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RESEARCH PAPER

Preparation and *in Vitro* Anti-Laryngeal Cancer Evaluation of Protopanaxadiol-Loaded Hollow Gold Nanoparticles

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Abstract: Protopanaxadiol (PPD) has inhibitory effects on a variety of tumor cells, but the poor water solubility and low availability limit its clinical application. By using polyethylene glycol (PEG)-modified hollow gold nanoparticles (HAuNs-PEG) as the transport carriers, we prepared here the PPD-loaded HAuNs-PEG (HAuNs-PEG-PPD). And then we conducted a number of experiments to investigate the in vitro anti-laryngeal cancer effect (Hep-2 cell as a model) of HAuNs-PEG-PPD, such as using high performance liquid chromatography (HPLC) to detect the sustained release effect, MTT assay to detect the inhibitory effect on the proliferation of Hep-2 cells, and flow cytometry to determine the apoptosis of Hep-2 cells. The results showed that HAuNs-PEG-PPD had an obvious sustained release effect. Compared with those in the control group, HAuNs-PEG group and PPD group, the survival rate of Hep-2 cells decreased and the apoptosis rate increased significantly ($p < 0.01$) in the HAuNs-PEG-PPD group. The HAuNs-PEG- PPD could significantly enhance the anti-laryngeal cancer effect of PPD and promote the apoptosis of tumor cells. Thus, the HAuNs is a promising drug delivery system of PPD in clinical treatment of laryngeal cancer.

Key Words: Protopanaxadiol; Hollow gold nanospheres; Laryngeal cancer; Cell apoptosis

1 Introduction

As a main component of ginseng, protopanaxadiol (PPD) has an obvious inhibitory effect on a variety of tumors[1–8]. However, its clinical bioavailability is very low owing to the poor water solubility of PPD. As a novel drug delivery vehicle, nano-drug delivery system has drawn much attention due to the less side effects, higher drug bioavailability and better therapeutic effect. In this regard, several kinds of nanomaterials containing high Z elements (gold, silver, iodine, gadolinium, etc.) have been widely studied as the drug delivery vehicles in recent years[9–11]. Especially, gold nanoparticles (AuNPs) show significant advantages in the field of drug delivery systems, based on their good biocompatibility and bioinertness, high atomic number, ease of synthesis and surface modification[12–20]. Recently, You *et al*[21] loaded DOX onto the PEG-modified hollow gold nanoparticles (DOX@PEG-HAuNs) to investigate the combined effect of photothermal therapy and chemotherapy of the drug delivery system. The results showed that DOX@PEG-HAuNs had stronger antitumor activity and lower cardiotoxicity than the free DOX and DOX liposomes.

In view of the distinctive advantages of hollow structure, large volume, thin and sturdy shell and so on, the HAuNs were used for loading and carrying the small molecule, PPD, and ingeniously combined with PPD to construct a novel drug delivery system for the first time. After being loaded on HAuNs, the water solubility and bioavailability of PPD was easily increased, and the obvious sustained release effect could make it more suitable for anti-tumor therapy. Meanwhile, the modification of PEG molecules onto the surface of HAuNs enhanced the biocompatibility of nanomaterials. In addition, we successfully used PEG-modified HAuNs as a PPD carrier to construct a novel drug delivery system for PPD and systematically studied its anti-laryngeal cancer cells effect in vitro.

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2 Experimental

2.1 Instruments and reagents

FEI Tecnai G2 F20 S-Twin Transmission Electron Microscope (American FEI company), X'Pert Pro x-ray diffractometer (Dutch PANalytical B.V), Vertex Perkine-Elmer 580BIR Fourier Transform Infrared Spectroscopy (American VARIAN company), Prominenece UFLC High Performance Liquid Chromatograph (Japanese SHIMADZU corporation), U-3310 UV-Visible-NIR Spectrophotometer (Japanese Hitachi Limited), M630 Multifunctional Microplate Reader (American Hercules company, FACSCanFlow Cytometry (American Becton Dickinson company) and MCO-20AIC CO2 Incubator (Chinese Sanyo company) were used in this experiment.

Chlorauric acid (III) (analytical grade) was purchased from Shanghai Sahn Chemical Technology Co., Ltd. $CoCl_2$•$6H_2O$ (analytical grade), trisodium citrate (analytical grade) and $NaBH_4$ (analytical grade) were purchased from Beijing Chemical Co., Ltd. PEG-SH and Hep-2 cells lines were purchased from American Nanocs company and Shanghai Institutes for Biological Sciences, respectively. PPD (> 99% purity) was a gift from the Organic Chemistry Laboratory of Jilin University. RPMI-1640 medium and fetal calf serum were purchased from American Hyclone company. DMSO, Penicillin and streptomycin were purchased from Shanghai Ding Guo Biotechnology Co., Ltd. MTT was purchased from American Sigma company. TUNEL Apoptosis Detection Kit was purchased from Swedish Roche Group. Experimental water was the ultrapure water (18.2 MΩ cm) prepared by Milli-Q (American Millipore company).

2.2 Synthesis of hollow gold nanoparticles (HAuNs)

The HAuNs were synthesized according to the previous work[22]. Cobalt nanoparticles were first prepared. The cobalt chloride solution (0.1 mL, 0.4 M) was added into the deoxygenating deionized water containing the fresh prepared sodium borohydride solution (0.1 mL, 1 M) and sodium citrate solution (0.4 mL, 0.1 M). The mixture solution reacted for 60 min under constant nitrogen flow. Then, the as-prepared sodium borohydride-free cobalt nanoparticles solution (30 mL) was injected into the deoxygenating chloroauric acid aqueous solution (10 mL, 1 μM). After reacting for other 10 min under nitrogen flow, the solution was exposed to air until the cobalt was completely oxidized. The products were separated by centrifugation at 10000 rpm for 5 min and washed three times with deionized water.

2.3 Synthesis of HAuNs-PEG

HAuNs-PEG was obtained by ligand exchange method[23]. The as-synthesized HAuNs were dispersed in deionized water (1.0 mg mL^{-1}, 40 mL). Then, 150 mg PEG-SH was added and the mixture was stirred overnight at room temperature. After the reaction, the product was washed three times with deionized water to remove the unreacted PEG and dispersed in 40 mL ethanol.

2.4 PPD loading and drug release in vitro

The free PPD solution (1.0 mg mL^{-1}, 40 mL, ethanol) was added into an ethanol solution of HAuNs-PEG (1.0 mg mL^{-1}, 40 mL). And the mixtures were stirred at 37 °C for 24 h. The precipitate was separated by centrifugation and washed with ethanol three times. With all supernatants collected together, the actual drug loading content of PPD was calculated by the reduced weight of the PPD in the supernatant. The PPD loading capacity (LE) was 30% according to the following equation:

$$LE\ (\%) = (m_{\text{original PPD}} - m_{\text{remaining PPD}})/m_{\text{HAuNs-PEG}} \times 100\% \quad (1)$$

Chromatographic conditions: chromatographic column CORTECS C_{18} (100 mm × 4.6 mm, 2.7 μm) was chosen. The mobile phase A was 0.1% glacial acetic acid, and mobile phase B was acetonitrile. Isocratic elution was conducted at a volume ratio of A: B = 20:80, at flow rate of 0.5mL $min^{-1}$and at room temperature, sample size was 10 μL. The investigation of linear relationship: PPD was weighed with precision, dissolved in ethyl alcohol, diluted with ethyl alcohol to 10 mL (5, 10, 25, 40, 100 and 200 μg mL^{-1}) and preserved at 4 °C. Then 10 μL the sample was injected and measured the peak area. At last, by taking the concentration of PPD as the X-axis, the peak area as the Y-axis, the standard curve was drawn and the count correlation regression equation was obtained. Preparation of the sample under test: 500 μL of HAuNs-PEG-PPD was added into 4 mL RPMI-1640 medium, and then was placed in the incubator with the CO_2 volume fraction of 5% at 37°C. After that 100 μL of supernatants was stored at 4 °C for 30, 60, 120, 180 and 240 min, respectively.

Data processing: The peak area was introduced into the regression equation, then the dosage was obtained and the PDD release rate of the drug over time was calculated by taking the amount of PPD in the 500 μL compound as 100%.

2.5 Experimental grouping and cell culture in vitro

Hep-2 cells were divided into 4 groups: the control group, the HAuNs group, the PPD group, and the HAuNs-PEG- PPD group (The concentration of PPD, HAuNs-PEG-PPD and HAuNs corresponding with loading concentration were used for unified concentration unit when the drug was exposed). Hep-2 cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Gibco) and 1% antibiotics (100 IU mL^{-1} of penicillin and 100 mg mL^{-1} of streptomycin), and placed in the incubator with the CO_2 volume fraction of 5% at 37 °C.