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Studies on In Vitro Slow-Release Characteristics and Anticancer Effect of 5-Fluorouracil-Loaded Immuno-Nanoparticles

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OBJECTIVE To investigate slow-release features of biodegradable anticancer 5-fluorouracil-loaded immunonanoparticles (5-FU INPs), and to assess their tumor cell killing activity in vitro.

METHODS The method of vibrating dialysis at a constant temperature, and first-order derivative ultraviolet spectrophotometry were used to determine the drug-releasing character of 5-FU INPs. The methyl thiazolyl tetrazolium (MTT) colorimetric method was employed to assay the killing activity of 5-FU INPs on 5 tumor cell lines at different phases.

RESULTS The 5-FU INPs had a favorable slow-release function, with a $t_{1/2}$ release time of 10.4 days. The 5-FU INPs had a rather strong lethal effect on 5 tumor cell lines resulting in a positive correlativity between the killing activity and the action time and amount of the drug released.

CONCLUSION The drug disposition is uniform from the 5-FU INPs, and there is no impact on efficacy of the 5-FU during preparation and degradation of the 5-FU INPs. The 5-FU INPs have a favorable function for drug release, and can maintain an effective killing activity over a long period of time.

KEYWORDS: 5-FU-loaded immuno-nanoparticles, slow release, anticancer effect.

INTRODUCTION

The immuno-nanoparticles, which have the function of targeted administration, can effectively localize a drug at a homologous antigen position under the guidance of an antibody, and thus reduce phagocytosis of the particles by the reticuloendothelial system[1]. In this study, 5-fluorouracil (5-FU) was used as the delivered drug, employing biocompatible polylactic acid that undergoes degradation and slow release of the 5-FU. Anti-VEGF121 antibodies were used as the targeting antibody to prepare the 5-FU INPs. The 5-FU INPs are implanted in an oriented fashion and are slowly degraded for drug release.This method of drug delivery enhances the availability of the drug at the target site, improves permeability of the tumor cells, thus maintaining the effect to increase the antitumor efficacy. Moreover, adverse side effects of drugs can be reduced providing good prospects for development and application[2]. We have explored features of in vitro drug release and the tumor-killing activity of the 5-FU INPs, so as to provide an experimental basis for use of 5-FU INPs in interventional treatment of tumors.

MATERIALS AND METHODS

Laboratory apparatus and materials

A JY92-II sonifier was purchased from the Shanghai Xinzhi BioTech Institute, a HITACHIX-650 scanning electron microscope was the product of the Philips Co., and a 100CX-II transmission electron microscope and Geol optical microscope were products of the Olympus Optical Industry Co., Japan. A PHI-1600 photoelectron spectroscope was acquired from the Perkin-Elmer Co., and a UV-9100-type ultraviolet-visible spectrophotometer was the product of the Beijing Rayleigh Analytical Instrument Corp. The 5-FU crude drugs were bought from the Tianjin Xudong Haipu Pharmaceutical Co., Ltd., polylactic acid (DL type, MW=30000) was obtained from the Shandong Jinan Pharmaceutics Institute, and bovine serum albumin from the Tianjin Hematology Institute. MTT (methyl thiazolyl tetrazolium) was the product of the Sigma Co.

The preparation of the 5-FU INPs was as follows: The 5-FU-loaded nanoparticles were prepared by the Department of Gastroenterology, the Second Affiliated Hospital of Zhongshan University, using supersonic emulsification,[2] and coupling with anti-VEGF monoclonal antibodies[3]. The particles had a mean diameter of 210 nm and a drug content of 15.2%.

Pancreatic SW1990 cancer cells, Hep G2 human liver cancer cells, MGC803 gastric cancer cells and the colon SW480 carcinoma cell line, as well as the ECA9706 esophageal cancer cell line, were all purchased from the Shanghai Cytology Institute.

Determination of the nature of in vitro drug release

The 5-FU INPs (50 mg) were accurately weighed and placed into a dialysis bag. The bag was sealed and emmersed in 100 ml PBS (pH7.4) in a beaker which was placed in an oscillator at a constant temperature of 37℃, (frequency: 60 r/min). At specific times a 5 ml sample was removed and replaced with 5 ml of PBS. The 5-FU content of the removed samples was assayed by UV spectrophotometry and a mean of 3 experiments and release time ($t_{1/2}$) calculated.

Determination of the tumor-killing activity of 5-FU INPs

Tumor cells in their growth phase were used to prepare 1×10^5/ml suspensions from which 0.1 ml was added into each well of a 96-well plate. The 5-FU INPs in PBS at various dilutions were added to each well (0.1 ml) in triplicate along with INPs containing no 5-FU and PBS blanks. The cells were cultured under 5% CO_2 at 37℃ for 2, 4 and 7 days, when 0.01 ml/well of an MTT solution was added 4 h before determination. The supernatant in the plate was dried out once, DMSO (0.2 ml) was added to each well and the absorbance (A) measured at 570 nm using a microplate scanning spectrophotometer. The percentage of tumor cells killed was calculated using the A as follows:

The killing percentage = The A value of the blank group − the A value of the trial group/the A value of the blank group×100%

RESULTS

5-FU release from the 5-FU INPs

These experiments measuring 5-FU release showed that the $t_{1/2}$ was 5.3 days. The release at 0.5, 1st, 2nd, 3rd, 5th, 7th, 9th and 11th day was 23.8%, 26.3%, 31.6%, 38.8%, 48.2%, 65.8%, 92.7% and 94.9%, respectively.

Lethal effect on the tumor cells

The killing activity of the 5-FU INPs on 5 tumor cell lines varied considerably. Table 1 shows the killing activity of 5-FU at day 4. The ability of the 5-FU INPs to kill the tumor cells showed a dose-response effect. Nanoparticles devoid of 5-FU and tumor cells

Table 1. The killing activity of 5-FU INPs on 5 tumor cells lines (%)

Cancer Cells	Drug Concentration (mg/ml)					
	0.32	0.64	1.28	2.56	5.12	10.24
Hep G2	2.1±1.2	4.7±1.5	11.5±1.4	27.8±1.8	31.4±2.0	39.9±2.8
MGC803	11.4±3.5	26.8±3.0	38.9±4.1	54.9±4.8	76.8±3.2	91.5±4.4
SW480	14.6±4.6	29.5±3.1	41.7±4.8	64.2±3.4	83.9±5.7	93.8±4.6
SW1990	6.7±3.5	10.2±3.7	22.3±3.0	37.9±4.3	54.6±4.7	78.2±5.1
Eca9706	8.9±3.5	16.8±4.8	27.9±5.8	42.8±3.6	66.2±4.6	85.6±6.2

Values show the percentage of cells killed after 4 days of incubation.

of the control group grew well without an inhibitory effect.

Kinetics of 5-FU INPs killing of tumor cells

Although the anticancer activity of 5-FU INPs varied greatly based on the types of tumors, the release of 5-FU increased with prolonged incubation of the cells. The killing rate of 5-FU INPs on all 5 tumor cell lines significantly increased with time. There was a obvious positive correlation between the killing activity and the time of incubation related to the amount of drug released (Fig.1).

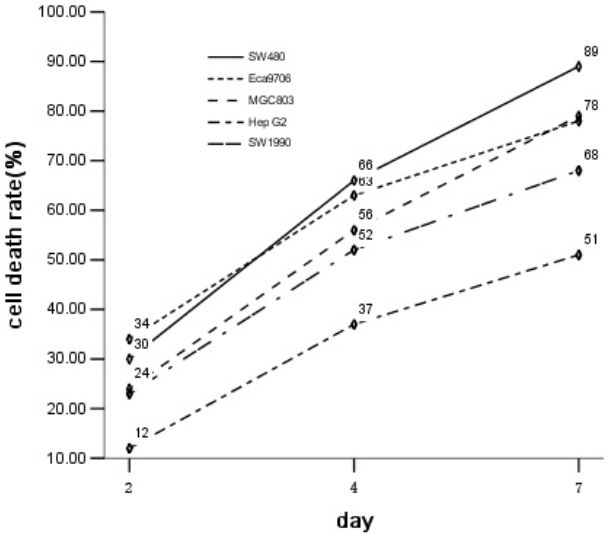

Fig.1. There was a positive correlation between the anticancer activity of 5-FU INPs and the time of incubation

DISCUSSION

In vitro delayed release by the 5-FU INPs

The drug-loaded nanoparticle system is comprised of solid sol-like particles with diameters ranging from 10 to 500 nm. Their active components (drug and bioactive materials etc.) are held in the particles via solution and encapsulation. The system makes for solubilizing hydrophobic drugs, safeguarding drug activity, improvement of medicamentous stability and an increase in accumulation of the drugs in the target organs. These features enhance the therapeutic index and decrease adverse reactions[4]. As a biodegradable biomedical high-molecular material, the final degradation products of the polylactic acid (PLA) in human body are CO_2 and water, with a favorable biocompatibility. The degradation products can be eliminated through the kidneys[5]. As an antimetabolic drug, 5-FU has a broad anticancer spectrum, and is one of the common chemotherapeutics for tumors. Conventionally, 5-FU is administered by mouth or intravenous drop infusion, however, continuous administration may bring about adverse reactions such as nervous system toxicity, thus restricting its dosage and cycle time[6].

In our study, supersonic emulsification was used to prepare the biodegradable 5-FU-PLA nanoparticles, with a particle diameter of no more than 200 nm. It was found, after determination, that there was a uniform distribution of the 5-FU in the microspheres which showed a satisfactory slow release[7]. Anti-VEGF monoclonal antibodyes were chosen to combine with 5-FU-NPs, forming a "bullet" drug. This resulted in successful preparation of the 5-FU INPs with target and immune functions. A liquid form of the carrier can be used for i.v. administration, allowing easy permeation through natural membranes, and an increasing local antitumor drug concentration at the target site and low general toxicity. Therefore the anticancer drugs are released slowly providing high tumor killing. These particles have an important significance for interventional tumor therapy.

The anticancer activity of 5-FU INPs

The clinical dosage of 5-FU is 750 mg/week, 100 mg/d reaching a blood concentration of 0.02 g/L. The plasma half life of 5-FU is approximately 6 min, and 80% of the metabolic products can be excreted within 12 h[8]. Therefore an examination of the 5-FU drug with different action time (2, 4 and 7 d) and homologous release dose was conducted based on the character of the drug release, as anticancer activity of the 5-FU INPs was designed and determined. The results showed that the preparation of the nanoparticles and release of the 5-FU had no impact on the effectiveness of the 5-FU.

All tumors of the human digestive system are sensitive to the killing effect of 5-FU, however, the degree of sensitivity to 5-FU varies greatly with the tumor cell type. All of the following tumor cells are sensitive to the 5-FU-loaded INPs: SW1990 pancreatic cancer cells; Hep G2 hepatoma, MGC803 gastric cancer, SW480 colon carcinoma cell line and Eca9706 esophageal cancer cell line. Among these tumor cells, the gastric cancer and colon carcinoma cells are more sensitive to 5-FU compared to the others[9].

It was found, after a comparison of the tumor-killer kinetics between 5-FU INPs and the 5-FU drug, that the duration of the two drug actions are 2 and 4 days, respectively. The killing activity of the 5-FU INPs is slightly lower than or equivalent to that of the 5-FU, suggesting that the 5-FU is a time-effect drug, and the slow release of 5-FU INPs can retain a very long pe-

riod of antitumorous effect[10]. In the blood, the half-life of 5-FU is short, requiring a large-dose and frequent administration to achieve a therapeutic effect. Obvious adverse side effects often occur with 5-FU treatment, so 5-FU INPs can resolve this problem owing to specific targeting and slow 5-FU release. The findings of our study have provided a preliminary experimental basis for the clinical application of 5-FU.

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