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Antitumor activity of hyaluronic acid–selenium nanoparticles in Heps tumor mice models

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ABSTRACT

In this study, hyaluronic acid–selenium (HA–Se) nanoparticles as novel complexes were synthesized and their antitumor activities *in vivo* were investigated. The mice inoculated with Heps tumor were orally administered with HA–Se nanoparticles at 86.45 mg/kg (H) and 4.32 mg/kg (L) body weights as high and low doses respectively (2.20% selenium content in the HA–Se nanoparticles samples by ICP-AES) for 10 days. The transmission electron microscopy (TEM) results indicated that the HA–Se nanoparticles were spherical with mean size of 50–70 nm. The HA–Se nanoparticles could significantly reduce tumor weights at the tumor inhibition ratios of 46.92% (H) and 49.12% (L) respectively. However, in the 5-fluorouracil positive group (25 mg/kg), the tumor inhibition ratio was 61.71%. From the study, the HA–Se nanoparticles (4.32 mg/kg) significantly increased thymus and spleen relative weights, enhanced the activities of superoxide dismutase (SOD), reduced the formation of malondialdehyde (MDA) and the activities of aspartate transaminase, alanine transaminase and crea in Heps tumor mice. The results of the study indicated that the HA–Se nanoparticles are potential antitumor candidate for cancer treatment.

1. Introduction

Selenium was discovered in 1817 and considered as an essential trace element for humans and animals. It can improve the activities of seleno-enzyme and glutathione peroxidase (GPx), and also prevent free radicals from damaging cells and tissues *in vivo*. Selenium has additional important health effects particularly in relation to the immune response, cancer prevention and anti-diabetic effects [1–3].

Selenium deficiencies among human beings as well as animals are recognized to be related to a number of pathologies. Low or lack of Se in humans could be the possible cause of diseases including cancer, cardiovascular sclerosis, cirrhosis, diabetes, *etc.* [4]. Epidemiological data has shown a good correlation between the concentration of selenium in environment and the incidence of Se-responsive human diseases [5]. The supplementation of food with selenium concludes organic and inorganic selenium-containing compounds. With the current development of nanoscale materials, more and more studies now focuses on synthesizing of selenium nanoparticles. Selenium nanoparticles are less toxic than selenite (inorganic selenium) and high-selenium protein (organic selenium) [6]. Furthermore, studies have shown that Se nanoparticles have excellent bioavailability which exhibit novel antioxidant activities *in vitro* and *vivo* and also protect against hepatic injury [7–10].

Hyaluronic acid (HA) is a linear, negatively charged polysaccharide, consisting of two alternating units of D-glucuronic acid and N-acetyl-D-glucosamine. It is considered as a good candidate for pharmaceutical applications due to its diverse sources, biocompatibility, biodegradability, non-toxicity, non-immunogenicity and abundance of functional groups (—COOH, —OH) for modification or functionalization [11–14]. HA has been used for the design of tumor-targeting drug delivery vehicles for anticancer drug such as paclitaxel and doxorubicin [13,15,16].

Methods of synthesizing selenium nanoparticles including chemical reduction, sonochemical process, γ-radiolytic reduction and wet chemical method [17–19]. Nowadays, synthesizing of selenium nanostructures with biomacromolecules has attracted much attention. Researches have shown that selenium nanoparticles can be produced using protein BSA, L-cysteine, glucose, sucrose, chitosan and sulfated polysaccharides as stabilizer in the redox system with mean particle size diameter ranging from 24 nm to 200 nm [20–22]. In this study, hyaluronic acid was used as a stabilizer

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to synthesize selenium nanoparticles. Moreover, the antitumor activity of HA–Se nanoparticles in Heps mice model was also investigated.

2. Materials and methods

2.1. Materials

Hyaluronic acid natamycin (molecular weight is 1.06×10^6 Da) was obtained from Jiangsu Hai Hua biotech Co., Ltd. (Jiangsu), scorbic acid and sodium selenite were purchased from Sinopharm Chemical Reagent Co., Ltd. All the solvents and other chemicals were the analytical grades and were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Assay kits for malondialdehyde (MDA) and superoxide dismutase (SOD) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2. Animals and cell

ICR mice (female, 20 ± 2 g) were obtained from Comparative Medicine Center in Yangzhou University, China (the license number SCXK (SU) 2007-0001). The mice were kept in polypropylene cages and maintained under temperature (24 ± 1 °C) with a normal day/night cycle and humidity (55–60%). They are allowed free access to food (standard pellet diet) and drinking water. Before using for experiment, the mice were housed 3 days for adjustment to the environment after arrival.

Heps cells were obtained from Nanjing University. Heps tumor cells were passaged through the ICR mice before initiating an experiment. Seven days after the tumor cells injection, ascites cells from the mice were harvested and used for the experiments.

2.3. Preparation of HA–Se nanoparticles

The hyaluronic acid–selenium nanoparticles were prepared by reducing sodium selenite with ascorbic acid in the presence of hyaluronic acid natamycin. The HA–Se nanoparticles were prepared following the method described by Zhang Lina et al. with little modifications [23]. During the synthesis process, hyaluronic acid natamycin aqueous (0.25%, 200 mL) was added into a 250 mL beaker. The solution was then mixed with sodium selenite aqueous (0.1 mol/L, 2.5 mL) under magnetic stirring overnight. Ascorbic acid (0.1 mol/L, 10 mL) was then drop-wisely added into the resulting mixture. This was further stirred at room temperature. After the reaction mixture was milled for 30 min, an orange-red product was obtained. This product was dialyzed using regenerated cellulose tubes (Mw cutoff 7000) against ultrapure water for 48 h. The product was finally freeze-dried with a lyophilizer and the powdered of HA–Se nanoparticles obtained.

A drop of the HA–Se nanoparticles solution was placed on Cu grids pre-coated with carbon film. The transmission electron microscopy (TEM) image was taken on a JEM-2100 transmission electron microscope operated at accelerating voltage of 200 kV. Mean diameter and size distribution of the HA–Se nanoparticles were measured by a Fiber Optic Quasi-Elastic Light Scattering.

2.4. Determination of Se content by ICP-AES

The samples (0.1000 g of each HA–Se nanoparticles) were dissolved with 5 mL mixture of HNO_3 and $HClO_4$ (v/v, 4:1) in a conical flask and then treated with 12 hrs of pre-digestion at room temperature and then heated until white fumes occurred. The solutions were kept for a while to allow evaporation to take place till almost drying point. 6 mL of HCl (6 mol/L) was added and the solution kept for 20 min. The content was then transferred to a volumetric flask with water which increased the final volume of the solution to 25 mL. Se content in the samples was then determined with ICP-AES analysis. The matrix-matched standard solutions were prepared before used.

2.5. Antitumor activity assay

2.5.1. Animals grouping and experiment design

Cell suspension was collected from peritoneal lavage of a 7-day-old mouse inoculated with Heps tumor. The cells were stained with trypan blue and counted in a hemocytometer to ensure that there were >98% live cells. Cell suspension (0.2 mL; 1×10^7 cells/mL) was implanted subcutaneously into the right frontal groin of the 40 experimental mice. After inoculation, these 40 mice were divided randomly into four groups of 10 mice each. The group include, model group (oral administration, normal saline), 5-Fu positive group (injected intraperitoneally, 25 mg/kg), the high dose of HA–Se nanoparticles group (oral administration, 86.45 mg/kg), the low dose of HA–Se nanoparticles group (oral administration, 4.32 mg/kg). The other 10 mice without tumor inoculation were used as the normal group (oral administration, normal saline). All the mice were allowed free access to standard solid diet and drinking water.

2.5.2. Antitumor activity of HA–Se nanoparticles in Heps-bearing mice

Blood was obtained from all the mice through retro-orbital puncture on the 10th day post-inoculation. The tumors were excised and the internal organs including thymus, spleen, liver and kidney weighted. The inhibition rate of tumor growth was calculated as flows:

$$\text{Tumor inhibition ration } (\%) = \left[\frac{M - T}{M}\right] \times 100,$$

where M is the average tumor weight of the model group, T is the average tumor weight of the treated group.

Relative thymus/spleen/liver/kidney weights were expressed as the ratio of the thymus/spleen/liver/kidney to body weight (mg/g).

2.5.3. Hematological and biochemical assay

For the hematological analysis, total count of leukocytes was carried out using analytical hematology system Symex XE-2100 (Symex Cooperation, Japan). In the case of the biochemical assay, blood serum was separated by centrifuging at 3000 rpm for 15 min using Olympus AU2700 equipment (Olympus Cooperation, Japan).

2.5.4. Detection of SOD activity and MDA level in Heps-bearing mice

Kidney and liver were homogenized with 7 volumes of saline solution using a motor-driven instrument to yield a 5% (w/v) homogenate. The homogenate was then centrifuged for 10 min at 3000 rpm at room temperature. The supernatant obtained was used for assays of the activities of SOD and level of MDA which can be determined by commercial reagent kits in accordance with the instruction manuals.

2.6. Statistical analysis

All data were presented as mean ± standard deviation. The differences between experimental groups were compared by one-way ANOVA (analysis of variance) followed by Student Newman Keuls (SNK). p-Values of less than 0.05 were considered to be statistically significant. Statistical analyses were carried out by SPSS version 16.0 (SPSS Inc., Chicago, USA).

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