cells in each sample was quantified using a Neubauer chamber. The cells were then centrifuged and subsequently dissolved in aqua regia overnight. Finally, samples were diluted 1000 times and measured by ICP-MS to determine the cellular Au content. The cells incubated in particle free medium were used as a control.

In order to track DOX inside cells, HepG2 cells were seeded in 24-well plate at a density of 5×10^4 cell/well and cultured overnight. The cells were incubated with 20 μg/mL AuNRs@DOX for different periods of time. Cells were washed with PBS, stained with lysotracker Green at 37 °C for 15 min, and then observed under confocal laser scanning microscopy (CLSM, TCS SP5, Leica) [39].

Combinational therapy in vitro. Cells were plated in a 96-well plate and incubated overnight for cell adhesion. The medium was replaced with fresh one containing varying concentrations of the AuNRs. After 24 h incubation, the cells were carefully washed with PBS and irradiated using a NIR laser at 808 nm with a beam spot of ~6 mm in diameter at a power of 0.1 W/cm^2 for different time.

After another 24 h incubation, the cell viability was measured using the MTT method [36–38]. The absorbance that has a proportional relationship with the number of living cells and cell viability was recorded at a wavelength of 570 nm by a microplate reader (Model 680, Bio-rad).

The cell apoptosis of HepG2 cells was measured by using a flowcytometry assay based on Annexin V-FITC and PI staining. The cells were incubated with samples for 24 h and irradiated for 1 min.