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Research article

Enhanced apoptosis and inhibition of gastric cancer cell invasion following treatment with LDH@Au loaded Doxorubicin

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ABSTRACT

Background: The suppression of cancer cell growth and invasion has become a challenging clinical issue. In this study, we used nanotechnology to create a new drug delivery system to enhance the efficacy of existing drugs. We developed layered double hydroxide by combing Au nanosol (LDH@Au) and characterized the compound to prove its function as a drug delivery agent. The anti-cancer drug Doxorubicin was loaded into the new drug carrier to assess its quality. We used a combination of apoptosis assays, cell cycle assays, tissue distribution studies, cell endocytosis, transwell invasion assays, and immunoblotting to evaluate the characteristics of LDH@Au as a drug delivery system.
Results: Our results show that the LDH@Au-Dox treatment significantly increased cancer cell apoptosis and inhibited cell invasion compared to the control Dox group. Additionally, our data indicate that LDH@Au-Dox has a better target efficiency at the tumor site and improved the following: cellular uptake, anti-angiogenesis action, changes in the cell cycle, and increased caspase pathway activation.
Conclusions: Our findings suggest the nano drug is a promising anti-cancer agent and has potential clinical applications.

1. Introduction

Gastric cancer is the third most common cause of cancer-related mortality worldwide, and the 5-year survival rate is less than 30% [1,2]. There is a high incidence rate in of gastric cancer in Asia. Surgical resection is currently the most effective treatment and chemotherapy has a crucial role following surgical resection [3,4,5]. Anthracycline treatment and the combination of paclitaxel and thymidine phosphorylase are the key chemotherapeutic options. However, these treatments have different complications. Thus, potent antitumor agents with limited toxicities are needed in the clinic. Doxorubicin (Dox) is a chemotherapeutic drug that induces cell senescence at a low concentration and can induce apoptosis at higher concentrations [6]. Approximately 10% of Dox-treated patients have a risk of developing cardiac complications. Therefore, improving the efficacy and reducing the toxicity of Dox could provide a new possible treatment option [7].

The medical application of nano materials has increased, and there have been several achievements in both disease diagnosis and therapy [8,9]. Gold nanoparticles (Au nanoparticles, AuNPs) are considered as promising candidate tools for nanobased medicines and can be used in biosensors, bioimaging, photothermal therapy, and targeted drug delivery. AuNPs have high chemical stability, well-controlled size, and surface functional properties. Thus, these compounds are emerging as promising agents for anti-cancer treatment [10,11].

Layered double hydroxide (LDH) has the general formula of $[M^1\_x2 + Mx3 + (OH)_2]$ $[Ax/n]$ mH_2O. These materials are highly biocompatible, show low toxicity and have satisfactory loading efficiency. Several previous studies reported that LDH has acceptable controlled-release properties, which make it a suitable tool for drug delivery [12,13,14].

In this study, we synthesized high-quality LDH and combined Au nanosol to form a new nano-carrier system (LDH@Au). LDH@Au can take full advantage of the two nano materials and performed better as a carrier of anti-cancer medicine. LDH@Au bound Dox (LDH@Au-Dox) using ionic-exchange and showed significantly improved antitumor efficacy in gastric carcinoma cells compared to the control drug.

2. Methods

2.1. Materials and cell culture

The gastric cancer cells SGC-7901 were obtained from the Institute of Cell Biology (Shanghai, China) and cultured in the RPMI-1640 medium (HyClone, MA, USA) with 10% deactivated fetal bovine serum

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(FBS, Gibco, NY, USA) and 1% penicillin–streptomycin. The cells were incubated at 37°C with 5% CO_2.

2.1. Preparation of LDH, LDH@Au, and LDH@Au-DOX

The Mg–Al/LDH was synthesized by hydrothermal treatment. Briefly, Al $(NO_3)_3 \cdot 9H_2O$ (0.372 g, 0.002 M) and Mg $(NO_3)_2 \cdot 6H_2O$ (0.769 g, 0.006 M) were dissolved in 10 mL deionized ddH_2O and then the mixed salt solution was added into 40 mL NaOH (0.272) solution. The mixed solution was stirred at 60°C for 0.5 h in an N_2 atmosphere.

The solutions of $HAuCl_4 \cdot 4H_2O$ (2.44×10^{-3} mol/L), $Na_3C_6H_5O_7 \cdot 2H_2O$ (3.43×10^{-2} mol/L), polyvinylpyrrolidone (PVP, 1.00×10^{-4} mol/L) and $NaBH_4$ (0.391 mol/L) were pre-made for use.

We added 10 mL $HAuCl_4 \cdot 4H_2O$ solution to 80 mL ddH2O and stirred at 600 r/min for 2 min before heating to 75°C. A reducing agent ($Na_3C_6H_5O_7$ or $NaBH_4$) was quickly added into the mixed solution and heated for 9 min before stirring for 5 min. The mixed solution was cooled to room temperate, which led to the formation of Au nanosol.

The Mg–Al/LDH and Au nanosol were mixed in ddH_2O and stirred at room temperate in an N_2 atmosphere for 24 h. The LDH@Au composite nanomaterial was formed through anion exchange.

The Dox (10 mg) and LDH@Au (30 mg) were mixed in 20 mL deionized ddH_2O. The mixture was stirred at room temperate for 24 h and centrifuged twice. The supernatant was then removed and the precipitate was lyophilized to obtain the LDH@Au-Dox.

2.2. Transmission Electron Microscopy (TEM)

The surface morphology of LDH, Au, and LDH@Au was examined using a transmission electron microscope (JEOL, Tokyo, Japan). Drops of LDH, Au, and LDH@Au solutions were placed on the carbon-coated copper TEM grids (150 mesh, Ted Pella Inc., Rodding, CA, USA). The samples were observed at 100 kV under a microscope.

2.3. Size distribution by intensity study (DLS)

The size distribution of LDH@Au was determined by DLS. The LDH@Au (1 mg/mL) was prepared by diluting 100 μL samples in a 1-mL aqueous solution. The distribution was measured at 25°C by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK).

2.4. Zeta potential

The zeta potential values of LDH@Au were measured based on the electrophoretic mobility using samples with concentrations (10 μg/mL) defined by photon correlation spectroscopy (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 25°C.

2.5. Cellular cytotoxicity

SGC-7901 cells were seeded into a 96-well plate at a density of 1×10^4/per well. The cells were incubated overnight and then treated with Dox, LDH@Au, and LDH@Au-Dox at various concentrations (0–4 μg/mL) for 24 h. The control group received no treatment. All the cells were incubated with 10 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution for 4 h at 37°C. Each well was then treated with 150 μL dimethyl sulfoxide (DMSO). The absorbance was measured at 490 nm using a microplate reader and the cell viability was calculated [15].

2.6. Cell cycle assay

Cell cycle status was measured in SGC-7901 cells treated with 3 μg/mL Dox or LDH@Au-Dox for 24 h. The cells were then fixed with 70% ethanol for 2 h. The control group received no treatment. The cells were then stained with propidium iodide (PI), and the cell cycle distribution was analyzed by flow cytometry [16].

2.7. Apoptosis assay

The in vitro apoptosis of SGC-7901 was analyzed by flow cytometry. Briefly, SGC-7901 cells were seeded onto 6-well plate at 1.5×10^6 cells per well and incubated overnight to form monolayer cells. The cells were divided into different groups and each group was incubated with LDH-Au, Dox, and LDH-Au-Dox at concentrations ranging from 0.5 μg/mL to 4.0 μg/mL or without any treatment (control) at 37°C for 24 h. The cells were collected and stained with Annexin V APC and PI before analysis by flow cytometry (Becton Dickinson, San Jose, CA).

2.8. Cell endocytosis and TEM observation of cellular uptake

Endocytosis was investigated by confocal microscopy (Leica TCS SP5, Leica Microsystems GmbH, Germany). SGC-7901 cells were seeded on confocal dishes at a concentration of 0.8×10^6 per plate and then incubated overnight to form monolayer cells. The cells were treated with either Dox or LDH@Au-Dox at same concentration (4.0 μg/mL) for 24 h. The cells were washed twice with PBS and stained with DAPI to localize the nuclei.

SGC-7901 cells were treated with different concentrations of LDH@Au-Dox for 2 h and centrifuged at 1000 r/min for 5 min to observe cellular uptake by TEM. The pellet was washed by PBS and fixed with 2% glutaraldehyde. Then, the cells were washed and evaluated using the TEM assay described in Section 2.2.

2.9. Tissue distribution

We subcutaneously injected 1×10^6 SGC-7901 cells in nude mice to form tumors. The xenograft animals were then treated with Dox or LDH@Au-Dox (10 mg/mL) by i.p. injection. The treated mice were sacrificed after 24 h. The organs and tumor tissue were homogenized and extracted. The drug level was measured by HPLC.

2.10. Transwell invasion assay

The Matrigel™ Matrix (BD Biosciences, San Jose, CA, USA) was diluted in serum-free medium and used to coat 24-well transwell inserts (BD Biosciences). SGC-7901 cells were serum-starved for 4 h and then collected and resuspended in 200 μL serum-free medium at a concentration of 1×10^5 cells with 5 μg/ml of LDH@Au, Dox, or LDH@Au-Dox. The cells were loaded into the upper chamber of the wells. We then placed 600 μL of medium containing 10% FBS in the lower chamber. The cells on the upper surface of the filters were gently removed and the invasive cells on the lower surface were fixed with ethanol after 24 h. The cells were stained with AM-calcein. We counted five fields for each group using a microscope [17].

2.11. Western blotting

SGC-7901 cells were treated with 4 μg/mL Dox or LDH@Au-Dox. The cells were collected and washed with PBS after 16 h. We then separated 20 μg of protein lysate by sodium dodecyl sulfate gel electrophoresis and transferred the proteins to PVDF membranes. The membranes were probed for β-actin, cytochrome-C, caspase-3, and caspase-9 (Cell Signaling Technology, MA, USA). The protein signals were detected using the ECL detection system and quantified by BandScan.

2.12. Statistical analysis

All data are expressed as the mean ± SD and analyzed with T-test. All values of $P < 0.05$ were considered statistically significant.