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Preparation of Sodium New Houttuyfonate Nano-particles and the Efficacy Against Esophageal Cancer

Linsong Yang[1,2], Meijun Shi[1], Luyao Wang[2], and Xiaolin Zhu[2]

[1] Biomedicine Laboratory, School of Pharmaceutical Engineering and Life Science, Changzhou University, Changzhou 213164, China

[2] Changzhou's Key Laboratory of Pharmaceutical Manufacture and Quality Control Engineering, Changzhou 213164, China

Abstract

The aim of this is a sodium new houttuyfonate(SNH) hyaluronic acid-(HA)-poly(lactic acid)-hydroxyacetic acid(PLGA) copolymer nanoparticl-es delivery system constructed to improve the solubility, biocompatibility, stability, and efficacy of hydrophobic drugs. In the research, hyaluronic acid(HA)-targeted sodium new houttuyfonate(SNH) nanoparticl-es were synthesized to investigate the proliferation inhibition of KYSE450, TE1, and ECA109 esophageal cancer cells. Methods: HA-SNH-PLGA nanoparticles were synthesized by evaporation with an emulsifying solvent, and their entrapment efficiency and drug loading were calculated by a UV spectrophotometer. Meanwhile, Malvern particle size analyzer to determine the particle size and PDI value. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used to analyze the proliferation inhibition effects of HA-PLGA nanoparticles, SNH, and SNH nanoparticles on KYSE450, TE1, and ECA109 cells. Hoechst- 33258 staining assay was to verify the effect of drugs and drug-loaded nanoparticles on cell apoptosis. The results showed that the synthesized HA-SNH-PLGA NPs were spherical with an average size of 150-250nm, the polydispersion coefficient (PDI) was 0.141, and the loadingefficiency of HA-SNH-PLGA nanoparticles was approximately 25%, the entrapment efficiency of HA-SNH-PLGA nanoparticles was 57%. In ad-dition, The proliferation inhibition of HA-SNH-PLGA nanoparticles on KYSE450, TE1, and ECA109 cells was significantly enhanced than SNH.The results demonstrated that HA-SNH-PLGA nanoparticles improve the selectivity of drugs against esophageal cancer cells and have en-hanced antitumor activity.

Keywords

Sodium New Houttuyfonate; Targeted; Hyaluronic Acid-polylactic Acid-hydroxyacetic Acid; Esophageal Cancer.

1. Introduction

Sodium new houttuyfonate(SNH), an aldehyde compound in the volatile oil of the cordate houttuynia plant, which was used to treat upper respiratory tract infections [1], pneumnia [2], chronic cervicitis [3], and other inflammation of the treatment, and has enhanced anti-inflammatory effects. It has an obvious growth inhibition effect on Staphylococcus aureus, Escherichia coli, Typhoid bacillus, etc., can effectively improve the immune capacity of the body, enhance the phagocytosis ability of leukocytes in patients, and also has certain antitumor activity [4], but its low water solubility makes it difficult to improve the effective concentration of tumor target cells. PLGA is a nano-carrier with good biocompatibility. Encapsulation of insoluble drugs can effectively improve their bioavailability in vivo and enhance efficacy. Meanwhile, surface modification ligand of nanoparticles is a significant

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way to construct actively targeted nanoparticles, which target specific tissues [5]. Hyaluronic acid(HA), a natural polysaccharide, is an active targeting ligand that has been widely studied. There are many HA receptors in the human body, such as CD44 receptor, HA mediated motility receptor (RHAMM), HA endocytosis receptor (HARE), etc., among which CD44 is the most widely and deeply studied [6]. Studies have shown that CD44 is closely related to the growth, development, metastasis, and prognosis of a variety of tumors, including adenocarcinoma, ovarian cancer, breast cancer, esophageal cancer, etc. CD44 receptor can specifically bind with HA to achieve targeted drug delivery through receptor-ligand mechanism [7]. Therefore, nanoparticles modified by HA and its derivatives on the surface can deliver Antineoplastic drugs to tumor cells and tissues with high expression of HA receptors. In this study, PLGA nanoparticles loaded with SNH were modified with HA as an active targeting ligand to increase the antitumor effect of SNH.

2. Materials and Methods

2.1 Regents

Sodium new houttuyfonate (SNH,purity ≥99%) was purchased from Xi 'a Kelai Biological Engineering Co., Ltd. Polylactic acid-glycolic acid copolymer (PLGA MW=7000) was purchased from Jinan Daigang Biological Engineering Co., Ltd. Sodium hyaluronate (HA, MW=7521) was purchased from Huaxi Furuda Biomedical Co., Ltd. Hexadecyl trimethyl ammonium bromide(CTAB, purity≥98.0%).Hoechst33258 apoptosis staining kit was purchased from Biyuntian Biotechnology Co., Ltd. Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Bioengineering Materials Co., Ltd. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), DMEM medium, and RPMI1640 medium were all purchased from Sigma Company in the United States. All other chemicals and solvents used were ofanalytical grade.

2.2 Preparation of Sodium New Houttuyfonate Nanoparticles

HA-SNH-PLGA nanoparticles were synthesized by a simple evaporation with an emulsifying solvent method [8]. Typically, 20mg of PLGA and 10mg of SNH were completely dissolved in 2ml of acetone, separately, and then mixed together. the mixture solution was slowly added to the 20ml of 1.5%CTAB aqueous phase. The mixture was stirred at an at-normal temperature.PLGA nanoparticles were formed after the organic solvent was volatilized. At normal temperature,The 3ml of 2%HA solution mixture was slowly added to the mixture solution for 5 minutes, stirring was continued overnight, and 0.45μm microporous filter membrane was filtered, and HA-SNH-PLGA nanoparticles were obtained.

2.3 Drawing the Standard Curve of Sodium New Houttuyfonate

10mg sodium new houttuyfonate was dissolved in the above method to prepare the unloaded HA-PLGA nano liquor, and diluted to the concentrations of 200, 400, 600, 800, and 1000μg/ mL, respectively. The absorbance of sodium new houttuyfonate was determined at a specific wavelength of 283nm using a quartz plate containing the HA-PLGA solution as a blank control.

2.4 Determination of Entrapment Efficiency, Drug Loading, Particle Size, and PDI Value of HA-SNH-PLGA Nanoparticles.

The amount of non-entrapped drug (free drug) was measured in the clear supernatant using UV spectrometry [9]. The HA-SNH-PLGA nanoparticles were placed in a centrifuge tube and centrifuged at 10000 r/min for 10 min. The lower precipitated part was the prepared SNH nanoparticles. The upper layer solution containing free sodium new houttuyfonate was carefully poured out and absorbed into a quartz dish, and absorbance at 283nm was measured. The standard curve of sodium new houttuyfonate was used to calculate the content of free sodium new houttuyfonate, which the entrapment efficiency and drug loading of HA-SNH-PLGA nanoparticles calculate.

$$\text{Entrapment Efficiency (EE)} = \text{Total drug-free drug/Total drug amount} \times 100\%$$

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Drug loading (DL) = Total drug-free drug/Nanoparticle weight×100%

The particle size and PDI values were measured using Zetasizer Nano2019.

2.5 Cell Line and Culture

KYSE450, TE1, and ECA109 esophageal cancer cells were kindly donated by Professor Zhigang Tu (Institute of Life Sciences, Jiangsu University). The cells were grown in RPMI1640/DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/ml streptomycin in a humidified incubator at 37 °C and 5% CO^2.

2.6 Cytotoxicity Test of HA-SNH-PLGA Nanoparticles

KYSE450, TE1, and ECA109 cells were cultured and inoculated into 96-well plates at 7000 cells/well plates. 24 H later, when the cells were completely adherent and in the logarithmic growth phase, cell suspension without drug treatment was used as the negative control group. SNH, HA-PLGA nanoparticles, and HA-SNH-PLGA nanoparticles with three concentrations (50, 100, and 200μg/ mL) were added. After continued culture for 24h, Next, 5 mg/mL of MTT was added to each well and incubated at 37°C for half an hour. Subsequently, the medium was discarded and 150 μL of DMSO was added to each well. After 4 hours of incubation at 37°C, the absorbance was measured at 490nm using an ELISA reader and was compared with the control cultures without compound. Results were generated from 3 independent experiments and each experiment was performed in duplicate. The cell survival rate of SNH, blank nanoparticles, and SNH nanoparticles on KYSE450, TE1, and ECA109 were calculated as follows:

Cell survival rate = experimental group light absorption value/control group light absorption valueabsorption value

2.7 Hoechst33258 Staining

KYSE450, TE1, and ECA109 cells were respectively cultured with trypsin and digested, centrifuged for 300g×5min, discarded supernatant, and suspended with the proper amount of conventional cell complete medium. An appropriate amount of complete medium was added to adjust the cell density to 2×105 cells/well, and evenly hit. 2ml cells/well were inoculated into 6-well plates. When the cells in the pore plate were completely adherent and in the logarithmic growth phase, the cell suspension without drug treatment was taken as the negative control group, and the concentration of SNH, HA-PLGA nanoparticles, and HA-SNH-PLGA nanoparticles were added to the culture for 24h. 0.5 mL fixative solution was added to each well, fixed for 10min, 0.5ml Hoechst33258 staining solution was added to each well, and photos were taken with a fluorescence microscope.

2.8 Statistical Analysis

The data are presented as mean ± standard deviation (S.D.). Differences between control and test groups wereassessed by one way analysis of variance (ANOVA) and Student's t-test. A probability (P) value of <0.05 and0.01 is considered to be significant and very significant.

3. Results

3.1 Determination Results of HA-SNH-PLGA Nanoparticles Size

An appropriate amount of nanoparticles was taken, diluted with ultrapure water, and the particle size was measured by a dynamic light scattering particle size analyzer. The average particle size and polydispersion coefficient (PDI) were measured, and the results were shown in Figure 1. The average particle size was 182.1nm, and PDI was 0.141. The particle size distribution was narrow, the particle size was uniform, which met the conditions of being used as drug-carrying nanoparticles.

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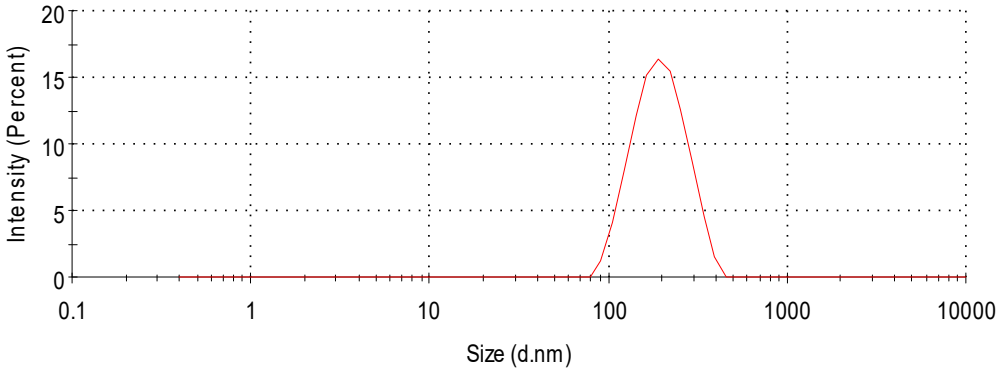

Figure 1. Particle size map

3.2 Standard Curve of SNH

The standard curve of SNH was plotted with the OD value of SNH as the ordinate and the concentration as the abscissa. The standard curve equation of SNH was Y=0.0011x-0.017, R2=0.9928, and the linear relationship was good.

Table 1. Uv absorption of sodium new houttuyonate at different concentrations

Concentration(μg/mL)	0	200	400	600	800	1000
Absorbance value	0	0.227	0.386	0.603	0.830	1.12

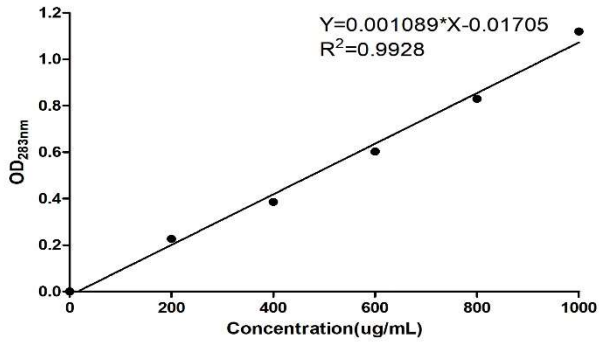

Figure 2. The standard SNH curve

Entrapment Efficiency (EE) = Total drug-free drug/Total drug amount×100%
=5.704mg/10mg×100%=57%
Drug loading (DL) = Total drug-free drug/Nanoparticle weight×100%
=5.704mg/23mg×100%=25%

3.3 Antitumor Activity of HA-SNH-PLGA Nanoparticles

3.3.1 Effect of HA-SNH-PLGA Nanoparticles on KYSE450 Cell Proliferation

As can be seen from Figure 3, compared with the control group, cell activity of the no-load carrier group with different concentrations remained unchanged. With the increase of drug concentration, SNH and HA-SNH-PLGA nanoparticles enhanced the inhibition rate of cell proliferation, showing a certain dose dependence ($P<0.05$), and the same concentration of drug-loaded nanoparticles had a more significant inhibition effect on tumor cell activity than SNH.

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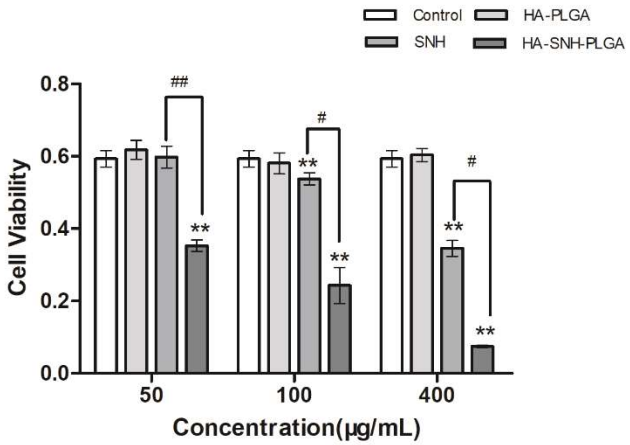

Figure 3. The HA-SNH-PLGA nanoparticles inhibited the proliferation of KYSE450 cells. Data were analyzed using independent sample T test, *P<0.05, **P<0.01 compared with the control group, #P<0.05, ##P<0.01 compared with the active drug group, data were expressed as mean ± standard deviation.

3.3.2 Effect of HA-SNH-PLGA Nanoparticles on TE1 Cell Proliferation

As can be seen from Figure 4, compared with the control group, cell activity of the no-load carrier group with different concentrations remained unchanged. With the increase of drug concentration, SNH and HA-SNH-PLGA nanoparticles enhanced the inhibition rate of cell proliferation, showing a certain dose-dependent (P<0.01), and the same concentration of drug-loaded nanoparticles had a more significant inhibition effect on tumor cell activity than SNH.

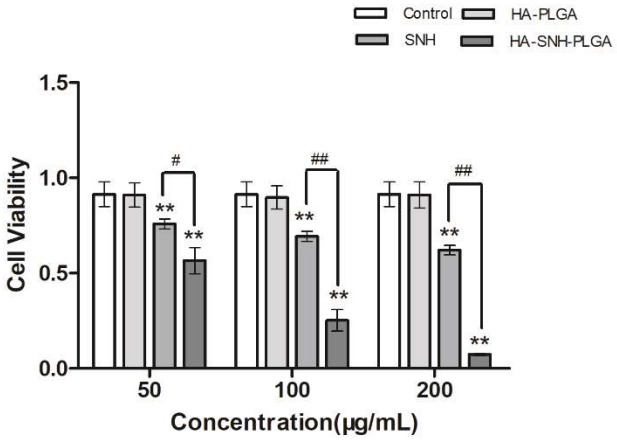

Figure 4. HA-SNH-PLGA nanoparticles inhibit the proliferation of TE1 cells. Data were analyzed using independent sample T test, *P<0.05, **P<0.01 compared with the control group, #P<0.05, ##P<0.01 compared with the active drug group, data were expressed as mean ± standard deviation.

3.3.3 Effect of HA-SNH-PLGA Nanoparticles on ECA109 Cell Proliferation

As can be seen from Figure 5, compared with the control group, cell activity of the no-load carrier group with different concentrations remained unchanged. With the increase of drug concentration, SNH and HA-SNH-PLGA nanoparticles enhanced the inhibition rate of cell proliferation, showing a certain dose dependence (P<0.05), and the same concentration of drug-loaded nanoparticles had a more obvious inhibition effect on tumor cell activity than SNH.

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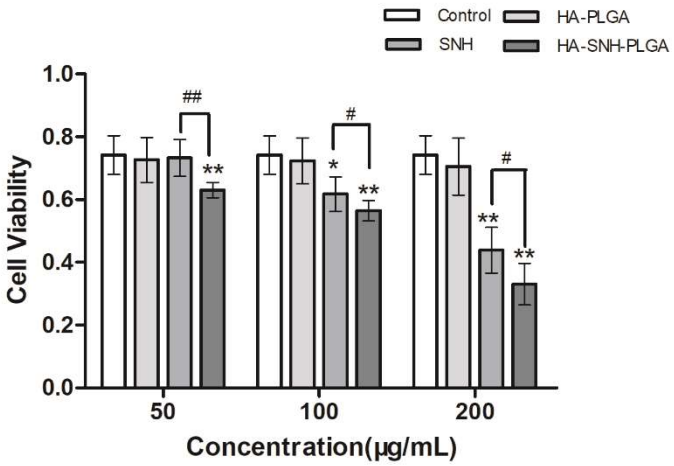

Figure 5. HA-SNH-PLGA nanoparticles inhibit the proliferation of ECA-109 cells. Data were analyzed using independent sample T test, *P<0.05, **P<0.01 compared with the control group, #P<0.05, ##P<0.01 compared with the active drug group, data were expressed as mean ± standard deviation.

3.4 Results of Hoechst Cell Staining Experiment

Fluorescence photos of KYSE450, TE1, and ECA109 cells under the action of SNH group and HA-SNH-PLA drug-loaded nanoparticles (concentration and time) are shown in Figure 6. SNH can promote cell apoptosis, and HA-SNH-PLA is more obvious.

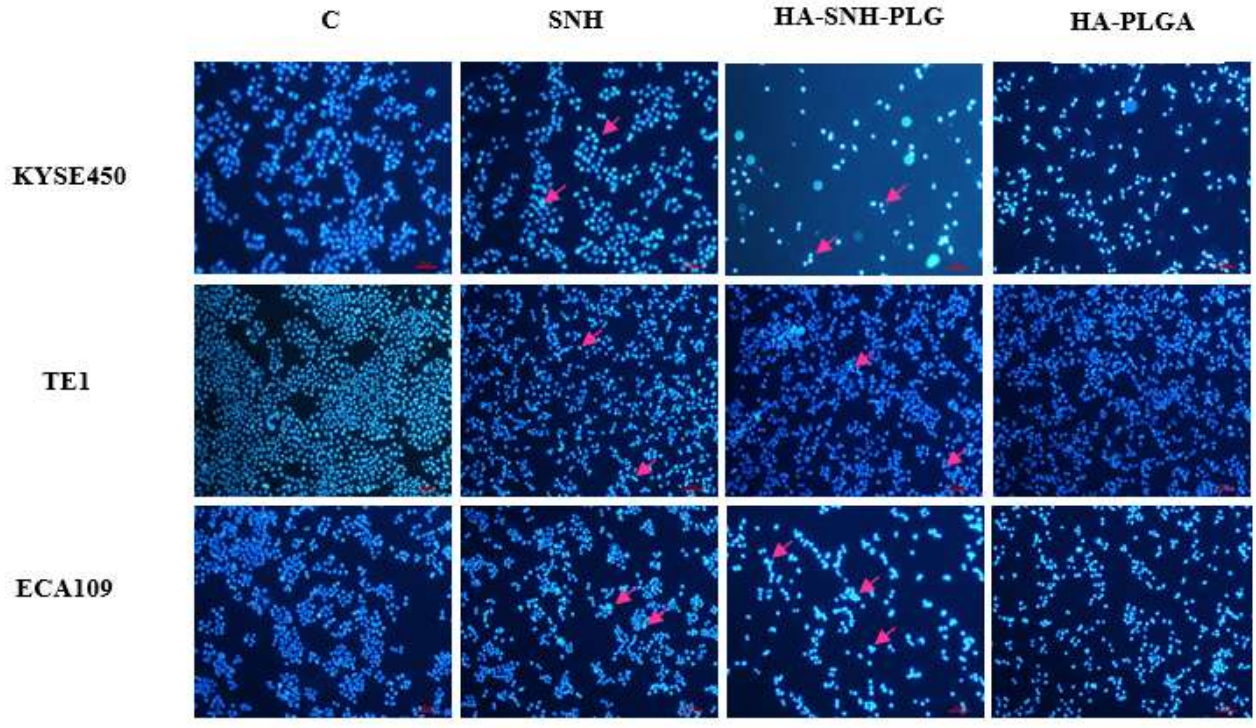

Figure 6. Hoechst nuclei staining

4. Discussion

Malignant tumor has become the second majorly disease threatening human life safety and presents a high incidence trend. At present, the methods of tumor treatment mainly include surgery, radiotherapy, and chemotherapy. However, the adverse reactions of radiotherapy chemotherapy are

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strong and drug resistance is easy to occur. Traditional Chinese medicine and its components can play the role of multiple approaches and multiple targets in the treatment of tumors, and have the advantages of fewer adverse reactions and good tolerance, making it a hot topic in tumor treatment. New sodium Houttuyfonate is a kind of an aldehyde compound in volatile oil on the cordate houttuynia plant. It has shown that it can play an anti-tumor role by inducing apoptosis, inducing cell differentiation, and inhibiting various enzymatic activities related to apoptosis.

In this study, hyaluronic acid (HA) was used to modify the drug delivery system of polylactide-glycolic acid copolymer (PLGA) containing sodium new houttuyfonate(SNH) to construct HA-modified SNH nanoparticles to improve the limitations of the physical and chemical properties of SNH, to enhance the antitumor effect of SNH. The results showed that compared with SNH, SNH coated nanoparticles enhanced the cytotoxicity of SNH to KYSE450, TE1, and ECA109 cells. Hoechst cell staining experiment also proved that HA-SNH-PLGA could enhance cell uptake rate and thus improve efficacy, indicating that the drug-carrying particles have a certain targeting ability. The active targeting pathway of HA-SNH-PLGA nanoparticles and its tumor inhibition ability in vivo remains to be further studied. These results indicate that HA-SNH-PLGA is a promising targeted anti-tumor drug delivery system.

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No potential competing interest was reported by the authors.