Enhanced antitumor efficiency of docetaxel-loaded nanoparticles in a human ovarian xenograft model with lower systemic toxicities by intratumoral delivery

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Abstract. As successful chemotherapy with the taxanes needs to reduce the toxic side effects against normal tissues and avoid the detrimental effects caused by intolerable solvents, drug delivery system using soluble polymeric micelles tends to be the focus. Docetaxel (Doc) has demonstrated extraordinary activities against a variety of solid tumors. However, the clinical efficacy is contrasted by its toxicity profile. To reduce the toxicity and enhance the circulation time of Doc, core-shell structure nanoparticles were prepared from block copolymer of methoxy poly(ethylene glycol)-polycaprolactone (mPEG-PCL). It was found that Doc can be incorporated into the nanoparticles with high encapsulation efficiency of more than 90%. *In vitro* release study showed that Doc was released from Doc-np in a sustained manner. *In vitro* cytotoxicity studies indicated that IC50 of docetaxel-loaded nanoparticles (Doc-np) against SKOV3 cells is significantly lower than that of free Doc. Furthermore, intratumoral administration was applied to improve the tumor-targeted delivery in the *in vivo* evaluation. Compared with free Doc, Doc-np exhibited superior antitumor effect by delaying tumor growth when delivered intratumorally. Blood test, as well as liver and kidney function, showed that Doc-np had little toxicity while free Doc induce severe anemia and liver damage. These results suggest that Doc-np are effective in inhibiting the growth of human ovarian cancer with little toxicity to normal tissues, and intratumoral delivery of Doc-np could be a clinically useful therapeutic regimen and merit more research to evaluate the feasibility of clinical application.

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Introduction

One of the most common serious forms of gynecologic cancers is ovarian cancer, which accounts for around 3% of all cancers among women. In 2008, nearly 15520 deaths occurred and it is estimated that about 21550 new cases will be diagnosed with a predictable 14600 deaths in 2009 (1). According to the statistics, the 1- and 5-year relative survival of ovarian cancer patients is 75 and 45%, respectively. With tumor progression, the survival rates decrease. For instance, 5-year survival rates are 71 and 30%, respectively, for women with regional and distant disease (1,2).

Doc, which is more potent than paclitaxel with regard to the promotion of tubulin polymerization and inhibition of depolymerization (3), has demonstrated extraordinary anticancer effects *in vitro* and *in vivo* against a variety of tumors, including lung, ovaries, breast, leukemia and malignant melanoma (4-6). As suggested by NCCN, Doc is recommended as first-line chemotherapy in ovarian cancer (7). However, the clinical application of Doc is limited by several problems. For instance, the limited water solubility of Doc requires a specific solvent, ethanolic solution containing polysorbate 80, to facilitate its clinical use while the solvent system elicits hypersensitivity reactions that necessitate premedication, again limiting the maximum tolerable dose of the drug (8). Another limitation is the non-specific distribution throughout the body, which contributes to drug related side effects, such as neurotoxicity, musculoskeletal toxicity and neutropenia (9). Thus, novel formulation of Doc that is less toxic and better targets tumor site is desirable.

Drug delivery systems for chemotherapeutics based on polymer science have provided an alternative way to maximize localization of the drug towards the tumor while minimizing systemic toxicity (10). Among these drug carriers, amphilic block copolymers, which are composed of a hydrophilic segment and a hydrophobic segment, attract most interests with the capacity to self-assemble into nanoscale spherical structures with a hydrophilic outer shell and a hydrophobic inner core (11-16). Hydrophobic drugs could be incorporated into the hydrophobic core of the nanosphere, while its hydrophilic outer shell still exists as a stabilizer for the system. In most studies, PEG is used as the hydrophilic part (outer shell)

due to its hydrophilicity, biocompatibility, low toxicity, negligible antigenicity and immunogenicity (17,18). Moreover, the core-shell structure with PEG enables the nanoparticles to escape from the scavenging of the reticulo-endothelial systems (RES) effectively after systemic administration (19). Additionally, previous reports have demonstrated that drug-loaded polymeric nanoparticles preferentially accumulate in tumors due to the enhanced permeability and retention (EPR) effect (also called passive targeting), and, also, exhibit significantly lower systemic toxicity as compared to conventional drug formulation (20,21). Therefore, drug delivery systems based on the amphilic block copolymers are promising candidates for anticancer drug delivery.

As one of the most commonly used methods, intravenous administration is frequently adopted in the *in vivo* evaluation of anticancer agents. However, due to the diverse growth patterns of different tumors, it is difficult for systemic delivery of chemotherapeutics to achieve ideal effective concentration locally. To overcome this problem, we evaluated the anticancer efficiency of drug loaded nanoparticles by intratumoral delivery in our previous reports. Cisplatin and Doc was used as model drugs incorporated into nanoparticles formed by amphilic copolymers, demonstrating exciting *in vivo* anticancer efficacy (14,22). Compared with free drugs, nanoparticle-based drug delivery systems are characterized by the controlled release of incorporated drugs, which may overcome the deficiency of necessary drug retention in the tumor and may reach a satisfying outcome in improving antitumor efficacy when combined with intratumoral delivery instead of intravenous route.

In the present report, Doc loaded nanoparticles were prepared and characterized by dynamic light scattering (DLS), transmission electron microscopy (TEM), and atomic force microscopy (AFM). To assess the potential anticancer efficacy of the nanoparticulate system, SKOV3 cell line was used to test its *in vitro* cytotoxicity. Meanwhile, the *in vivo* antitumor efficiency of Doc-np was evaluated by intratumoral delivery in the xenograft model. At the end of *in vivo* experiment, mice were sacrificed to detect the influence of Doc-np on the peripheral blood parameters and liver and kidney functions.

Materials and methods

Materials. Doc was kindly provided by Jiangsu Hengrui Pharmceutical Co., Ltd. All other chemicals were of analytical grade and used without further purification. Human ovarian cancer cell line SKOV3 was obtained from Shanghai Institute of Cell Biology (Shanghai, China).

Male and female nude mice (nu/nu; 6-8 weeks old and weighing 18-22 g) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed and maintained in the animal facility of the Animal Center of Nanjing Medical University. The animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Nanjing Medical University.

Synthesis of mPEG and PCL block copolymers and preparation of mPEG-PCL nanoparticles. mPEG-PCL block copolymers were synthesized by a ring opening copolymerization as previously described (14). Doc-loaded nanoparticles were prepared by a nano-precipitation method as described previously with minor modification (22). Briefly, 10 mg of mPEG-PCL block copolymers and a predetermined amount of Doc were dissolved in an aliquot of acetone. The obtained organic solution was added dropwise into 10-time volumes of distilled water under gentle stirring at room temperature. The solution was dialyzed in a dialysis bag (MWCO 12000) to remove acetone thoroughly. The resulted bluish aqueous solution was filtered through a 0.22-μm filter membrane to remove non-incorporated drugs and copolymer aggregates. Drug-free nanoparticles were produced in a similar manner without adding drugs. Solutions of drug-loaded nanoparticles and blank nanoparticles were then lyophilized for further characterization and utilization.

Size and zeta potential analysis of the nanoparticles. Mean diameter and size distribution were measured by photon correlation spectroscopy (DLS) with a Brookheaven BI-9000AT instrument (Brookheaven Instruments Corp., NY, USA). Zeta potential was measured by the laser Doppler anemometry (LDA) from Brookheaven Instruments Corp. (Zeta Plus, Zeta Potential Analyzer).

Transmission electron microscopy (TEM), atomic force microscopy (AFM). Morphological examination of the nanoparticles was conducted with JEM-100S (Japan) transmission electron microscope (TEM). One drop of nanoparticle suspension was placed on a copper grid covered with nitrocellulose membrane and air-dried before negative staining with phosphotungstic sodium solution (1% wt/vol). Atomic force microscope (AFM) (SPI3800, Seiko Instruments, Japan) was used to study the surface morphology of nanoparticles in a greater detail. One drop of properly diluted nanoparticle suspension was placed on the surface of a clean silicon wafer and dried under nitrogen flow at room temperature. The AFM observation was performed with a 20-μm scanner in tapping mode.

Stability evaluation. Doc-np was kept at room temperature. Particle sizes were determined by DLS every 2 days for 15 days to evaluate stability.

Drug loading content (DLC) and encapsulation efficiency (EE). Doc loading content was determined by HPLC with minor modification (22). The concentration of Doc was assayed on a Shimadzu LC-10AD (Shimadzu, Japan) HPLC system equipped with a Shimadzu UV detector and an agilent C-18, 5 μ, 200 mm x 4.6 mm RP-HPLC analytical column. The mobile phase consisted of acetonitrile (spectral grade, Merck, Germany)/double-distilled water (50/50, v/v) pumped at a flow rate of 1.0 ml/min with determination wavelength of 227 nm. The concentration of Doc was determined based on the peak area by reference to a calibration curve. The following equations were applied to calculate the drug loading content and encapsulation efficiency. Drug loading content (%) = weight of the drug in nanoparticles/weight of the nanoparticles x 100% and encapsulation efficiency (%) = weight of the drug in nanoparticles/weight of the feeding drugs x 100%

In vitro release of Doc-loaded nanoparticles. For *in vitro* release detection, 10 mg lyophilized Doc-loaded nanoparticles were suspended in 1 ml of 0.1 M phosphate-buffered saline (PBS, pH 7.4). The solution was then placed into a pre-swelled dialysis bag with a 12-kDa molecular weight cut-off (Sigma) and immersed into 20 ml 0.1 mol/l PBS, pH 7.4, at 37˚C with gentle agitation. Samples (1 ml) were withdrawn from the incubation medium and measured for Doc concentration as described above. After sampling, equal volume of fresh PBS was immediately added to keep the incubation medium in a constant volume. The concentration of Doc released from the nanoparticles was expressed as a percentage of the total Doc in the nanoparticles and plotted as a function of time. It should be indicated that the concentration of released Doc was corrected for the dilution due to the adding of fresh PBS.

In vitro cytotoxicity studies. Cytotoxicity of Doc-loaded nanoparticles against SKOV3 cells was assessed by MTT assay (23). Briefly, cells were seeded in 96-well plates with a density around 5000 cells/well and allowed to adhere for 24 h prior to the assay. Cells were exposed to a series of doses of free Doc, blank nanoparticles, or Doc-loaded nanoparticles alone at 37˚C. After 48 h incubation, 50 μl of MTT indicator dye (5 mg/ml in PBS, pH 7.4) was added to each well and the cells were incubated for another 2 h at 37˚C in the dark. The medium was withdrawn and 200 μl acidified isopropanol (0.33 ml HCl in 100 ml isopropanol) was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read on a microplate reader (Bio-Rad, Hercules, CA, USA). Absorption was measured at a wavelength of 490 nm with 620 nm as a reference wavelength in Microkinetics reader BT2000 and obtained values were expressed as a percentage of the control cells to which no drugs were added. All experiments were repeated three times.

In vivo antitumor efficacy. Nude mice implanted with SKOV3 cells were used to qualify the relative efficacy of Doc-np through intratumoral administration. The mice were raised under specific pathogen-free (SPF) conditions and all of the animal experiments were performed in full compliance with guidelines approved by the Animal Care Committee of Nanjing Medical University. The mice were subcutaneously injected at the left axillary space with 0.1 ml of cell suspension containing 4 to 6x10^6 SKOV3 cells. Treatments were started after 7-8 days of implantation. The mice whose tumor reached a volume of 100 mm^3 were selected and this day was designated as 'day 0'.

For intratumoral injections, animals received a single injection volume of ~0.2 ml with a 21-gauge needle placed in the center of the tumor. The intratumoral injections were infused for about 10 sec, and the needle was allowed to remain in place for an additional 10-15 sec and removed through another direction. On day 0, the mice were randomly divided into eight groups, each group was composed of 6 mice. The mice were treated intratumorally with free Doc, Doc-loaded nanoparticles, blank nanoparticle and saline, respectively. Doc solution was administered at doses of 5 and 10 mg/kg, respectively. Doc-loaded nanoparticles were administered as a saline solution at the equivalent Doc doses of 5 and 10 mg/kg. All mice were tagged, and tumors were measured every other day with calipers during the period of study. The tumor volume was calculated by the formula $(W^{2*}L)/2$, where W is the tumor measurement at the widest point, and L is the tumor dimension at the longest point. Relative tumor volume (RTV) was calculated by the formula (V_n/V_0), where V_n is the tumor volume measured at the corresponding day, and V_0 is the tumor volume measure at day 0. Another antitumor indicator is T/C%, which was calculated by the formula (T_{RTV}/C_{RTV}), where T_{RTV} is RTV of the experimental group, and C_{RTV} is RTV of the control group.

Each animal was weighed at the time of treatment so that dosages could be adjusted to achieve the mg/kg amounts reported. Animals also were weighed every other day throughout the experiments. After 15 days of injections, the mice were sacrificed for the detection of peripheral blood parameters as well as liver and kidney functions.

Statistical analysis. Results are presented as mean ± SD. Statistical comparisons were made by t-test or ANOVA analysis. The accepted level of significance was $p<0.05$.

Results

Characterization and in vitro release of Doc-np. mPEG-PCL block copolymers were prepared by ring opening polymerization of ε-caprolactone in the presence of mPEG with a small amount of stannous octoate as catalyst. The molecular weight of the polymers obtained from 1H-NMR data and GPC were in a good agreement with that reported in the previous work (14,21).

The appearance of Doc-np is showed in Fig. 1A. The Doc-np features a bluish look due to the light scattering by the nanoparticles in the solution. Fig. 1B indi-cates the change of particle size of Doc-np at room tempe-rature. With progress of time, the size of Doc-np showed less variation during the observation time, which suggested satisfactory stability of Doc-np. The microscopic morphology images of the Doc-np obtained from AFM (Fig. 1C) and TEM (Fig. 1D) indicate that the nanoparticles, estimated less than 100 nm in size, are in spherical shape with a smooth surface, which is in accordance with the DLS measurement (88 nm) presented in our previous report (22).

As reported previously, the highest drug loading content of Doc into mPEG-PCL nanoparticles was detected as 19.4±2.4% while the encapsulation efficiency is >90% by varying the feeding ratio of copolymer and Doc (22).

Fig. 2 shows the sustained release profile of Doc-np. The release of Doc from the core-shell polymeric nanoparticles in phosphate-buffered saline (PBS, pH 7.4) was evaluated by dialysis method. Doc-np revealed a more sustained release pattern with a burst release >30% in the first 5 h. During the following 5 days, no more than 60% of the total Doc was released from the solutions of Doc-np. Fig. 2 features the controlled release pattern of Doc-np, which might contribute to the prolonged circulation of Doc *in vitro* and *in vivo*.

In vitro cytotoxicity of Doc-np against SKOV3 cells. Cytotoxicity of Doc-np against human ovarian cancer cell line

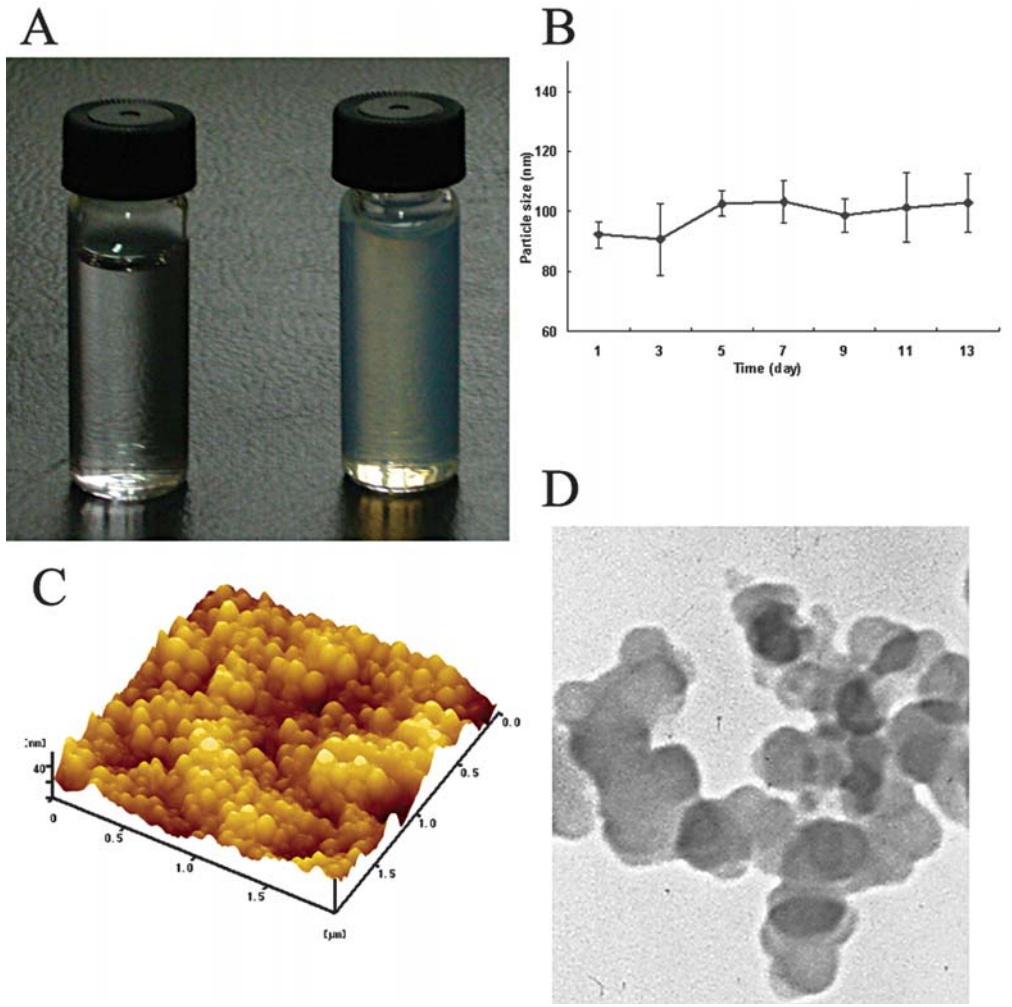

Figure 1. (A) The solution of DOCNP in glass bottles. Left, Doc-np solution in water. Right, free water. (B) Change of particle size of Doc-np. (C) AFM image of Doc-np. (D) TEM image of Doc-np.

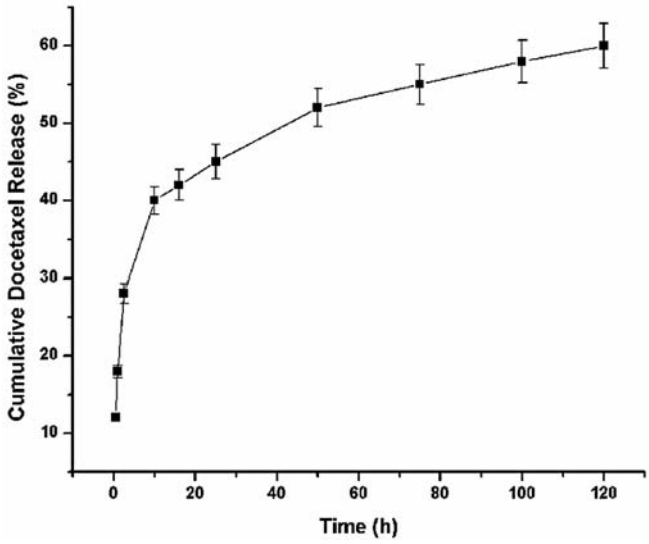

Figure 2. Cumulative *in vitro* release profile of Doc from mPEG–PCL nanoparticles.

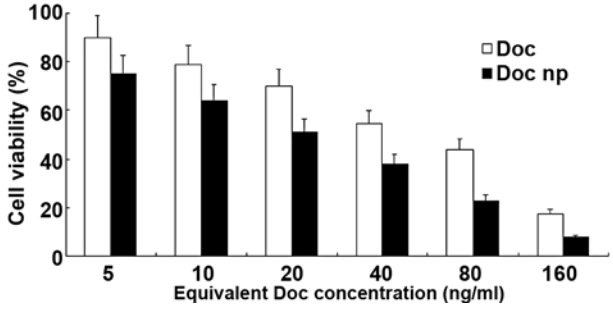

Figure 3. Cytotoxicity of Doc-np against SKOV3 cells.

SKOV3 was measured by MTT assay (Fig. 3). Preliminary results showed that blank nanoparticles are nearly non-toxic to SKOV3 cells with the inhibition rate <15% even at a high nanoparticle concentration of 400 μg/ml. Fig. 3 clearly indicated that Doc-np show similar dose- and time-dependent cytotoxicity again SKOV3 cell with of both free Doc and Doc-np at an equivalent dose from 5 to 160 ng/ml. However, equivalent dose of Doc-np induced more cell death than free Doc. The MTT results (Table I) suggested that the IC50 value of Doc-np (35.1±5.6 ng/ml) is significantly lower than that of Doc (73.8±8.2 ng/ml).

Table I. *In vitro* cytoxicity of free docetaxel and docetaxel-loaded nanoparticles against SKOV3 cell line.

SKOV3	IC50 values (ng/ml)[a]
Doc[b]	73.8±8.2
Doc np[c]	35.1±5.6

[a]Represents 50% inhibitory concentration evaluated by MTT assay. Data are presented as mean ± SD. [b]Doc, Docetaxel; [c]Doc np, Doctaxel-loaded nanoparticles.

In vivo antitumor evaluation of Doc-np by intratumoral delivery. Antitumor efficacy of Doc-np was investigated in SKOV3 human ovarian cancer xenografts in nude mice. The focus of this study was to evaluate the antitumor efficiency of Doc-np by intratumoral delivery. The mice were treated i.t. with Doc injection (10 mg/kg), Doc-np (5 and 10 mg/kg), empty nanoparticles and saline. During the experimental process, RTV and T/C% were calculated to compare the antitumor efficacy of different therapeutic protocols, with the change of body weight as an indicator for toxicity.

RTV and T/C analysis shows that the treatment groups (Doc and Doc-np) demonstrated significantly higher anti-tumor efficiency ($p<0.05$ vs. control) while E-np had no effect on tumor growth. Among the five groups, the group that received 10 mg/kg Doc-np was observed to maintain the greatest amount of antitumor activity (Fig. 4A). At the end of treatment, the RTV and T/C% is 6.3±2.1 and 37%, which is the lowest among all the groups indicating the strongest tumor inhibition. Statistical analysis reveals the significant differences between the group receiving 10 mg/kg Doc-np and the group receiving 10 mg/kg Doc ($p<0.05$) or 5 mg/kg Doc-np ($p<0.05$) (Table II). Fig. 4C showed the shrinkage of tumors in the treatment groups. It was observed clearly that the tumors taken from the mice receiving 10 mg/ kg Doc-np were smaller than those of other groups. Fig. 4A also shows that 5 mg/kg Doc-np demonstrated similar antitumor efficiency compared to 10 mg/ kg Doc with their tumor volume curves nearly overlapping, which revealed that the antitumor effects were elevated when Doc was incorporated into nanoparticles.

An analysis of body weight variations generally defined the adverse effects of the different therapy regimens (Fig. 4B). Doc-np at two doses demonstrated favorable results without any obvious body weight loss, whereas Doc induced severe weight loss in nude mice. These results partially indicate that Doc contributes more to toxicity than Doc-np.

Influence on Doc-np on peripheral blood parameters. As shown in Table III, E-np had no adverse effect on the levels of peripheral blood parameters. On the contrary, 10 mg/kg Doc induced significant reduction of WBC (16.5±2.9x10^9/l) and Hb (89.5±14.3 g/l) while 5 or 10 mg/kg Doc-np showed little undesirable influences on WBC and Hb. Determination of liver and kidney parameters (Table IV) featured the abnormality of liver function with increased ALT (88.2±11.4) in mice receiving 10 mg/kg Doc while Doc-np induced no damage on liver function. In addition, Doc and Doc-np showed no side effects on the kidney function of mice with the parameters remaining within normal ranges.

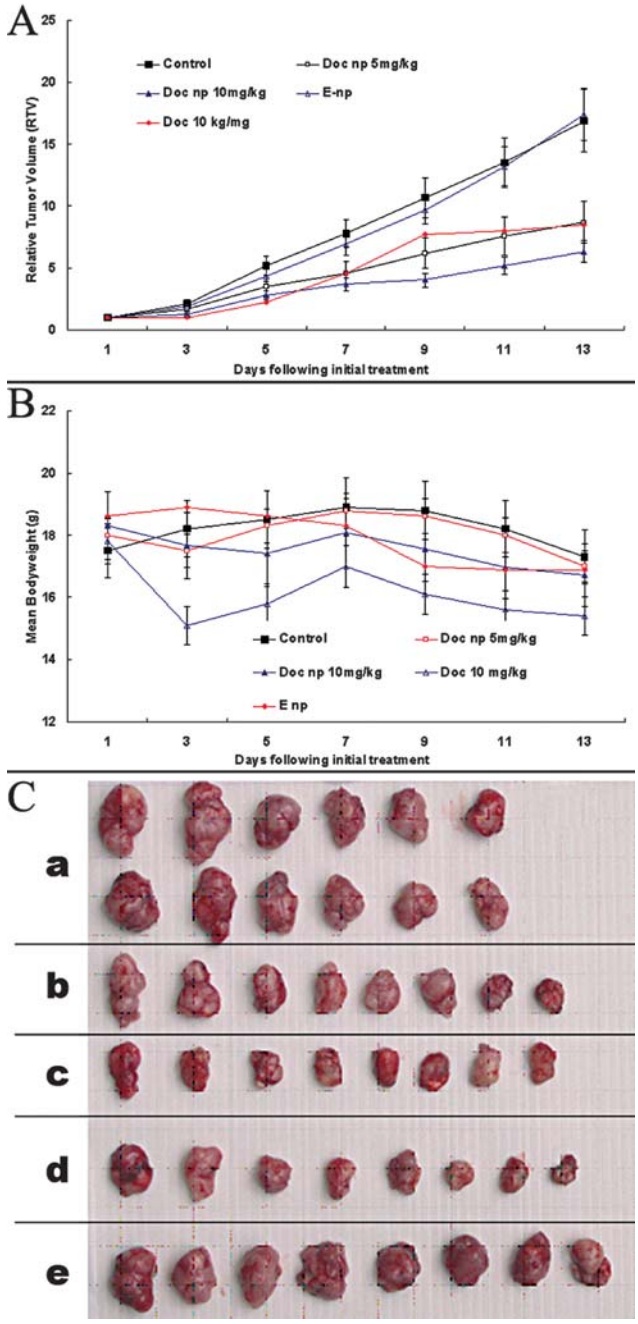

Figure 4. (A) Tumor volume of established SKOV3 xenografts in nude mice during therapy under different treatments. Mice were treated with different protocols on Day 0 (arrow) as showed in the figure. Saline, vehicle; E-np, empty nanoparticles; Doc 10, free Doc at a dose of 10 mg/kg; Doc-np, docetaxel-loaded nanoparticles in a saline solution at equivalent Doc doses of 5 and 10 mg/kg, respectively. Different agents were delivered through intratumoral pathway when tumor volume measured 100 mm^3. Data are presented as mean ± SD (n=6). The difference between tumor volumes in the group of saline and Doc-np is significant ($p<0.05$). Significant difference ($p<0.05$) also is observed between the group of free Doc and Doc-np at the equivalent dose. $^*p<0.05$ vs. the saline group. $^\#p<0.05$ vs. the group receiving 10 mg/kg free Doc. (B) Bodyweight change of nude mice receiving different treatments during therapy. Data are presented as mean ± SD (n=6). (C) The images of excised tumors at the time of sacrifice from the subcutaneous SKOV3 ovarian adenocarcinoma xenograft-bearing male nude mice after 15 days of single dose therapy. (a) Saline. (b) Doc-np at a dose of 5 mg/kg. (c) Doc-np at a dose of 10 mg/kg. (d) Doc at a dose of 10 mg/kg. (e) E-np.

Table II. Tumor growth inhibition effect of docetaxel-loaded nanoparticles against SKOV3 xenografts.

Groups	Dose of Doc (mg/kg)	Tumor volume (mm^3)		RTV (x±SD)	T/C (%)	P-value vs. control
		d0	dn			
Control	-	167±43	2685±994	16.9±7.3	-	-
E-np	-	140±25	2362±452	17.4±4.6	103	-
Doc np	5	156±46	1290±444	8.7±3.6	51	<0.05
Doc np	10	158±21	967±254	6.3±2.1	37	<0.01
Doc	10	122±37	1004±598	8.5±5.5	50	<0.05

Table III. Influence of docetaxel-loaded nanoparticles on peripheral blood parameters.

Groups	WBC (10^9/l)	RBC (10^9/l)	Hb (g/l)	Plt (10^9/l)
Saline	26.5±2.8	7.4±0.9	108.5±12.3	165.4±21.5
E-np	24.2±3.1	7.5±1.1	112.5±15.6	139.1±19.4
Doc 10	16.5±2.9[a]	7.2±1.3	89.5±14.3[a]	146.5±25.6
Doc np 5	23.9±3.9	7.6±0.8	124.5±19.5	136.5±15.5
Doc np 10	21.5±2.5	7.4±1.4	130.1±14.3	149.3±23.3

[a]Represents p<0.05 vs. saline.

Table IV. Influence of docetaxel-loaded micelles on liver and kidney parameters.

Groups	ALT (U/l)	BUN	Cre
Saline	52.5±12.3	7.6±1.2	38.5±5.2
E-np	54.3±11.5	7.3±1.5	39.5±4.6
Doc 10	88.2±11.4[a]	8.4±3.6	30.6±6.2
Doc np 5	42.3±10.6	5.9±0.6	28.9±9.2
Doc np 10	46.5±13.2	8.9±1.9	34.5±4.9

[a]Represents p<0.05 vs. saline.

Discussion

Herein we report that a spherical coreshell structure Doc-np formed by amphilic mPEG-PCL block copolymers demonstrated superior antitumor efficacy against ovarian cancer both *in vitro* and *in vivo*.

Previous studies mainly concentrated on developing nano-delivery systems for paclitaxel and few published reports focused on the study of Doc-loaded nanoparticles (24,25). This study may pave the way for future research with Doc nano-delivery systems. Accordingly, the introduction of Doc-loaded nanoparticles provides significant progress in at least two aspects. Firstly, it improves the selectivity of Doc delivery towards the tumor site. As a result, there is an eventual reduction in the toxicity of Doc to normal tissue. Secondly, the specific solubility of Doc-loaded nanoparticles can reduce the use of Tween-80/ethanol, which further decreases the toxicity of the current Doc formulation. In additional, the favorable results of Doc-loaded nanoparticles indicate that the physical entrapment of chemotherapeutics into polymeric nanoparticles may shed light on future developments in application of Doc.

Doc-loaded nanoparticles reported in our previous and the current work showed a bluish appearance under the light, which indicated the characteristic manifestation of nano-sized particles. AFM and TEM confirmed the size of nanoparticles as <100 nm, with their morphology being roughly spherical. Determination of DLC revealed a high loading of Doc. The strong affinity between Doc and PCL led to the slow dissociation of Doc from the polymeric nanoparticles in a sustained release pattern. Cytotoxicity test indicated Doc-np caused significantly more cell death of SKOV3 cells than free Doc. Possible mechanism underlying the enhanced efficacy of Doc-loaded nanoparticles against SKOV3 cells may include the enhanced intracellular drug accumulation by nanoparticle uptake as reported (22).

In the current research, a xenograft model of human ovarian cancer was established in nude mice to evaluate the efficiency and toxicity of Doc-np. Intra-tumoral administration was adopted instead of intravenous administration. Previous report from our laboratory demonstrated satisfactory antitumor effect of Doc-np against murine malignant melanoma by intratumoral delivery (22). The current research showed that, when administered intratumorally, Doc-np exhibited

more powerful anticancer efficiency than free Doc against human ovarian xenografts with lower toxicity towards bone marrow, liver and kidney.

The enhanced efficiency of intratumoral delivery may be related to the characteristic pharmacodynamics and pharmacokinetics profile. Intratumoral administration is regarded as a site-specific delivery to tumor nodule, which will accordingly induce a higher local drug concentration than systemic delivery (26). Possible mechanisms underlying the superiority of Doc-np against free Doc delivered intratumorally may include the continuous exposure of tumor mass to released Doc from the nanoparticles. Though Doc-np by intratumoral administration cannot achieve as high initial concentration as free Doc, the sustained release of Doc is capable of delivering its antitumor efficacy constantly (27). As a result, it is highly reasonable that released Doc from the intratumorally delivered nanoparticles retain in the interstitial space of the tumor for a longer time compared to normal tissue and exerts protracted tumor-eliminating effect locally (28). Besides, the relatively lower drug concentration in plasma by local delivery, compared to systemic administration, means less toxicity to normal tissues. This could be observed from the measurement of peripheral blood parameters and liver and kidney parameters. Free Doc induced severe WBC reduction and anemia together with hepatic damage while Doc-np had no adverse effects.

Planned modifications with the nano-drug delivery system utilized in this study are under active consideration as a part of this ongoing research. In addition, further development with the targeted release of chemotherapy drugs, combined with various targeting methods, will be more fully reviewed in order to further expand the parameters of this current research. A new emphasis has been placed on specific modifications in the targeting ability of this nano-delivery system by introducing receptor-targeting peptides. Since chemotherapy drugs are physically entrapped within the polymer matrix, and these drugs have no known chemical interaction with polymers, it is hypothesized that a combination of passive targeting, with receptor targeting peptides, may significantly amplify the antitumor activity of the drug delivery system. Physically, the EPR effect enables the relatively high drug accumulation in tumor site through the vascular system. Due to the molecular recognition of peptides by tumor cell surface receptors, site-specific drug uptake by tumor cells may be raised, which may then lead to enhanced cytotoxicity. This hypothesis is under active current review and requires further study.

The current research shows satisfactory antitumor efficiency of a spherical core-shell structure Doc-loaded nanoparticle formed by amphilic mPEG-PCL block copolymers. *In vivo* evaluation further demonstrated the superior anticancer efficacy of Doc-loaded nanoparticles compared to free Doc by intratumoral delivery in an established B16 transplanted mice model. Moreover, Doc-np showed little toxicity to normal tissues including bone marrow, liver and kidney at its therapeutic dose. It is concluded that nanoformulation of Doc delivery is a most promising way in countering the spread of ovarian cancer, and continuing research will advance the current study. It is fully understood and appreciated that the development of nanoscale drug formation of chemotherapeutics warrants more intensive research in order to further characterize the detailed mechanism in the uptake of drug-loaded nanoparticles, the interaction between drug release and tumors, and ultimately, the feasibility and advantages of clinical applications.

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