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Full Length Research Paper

Biodegradable methoxy poly (ethylene glycol)-poly (lactide) nanoparticles for controlled delivery of dacarbazine: Preparation, characterization and anticancer activity evaluation

Bao-yue Ding[1,2†], Wei Zhang[1,3†], Xin Wu[1], Xiang Wang[1], Wei fan[1], Shen Gao[1]*, Jing Gao[4], Lu-lu Ma[4], Xue-ying Ding[4] and Qiang Hao[5]

[1]Department of Pharmaceutics, Changhai Hospital, Second Military Medical University, Shanghai, PR China.
[2]Department of Pharmaceutics, Medical College of Jiaxing University, Jiaxing, PR China.
[3]Department of Pharmacy, No. 522 Hospital of PLA, Luoyang, Henan, PR China.
[4]Department of Pharmaceutics, School of Pharmacy, Second Military Medical University, Shanghai, PR China.
[5]Department of Radiology, Changhai Hospital, Second Military Medical University, Shanghai, PR China.

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Dacarbazine (DTIC) loaded or blank methoxy poly (ethylene glycol)-poly (lactide) (MPEG-PLA) nanoparticles (NPs) were prepared by modified w/o/w double emulsion-solvent evaporation method through ultrasonic processor without any additional additives. The hemolytic test and cytotoxicity test of blank MPEG-PLA NPs demonstrated that the obtained drug delivery system is safety. Its particle size distribution, morphology, drug loading, drug release profile and anticancer activity *in vitro* were studied in detail. The small sized nanoparticles (NPs) with a particle size of 144.2±7.8 nm in diameter and drug encapsulation efficiency of 70.1±2.3% are easy to be dispersed in water and suitable for vascular administration. The drug release pattern was biphasic with a fast release rate followed by a slow one. The *in vitro* and *in vivo* study results demonstrated that compared with free DTIC, DTIC loaded MPEG-PLA NPs could induce more apoptosis of cancer cell and showed enhanced antitumor activity. The described DTIC loaded MPEG-PLA nanoparticle in this paper might be a novel potential formulation for metastatic melanoma therapy.

Key words: Malignant melanoma, dacarbazine, methoxy poly (ethylene glycol)-poly (lactide), double emulsion and solvent evaporation method, nanoparticles, anticancer activity.

INTRODUCTION

Malignant melanoma is one of the most lethal and aggressive human malignancies. Although melanoma accounts for only 4% of all dermatological malignancies, it is responsible for 80% of mortalities from skin tumors (Jemal et al., 2007). Moreover, the incidence of melanoma has been steadily increasing worldwide, resulting in an increasing public health problem. Common treatments for malignant melanoma involve a combination of therapies including surgical removal, chemotherapy, radiotherapy and so on. Therapy for early disease is predominantly surgical, with a minor benefit noted with the use of adjuvant therapy. Management of systemic melanoma is a challenge because of a paucity of active treatment modalities (Svetomir et al., 2007). The most commonly utilized chemotherapeutic regimens have been tried without significantly increasing overall survival rates among metastatic melanoma patients (Soengas et al., 2003). Alkylating agents are among the most widely used chemotherapeutic agents for the treatment of malignant melanoma (Mhaidat et al., 2007). Dacarbazine or dimethyl-triazeno-imidazol carboxamide (DTIC) is the only US Food and Drug Administration (FDA) approved chemotherapeutic agent for melanoma. It is so far the

*Corresponding author. E-mail: liullk@126.com. Tel: +86 21 81871293. Fax: +86 2181873715, +86 21 81871293-803.

† These authors contributed equally to this work.

most active single agent for treatment of metastatic melanoma (Quirin et al., 2007; Lillehammer et al., 2007). The drug exerts its anti-tumor activities by methylation of nucleic acids or direct DNA damage and results in growth arrest and subsequent cell death (Loo et al., 1976). However, its clinical application and therapeutic effect has been limited greatly due to the rapid degradation and metabolism of DTIC after intravenous injection as well as drug resistance (Xie et al., 2009). Presently, the only available formulation for clinical use is ordinary sterile powder for injection, whose therapeutic efficiency is only 20% and has serious adverse effects. Therefore, there is an urgent need for developing alternative DITC formulation for malignant melanoma.

Colloidal drug delivery systems such as liposomes, micelles or nanoparticles have been intensively investigated for their use in tumor therapy. The effectiveness of drug delivery systems can be attributed to their small size, reduced drug toxicity, controlled drug release and modification of drug pharmacokinetics and biodistribution (Joanna et al., 2006). For example, entrapping cytotoxic agents in nanoparticles or liposomes can improve drug stability, influence pharmacokinetics and reduce overall toxicity. Tumor vasculature has been described as “leaky” due to the presence of interendothelial junctions and transendothelial channels, therefore, it has been demonstrated that the use of colloidal systems improves tumor therapy due to the enhanced permeability and retention (EPR) effect within the tumor site (Yuan et al., 1995; Hobbs et al., 1998; Mojgan et al., 2010). Among these carriers, Nano-technology shows promising application in drug delivery system that accounts for the main part of nanomedicine (Leach et al., 2005; Ricci et al., 2005; Ferrari, 2005; Jemal et al., 2008; Gou et al., 2008b; Farokhzad and Langer, 2009). It provided a novel method to overcome the poor water solubility and instability of hydrophobic drugs, after hydrophobic drug entrapped into amphiphilic polymeric nanoparticles, drug loaded nanoparticles could be well and stably dispersed in water solution to meet the requirement of intravenous injection (Saffie-Sieverb et al., 2005). There were also some reports about novel formulations of DTIC, such as nano-emulsion, cubosomes and so on, which could prolong the shelf life, increase safety as well as enhance the anticancer activity of DTIC (Di Bei et al., 2010; Srikanth Kakumanu et al., 2011). These studies could provide alternative DITC formulation for malignant melanoma.

Recently, biodegradable polymeric nanoparticles are highlighted as advanced drug delivery system for cancer therapy because of their properties of low toxicity, long-circulation, and tumor localization that are associated with such polymer carriers, many studies of anticancer drugs based on biodegradable polymer nanoparticles have been performed in the preclinical and clinical research (Service, 2005; Ganta et al., 2008; Wang et al., 2008). Methoxy poly (ethylene glycol)-poly (lactide) (MPEG-PLA) diblock copolymer with properties of long-circulation, great biodegradability and compatibility has been widely applied in drug delivery system. MPEG-PLA nanoparticle was regarded as safe drug vectors and widely used in drug delivery, which was also one widely studied intravenously injectable drug vectors (Gref et al., 1994; Matsumoto et al., 1999; Dong and Feng, 2004; Lu et al., 2005; Zhang et al., 2006). Meanwhile, there is still nano-drug based on MPEG-PLA nanoparticles that has been paid clinical study and some were proved by FDA to be applied in clinic (Kim et al., 2004; Rapoport, 2007).

To overcome the limitations of the current therapies for malignant melanoma, we developed a novel dacarbazine formulation based on MPEG-PLA diblock copolymer with great biodegradability and compatibility. The prepared dacarbazine loaded MPEG-PLA nanoparticle was characterized, and drug release profile has been studied. Meanwhile, the hemolytic test and MTT had been done to evaluate the safety of MPEG-PLA nanoparticle as intravenous drug vector. And the therapeutic effect of DTIC loaded MPEG-PLA nanomedicine on malignant melanoma has been evaluated *in vitro* and *in vivo*.

MATERIALS AND METHODS

Methoxy poly (ethylene glycol)-poly (lactide) (MPEG-PLA) (3000:30000, Mw=33,000) was obtained from Shandong Institute of Medical Instrument (Shangdong, China). Human serum albumin (HSA) and 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (MO, USA). Dacarbazine (DTIC) was purchased from Suzhou Lixin Pharmaceutical Co. (Suzhou, China). The human malignant melanoma cell lines A375 and mice fibroblasts cell lines NIH were maintained in the International Joint Cancer Institute, Second Military Medical University (Shanghai, China). DMEM medium or fetal bovine serum (FBS) was purchased from Hycolne Co. (UT, USA). Dimethyl sulfoxide (DMSO), dichloromethane (DCM), acetone and methanol were purchased from China National Medicine Corporation Ltd. (Shanghai, China). The other chemicals used in this work were all analytical pure grade and used as received.

Preparation of blank or dacarbazine loaded MPEG-PLA nanoparticles

The DTIC-loaded MPEG-PLA NPs were prepared using the double emulsion (w/o/w) and solvent evaporation method with minor modification (Lu et al., 2005). Briefly, 100 µl of an aqueous solution of DTIC (w1) was emulsified with 1 ml mixture of methylene dichloride and acetone (3:2, v/v) containing 10 mg of polymers (MPEG-PLA) by an ultrasonic processor (100 W, 60 s) (Branson Sonicator® 450) in an ice bath to form the first emulsion (w1/o). Then, the w1/o emulsion was poured into 4 ml of HSA solution (w2, 1%, w/v). And the mixture was sonicated for 1 min (100 W, 30 s × 2) to form double emulsion (w1/o/w2). The w1/o/w2 double emulsion was then diluted into 30 ml 0.3% HSA solution and stirred at room temperature for 4 h to evaporate the organic phase. The resultant NPs were purified and obtained by centrifugation at 16,000 rpm for 30 min (L-100XP, Beckman USA) and washed thrice before lyophilization (V2K Virtis, USA) to remove the free drugs on the surface of NPs. All the following experiments utilized the Freeze-dried NPs obtained from above process, which has already removed the free drugs on the surface of NPs. Blank MPEG-PLA

NPs were prepared according to the above mentioned method except omitting the DTIC.

Particle size, zeta potential and morphology

Freeze-dried NPs were dispersed in deionized water (pH=7.0). Average size and zeta potential of MPEG-PLA NPs and DTIC-MPEG-PLA NPs were analyzed using a dynamic light-scattering detector (Zeta sizer ZS 90, Malvern, UK). At least three different batches were analyzed to give an average value and standard deviation for the particle diameter and zeta potential. The morphological examination of DTIC-loaded MPEG-PLA NPs was performed by transmission electron microscope (TEM). Briefly, after the DTIC-MPEG-PLA NPs were lyophilized, the dried NPs were resuspended with deionized water. Samples were prepared by dropping one drop of dilute dispersion onto a copper grid coated with a carbon membrane. The surface morphology of the DTIC-MPEG-PLA NPs samples was then visualized under the TEM (H600, HITACHI, Japan).

Evaluation of drug contents

The concentration of DTIC was determined by high performance liquid chromatography (HPLC) Instrument (Hitachi D-2000, Japan). Chromatographic separations were performed on a reversed phase-C18 column (4.6 × 200 mm, 5 µm, Dikma Analysis column). Methanol/water (30/70, v/v) was used as eluent at a flow rate of 1 ml/min. Detection wavelength was 319 nm. Drug loading and entrapment efficiency were determined as follows. Briefly, 10 mg of drug loaded MPEG-PLA nanoparticles were introduced into EP tube and were dissolved in 1 ml acetonitrile and diluted by 0.1 M citric acid. Meanwhile, the amount of DTIC in the solution was determined by HPLC. Drug loading (DL) and encapsulation efficiency (EE) of drug loaded nanoparticles were calculated according to Equations 1 and 2:

$$DL\ (\%) = \frac{\text{amount of drug in nanoparticles}}{\text{amount of the nanoparticles}} \times 100 \quad (1)$$

$$EE\ (\%) = \frac{\text{amount of the drug in nanoparticles}}{\text{amount of the feeding drug}} \times 100 \quad (2)$$

***In vitro* release study**

The *in vitro* release profile of DTIC from MPEG-PLA NPs was determined by measuring the residual amount of DTIC present in the nanoparticles. For that purpose, several aliquots (1 ml) of the same DTIC loaded MPEG-PLA nanoparticle suspension were diluted with phosphate buffered saline solution (PBS, pH = 7.4, final volume of 20 ml) in a capped centrifuge tube. The tubes were incubated at 37°C and shaken horizontally at 120 strokes/min. At specified time periods (ranging from 1 to 168 h, three tubes of each formulation were withdrawn and centrifuged at 16,000 rpm, 4°C for 30 min (L-100XP, Beckman USA), to obtain the nanoparticle precipitation by centrifugal force. After removing the supernatant, the precipitation was washed twice with distilled water and lyophilized. The amounts of residual DTIC in the nanoparticles were determined by HPLC using the same procedure as described above. The released drug was quantified and the cumulative release profile with time was demonstrated. The amount of drug released with time was expressed as a percent of the initial drug load in the nanoparticles.

Hemolytic test *in vitro*

The hemolytic study was performed on MPEG-PLA nanoparticle *in vitro* (Gou et al., 2009). Briefly, 0.5 ml sample at different concentrations in normal saline was diluted into 2.5 ml by normal saline and added into 2.5 ml of rabbit erythrocyte suspension (2%) in normal saline under 37°C. Normal saline and distilled water were used as negative and positive control, respectively. Three hours later, the erythrocyte suspension was centrifuged and the color of the supernatant was compared with controls. If the supernatant solution was absolute achromatic, it implied that there was no hemolysis. In contrast, hemolysis occurred when the supernatant solution was red.

Cell culture

The human malignant melanoma cell lines A375 and mice fibroblasts cell lines NIH were cultivated in monolayers to 80% confluence in DMEM medium supplemented with 10% fetal bovine serum at 37°C in humidified environment of 5% CO_2. The medium was replenished every other day and the cells were subcultured after reached confluence.

***In vitro* cytotoxicity**

Cytotoxicity was analyzed using cell titer 96 non-radioactive cell proliferation assay kit according to the manufacturer's protocol (Promega, Madison, WI) (Wang et al., 2007). Briefly, A375 cells and NIH cells (5×10^3) were cultured for 24 h before treatment. A375 cells were then treated with various concentrations of free DTIC, Drug-free MPEG-PLA NPs as well as DTIC-MPEG-PLA NPs for 3 days at 37°C in a CO_2 incubator. The concentrations of DTIC used in this assay were varied from 0.001 to 8 µg/ml (equivalent to MPEG-PLA concentration of up to about 0.5 mg/ml). NIH cells (5×10^3) were treated with drug-free MPEG-PLA NPs with different concentrations (from 137.5 to 2200 µg/ml) to evaluate the cytotoxicity of drug delivery system. Then, 20 µl of MTT reagent (3-(4, 5-Dimethylthia-zol-2-yl)-2, 5-diphenyltetra-zolium bromide, 5 mg/ml) was added to each well followed by 150 µl of DMSO to dissolve the crystal for 4 h later. The absorbance of each well was measured at 570 nm using a microplate reader. Viability of untreated cells was set at 100%, and absorbance of wells with medium and without cells was set as zero. All of the results were from at least triplicate experiments.

Detection of cell apoptosis

A375 cells (1×10^5 cells/well) were pre-incubated in 24 well plates and incubated at 37°C in a 5% CO_2 incubator for 24 h, after treatment with DTIC at different concentrations (1, 8 and 32 µg/ml) or the MPEG-PLA NPs loaded with the same amount of DTIC (untreated cells were used as controls) for 72 h, the cells were washed with 0.1 M PBS, trypsinized and centrifuged, then the cell pellet was resuspended in 490 µl of the cold binding buffer and aliquots of 5 µl of diluted Annexin V-FITC and propidium iodide (PI) solutions were added to the tubes containing 490 µl of the A375 malignant melanoma cells suspensions. Then, the mixtures were vortexed for 5 s and kept for 10 min on ice in the dark. After the incubation, the cell samples were analyzed by FAC Scan flow cytometer (Becton Dickinson, San Jose, CA).

***In vivo* antitumor study**

Antitumor activity of various agents was determined in BALB/c nude

Table 1. The particle size and zeta potential of NPs.

Variable	Blank-NPs	DTIC-NPs
Size (nm)	128. 0±5.6	144. 2±7.8
Zeta potential (Mv)	–24. 6±2.5	–32.1±5.4

Data are expressed as the mean±SD (n=6).

bearing human malignant melanoma. Tumor growth was induced by subcutaneous (s.c.) injection of 1×10^6 A375 cells on the back. After 14 days when the tumor growth was visible (about ~100 mm^3), mice were injected with various agents (PBS, Blank-NPs, DTIC, DTIC-NPs, given at doses of 25 mg/kg) through the tail vein once every other day for seven days. Tumors were measured with a caliper every 2 days for an additional 16 days and tumor volume was calculated using the following formula: tumor volume (mm^3) = [length × $(width)^2$]/2. To 16 days, mice were killed by cervical dislocation, then the final tumors were stripped and weighed. Tumor weight inhibition rate was calculated according to the following formula: Tumor weight inhibition rate (%) = (average tumor weight of control group- average tumor weight of test group)/ average tumor weight of control group×100%.

Data analysis

The data were presented as mean ± SD. Statistical significances were determined using two-sample t test and analysis of variance (ANOVA) with $P<0.05$ as a significance level.

RESULTS AND DISCUSSION

Particle size, zeta potential and morphology

In the present study, NPs were prepared from a MPEG-PLA polymer which has great biodegradability and compatibility, using a modified double emulsion and solvent evaporation method. As the HSA with great compatibility and emulsification was utilized as external phase, there is no additional toxic emulsifier in the whole preparation process. So the drug delivery system blank-NPs are expected to be low toxicity.

As the size of the NPs is important for establishing drug delivery strategies to specific sites of the body, the smaller NPs may be prone to minimize the particle uptake by non-targeted cells, including their premature clearance by the MPS (mononuclear phagocytic system), resulting in long circulation *in vivo* (Brigger et al., 2002; Mo et al., 2007). Orthogonal design was applied to optimize the preparation technology on the basis of the single factor evaluation. The optimal conditions for preparation of NPs were as follows: 10 and 3 mg/ml were the concentration of MPEG-PLA and DTIC, the methylene dichloride/ acetone ratio was 3:2 (v/v), the concentration of HSA was 1% and the volume ratio of O/W was 1/10 (v/v).

The resulting DTIC-NPs were sized at 144.2±7.8 nm (mean ± SD; n=6) (Table 1 and Figure 1) with a polydispersity index (PDI) less than 0.3, which was not much larger than drug-free MPEG-PLA-NPs (128.0±5.6 nm). The mean zeta potential of NPs was -32.1±1.6 mV (mean ± SD; n=6), its absolute value was larger than drug-free MPEG-PLA NPs (–24.6±2.5 mV). As shown in Figure 1, DTIC-NPs appeared spherical in shape with a relatively mono-dispersed size.

Evaluation of drug contents

We had optimized formulations to control the size of NPs as described above. Furthermore, drug contents also play a critical role in targeted drug delivery. To obtain large drug loading to meet therapeutic needs, high concentration of DTIC (≈3 mg/ml, the solubility of DTIC in water was about 4 mg/ml) was chosen as the concentration of inner water phase. The prepared DTIC-loaded MPEG-PLA NPs with a drug loading of 15.0±0.3 μg/mg (mean ±SD; n=3) and an encapsulation efficiency of 70.1±2.3% (mean ± SD; n=3) was used in subsequent experiments except otherwise stated.

In vitro release study

The release profile *in vitro* was evaluated. A sustained release manner could be visibly observed when DTIC released from MPEG-PLA nanoparticle, as shown in Figure 2, the release behaviour of DTIC from the polymer matrix exhibited a biphasic pattern characterized by a burst-release in the first 4 h, which can be ascribed to the drug located in or close to the NPs surface, then followed by a slower and continuous release. About 43.3% DTIC released from the nanoparticles within 24 h and the cumulative release could be up to 80% in the 72 h, after that, a slower release rate could be observed and nearly 90% DTIC was released in 7 days. These physical properties indicated that the prepared DTIC loaded MPEG-PLA NPs was a novel dacarbazine formulation which could meet the requirement of intravenous injection.

Hemolytic and cytotoxicity evaluation of MPEG-PLA nanoparticles *in vitro*

Hemolytic test was performed on MPEG-PLA NPs. As shown in Figure 3, MPEG-PLA NPs at the concentration of 50 mg/ml did not cause any hemolysis on rabbit erythrocyte comparing with the negative control (normal saline). Meanwhile, the cytotoxicity of blank MPEG-PLA was evaluated by cell viability assay in NIH cells which was shown in Figure 4. The NPs were found to be non-toxic at each of the tested concentrations. We did not observe a significant difference in the toxicity of MPEG-PLA NPs, although there was a slight reduction in cell viability at higher concentrations. Average cell viability ranged from 81.7±4.7 to 96.6±2.2% (mean ± SD, n=5) of control viability at the concentrations studied. The above results suggest that MPEG-PLA NPs is safety and may

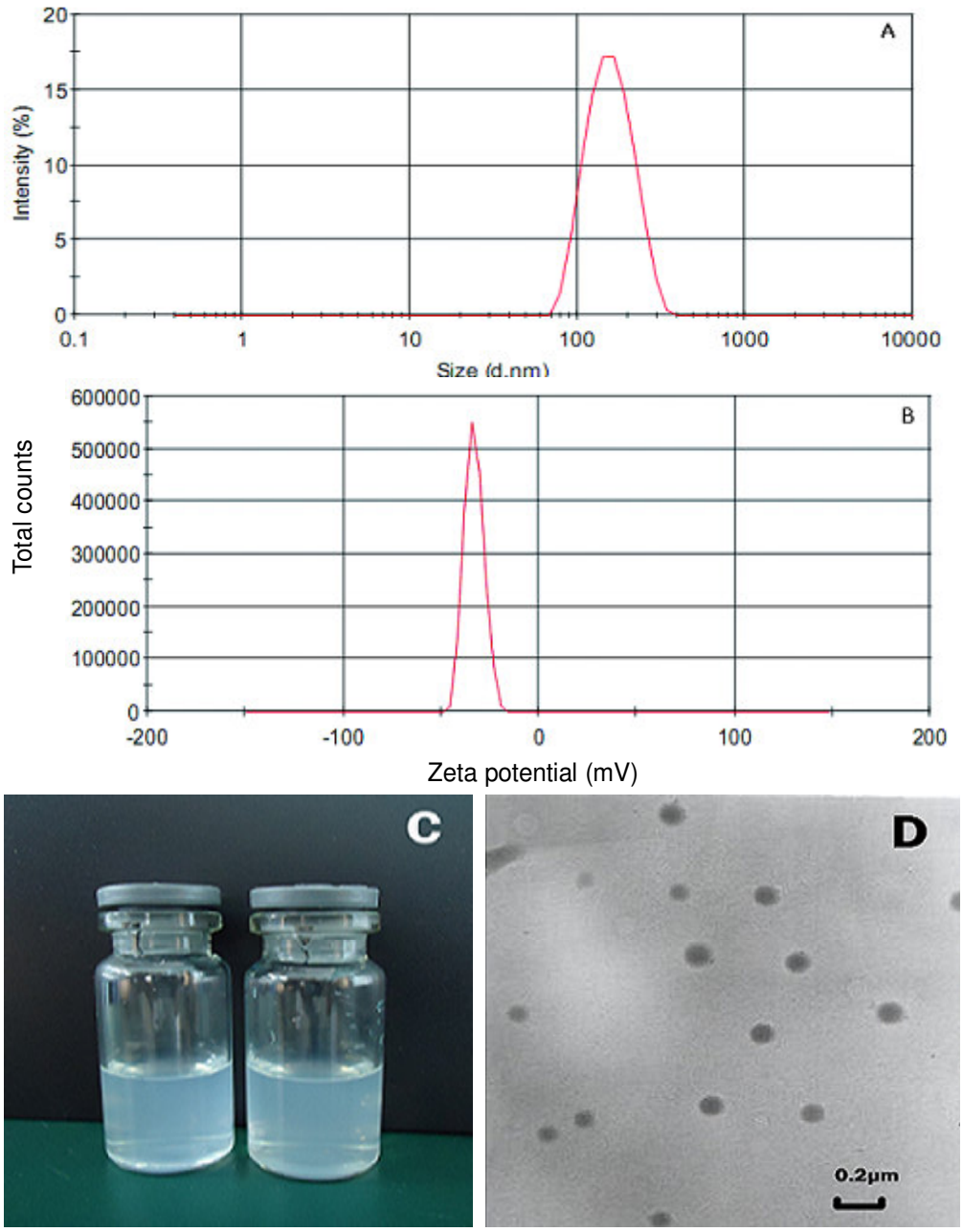

Figure 1. Characterization of nanoparticles. (A) Size distribution spectrum determined by laser diffraction size detector, mean size 144.2±7.8 nm; (B) Zeta potential determined by laser diffraction zeta detector, mean zeta potential -32.1±5.4 mV, (C) Optic image; (D) Morphology of DTIC-MPEG-PLA NPs determined by TEM.

be used as a delivery carrier.

Anticancer activity of dacarbazine loaded MPEG-PLA nanoparticle

Free DTIC and DTIC-MPEG-PLA NPs were tested for cytotoxic activity against human malignant melanoma cell lines A375 using MTT assay. Figure 5 showed the influence of drug concentration and nanoparticle on cell viability. Free DTIC and DTIC loaded nanoparticles significantly decreased the viability of A375 cells with increase in dacarbazine concentration. Meanwhile, the advantages of incorporating DTIC in the developed MPEG-PLA NPs are illustrated in Figure 6. Incubation of the cells with 8 μg/ml of free DTIC contributed to only

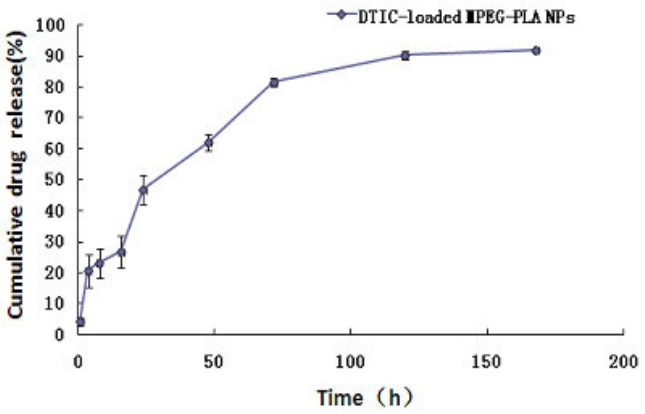

Figure 2. *In vitro* cumulative release profile of DTIC from drug loaded MPEG-PLA nanoparticles in PBS (pH= 7. 4) at 37°C.

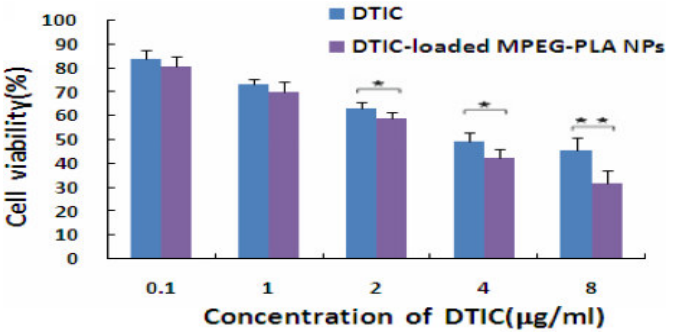

Figure 5. Cytotoxicity of DTIC-MPEG-PLA NPs in human malignant melanoma cell lines A375. The cells were incubated using different concentrations NPs (contain DTIC in the range from 0. 001 to 8 μg/ml) for a time period of 72 h (mean ± SD; n = 5), *: P<0.05, **: P<0.01.

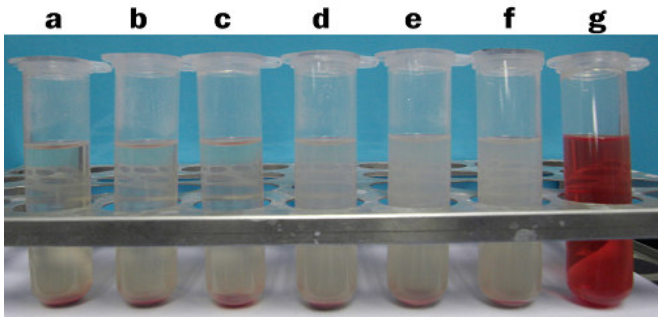

Figure 3. Hemolytic test on the MPEG-PLA NPs. The images were taken on 3 h after reaction. The concentration of MPEG-PLA NPs is (a) 5 mg/ml, (b) 10 mg/ml, (c) 20 mg/ml, (d) 30 mg/ml, (e) 50 mg/ml, (f) Sample is normal saline used as negative control and (g) sample is distilled water used as positive control.

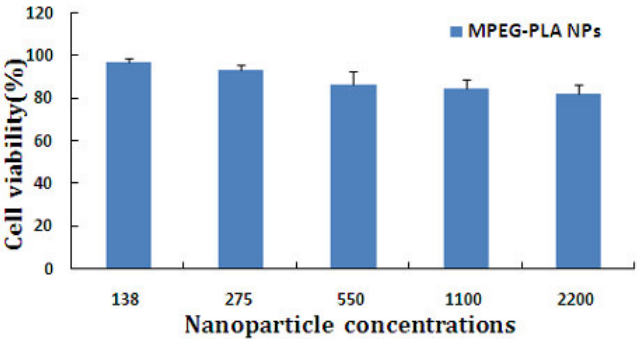

Figure 4. Cytotoxicity of blank MPEG-PLA nanoparticles in NIH cells. The cells were incubated using different concentrations NPs in the range from 137.5 to 2200 μg/ml for a time period of 24 h (mean ± SD; n = 5).

54.19±5.23% reduction in cell viability, while the same concentration of the drug provided as MPEG-PLA-NPs allowed a cytotoxic effect of 67.8±5.34% (P<0.01). It might be related to the enhanced stability and improved cellular uptake of DTIC being wrapped by nanoparticle which also can promote a sustained chemotherapy. The results imply that DTIC loaded MPEG-PLA nanoparticle might have great potential application of anticancer effect on human malignant melanoma cells *in vitro*.

Cell apoptosis

Cell apoptosis was evaluated by flow cytometry. Specifically, the A375 cells were treated for 72 h with free DTIC or DTIC-loaded MPEG-PLA NPs containing the same concentration of the free DTIC. As shown in Figure 6, the apoptotic cells incubated with free DTIC were about 14.15±0.87, 17.23±1.27 and 25.57±3.56% when the concentration of DTIC was 1, 8 and 32 μg/ml, respectively. In contrast, the number of apoptotic cells increased to about 16.57±1.86, 21.32±3.47, and 43.64±5.62% when the A375 cells were incubated with 1, 8 and 32 μg/ml of DTIC-loaded MPEG-PLA NPs, respectively (P<0.05). Therefore, DTIC-loading in MPEG-PLA NPs may induce more cancer cell apopotosis when compared with the direct administration of DTIC, and it is more apparent when the cells were treated with high concentration drug. DTIC-loaded MPEG-PLA NPs can significantly enhance the efficiency of the intracellular delivery and the apoptosis-inducing effect of DTIC.

In vivo antitumor study

The antitumor efficacy of the drug-loading nanoparticles was evaluated using xenograft models of breast cancer developed by s.c. injections of A375 cells on the back of BALB/c nude. As shown in Figure 7, the tumors of BALB/c nude PBS treated group grew rapidly, the tumors of DTIC treated group grew slowly and DTIC-NPs treated

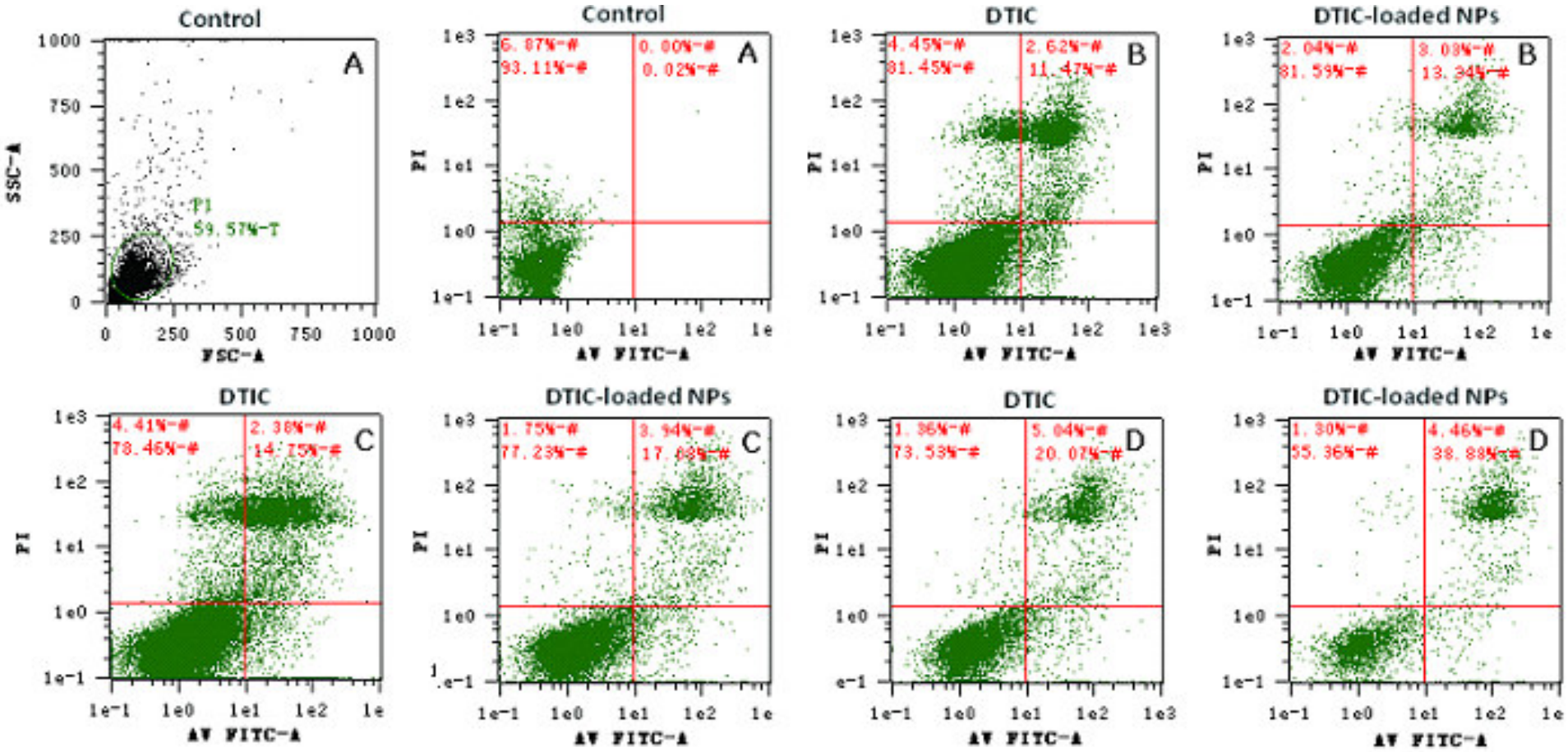

Figure 6. Dacarbazine (DTIC) and DTIC-loaded MPEG-PLA nanoparticles (DTIC-loaded NP_S) induced apoptosis. (A) Untreated A375 cells were used as controls; (B)(C)(D) Pictures were representative results from flow cytometry of propidiumiodine (PI) and Annexin V-FITC stained A375 cells treated with DTIC at different concentrations (1, 8, and 32 ug/ml) or the MPEG-PLA NPs loaded with the same amount of DTIC for 72 h.

group grew even more slowly. Compared with DTIC the antitumor activity of DTIC-NPs was markedly improved: The final mean tumor load was 485.85±133.74 mm^3 (mean ±SD; n=6, significantly smaller than proportional DTIC treated group (687.57±123.31 mm^3), as shown in Figure 8. The tumor growth inhibition rate also indicated that DTIC-NPs could significantly inhibit the tumor growth in tumor-bearing mice (Table 2). The *in vivo* experimental results presented were consistent with the effects that DTIC-NPs have in *in vitro* experiments.

Conclusions

In this study, small sized DTIC loaded MPEG-PLA NPs were successfully prepared. We used biodegradable MPEG-PLA polymers with great compatibility to develop DTIC-loaded MPEG-PLA

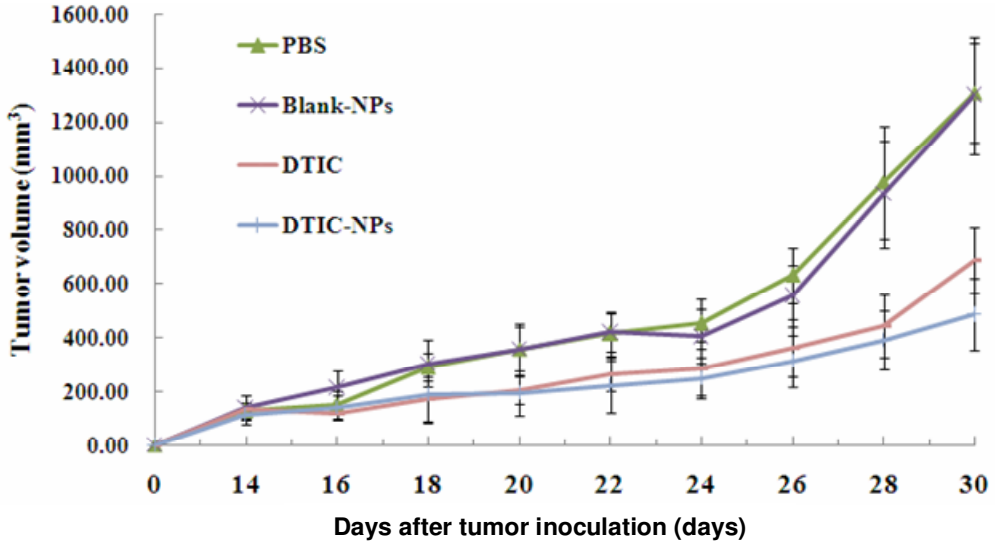

Figure 7. Growth curve of tumor in nude in therapeutic experiments (PBS, Blank-NPs, DTIC and DTIC-NPs were given on the 14th day, agents were given at doses of 25 mg/kg).

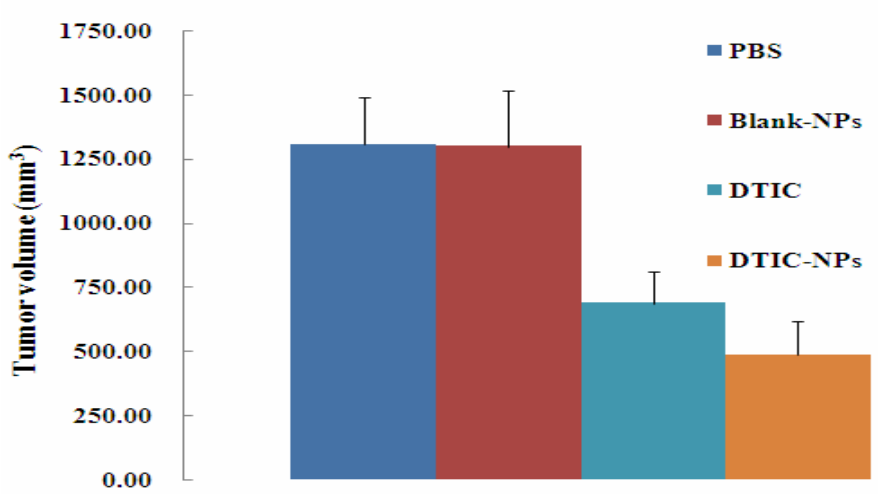

Figure 8. Final tumor volume of tumor-bearing nude in therapeutic experiments.

Table 2. The average weight of tumors and tumor growth inhibition rate (n = 6).

Group	Average weight of tumors (g)	Tumor growth inhibition rate (%)
DTIC-NPs	0.67±0.25$^{*,\#}$	57.30
DTIC	0.89±0.11$^{*,\#}$	43.53
Blank-NPs	1.44±0.27$^{\#}$	8.65
PBS	1.57±0.35$^{\#}$	-

*P < 0.05, vs PBS group; #P < 0.05, vs DTIC-NPs group.

NPs without any additional additives. The hemolytic test and cytotoxicity test of blank MPEG-PLA NPs demonstrated that the drug delivery system is safety. As the NPs easy to be dispersed in water, encapsulating dacarbazine in MPEG-PLA nanoparticle made hydrophobic dacarbazine to be injectable. Most strikingly, compared with free DTIC, DTIC loaded MPEG-PLA NPs showed enhanced antitumor activity and could induce more

apopotosis of cancer cell, which may be owing to the sustained release of DTIC from nanoparticles as well as the improved stability and cellular uptake of DTIC being wrapped by nanoparticle. These results suggested that the MPEG-PLA-NPs could be used as an efficient delivery system. This dacarbazine loaded MPEG-PLA NPs have great advantages versus the original drug, which might be a novel dacarbazine formulation for malignant melanoma with much fewer side effects than conventional cytotoxin.

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