Study on Mechanism of Action and Optimization of Delivery System of Antitumor Drugs based on Molecular Targeting

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Abstract. In response to the shortcomings of molecularly targeted drug delivery systems, this article designs a supramolecular self-assembled nanodrug delivery system. The system uses polylactic-co-glycolic acid (PLGA) and polyethylene glycol (PEG) as main materials, and through careful design and optimization, achieves efficient loading and stable release of doxorubicin hydrochloride (Doxorubicin HCl). The experimental results show that the nano-drug delivery system has uniform particle size distribution (about 100-200nm), high drug loading efficiency and good stability. In vitro experiments, using human breast cancer cell line (MCF-7) as a model, it is verified that the system can significantly improve the drug targeting and bioavailability, and enhance the proliferation inhibition and apoptosis induction of tumor cells. Compared with free drugs, nano-drugs show stronger killing effect on tumor cells at the same concentration, and significantly reduce the toxicity to normal cells. This study not only provides a new perspective for understanding the mechanism of molecular targeted drugs, but also provides a new strategy for improving the clinical efficacy of molecular targeted therapy by optimizing the delivery system.

Keywords: Molecular targeting, antitumor drugs, delivery system.

1. Introduction

As a global medical and health problem, tumor's complexity and diversity bring great challenges to treatment. Although traditional chemotherapy can inhibit the growth of tumor to a certain extent, it is often accompanied by serious side effects and easy to produce drug resistance [1]. Small molecule drugs play an important role in tumor treatment because of their easy synthesis and transformation. However, their nonspecific distribution and damage to normal tissues limit their clinical application.

With the rapid development of biomedicine, molecular targeted therapy, as a new treatment strategy, is gradually changing the pattern of tumor treatment. Molecular targeted therapy specifically acts on key molecules in tumor cells, such as gene mutation and over-expressed protein, so as to achieve precise attack and minimize the damage to normal tissues [2-3]. This treatment strategy not only improves the efficacy of drugs, but also hopes to overcome the problem of drug resistance in traditional chemotherapy. Although molecular targeted therapy has made remarkable progress in the field of tumor, there are still many problems to be solved urgently. How to deeply understand the mechanism of molecular targeted drugs in order to design drugs more accurately? How to optimize the drug delivery system to ensure that drugs can reach the target efficiently and safely? These problems are very important for further improving the effect of molecular targeted therapy.

To explore the mechanism of molecular targeted antitumor drugs and optimize the existing delivery system. Through the comprehensive application of interdisciplinary methods such as biology, pharmacology and nanotechnology, the behavior mode of molecular targeted drugs in cells is revealed, and an efficient and low-toxic nano-drug delivery system is constructed.

2. Mechanism of Molecular Targeted Antitumor Drugs

As an important strategy of contemporary tumor therapy, the core of molecular targeted therapy is to accurately intervene specific molecular abnormalities in tumor cells [4]. These abnormal molecules may be the key factors to promote the proliferation of tumor cells, inhibit apoptosis or participate in invasion and metastasis. By designing and developing drugs that can specifically bind to these targets,

molecular targeted therapy aims to achieve selective killing of tumor cells and reduce damage to normal cells.

Epidermal Growth Factor Receptor (EGFR) inhibitors are drugs that can specifically bind to EGFR, block its downstream signaling pathways, and thus inhibit the proliferation and migration of tumor cells [5-6]. Another class is HER2 inhibitors, which target tumor cells with overexpressed HER2, preventing tumor growth and spread by interfering with HER2 function [7].

The mechanism of molecular targeted drugs involves not only the direct inhibition of the target, but also the induction of tumor cell apoptosis or re-entry into the normal cell cycle by affecting the intracellular signal network [8]. These complex biological processes need to be revealed by elaborate experimental design and advanced technical means. For example, using gene sequencing, protein omics and other analytical methods, we can more comprehensively understand the interaction between drugs and targets, and how these effects affect the fate of tumor cells. However, molecular targeted therapy is also facing the challenge of drug resistance. Under the pressure of drugs, tumor cells may undergo gene mutation or signal pathway remodeling, thus avoiding the killing effect of drugs. Therefore, it is necessary to pay attention to the mechanism of drug resistance and explore new strategies to overcome drug resistance.

3. Optimization of Delivery System for Molecular Targeted Drugs

3.1 Advantages of Nano-drug Delivery System

As a new drug delivery technology, nano-drug delivery system is gradually becoming a powerful tool to achieve this goal. Nano-drug delivery system uses nano-scale materials as drug carriers. Through careful design and optimization, it can significantly improve the targeting, stability and bioavailability of drugs, thus showing great application potential in molecular targeted therapy.

Nano-carriers have high plasticity and versatility, and can be customized according to treatment needs [9]. By modifying specific targeting molecules (such as antibodies, peptides or aptamers) on the surface of nanocarriers, the specific recognition of drugs to tumor cells can be realized, thus improving the targeting of drugs. Nanocarriers can protect drugs from the interference of complex environment in vivo, such as the degradation of enzymes or the elimination of immune system, thus prolonging the circulation time of drugs in vivo and improving the stability of drugs [10]. Nano-carriers can also control the release rate and position of drugs to achieve efficient drug release in tumor tissues, and further improve the bioavailability of drugs.

Nano-drug delivery system has shown a wide application prospect in molecular targeted therapy. Aiming at some specific gene mutation or protein overexpression, corresponding nano-drug carriers can be designed to realize accurate drug delivery. Nano-drug delivery system can also be used in combination therapy, which can improve the therapeutic effect and overcome drug resistance by delivering a variety of drugs with different mechanisms at the same time. More importantly, with the continuous development and improvement of nanotechnology, nano-drug delivery system is expected to realize personalized treatment, and customize the exclusive treatment plan according to the specific condition and genetic characteristics of patients.

3.2 Nano-drug Delivery System based on Supramolecular Self-assembly

A nano-drug delivery system based on supramolecular self-assembly is designed to improve the delivery efficiency and therapeutic effect of molecular targeted drugs. The system uses supramolecular self-assembly technology to construct a nano-carrier with specific structure and function for accurate drug delivery.

Poly(lactic-co-glycolic acid) (PLGA) (50:50 ratio, molecular weight approximately 20kDa) and polyethylene glycol (PEG) (molecular weight approximately 3.4kDa) were used as primary materials for preparing nanoparticles. Doxorubicin hydrochloride (Doxorubicin HCl) was selected as the model chemotherapeutic drug for loading. Acetate buffer (pH 5.0, 10mM) and phosphate-buffered saline (PBS, pH 7.4) were used, along with dialysis bags (molecular weight cutoff 12-14kDa). Equipment

involved in the experimental process included a magnetic stirrer, precision electronic balance, pH meter, ultrasonic disruptor, high-speed centrifuge, transmission electron microscope (TEM), dynamic light scattering (DLS) instrument, UV-visible spectrophotometer, and freeze dryer, which were used for synthesizing, analyzing, and characterizing the nanoparticles.

Weigh 100 mg of PLGA and 20 mg of PEG, dissolve them in 10 mL of dichloromethane (DCM), and then slowly pour the organic phase into 20 mL of polyvinyl alcohol aqueous solution, stirring at 1000 rpm for 1 hour to form an emulsion. The emulsion is stirred overnight at room temperature to evaporate DCM and solidify the nanoparticles. Next, use a high-speed centrifuge to centrifuge at 15,000 g and 4°C for 30 minutes, collect the nanoparticles, carefully remove the supernatant, resuspend the nanoparticles in PBS, and repeat the centrifugation and washing steps twice. Suspend the nanoparticles in 10 mL of acetate buffer, then add 5 mg of doxorubicin hydrochloride, and stir gently at room temperature for 24 hours in the dark to achieve surface modification. Gently stir at room temperature for 2 hours to achieve surface modification. Unreacted targeting ligands and unloaded drugs are removed through centrifugation and dialysis steps, and the size and morphology of the nanoparticles are analyzed using transmission electron microscopy (TEM) and dynamic light scattering (DLS), while the drug loading efficiency is determined using a UV-visible spectrophotometer. Finally, transfer the purified nanoparticle suspension to a dialysis bag and place it in a freeze dryer to obtain nanoparticle powder.

The nano-drug delivery system was characterized in detail by TEM and DLS. The results show that the nanocarriers have uniform particle size distribution (about 100-200nm), moderate surface charge and good stability. In addition, the drug loading efficiency is high, and the drug is evenly distributed in the carrier.

Through surface-modified targeting ligands, nanocarriers can specifically recognize and bind to receptors on the surface of tumor cells. This specific recognition ability enables nanocarriers to accurately deliver drugs into tumor cells and reduce the damage to normal cells. After nanocarriers enter tumor cells, the microenvironment (such as pH, enzyme activity, etc.) in cells is different from that in vitro, which can trigger the release of drugs. By designing responsive materials, the drug was released efficiently in tumor cells.

Because of its high specificity and accuracy, nano-drug delivery system can deliver drugs directly into tumor cells, thus reducing the exposure and damage to normal tissues. This local high-concentration drug release method can not only improve the therapeutic effect, but also significantly reduce the systemic toxic and side effects of the drug. By optimizing the material and structure of the nano-carrier, the immunogenicity and toxicity of the carrier are further reduced, and its biocompatibility and safety are improved.

4. Experimental Research

4.1 Experimental Materials, Methods and Steps

Using the human breast cancer cell line (MCF-7) as a model system, we explored the efficacy of gefitinib, an EGFR inhibitor, as a molecularly targeted drug. A nanodrug delivery system based on supramolecular self-assembly was designed to enhance the targeting and bioavailability of the drug. Cell culture reagents were prepared, including medium, fetal bovine serum, and antibiotics. Various assay kits were used to evaluate the effects of the nanodrug delivery system on cell proliferation, apoptosis, and toxicity.

Tumor cells were inoculated in suitable culture medium, fetal bovine serum and antibiotics were added, and cultured in an incubator with 37℃ and 5% CO2. Change the culture medium regularly to keep the cells in logarithmic growth phase.

In the experiment, firstly, the molecular targeted drug gefitinib was loaded into the designed nano-drug delivery system based on supramolecular self-assembly, so as to prepare nano-drugs. In order to evaluate the drug effect, several experimental groups were set up: free gefitinib treatment group, nano-drug treatment group and untreated control group. Three different concentrations were set for

free gefitinib and nano-drug: 1 μM, 5 μM and 10 μM, respectively, to explore the drug effect under different concentrations. These drugs with different concentrations were added to the cell culture medium and co-cultured with MCF-7 cells for 48 hours, and then the cell proliferation, apoptosis and toxicity were analyzed by using the corresponding detection kit.

Use cell proliferation kit to detect cell proliferation, record absorbance value, and calculate cell proliferation inhibition rate. Apoptosis was detected by apoptosis kit, and the proportion of apoptotic cells was analyzed by flow cytometry. Cytotoxicity kit was used to evaluate the toxic effect of drugs on cells and record the cell survival rate.

4.2 Experimental Result

Both free drugs and nano-drugs can inhibit the proliferation of tumor cells in a concentration-dependent manner. Compared with free drugs, nano-drugs show stronger proliferation inhibition at the same drug concentration (Table 1).

Table 1. Proliferation inhibition rate and cell survival rate of tumor cells treated with different concentrations of drugs

Drug type	Drug concentration (μM)	Proliferation inhibition rate (%)	Cell survival rate (%)
Free gefitinib	1	18.7	83.5
	5	43.2	58.9
	10	63.8	34.1
Nanomedicine	1	29.6	74.3
	5	58.4	42.7
	10	82.1	13.6
control group	0	0	100

With the increase of drug treatment time, the proportion of apoptotic cells in both free gefitinib and nano-drug increased gradually. This shows that both drugs can effectively induce apoptosis of tumor cells, and the apoptosis effect is proportional to time. Under the same drug treatment time, the proportion of apoptotic cells induced by nano-drugs was significantly higher than that induced by free gefitinib. This difference is consistent at all time points of drug treatment, which shows that nano-drugs have a more significant effect in inducing tumor cell apoptosis. This may be related to the nano-drug delivery system enhancing the targeting and bioavailability of drugs, thus improving the killing effect of drugs on tumor cells (Figure 1).

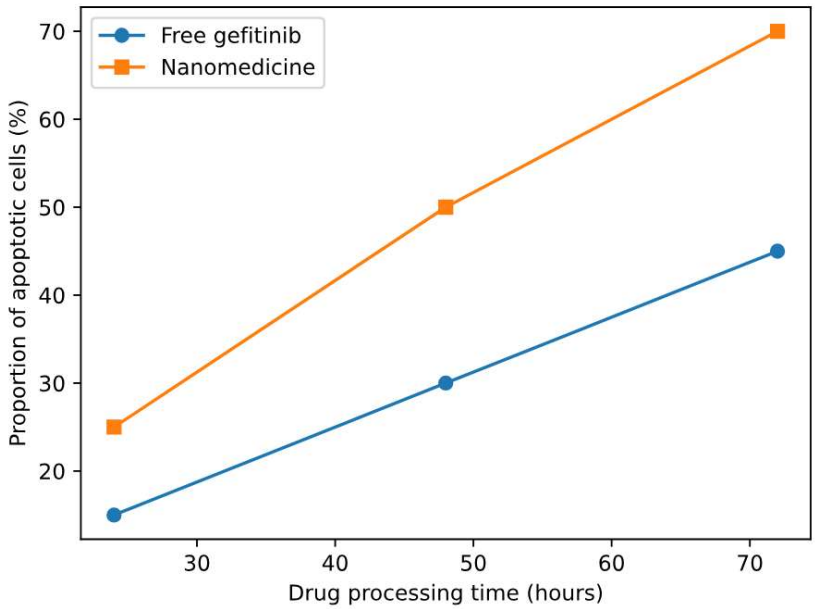

Figure 1. Relationship between drug treatment time and apoptosis induction

Nano-drug delivery system can significantly improve the therapeutic effect of molecular targeted drugs and reduce the toxicity to normal cells. This is mainly due to the slow release and specific recognition ability of nano-carriers, which enables drugs to accumulate efficiently in tumor cells. In addition, the biocompatibility and biodegradability of nanocarriers also improve the safety of drugs. The nano-drug delivery system designed in this study successfully realized the optimal delivery of molecular targeted drugs, improved the therapeutic effect and reduced the side effects. This provides new ideas and methods for future tumor treatment, and is expected to promote the development of molecular targeted therapy.

5. Conclusion

Nano-drug delivery system has great potential in improving drug targeting, stability and bioavailability. By designing nanocarriers with specific structures and functions, drugs can be protected from the interference of complex environment in vivo, and drugs can be released efficiently in tumor tissues. Experiments with MCF-7 show that compared with traditional free drugs, nano-drug delivery system can significantly improve the ability of drug proliferation inhibition and apoptosis induction. In addition, the system can also reduce the toxicity to normal cells and improve the safety. The nano-drug delivery system designed in this study successfully realizes the optimal delivery of molecular targeted drugs, which provides new ideas and methods for future tumor treatment. With the continuous development of nanotechnology, it is expected to promote the realization of personalized treatment, and customize the exclusive treatment plan according to the specific condition and genetic characteristics of patients.

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