Study on fluorouracil–chitosan nanoparticle preparation and its antitumor effect

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Abstract To successfully prepare fluorouracil–chitosan nanoparticles, and further analyze its anti-tumor activity mechanism, this paper makes a comprehensive study of existing preparation prescription and makes a detailed analysis of fluorouracil–chitosan in vitro release and pharmacodynamic behavior of animals. Two-step synthesis method is adopted to prepare 5-FU–CS–mPEG prodrugs, and infrared, 1H NMR and differential thermal analysis are adopted to analyze characterization synthetic products of prepared drugs. To ensure clinical efficacy of prepared drugs, UV spectrophotometry is adopted for determination of drug loading capacity of prepared drugs, transmission electron microscopy is adopted to observe the appearance, dynamic dialysis method is used to observe in vitro drug release of prepared drugs and fitting of various release models is done. Anti-tumor effect is studied via level of animal pharmacodynamics. After the end of the experiment, tumor inhibition rate, spleen index and thymus index of drugs are calculated. Experimental results show that the prepared drugs are qualified in terms of regular shape, dispersion, drug content, etc. Animal pharmacodynamics experiments have shown that concentration level of drug loading capacity of prepared drugs has a direct impact on anti-tumor rate. The higher the concentration, the higher the anti-tumor rate. Results of pathological tissue sections of mice show that the prepared drugs cause varying degrees of damage to receptor cells, resulting in cell necrosis or apoptosis problem. It can thus be concluded that ion gel method is an effective method to prepare drug-loading nanoparticles, with prepared nanoparticles evenly distributed in regular shape which demonstrate good slow-release characteristics in receptor vitro and vivo. At the same time, after completion of drug preparation, relatively strong anti-tumor activity can be generated for the receptor, so this mode of preparation enjoys broad prospects for development.

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1. Introduction

In recent years, constant social and economic development results in people’s accelerating pace of life. Meanwhile, environmental problems deteriorate (He et al., 2008). As a result, global cancer incidence and mortality rates stay a high level, posing a serious threat to people’s health, wherein, liver
cancer, stomach cancer, esophageal cancer and colorectal cancer and other gastrointestinal cancers have a high incidence (Chao and Zhang, 2012). Currently, chemotherapy is one of the primary means for treatment of cancer, but its treatment effect is subject to drug side effects and drug resistance. In recent years, with the blend of molecular biology, molecular pharmacology, polymer materials, thermal chemistry and other subjects, the researchers have developed controlled release preparations and targeting preparation which can be intelligently controlled according to tumor site characteristics (Wang et al., 2010; Li et al., 2012a,b; He et al., 2011). Chitosan (shown as Fig. 1) (CS), the product of deacetylation of chitin, is the only alkaline polysaccharide in nature. With wide range of sources, good biodegradability, biocompatibility and low toxicity, it is widely used in such aspects as pharmaceutical, textile, environmental monitoring and tissue repair. But intermolecular and intramolecular hydrogen-bond interaction reduces its solubility, only partially soluble in acid, such as acetic acid, hydrochloric acid, methane sulfonic acid. In order to make excellent the properties of chitosan which are benefit for preparing nanoparticles, people have done a lot of chemical modification work on 5-FU, to effectively reduce toxicity, over the years, people have done a lot of chemical modification work on 5-FU, to effectively reduce side effects of these drugs (Xu et al., 2014). Nanoparticles (NP) is ultra fine particle decentralized administration system formed by aggregate, poly charge of natural or synthetic polymer materials, which belongs to colloid administration system; its shape is mostly solid colloidal particles with diameter of 10–1000 nm (Li et al., 2012a,b). Nanoparticles have significant medicinal property transport advantages, so preparation of nanoparticles has always been the focus of research questions. With constant progress and development of chemical preparation level, preparation mode of nanoparticles demonstrates significant diversification characteristics. In this paper, preparation effect of fluorouracil-chitosan nanoparticles with ion gel method is deeply analyzed, and its inhibitory effect for cancer tumors is explored.

2. Materials and methods

2.1. Fluorouracil–chitosan preparation

Chitosan (molecular weight 5.0 × 104), 5-fluorouracil, polyethylene glycol monomethyl ether 5000 (mPEG), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide salt, silicate (EDC s HCl), N-hydroxysuccinimide (NHS), succinic anhydride, 4-dimethylaminopyridine (DMAP), N,N-dimethylformamide (DMF), anhydrous ether, chloroacetic acid, methyl orange/phenolphthalein, dialysis bags, and deuterated reagent.

Acid/alkaline buret, 8LGJ-18A freeze-drying machine, WQF-410 Fourier transform spectrometer, 500 MHz fully digital superconducting NMR spectrometer, UV-1700 UV spectrophotometer, DF-101S heat collection constant temperature magnetic stirrer, constant temperature magnetic stirrer, differential scanning calorimeter.

Determination of degree of deacetylation of chitosan: (1) Determine degree of deacetylation of chitosan; (2) demarcate acid or alkaline solution with primary standard substance anhydrous Na₂CO₃; and (3) Calculate the degree of deacetylation: formula is as follows:

\[
(-\text{NH}_2)\% = \left\{ \left[ \frac{C_1 V_1 - C_2 V_2}{G} \right] \times 0.016 \right\} \times 100\%
\]

\[
\text{DD} = \left\{ \frac{(-\text{NH}_2)\%}{9.94\%} \right\} \times 100\%
\]

wherein \(C_1\) represents concentration of HCl, \(V_1\) represents HCl volume needed, \(G\) represents mass of chitosan, \(C_2\) represents concentration of NaOH standard solution, and \(V_2\) represents volume of NaOH.

Preparation of polyethylene glycol monomethyl ether chitosan, with synthetic method is shown in Fig. 3 below (Yan et al., 2011a,b).

Structural characterization of polyethylene glycol monomethyl ether chitosan of 5-fluorouridine conjugate: (1) Weigh 1–2 mg sample after drying treatment with infrared spectrophotometry (FT-IR), after mixed compression with KBr, measure on infrared instrument, with scan range at 4000–400 cm⁻¹; (2) nuclear magnetic resonance spectroscopy (¹H NMR), measure ¹H NMR of substance with AVANCE III 500 MHz NMR instrument, measuring solvent of 5-FUA is DMSO-d₆, measuring solvent of mPEG is D₂O, and measuring solvent of other substances is mixed solution of deuterated water and deuterated hydrochloric acid (D₂O/DCI) (Yan et al., 2012). Degree of substitution of mPEG on chitosan is calculated with integration of characteristic peaks in ¹H NMR. (3) Differential thermal analysis method employs Perkin ElmerDSC4000 for determination: Weigh 5 mg substance for die determination, nitrogen flow rate: 20 ml/min, measuring range: 20–300 °C.
2.2. Preparation of nanoparticles

5-FU–CS–mPEG, mPEG–CS, sodium polyphosphate, phosphate buffer.

SHZ-82 constant temperature oscillator, LGJ-18A freeze-drying machine, laser particle size analyzer, KQ5200DB CNC ultrasonic cleaner, and JEM-2010HR TEM.

Ion induction method, with its simple, gentle features, becomes the most popular method for preparation of chitosan nanoparticles, which takes advantage of sodium tripolyphosphate (TPP) with non-toxic side effects for ion induction of chitosan and derivatives to prepare chitosan nanoparticles (Yan et al., 2011a,b). Sodium tripolyphosphate contains multiple-PO-Na+ groups, while chitosan dissolved in acetic acid and derivative molecular chain contains NH3+ structure, positive and negative charges between the two interacts, which triggers intermolecular and intramolecular cross-linking and then produces nanoparticles.

Weigh appropriate amount of 5-FU–CS–mPEG, to be dissolved in 1% acetic acid solution, perform magnetic stirring until completely dissolved, adjust the pH (range 4–6) with 0.1 mol/L NaOH; under magnetic stirring, add sodium tripolyphosphate (TPP) solution filtered with 0.22 μm membrane into chitosan derivative solution in drops, stir and allow a period of reaction, and observe appearance of reaction system. Centrifuge the resulting suspension at high speed and low temperature, discard the supernatant, add cryoprotectant, freeze and obtain drug-loaded nanoparticles. Determination of drug loading capacity: nanoparticles drug loading capacity = fluorouracil content/total mass of drug-loaded nanoparticles / 100%.

2.3. Animal pharmacodynamics experiment

BL-220H electronic scale, 1 ml, 5 ml disposable syringe, H-88 thermostat water bath, optical microscope, and vacuum dryer.

5-Fluorouracil, mPEG–CS nanoparticles, 5-FU–CS–mPEG nanoparticles, 0.9% saline, and formaldehyde solution.

120 healthy adult mice of clean grade, half and half of male and female weigh 18–22 g. Also, mice H22 hepatoma cell lines need to be bought.

(1) Establish transplanted H22 hepatoma solid tumor model. Inoculate H22 cells with good growth after recovery to abdominal cavity of Kunming mice. After 5 d of intraperitoneal subculture, mice abdomen swelled slightly. By 10 d, mice abdomen significantly mustered.

Abdomen of mice passaged to 10 d received 75% alcohol disinfection. Then extract milky ascites with 5 ml syringe under sterile conditions, blend and inoculate to armpit skin of right forelimb of mice with 1 ml syringe under 0.2 ml/one. Conduct experiment after 3 d.

(2) Conduct animal grouping and administration. Kunming mice with H22 solid tumor successfully established (i.e., mice with palpable solid tumor block under armpit) were randomly divided into six groups, half male and half female, 20 in each group. All groups were intraperitoneally administered once every other day, with total dosage of 5 times; negative control group: normal saline, positive control group: 5-fluorouracil solution (25 mg kg⁻¹ d⁻¹); blank vector group: mPEG–CS nanoparticles; experimental group: 5-FU–CS–mPEG nanoparticles group, divided into groups of high, medium and low concentrations, with 5-FU concentration respectively at 50 mg kg⁻¹ d⁻¹, 25 mg kg⁻¹ d⁻¹, 12.5 mg kg⁻¹ d⁻¹. During the period, regularly observe activities of tumor-bearing mice and make records.

Tumor volume was calculated while tumor weight was weighed and tumor inhibition rate was calculated.

Tumor inhibition rate = (tumor weight of saline group – tumor weight of experimental group)/tumor weight of saline group × 100%.

Observe apoptosis and necrotic conditions of mice histopathologic slide.

2.4. Statistical methods

Use SPSS 19.0 statistical software for data analysis and comparison.

3. Results

Average particle size of drug-loaded nanoparticles (5-FU–CS–mPEG) is respectively 169.2 nm and 259.8 nm, and Zeta potentials are respectively +42.55 mv and +39.27 mv; in electron microscopy observation, nanoparticles are in regular shapes with good dispersion; in vitro release test found that drug-loaded nanoparticles show certain release effect in pH = 5.5, pH = 7.2 buffer.

Animal pharmacodynamic experiments have shown that tumor inhibition rates of high, medium, low concentration group of drug-loaded nanoparticles are respectively 62.05%, 48.79% and 36.14%. Compared with negative control group,
there is significant difference ($P < 0.05$), and tumor inhibition rate of high dose group is comparable with positive control group (5-FU) ($P > 0.05$); histopathologic slide shows that cells of administration group are with different degrees of necrosis and apoptosis.

4. Discussion and conclusion

Drug-loaded nanoparticles prepared by ion gel method enjoy significant advantages, mainly uniform particle size, regular shape and strong sustained release characteristics. The results of animal experiments show that drug concentration exerts clear impact on anti-tumor effect. Therefore, during the course of drug treatment, disease parting of patients should be fully identified to develop reasonable therapeutic regimens.

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