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Adoption of ultrasound-guided polymer nanocarriers in tumor chemoradiotherapy and oxidation treatment

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ABSTRACT

The research was aimed at discussing the effectiveness of ultrasound-guided polymer nanocarriers in the clinical treatment of tumors by chemoradiotherapy and oxidation treatment. Twenty female Balb/cAnN (BALB/C) mice were selected as the research objects in the experiment. These mice were set up as tumor-bearing mice, and then ultrasound-guided polymers with different doses, including polyethylene glycol-poly 2-bromoethyl methacrylate (PEG-PBEMA) (Micelle group), free small molecules called l-ascorbyl palmitate (PA) (PA group), PA-micelle micellar particles (PA-Micelle group) prepared in the research, and phosphate buffer solution (PBS) (PBS group) were adopted. Besides, the growth of mice was recorded and compared after each operation. Meanwhile, different concentrations of PA-Micelle micellar particles and free small molecules of PA were added to the breast cancer cells of mice, and the concentration changes of glutathione (GSH) were detected to test the oxidation treatment ability of this method. According to the results of the experiment, the tumor volume of mice in the PA-Micelle group prepared in the research was the smallest followed by the PA group, and the tumor volume of mice in the Micelle group was the third smallest. The mice in the PBS group had the largest tumors among mice in all four groups. In oxidation treatment, the GSH concentration of mice in the PA-Micelle group was the lowest, while the GSH concentration of mice in the PA group was almost unchanged. The results of this experiment proved that the therapeutic effect of polymer nanocarriers in tumor chemotherapy and oxidation treatment was more significant than in traditional drug treatment.

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Introduction

A tumor refers to the neogrowth that is formed by abnormal hyperplasia in cells, which is caused by various types of tumorigenic factors. The form of neogrowth is usually space occupying a bulge, which is called a neoplasm. All types of tumors had three features, including immortality, migration, and loss of contact inhibition (1). According to the damage level of neogrowth to the human body, the tumor is categorized as benign tumors and malignant tumors. The major differences between the two types of tumors include growth rate (benign tumors grow slowly, while malignant tumors grow fast), envelope metastasis (no envelope occurs in benign tumors, while envelope metastasis frequently occurs in malignant tumors), prognosis effect (the prognosis effect after benign tumor treatment is satisfactory, while malignant tumors adhere to peripheral tissues, and it usually recurs after treatment and even results in the death of patients) (2). Based on growth source, malignant tumors consist of cancer and sarcoma. In general, cancer refers to malignant tumors that grow in epithelial tissues, while sarcoma is malignant tumors growing in connective tissues, fat, muscle, blood vessels, skeleton, cartilage tissues and other mesenchymal tissues (3,4). Related studies showed that tumor is the most fatal disease causing great damage to human life health currently (5). In the United States, the mortality of malignant tumors is ranked in second place, following that of cardiovascular and cerebrovascular diseases. In China, a malignant tumor is the deadliest symptom for citizen health (6,7).

At present, surgical treatment is adopted as a basic treatment plan for tumor treatment, while other treatment plans, such as radiation therapy and chemotherapy, are often adopted as auxiliary methods of surgical treatment. In the current academic circle, there are two major opinions about tumor treatment. One opinion is the complete eradication of all or most of the tumor cells in patients' bodies to avoid postoperative tumor recurrence, while another opinion is the manipulation of tumor cells to postpone and even terminate the development of the disease, which can be achieved by changing the features of tumor cells. Scholars holding this opinion view tumor cells as a kind of normal cell out of control (8-10). The former opinion is the description of the major treatment method at present. Surgical treatment and tumor chemotherapy are aimed both at eradicating tumors. In contrast, the latter opinion is put forward recently. Most of the treatment methods are studied. In terms of tumor treatment methods, immunotherapy and gene therapy are included in advanced research. Besides, the therapeutic effects of many drugs on tumor cells are obvious. However, drug therapy alone results in many toxic and side effects throughout the human body, which also eradicate cells in some normal tissues. There-

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fore, clinical application of this treatment method is not common (11-16).

With the rapid development of nanotechnology and further research into targeted drugs in recent years, nanomaterials are applied as the carrier of tumor treatment drugs, and different polymer nanocarriers are utilized to perform ultrasound-guided particular targeted transport on tumor tissue cells, which are released at particular positions to guarantee the maximum therapeutic effects of treatment drugs on particular tumor cells. In this way, therapeutic effects become more significant, while toxic and side effects are greatly reduced. This technology has become the research topic of domestic and foreign tumor drug treatment (17,18). At present, polymer nanocarrier is divided into three types, including the potential of hydrogen (pH) responsive nano drug controlled release system, temperature responsive nano drug controlled system, and reduced responsive drug carriers according to different delivery medium (19-21). Because there is little related research into the clinical treatment effects of polymer nanocarriers on tumors at present, the specific therapeutic effects of polymer nanocarriers on tumor cells are studied to identify their function in tumor chemotherapy and oxidation treatment. Besides, this research is expected to provide a more effective clinical treatment method with less toxicity and side effects for future clinical treatment of tumors. Twenty BALB/C (Balb/cAnN) mice were selected as research objects in the research. These mice were set up as tumor-bearing mice, and tumors on their bodies were treated by ultrasound-guided polymer nanocarriers, free small molecules, and l-ascorbyl palmitate (PA)-Micelle micellar particles. The application value in the clinical treatment of ultrasound-guided polymer nanocarriers is identified by the detection of tumor changes after treatment.

Materials and Methods

Experimental animals

FemaleBALB/C mice were the experimental models aged between 5 and 6 weeks, weighing 15 ± 5g. They were purchased from the Affiliated Hospital of Jiangnan University Animal Experimental Center. Besides, all mice were acclimated to the environment before the experiment. They were fed with food and water timely and wood debris was replaced.

Main experimental reagents and apparatus

Main experimental reagents and apparatus included mice breast cancer cells (Chinese Academy of Sciences Shanghai Branch), polymer PEG-PBEMA (Jinan Daigang Biomaterial Co., Ltd), PA (Alfa Aesar (China) Chemical Co., Ltd), phosphate buffer solution (PBS) (Shanghai Double-helix Biotechnology Co., Ltd), trypsin (SPH No.1 Biochemical & Pharmaceutical Co., Ltd), and glutathione (GSH)/oxidized glutathione (GSSG) detection kit (Shanghai Beyotime Biotechnology Co., Ltd).

Establishment of tumor-bearing mice model

The injection into tumor cells of BALB/C mice purchased was performed to establish a tumor-bearing mice model (22). Breast cancer cells of mice were re-suspended in a serum-free medium to generate the suspension, which contained 1×10^7 cells per milliliter. After that, each mouse was injected with 0.2mL of the suspension before the observation of their growth.

Preparation of polymer nanocarriers for experimental use

Purchased PA and polymer PEG-PBEMA were dissolved into the mixed solvent of carbinol and chloroform with a volume ratio of 2:1 and then dried slowly in a rotary evaporator for about 1.5 hours. Besides, phosphate solution was added to the mixed solvent for hydration at 25°C for about 3 hours. After that, the solution was filtered by a microfiltration membrane with the size of 0.45μM to remove impurities. Next, reverse high-efficiency fluid was applied to test the drug-loading capacity of polymer nanocarriers. The adjustment of the usage amount of PA or polymer PEG-PBEMA could help obtain polymer nanocarriers with different concentrations of loaded drugs.

Experimental grouping and methods

All 20 female tumor-bearing mice models were grouped randomly. All mice were divided randomly into the PA group, Micelle group, PA-Micelle group, and PBS group. The caudal veins of mice in the PA group were injected with PA (63mg/kg), the purchased polymer nanocarriers without drug loading were injected into the caudal veins of mice in the Micelle group at 35mg/kg, polymer nanocarriers and PA were injected into the caudal veins of mice in PA-Micelle group at 35mg/kg and 63mg/kg, respectively, and the mice in PBS group were injected with the equal amount of PBS into their veins. Re-consolidation injection was performed on all experimental mice after the last injection, 2 days, and 4 days after the last injection. In addition, the change in mice tumor size was recorded and compared three weeks after the experiment to identify the therapeutic effects of this method on clinical tumor treatment.

The calculation equation of tumor volume is expressed by equation 1 as follows.

$$V = \frac{L \times W^2}{2} \quad [1]$$

In equation 1, L represented the maximum length of the tumor, and W referred to the minimum length of the tumor.

To verify the therapeutic effects of these polymer nanocarriers in tumor oxidation treatment, and to test the consumption capacity of prepared PEG-PBEMA (PA-Micelle) loading PA of glutathione (GSH), the breast cancer cells of purchased mice were inoculated onto six-hole cell culture plates for totally 24 hours. Cells inoculated in each hole were added with drugs carrying different concentrations of medicine (the concentrations of PA were 0μM, 10μM,100μM, and 1000μM, respectively, and PA-Micelle concentrations of the above drug-loading polymer nanocarriers were 0μM, 0.25μM, 0.65μM, and 1.30μM, respectively), and they were processed for 24 hours at 37°C. After the processing, trypsin was adopted to digest cells, which were centrifuged in a high-speed centrifuge at 1000r/m for 5 minutes. Next, cells were collected to be washed with PBS three times. Then, GSH concentration in cells was tested by GSH/GSSG detection kit to identify the oxidation treatment capacity of the polymer nanocarrier.

Statistical research

Statistical product and service solutions (SPSS) were used as the analysis and statistics software for data processing. Measurement data was denoted by mean ± stan-

dard deviation ($\bar{x}\pm s$), enumeration data was expressed by percentage (%), and $P<0.05$ indicated that the differences had statistical meaning.

Results

Mice modeling results

The tumor-bearing mice models of all 20 mice were established, and the growth of breast tumors after the injection into mice breast cancer cells was identified. Judging by touch, the models of 20 mice were all established successfully. Figures 1 and 2 showed mice modeling results and tumor modeling results, respectively. Figure 1 presented mice tumors 3 days after modeling, and Figure 2 showed mice tumors 2 weeks after modeling. The ultrasound image of small tumors was demonstrated in Figure 3, which indicated that ultrasound could display the location and form of mice tumor lesions.

Testing of oxidation treatment effects of polymer nanocarriers

GSH concentration in mice bodies was measured by GSH/GSSG detection kit. When PA-Micelle concentrations of drug-loading polymer nanocarriers were 0μM, 0.25μM, 0.65μM, and 1.30μM, respectively. GSH concentrations in mice bodies were 13.72 ± 0.38 nmoL/mg, 9.61 ± 0.42 nmoL/mg, 5.84 ± 0.29 nmoL/mg, and 3.82 ± 0.26 nmol/mg, respectively. The differences among all data showed $P<0.01$. Figure 4 demonstrated specific data.

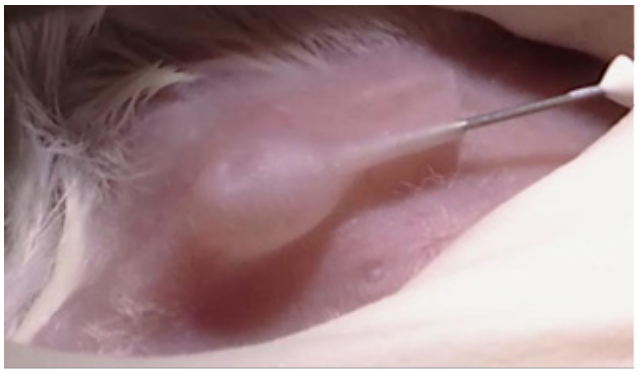

Figure 1. Tumor size 3 days after tumor-bearing mice modeling.

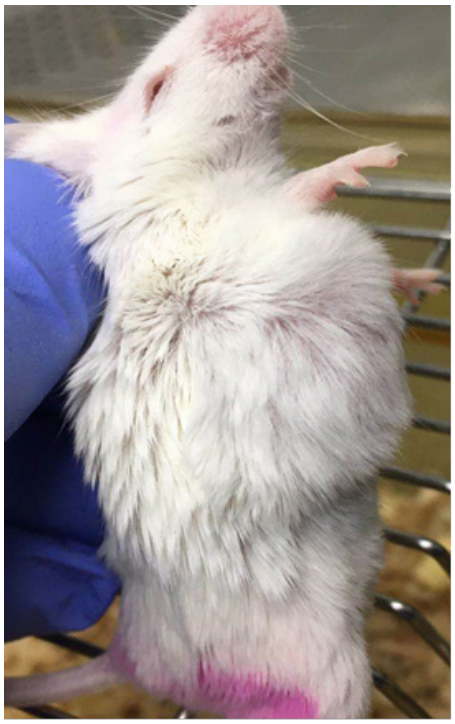

Figure 2. Tumor size 2 weeks after tumor-bearing mice modeling.

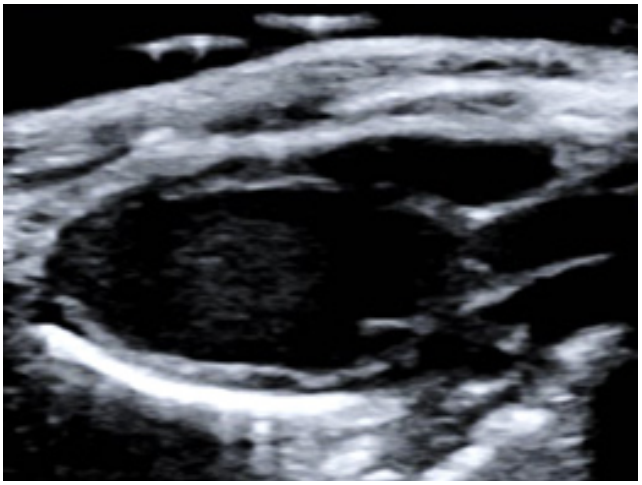

Figure 3. Ultrasound image of mice tumor.

Meanwhile, when only PA with the concentrations of 0μM, 10μM, 100μM, and 1000μM were used, GSH concentrations in mice bodies were 14.26 ± 0.41 nmoL/mg, 13.59 ± 0.27 nmoL/mg, 13.08 ± 0.22 nmoL/mg, and 12.87 ± 0.23 nmol/mg, respectively. Figure 5 presented specific data.

The comparison of data between the two groups showed that GSH concentration in mice bodies was significantly reduced after the injection of drug-loading polymer nanocarriers PA-Micelle, while the change of GSH concentra-

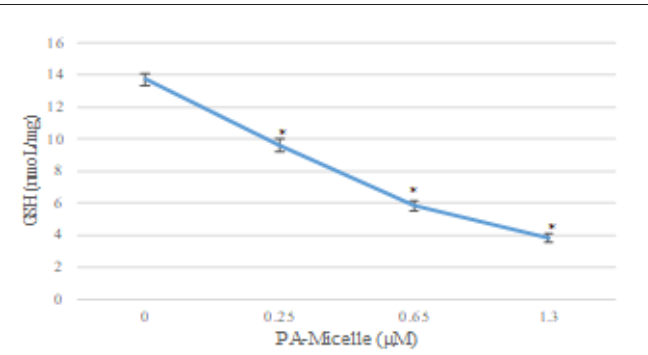

Figure 4. Changes of GSH concentration in mice bodies after the injection of different concentrations of drug-loading polymer nanocarriers PA-Micelle (the comparison with 0μM showed *$P<0.01$).

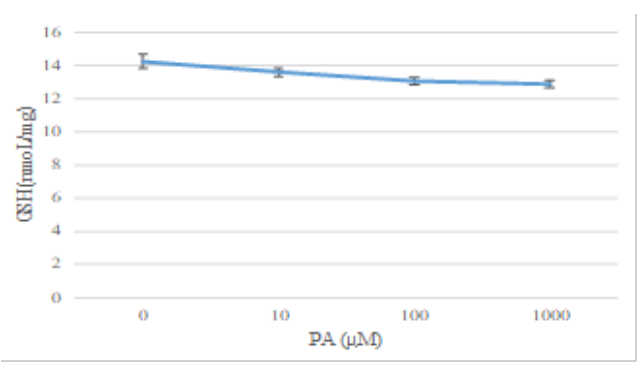

Figure 5. Changes of GSH concentration in mice bodies after the injection of different concentrations of PA.

tion in mice bodies was not obvious after the injection of free small molecules PA.

Testing of therapeutic effects on mice tumors

The volume of tumors in mice body was measured by grouping treatments. Mice in the control group were injected only with PBS into caudal veins, and the volume of tumors of these mice immediately after the experiment, 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, and 21 days after the experiment was 0.16cm^3, 0.27cm^3, 0.39cm^3, 0.62cm^3, 0.84cm^3, 1.37cm^3, 1.62cm^3, and 2.48cm^3, respectively. This experiment was conducted as a control group to be contrasted with other experimental results. The inhibition of PBS of tumors was less significant than PA. Figure 6 illustrated specific tumor changes at different time points.

Mice in the first group of the experimental group were injected only with PA into caudal veins, and the volume of their tumors immediately after the experiment, 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, and 21 days after the experiment were 0.16cm^3, 0.24cm^3, 0.35cm^3, 0.48cm3, 0.68cm^3, 0.72cm^3, 0.88cm^3, and 1.36cm^3, respectively. According to experiment results, the adoption of PA alone could inhibit tumor growth to some extent. Figure 7 showed tumor changes at different time points.

Mice in the second group of the experimental group were injected only with polymer PEG-PBEMA into caudal veins, and the volume of their tumors immediately after the experiment, 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, and 21 days after the experiment was 0.16cm^3, 0.25cm^3, 0.36cm^3, 0.51cm^3, 0.72cm^3, 0.91cm^3, 1.18cm^3, and 1.47cm^3, respectively. According to experiment results, the adoption of polymer PEG-PBEMA alone could inhibit mice tumor growth. However, the inhibition of polymer PEG-PBEMA of tumor growth was less significant than PA. Figure 8 showed tumor changes at different time points.

Mice in the third group of the experimental group were injected with polymer nanocarriers PA-Micelle prepared in the research into caudal veins, and the volume of their tumors immediately after the experiment, 3 days, 6 days, 9 days, 12 days, 15 days, 18 days, and 21 days after the experiment was 0.16cm^3, 0.17cm^3, 0.21cm^3, 0.25cm^3, 0.28cm^3, 0.32cm^3, 0.36cm^3, and 0.41cm^3, respectively. According to experiment results, the adoption of polymer nanocarriers PA-Micelle could inhibit mice tumor growth obviously, and its inhibition of tumor growth was more significant than the adoption of PA or PEG-PBEMA alone. Figure 9 showed specific tumor changes at different time points.

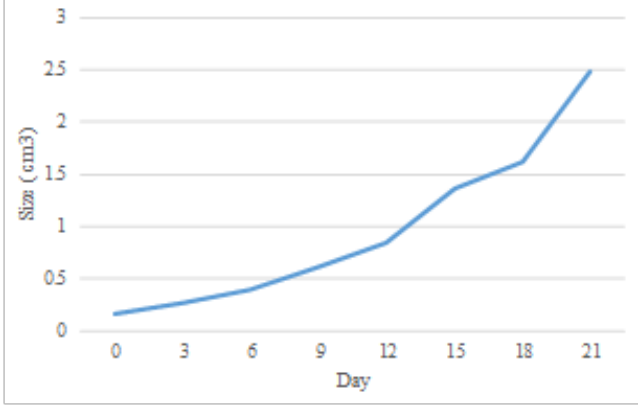

Figure 6. Mice tumor changes after the adoption of PBS alone.

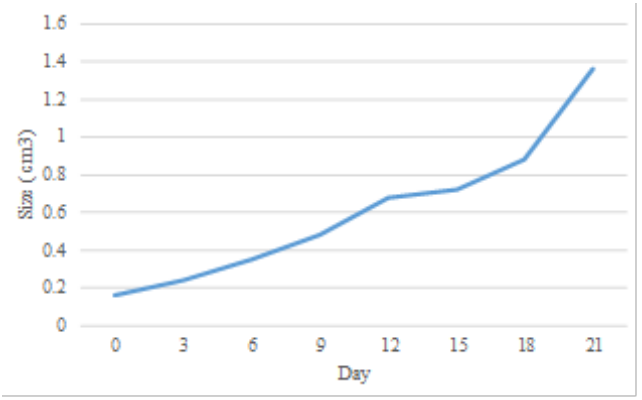

Figure 7. Mice tumor changes after the adoption of PA alone.

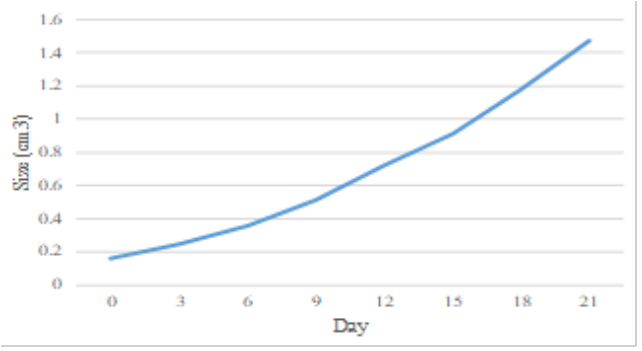

Figure 8. Mice tumor changes after the adoption of polymer PEG-PBEMA alone.

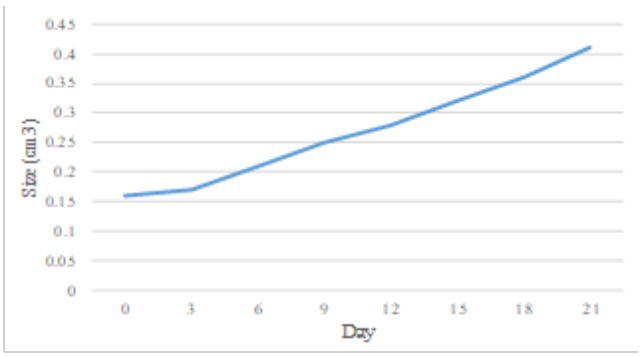

Figure 9. Mice tumor changes after the adoption of polymer nanocarriers PA-Micelle.

Discussion

As the most deadly disease harmful to all humankind, the tumor occurs among patients of all ages. This disease causes lots of emotional and financial burdens to patients and their family members. Although there are many feasible treatment plans at present, most of them have other toxic and side effects, which bring about permanent pains for patients during and after treatment. As a result, all tumor patients are looking forward to coming up with a treatment method that can improve tumor therapeutic effects and alleviate toxic and side effects after treatment. Based on the current situation, the treatment of currently novel polymer nanocarriers in the tumor was studied in animal experiments to identify its therapeutic effects in tumor chemoradiotherapy and oxidation treatment.

At first, the tumor-bearing mice models of twenty pur-

chased BALB/C mice were established. After successful modeling, all mice were grouped randomly for the preparation of tumor chemoradiotherapy and oxidation treatment experiment. Meanwhile, the purchased experimental reagents were processed and polymer nanocarriers (PA-Micelle) necessary for the research were prepared. To verify the effectiveness of polymer nanocarriers in tumor chemoradiotherapy, all mice were divided into control experiment PBS group injected with PBS, PA group injected with PA, Micelle group injected with polymer PEG-PBEMA, and PA-Micelle group injected with PA loading polymer PEG-PBEMA prepared in the research. The comparison of the changes in tumor volume of mice in the above four groups at different periods after the experiment showed that tumors of mice in the PA-Micelle group were inhibited most significantly, and their tumors grew at the slowest rate. The inhibition of tumors of mice in the PA group was the second most significant and the growth rate of tumors was the second slowest, which was followed by the Micelle group. In contrast, the inhibition of tumors of mice in the PBS group was the least significant, and they grew fastest among all tumors of mice in four groups. The experimental results proved that the loading and transportation of drugs by polymers could enhance drug use efficiency obviously, and inhibit tumors more significantly.

Secondly, the effectiveness of the polymer nanocarriers in tumor oxidation treatment was verified. GSH concentration of breast cancer cells of the purchased original mice was measured in vitro tests. The changes in GSH concentration were tested after the injection of PA and polymer nanocarriers PA-Micelle prepared in the research with different concentrations. The experimental results demonstrated that GSH concentration was greatly decreased as the increase of PA-Micelle concentration after the injection of PA-Micelle prepared in the research. In contrast, GSH concentration was slightly changed after the injection of PA with different concentrations. The experimental results proved that PA-Micelle prepared in the research could consume GSH in mice bodies effectively, and inhibit cellular anti-oxidation, which originated mainly from polymer PEG-PBEMA.

The research identified the significant effectiveness of polymer nanocarrier in tumor treatment, and its therapeutic effects in tumor chemoradiotherapy and oxidation treatment capacity by experimental verification, which laid the experimental foundation for future clinical experiments on this technology. Stras et al. (2020) (23) studied the poor prognosis of triple-negative breast cancer, which was caused by the uneven distribution of adopted drugs in targeted tumors according to the conclusion of their study. In the research, nano lipid carriers were adopted in the targeted delivery of drugs, which enhanced drug use efficiency effectively, extended drug administration time, and improved therapeutic effectiveness effectively. Zhou et al. (2019) (24) established a kind of nano small molecule drug carrier by camptothecin. Cancer drugs could be delivered to tumors by this kind of nanocarrier effectively. Because cancer is very complex and many causes have been reported for it, it is necessary to carry out numerous researches at the cellular, molecular, macro and nano levels in order to reach its promising treatments (25-29).

In the research, twenty tumor-bearing mice were selected for modeling to verify the effectiveness of polymer nanocarriers in tumor chemoradiotherapy and oxidation treatment. The results proved that the therapeutic effects of this technology in tumor treatment were more significant than traditional drug use. Because this technology is not perfect at present, the preparation of the drug and its toxic and side effects need to be verified in more experiments. Therefore, tumor-bearing mice were adopted to verify the effectiveness of polymer nanocarriers before experimental verification. It is expected that the technology will be adopted in clinical experiments as early as possible with continuous improvement of theory and technology, and more tumor patients will be offered superior treatment.

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