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Synthesis of polyethylene glycol bis-anti-cancer drug couplings and their anti-tumor activity based on tumor immune dynamic modeling

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Abstract: In order to make the drugs can cure the tumor precisely, this paper establishes the tumor immune dynamic model through the differential equation of tumor growth and analyzes the persistence of the tumor immune model. Research on dual anticancer drugs and commonly used coupling methods is carried out to complete the synthesis of polyethylene glycol dual anticancer drug couplers and the antitumor activity is analyzed to derive the degree of inhibition of polyethylene glycol dual anticancer drugs on tumor activity. From the four judging criteria, it was concluded that the polyethylene glycol bis-anti-cancer drug has a better curative effect on tumor cells. Evaluation of polyethylene glycol bis-anti-cancer drugs from four aspects: Apoptosis test, relative tumor growth rate, immunofluorescence staining of tumor tissue sections and antibody endocytosis, it can be found that polyethylene glycol bis-anti-cancer drugs play a role in inhibiting the activity of tumors by 20%, and the proportion of apoptosis is 97.6%, which effectively hinders the growth of tumor cells and reduces the multiplicity of tumor cells. Therefore, the polyethylene glycol bis-anti-cancer drug constructed according to the tumor immune model can effectively cure tumor cancer with targeting, can precisely find the location of the tumor and has high research significance for real-time tumor killing.

Keywords: Differential equations, immune dynamic model, dual anticancer drug, polyethylene glycol, apoptosis.

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INTRODUCTION

Cancer, as the number one problem in global medicine, has always attracted much attention in treatment and research. In recent years, due to the gradual rise of immunotherapeutic methods, tumor immunity has become the main direction of cancer treatment (Chen *et al*., 2021) (Yang *et al*., 2023). However, the prediction and screening tools for immunotherapeutic methods are not yet mature, and the tumor environment is extremely complex, which makes it difficult to accurately assess the efficacy of drugs in the human body (Morton *et al*., 2023; Yan *et al*., 2023). Therefore, tumor-like organ immune models are used to evaluate drugs and ensure their therapeutic efficacy. Tumor-like organs are three-dimensional cell clusters that simulate the characteristics of tumors in the human body, mainly reflecting various characteristics of tumors, including drug sensitivity, genotype and so on. This model is closer to the human body model, which improves the success rate of drug development, more accurately controls the drug properties and effects, reduces the risk to the human body, reduces the cost and time needed for drug development, and brings better therapeutic effects and improves the quality of life of patients. It provides a new angle and new direction for the research of tumor drugs, which has important theoretical significance and practical value (Meng *et al*., 2021).

In this paper, we first establish the differential equations of the tumor generating process, analyze the tumor immune model cycle and describe the interaction between the tumor and the immune system. Then hypothetical conditional equations are established to analyze the persistence of the pulse system. Next, three bispecific antibodies are described, and homogeneity of antibody drug couplings is achieved using the fixed-point coupling technique, which unfolds the coupling synthesis of polyethylene glycol PEG and HCPT as a way to enhance the inhibitory effect on cancer and tumors. Finally, the antitumor activity was analyzed to derive the curative ability and inhibitory effect of polyethylene glycol bis-anti-cancer drugs on tumors. By analyzing the polyethylene glycol bis-anti-cancer drug through multiple indicators, it can be concluded that the polyethylene glycol bis-anti-cancer drug has a better healing ability for tumors, and it can inhibit the growth of tumor cells and reduce the activity of tumor cells. The proposed research can accurately complete the inhibition of tumors, so that the chances of survival of patients rise and the life of patients is treated, in line with the needs of today's times.

In the study of tumor immune dynamics, Von Rueden, S. K., *et al*. generalized the process of the cancer-immunity cycle, pointing out key aspects in the process that contribute to cancer regression, as well as changes in the tumor microenvironment and associated immune

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phenotypes, providing new perspectives. ves on the study of immune responses between the tumor and host (Von Rueden and Fan, 2021). Lazebnik, T *et al*. addressed the complexity of the interaction between immunity and cancer and developed a new predictive method to predict individual treatment outcomes from patient data (Lazebnik *et al*., 2021). Alshebremi, M *et al*. In order to discover the mechanism of action for the therapeutic efficacy of cryoablation on tumors, local changes in tumors were assessed using a rhabdomyosarcoma cell line to observe inflammatory zonation factors and cytokine responses. It was noted that attention needs to be paid to the free inflammatory response triggered by cryoablation (Alshebremi *et al*., 2023). To reduce the resistance and side effects of anticancer drugs, Bai, Y. *et al*. reviewed studies on metallic anticancer drugs and summarized antitumor complexes such as platinum, ruthenium, and gold (Bai *et al*., 2023). Cardiac anticancer drugs cause damage to myocardium, pericardium and endocardium, Singh, S *et al*. discussed atherosclerosis and ischemic complications of anticancer drugs in their study (Singh and Singh, 2021). Sun, J *et al*. pointed out that polyethylene glycol has potential application in cancer therapy with properties such as reversal of multidrug resistance and synergistic effect with antitumor drugs to enhance antitumor activity (Sun *et al*., 2021). Cibotaru, S *et al*. established structure-antitumor activity relationship for the antitumor activity of drugs and found that polyethylene glycolization and dimethylation induced specificity for tumor cells (Cibotaru *et al*., 2023). Dong, Z *et al*. prepared a new type of metal-containing nanotubes based on poly (ethylene glycol) on folate-nickel nanotubes in order to block the cell cycle for a more desirable antitumor effect (Huang, 2020).

Polyethylene glycol was found to be a powerful drug through related studies, based on the tumor immune dynamic model, which can be exploited in the study of anticancer drugs and antitumor activity.

MATERIALS AND METHODS

Statistical analysis

In the statistics and analysis of this paper, spss26.0 is the main tool used. Spss26.0 can be used to conduct in-depth analysis of drug efficacy data under the dynamic model of tumor immunity, including key indicators such as anti-tumor activity of drugs, tumor cell apoptosis ratio, relative tumor growth rate, and explore the difference and significance of drug efficacy under different treatment conditions through hypothesis testing and variance analysis.

Establishment of tumor immune model

Tumors are divided into two main categories, benign and malignant, and are one of the major threats to human health in today's world. Generally speaking, benign tumors are not invasive and malignant in human body, and can be cured and restored to health by surgery and medicine. However, malignant tumors have strong malignant proliferation and invasiveness, often called cancer, and are extremely difficult to remove in humans, and after surgical resection, they often recur and metastasize and are difficult to eradicate (Yeo *et al*., 2022; Zhang *et al*., 2023). In order to be able to eradicate malignant tumors, the method of inputting immune cells is used to improve immunity to tumor cells, to enhance the sensitivity of benefit cells to kill tumor cells and to stimulate and enhance the body's anti-tumor immune response. Synergize the body's immune system to kill tumors and inhibit tumor growth. Differential equations are established for the process of tumor generation as follows:

$$\begin{cases} \dfrac{dV}{dt} = rV(1-\dfrac{V}{K}) - \beta VE \\ \dfrac{dE}{dt} = rV(E_0 - E) - \sigma E \end{cases} \tag{1}$$

Where, R represents the natural growth rate of the tumor, K represents the maximum volume that the tumor can reach and E_0 represents the maximum possible number of immune cells.

According to the established tumor growth equation, the input of immune effector cells was changed to interval transient pulse input to construct the tumor immune model, and the specific equation is as follows:

$$\begin{cases} \dfrac{dx}{dt} = s + \rho xy - \delta x, & t \neq (n-1)T + lT, t \neq nT \\ \dfrac{dy}{dt} = \alpha y(1-\beta y) - \rho xy, & t \neq (n-1)T + lT, t \neq nT \\ x(t^+) = (1-p_E)x(t), & t = (n-1)T + lT \\ y(t^+) = (1-p_T)y(t) & t = (n-1)T + lT \\ x(t^+) = x(t) + \mu, & t = nT \\ y(t^+) = y(t), & t = nT \end{cases} \tag{2}$$

where X is the number of immune cells, Y represents the number of tumor cells, S is the normal input rate of immune cells, PXY is the term describing the interaction of immune cells with tumor cells that leads to the production of immune cells, δ is the mortality rate of immune cells and σ is the endowment growth rate of tumor cells. 1/B is the environmental holding capacity, PXY represents the term describing the interaction of immune cells with tumor cells leading to tumor cell death, P_E and P_T represent the instantaneous killing rate of the drug on immune cells and tumor cells, respectively, at the moment of t = (n – 1)T+lT and μ represents the input of immune cells at the moment of t=nT. In addition, T>0 represents the cycle in which the pulse therapy strategy is implemented, 0<l<1. Some lemmas are used in the construction of the tumor immune model. Let y(t)=0, the subsystem of the impulse system is obtained as follows:

$$\begin{cases} \dfrac{dx}{dt} = s - \delta x, & t \neq (n-1)T + lT, t \neq nT \\ x(t^+) = (1-p_E)x(t), t = (n-1)T + lT \\ x(t^+) = x(t) + +\mu, \quad t = nT \end{cases} \tag{3}$$

The subsystem of the impulse system can be denoted as:

$$\begin{cases} x^*(0^+) = \dfrac{C_1}{1-(1-p_E)e^{-\delta T}} \\ x^*(lT^+) = (1-p_E)x^*(0^+)e - \delta^{lT} + (1-p_E)(1-e^{-\delta lT})\dfrac{S}{\delta} \end{cases} \tag{4}$$

Eq.

$$C_1 = \left[\left(1-e^{-\delta(1-l)T}\right) + (1-p_E)\left(e^{-\delta(1-l)T} - e^{\delta T}\right)\right]\frac{S}{\delta} + \mu .$$

There exists a unique tumor extinction period solution within the pulse system (x*(t), 0), where the period solution is:

$$x^*(t) = \begin{cases} \dfrac{s}{\delta} + \left(x^*(0^+) - \dfrac{s}{\delta}\right)e^{-\delta(t-(n-1))T}, (n-1)T < t \le (n-1)T + lT \\ \dfrac{s}{\delta} + \left(x^*(lT^+) - \dfrac{s}{\delta}\right)e^{-\delta(t-(n-1)T+lT)}, (n-1)T + lT < t \le nT \end{cases} \tag{5}$$

For any solution of the impulse system X(t) satisfies condition $\lim_{t\to+\infty}\left(x(t) - x^*(t)\right) = 0$. When $\delta > \dfrac{\rho}{\beta}$, there exists M_E, M_T>0 such that when t(>0) is sufficiently large, the following condition holds:

$$\begin{cases} x(t) < M_E \\ y(t) < M_T \end{cases} \tag{6}$$

Where (X(t),Y(t)) represents any solution of the impulse system.

Cycle analysis of tumor immunity models

The cycle of the tumor immune model is also the cancer immune cycle, which mainly describes the framework of the interaction between the tumor and the immune system, from which the antigen release and presentation of the tumor cells can be seen (Huang *et al*., 2022; Mirian *et al*., 2022). The cycle of the tumor immune model can be derived based on the tumor immune model, and the tumor immune model cycle is shown in fig. 1.

If condition $(1-p_T)e\int_{0^+}^{T}\left(\alpha - \sigma x^*(t)\right)dt < 1$ holds, then the tumor extinction cycle solution (X*(t),0) is locally asymptotically stable. From the behavior of the solution with small amplitude perturbations the following linear approximation of the impulse system can be obtained:

$$\begin{cases} \left.\begin{aligned} \dfrac{du(t)}{dt} &= -\delta u(t) + \rho x^*(t)v(t) \\ \dfrac{dv(t)}{dt} &= \left(\alpha - \rho x^*(t)\right)v(t) \end{aligned}\right\} t \neq (n-1)T + lT, t \neq nT \\ \left.\begin{aligned} u(t^+) &= (1-p_E)u(t) \\ v(t^+) &= (1-p_T)v(t) \end{aligned}\right\} t = (n-1)T + lT \\ \left.\begin{aligned} u(t^+) &= u(t) \\ v(t^+) &= v(t) \end{aligned}\right\} t = nT \end{cases} \tag{7}$$

The state transfer matrix of the tumor from t=0$^+$ to t =T under pulsed system observation can be obtained:

$$\Phi(T^+) = \begin{pmatrix} (1-p_E)e^{-\delta T} & * \\ 0 & (1-p_T)e^{\int_{0^+}^{T}\left(\alpha - \sigma x^*(t)\right)dt} \end{pmatrix} \tag{8}$$

The eigenvalues of $\Phi(T^+)$ can be derived from the state transfer matrix as follows:

$$\begin{cases} \lambda_1 = (1-p_E)e^{-\delta T} < 1 \\ \lambda_2 = (1-p_T)e^{\int_{0^+}^{T}\left(\alpha - \sigma x^*(t)\right)dt} \end{cases} \tag{9}$$

It follows that the cycle solution (X*(t),0) of the immunotumor model is locally stable.

Persistence analysis of pulse systems

The durability of the pulsed system is the durability of the tumor treatment, that is to say, in the process of the tumor treatment, it can continuously and effectively work on the tumor cells, so as to achieve the effect of long-term control or elimination of the tumor. To analyze the durability of the pulse system, if the following conditions hold:

$$\begin{cases} \delta > \dfrac{\rho}{\beta} \\ (1-p_T)e^{\int_{0^+}^{T}\left(\alpha - \sigma x^*(t)\right)dt} > 1 \end{cases} \tag{10}$$

Then the impulse system is persistent and positive periodic solutions exist. It follows from the above lemma that there exist M_E, M_T >0 and M_T >0 such that when M_T >0 can be sufficiently large, there is the following equation:

$$\begin{cases} m_E < x(t) < M_E \\ 0 < y(t) < M_T \end{cases} \tag{11}$$

It may be useful to set formula (6) to hold for any t ≥ 0. It is shown below that there exists M_T >0 such that when Y(t)≥m_T is sufficiently large, there is m_0(>0). According to Eqs. (8) and (5), there exist sufficiently small numbers ε_2(>0) and m_0(>0) to obtain the following equation:

$$\begin{cases} \rho m_0 < \delta \\ \varsigma > 1 \end{cases} \tag{12}$$

Table 1: Results of immunofluorescence staining

Tumor cell	Initial activity	Final activity	Activity description
NCI-H29	50%	20%	Low activity
NCI-H1975	60%	30%	Low activity
MDA MB-468	65%	35%	Low activity
HCC1954	70%	25%	Low activity
BXPC-3	75%	30%	Low activity
NCI-N87	80%	36%	Low activity

Table 2: Relative tumor growth rate

Point in time	The growth rate of the group treated with polyethylene glycol double anticancer drugs	Rate of increase in the untreated group
The first week	20%	50%
The second week	30%	60%
The third week	32%	65%
The fourth week	35%	70%
The fifth week	37%	75%
The sixth week	38%	80%

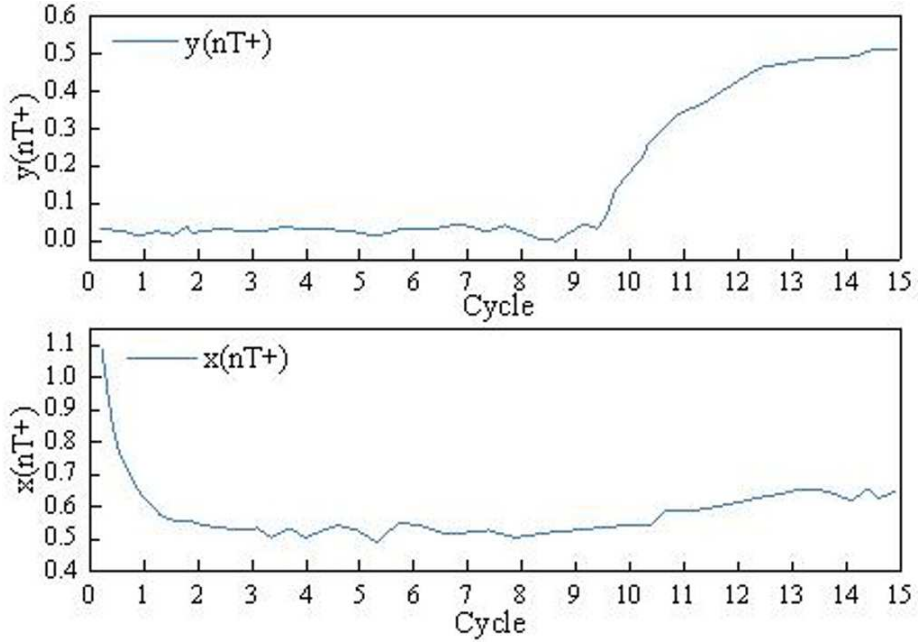

Fig. 1: Cycle of tumor immune model

By the above lemma one obtains $\lim_{t\to+\infty}\left(\hat{x}(t)-\hat{x}^{*}(t)\right)=0$, where x(t) is a positive periodic solution of the impulsive system and hence, in the presence of $t_2 > 0$, $t>t_2$, contains the condition $x(t)<x(t)\varepsilon_2$, from which it follows that there exists $t_3 > t_2$ and $y(t_3)>m_0$. From this, one can further set $n_1 \in Z_+$ such that $t^* \in((n_1-1)T+lT,n_1T+lT)$ and when $t \in(t^*,n_1T+lT)$, there exists $y(t)\leq m_0$.

Setting the persistence information of the impulse system to $t^{**}=\inf\{t/t^*, y(t)>m_0\}$, then $t^{**} \in (n_1T+lT,(n_1T+lT)+n_2T)$, and when $t \in(t^*,t^{**})$, there exists $y(t)\leq m_0$. If there exists $n_3 \in Z_+$ such that $t^{**} = n_3T +lT$, then by $y(t^{**})<m_0$, there exists $\Delta t>0$ such that when $t \in [n_1T+lT,t^{**}]$, there is $y(t)\leq m_0$, a contradiction. Thus, it follows from the continuity of y(t) at $t = t^{**}$ that $y(t^{**})=m_0$. For $t \in [n_1T+lT,t^{**}]$ assume $(n_1T+lT)+(n_4-1)T<t\leq(n_1T+lT)+n_4T$, where $1\leq n_4\leq n_2$.

The above process can be continued under condition $t>t^{**}$, since $y(t^{**})=m_0$. It follows from this that there exists a positive periodic solution to the impulsive system by Schauder's immovable point theorem.

RESULTS

Bispecific antibody construction methods

Bispecific antibodies are a form of dual anticancer drugs, primarily consisting of antibodies that recognize and bind two different antigens or epitopes simultaneously. The construction of bispecific antibodies is generally categorized into three types:

(1) cell-engineered bispecific antibodies are mainly produced by hybridoma or tetragenic hybridoma technology, which is also based on the Milstein and Cuello described methods (Underwood *et al*., 2022; Yang *et al*., 2021). Among them, the hybridoma method is simpler and produces bispecific antibodies that are naturally assembled. However, the disadvantage of this technique is that tetragenic hybridomas may produce multiple. Therefore, sophisticated techniques are required to separate bispecific antibodies from other antibody fragments.

(2) Chemically engineered bispecific antibodies are antibodies that are primarily from two different hybridomas and are generated using cross-linking reagents such as nitrogen-succinylcholine-2-pyridyldithiopropionate conjugation. However, due to the difficulty in qualifying cross-linked immunoglobulin molecules, it is necessary to utilize the sulfhydryl reaction properties between 2-nitro or o-cis-butenediimide and the hinge region of the IgG molecule to generate bispecific antibodies. Specifically, purified IgG or F(ab′)2 fragments prepared enzymatically are reduced to Fab′ in a temperature-suitable environment. The SH group of its free hinge region is covalently bound to DTNB or o-PDM, and another Fab′ segment containing the SH group of the free hinge region produced by the reduction of the secondary antibody is added and oxidation reaction is utilized to produce cross-linking of disulfide bonds to form a bispecific antibody molecule (Yilmaz and Ravandi, 2020).

(3) Current genetic engineering methods for bispecific antibodies fall into four main categories. One is the cross-linking of terminal cysteines and disulfide bonds of molecules functionally expressed in E. coli scFv by modification. The second is the joining of two different scFv monomers with sufficiently long collapsible linkers, i.e., the generation of the so-called bispecific single-chain Fv fragment Bs(scFv)2. The third is the use of fos-jun leucine zipper technology, which couples the scFv fragments together and generates a genetically bispecific antibody. Fourth, when the constructed E. coli scFv has no or only short linkers, it is replaced by interchain pairing, a phenomenon that arises due to the natural affinity between VH and VL, and in this way diabodies can be generated, i.e., the coding regions of the VH and VL chains of the two different antibodies are expressed on two separate chains, with a short linker within each one sufficient to prevent the binding of its own VH and VL regions and the two chains are expressed equimolarly at the same time.

Fixed-point coupling

In order to achieve homogeneity of antibody-drug couplings, the technique of targeted coupling becomes a key step. Among the targeted coupling, the most commonly used one is the Thiomab technology, which is to utilize the amino acid in the genetically engineered mutant antibody as a cysteine without affecting the affinity of the antibody, and form a thioether bond with the maleamide-modified cytotoxic drug to unfold the targeted coupling to generate homogeneous antibody-drug couplings, so as to enhance the therapeutic coefficients. Since this method requires a reduction-re-oxidation process, unpaired sulfhydryl groups are likely to be present. Moreover, the linker is unstable in the circulatory system and can exchange with the reactive sulfhydryl groups of free cysteine in plasma, leading to premature release of cytotoxic agents. Therefore, in the design of antibody-drug coupling fixed-point coupling, not only the homogeneity of antibody-drug coupling should be achieved, but also the stability of the linker in the circulatory system in vitro and in vivo should be ensured in order to avoid systemic toxicity brought by antibody-drug coupling, and the linker can be effectively disconnected and active cytotoxic drugs can be released to kill the tumor cells only after the antibody-drug coupling is endocytosed by the tumor cells (Clark and Stein, 2020; High and Carmon, 2023).

Another representative technique for targeted coupling is the introduction of non-natural amino acids into antibodies is. In order to produce homogeneous antibody-drug couplers, p-acetylphenylalanine is introduced into the antibody and p-acetylphenylalanine with an aldehyde group can selectively form a stable oxime bond with the alkoxylamines of cytotoxic drugs to realize the fixed-point coupling. However, the method is technically demanding, the process is relatively complex, and the generalizability has not been demonstrated so far. In addition, the use of amber codons in mammalian cells tends to lead to the erroneous introduction of unnatural amino acids, which complicates the purification of antibody-drug couplers and may also cause toxic side effects.

Polyethylene glycol bis-anti-cancer drug coupling composition

The composition of polyethylene glycol bis-anticancer drug was analyzed according to the constructed tumor immune model. Hydroxycamptothecin is a kind of alkaloid antitumor drug, which can inhibit the propagation of DNA topoisomerase and in this way prevent the growth of tumor cells and has good therapeutic effect on gastric cancer and pancreatic cancer, etc., and can cure the patients' diseases. However, it is difficult to be used in clinical treatment due to its low solubility and the unstable structure of the biologically active lactone ring, which is prone to hydrolysis and transformed into a carboxylate structure that is non-biologically active and contains toxic side effects. In order to improve the solubility of HCPT and stabilize the bioactive lactone ring structure, hydroxycamptothecin is shown in fig. 2.

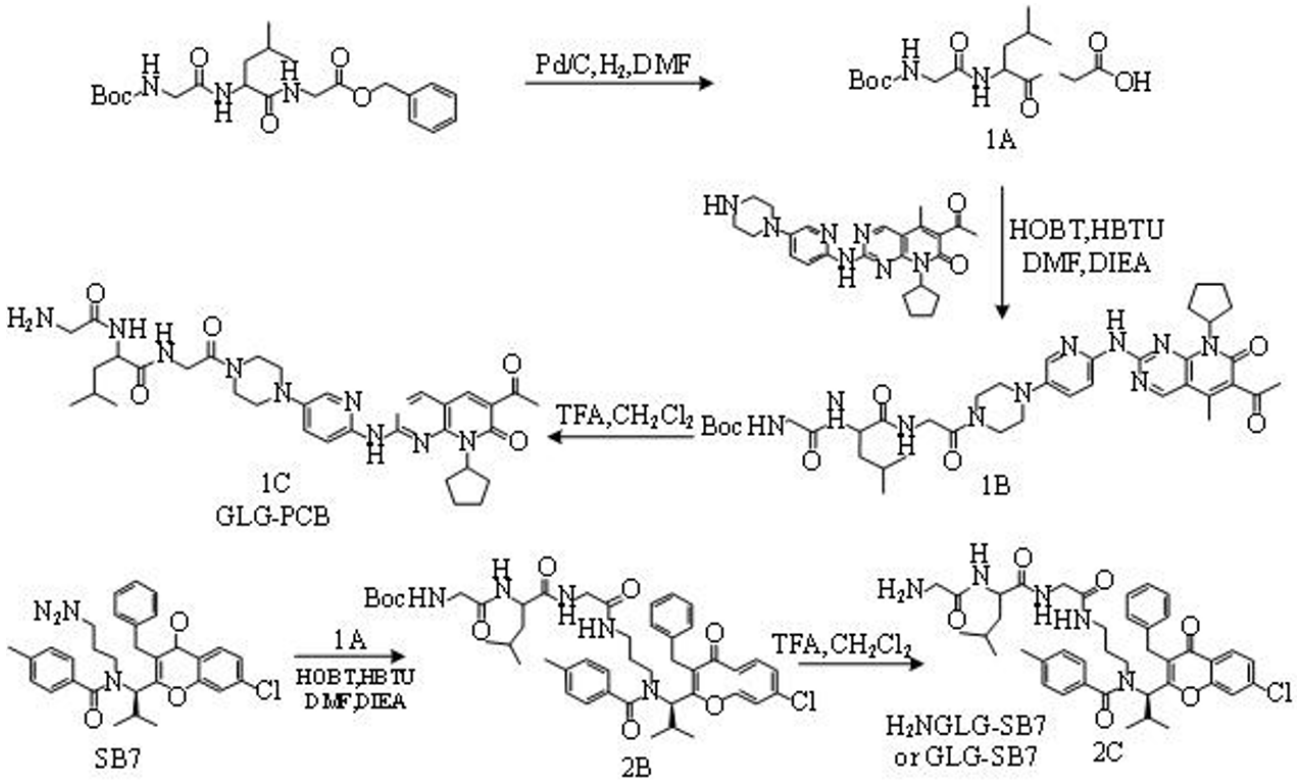

Fig. 2: Hydroxycamptothecin

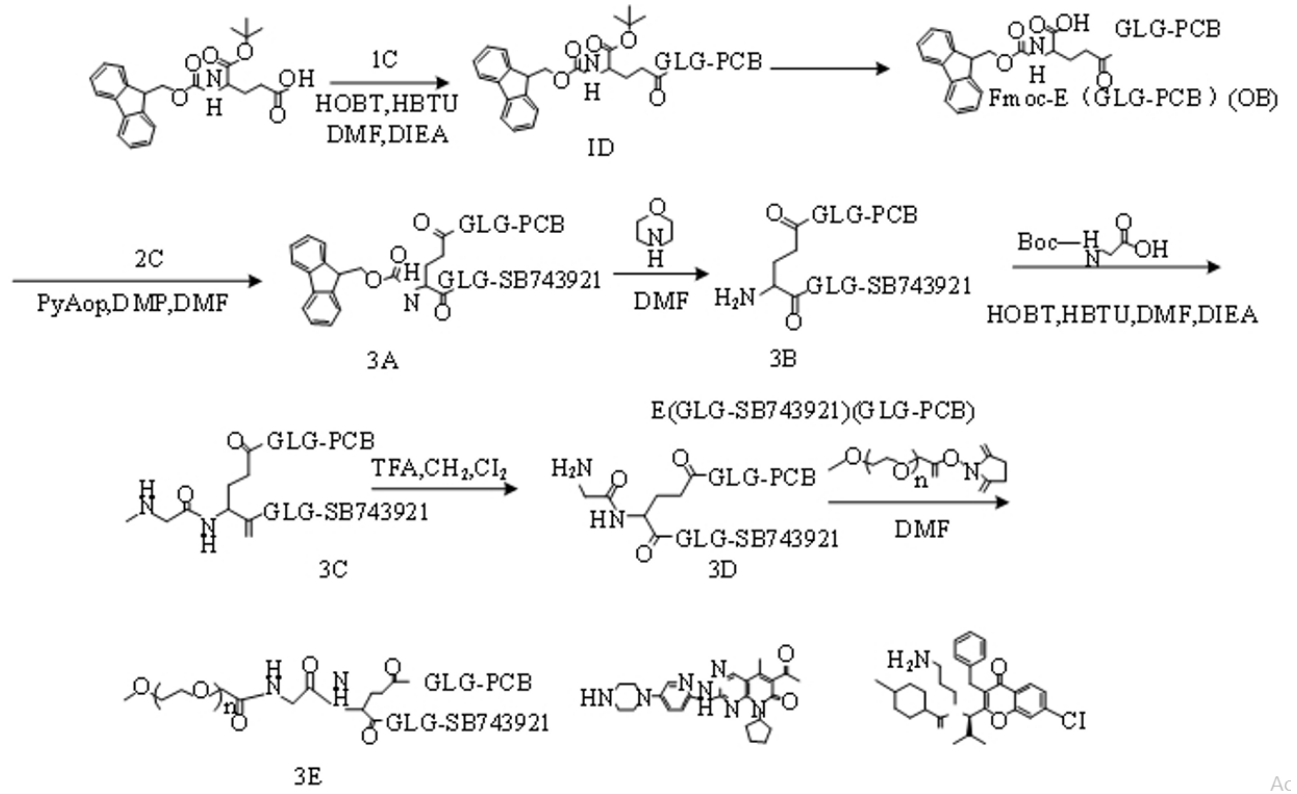

Fig. 3: Synthesis of MPEG-HCPT

Polyethylene glycol PEG and HCPT were coupled to enhance the water solubility and temperature utilization of the drug, reduce the immunogenicity and antigenic activity, prolong the circulation time and half-life, and give the drug passive targeting ability, etc., so as to improve the efficacy of the drug, and better cure the patient's disease.

Hydroxycamptothecin with a purity of about 99% and poly(ethylene glycol) monomethyl ether with a molecular weight of 750 and a purity of about 99% were chosen to initiate the synthesis and all other reagents were analytically pure.

Fig. 3 shows the synthesis of MPEG-HCPT, firstly, the terminal chlorinated MPEG-Cl was prepared, then the terminal iodinated MPEG was prepared by halogen interchange and finally, MPEG-I reacted with the 10-position hydroxyl group of HCPT to form ether, which gave the target product, MPEG-HCPT.

Add 11.4 g of MPEG in a 100 mL three-necked flask and add 90 ml of dichlorosulfoxide $SOCl_2$ under the protection of nitrogen, stirring and refluxing at 75°C for 24h; rotary evaporation to remove the excess of $SOCl_2$, and dissolve the obtained product in 40 mL of CH_2Cl_2, and wash with saturated saline twice times to remove the acid produced by $SOCl_2$ hydrolysis. After drying by anhydrous $NaSO_4$, the solvent was removed by spinning under reduced pressure to obtain light yellow MPEG-Cl with a yield of about 68.5%. 4.3g of MPEG-Cl was put into a 50mL three-necked flask, 30mL of anhydrous acetone was added, fully stirred to dissolve, 1.19g of KI was added, and the reaction was stirred and refluxed at 55°C for 3h, then 0.086g of KI was added, and the reaction was continued for 8h and the precipitate was filtered and removed and the solvent was removed by spin distillation. The residue was dissolved in 20 mL CH_2Cl_2, washed twice with saturated saline, dried by anhydrous $NaSO_4$ and then rotary evaporated to remove the solvent to obtain the yellow product MPEG-I, the yield was about 78.0%.

To a 250mL three-necked vial was added 3.51g of MPEG-I, 1.45g of HCPT, 150mL of N-dimethylformamide and 0.274 g of potassium carbonate, which was dissolved by thorough stirring and then reacted at reflux at 75°C for 24 h; DMF was evaporated under reduced pressure to obtain a dark brown gelatinous mixture. The mixture was dissolved in 30mL of methanol, ultrasonically dispersed for 10 min, insoluble inorganic salts were removed by centrifugation, and the upper layer of the clear liquid was spun to remove the solvent to obtain a solid mixture. The solid mixture obtained was ultrasonically dispersed in ethyl acetate to dissolve the unreacted MPEG. The solid crude product obtained by centrifugation to collect the bottom precipitation was dissolved with 2 mL of ethyl acetate/methanol mixed solvent, and was separated by column chromatography using a dry column loading with methanol as the eluent to obtain the brown powdery target product, MPEG-HCPT, with a yield of 33.8%.

According to the above synthesis process, the coupling of polyethylene glycol and HCPT was synthesized to enhance the solubility of the drug, so that the drug could give full play to its own characteristics and cure the patient's disease precisely.

Preparation of materials

Tumor activity analysis requires preparation of NCI-H1975 human lung adenocarcinoma cells, NCI-H292 human lung bronchial mucinous epithelioid carcinoma cells, MDA MB-468 human triple-negative breast cancer cells, HCC1954 human ductal carcinoma of the mammary glands, BXPC-3 human pancreatic carcinoma cells in situ, NCI-N87 human gastric carcinoma cells, HCC827 human non-small-cell lung cancer cells, NCI HCC827 human non-small cell lung cancer cells, NCI-H1650 human non-small cell lung cancer cells and other cell structures. The instruments to be prepared at the same time are a biosafety cabinet model SBC- 1300IIA2, a cell culture incubator model Thermo 311, a cell counter model CI1000, a flow cytometer model iQue 3-BR, a centrifuge model Sc-3610, a water bath model DK-8D, and a 1260Affinity liquid chromatograph. Experiments were carried out based on the established tumor immune model to test the degree of inhibition of tumor activity by the polyethylene glycol bis-anti-cancer drug, as well as the cure (Dai *et al*., 2023; Xiong *et al*., 2024).

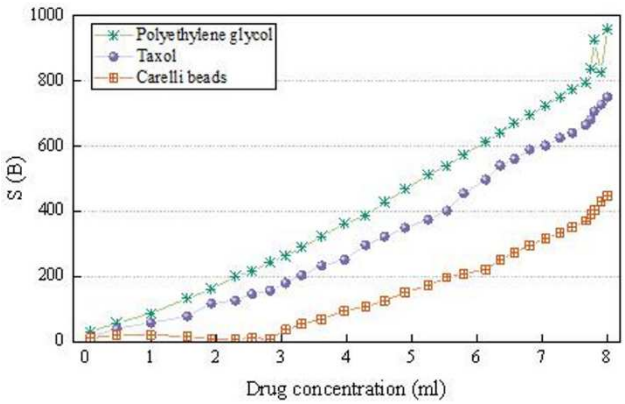

Fig. 4: Antibody endocytosis test

Immunofluorescence staining of tumor tissue sections

Immunofluorescence staining of tumor tissue sections is a commonly used assay to measure tumor activity and is used to detect the localization and expression of proteins and molecular markers in tumor tissue. Paraffin sections of the desired cancer cells were water-bathed at 40°C for 30 min to achieve that the sections were in the unfolded state and the sections were adhered to adhesive slides. The samples were deparaffinized in xylene solution for 10 min, and the above steps were repeated 2 times. The sections were washed in anhydrous ethanol for 5 min, and

the above steps were repeated once. The tissue samples were then sequentially hydrated in 95% ethanol, 70% ethanol, and purified water for 5 min. boiled pre-formulated citrate. Heat repair in citrate for 15min and cool naturally to room temperature. Rinse 3 times with PBS for 3 min each. 10% goat serum sealing solution was closed at 37°C for 30 min. After removing the sealing solution Phospho-c-Met was applied to the tissues and incubated for 30 min at 37°C. Sections were washed 3 times with PBS for 3 min each time.Tissues were coated with Goat Anti-RabbitIg GH&L solution and incubated at 37°C for 20 min. The sections were washed by PBS for 3 times, each time for 3 min. the Opal dye was applied to the tissues and incubated at 37°C for 15 min. the sections were washed by PBS for 3 times, each time for 3 min. the above operation was repeated to stain the tumor tissue sections using polyethylene glycol bis-anti-cancer drug coupler. Drops of DAPI staining solution were incubated for 10 min at 37°C and the sections were washed with PBS 3 times, each time for 3 min. The results were analyzed using a whole tissue section multicolor fluorescence quantitative analyzer and HALO unfolding.

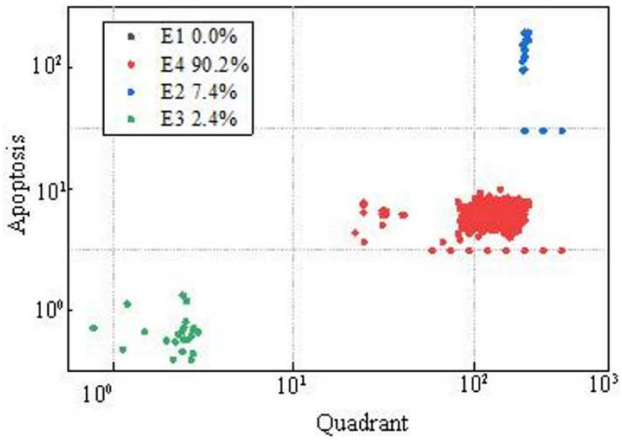

Fig. 5: Apoptosis test

Table 1 shows the results of immunofluorescence staining, polyethylene glycol bis-anti-cancer drug has a better inhibitory ability in different tumor cells, so that the activity of NCI-N87 was gradually reduced from the beginning of 80% to 36% and NCI-H29 was the most significant, from 50% to 20%. It proved that the polyethylene glycol bis-anti-cancer drug restrained the rate of tumor cell reproduction and growth. And the metabolism of tumor cells unfolded interference, which has a certain therapeutic effect on the tumor, can effectively kill or inhibit the growth of cells, with specific killing effect against tumor cells. Reduced the activity of tumor cells, enough to the location of the tumor precise cure, improve the chances of survival of patients, in line with the needs of today's pharmaceutical industry.

Relative tumor growth rate

Relative tumor growth rate is an important indicator of the therapeutic effect of antitumor activity, reflecting the growth of tumors receiving antitumor therapy, and the speed of growth of untreated tumors. Generally, a relative tumor growth rate of less than 40% proves that the drug contains antitumor activity and that treated tumors rise more slowly than untreated tumors. If the relative tumor growth rate is greater than 40%, it means that the drug has poor or insignificant antitumor properties. The relative tumor growth rate of the polyethylene glycol bis-anti-cancer drug was tested and table 2 shows the relative tumor growth rate. It can be seen that the relative tumor growth rate of polyethylene glycol bis-anti-cancer drug is low, below 40%, while for the treated tumor growth rate is high, at 80% role, proving that polyethylene glycol bis-anti-cancer drug has enough effect on the cure of tumor cells. The use of precise targeting effect, the drug to the location of tumor occurrence, to achieve the direct killing of tumor cells and polyethylene glycol double anticancer drug passive targeting and retention effect, reducing the toxic side effects produced by the combination of small molecule anticancer drugs. Effectively inhibited the growth tendency of the tumor that did not receive treatment. Since there is no drug to restrain the growth rate of the tumor and kill the tumor cells precisely, it leads to the increasing expansion of the tumor and faster growth rate. It is more able to highlight the inhibitory nature of polyethylene glycol double anticancer drug for tumor activity, which not only improves the cure rate of tumor disease, but also provides a new way and direction for tumor treatment.

Antibody endocytosis assay

Antibody endocytosis test is also an important indicator of anti-tumor activity, which is mainly used to assess whether the antibody drug, after binding to specific antigens of tumor cells, can be swallowed into the cell by the tumor cells to exert therapeutic effects. Tumor cells in logarithmic growth phase were digested by trypsin and collected in centrifuge tubes, centrifuged at 1200 r/min for about 3 min, the supernatant was discarded, and fresh complete culture medium was added and resuspended. NCI-H292 and NCI-H1975 cells were inoculated into 96-well flat-bottomed plates and placed in an incubator at 37°C with 5% CO_2 and MDA-MB-468 tumor cells were placed in an incubator at 37°C without CO_2. After 24h of cell wall attachment, the culture medium was discarded, and 50μL of fresh medium containing 2% fetal bovine serum was added to each well and another 50μL was added, and after labeling with Incucyte Fabfluor-pH Antibody Labeling Dyes dye, the antibody mixture was obtained and placed in the Incucyte apparatus for incubation. Fig. 4 shows the results of the antibody endocytosis experiments. The antibody endocytosis effect of polyethylene glycol bis-anti-cancer drugs was better, with an overall increasing trend, which proved that the polyethylene glycol bis-anti-cancer drugs had a stronger ability to enter the tumor cells and were able to be

absorbed by the tumor cells and could act quickly in the tumor cells. While the antibody endocytosis effect of paclitaxel and karelizumab drugs is poorer, the overall trend of the trend of polyethylene glycol bis anticancer drugs, the curative effect on tumor cells is not obvious, difficult to be absorbed by tumor cells, can not effectively inhibit the growth and reproduction of tumor cells, and there is no precise killing of tumor cells.

Apoptosis test

Apoptosis test is a test method mainly used to examine whether a cell is in a state of apoptosis or not, and to analyze the process and extent of apoptosis. The main principle of the test is to detect specific morphological and biochemical changes within the cell and through these changes to determine whether the cell is in apoptosis. Fig. 5 shows the results of the apoptosis test, which caused a high percentage of apoptosis in tumor cells and the percentage of apoptosis was 97.6%, respectively, with early apoptosis being 90.2% and late apoptosis being 7.4%. It proves that the polyethylene glycol bis-antidote has a significant effect on the treatment of tumor cancer, which can effectively induce apoptosis of tumor cells and improve the stability of the drug. It achieves specific recognition and killing of tumor cells, which improves the therapeutic effect, achieves the degree of inhibition of tumor cells, enhances the cure rate of tumor cancer, and provides a new technology and direction for the field of tumor cancer.

CONCLUSION

In this paper, we first establish the tumor immune dynamic model based on the tumor's growth process and analyze the cycle and persistence of the tumor immune model. In order to analyze the degree of tumor control by polyethylene glycol bis-anti-cancer drug, the degree of tumor inhibition by polyethylene glycol bis-anti-cancer drug is completed from the apoptosis test, relative tumor growth rate and immunofluorescence staining of tumor tissue sections, which can be concluded that polyethylene glycol bis-anti-cancer drug has enough inhibition of tumor cells, reduces the activity of tumor cells to 20%, hampers the growth and metabolism rate of tumor cells, and has a precise curative capacity for the tumor cells with precise curing ability. And the polyethylene glycol bis-anti-cancer drug limited the growth size of the tumor so that the growth rate of the tumor was below 40%, which proved that the polyethylene glycol bis-anti-cancer drug had a better therapeutic effect on the tumor and reduced the toxic side effects at the same time. The polyethylene glycol bis-anti-cancer drug, constructed using the tumor immune model, can target and remove tumor cells, inhibit their reproduction and growth, and quickly enter the cells to exert its effects, thereby enhancing the cancer cure rate.

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