

Original Article

In vitro and in vivo evaluation of hyaluronic acid-modified liposomes as a sustained-release carrier system for paclitaxel

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Abstract: Objective: We aimed to investigate the clinical value of hyaluronic acid (HA)-modified liposomes as a sustained-release carrier system for paclitaxel (PTX). Methods: PTX-loaded cationic liposomes (PCLs) were prepared using film dispersion method and HA-modified PCLs (HA-PCLs) were prepared by electrostatic interaction. The clinical value of HA-PCL was evaluated by characterization observation, release study, pharmacokinetics study, and antitumor efficacy study. Results: The average particle sizes of PCL and HA-PCL were 162.10 ± 6.77 nm and 239.30 ± 6.26 nm, and the average zeta potential of PCL and HA-PCL were 27.04 ± 5.89 mV and -22.76 ± 2.32 mV, respectively (all $P < 0.001$). An evident sustained release was observed in HA-PCL. The pharmacokinetics study revealed a similarity in bioavailability between HA-PCL and Taxol in vivo. PCL had a similar antitumor effect of PCL in the mice compared with Taxol, whereas HA-PCL showed better antitumor activity and less side effects than Taxol (all $P < 0.001$). Conclusion: HA-PCL can be a potential sustained-release drug delivery system for PTX.

Keywords: Hyaluronic acid, liposome, paclitaxel, antitumor activity, sustained-release drug delivery

Introduction

Cancer is the leading cause of death in developed countries and the second leading cause of death in developing countries with an increasing trend in the incidence worldwide [1]. Currently, the most common method for treating cancer is chemotherapy. Paclitaxel (PTX), one of the most effective broad-spectrum chemotherapeutic agents, has been widely used for the treatment of various tumors, including non-small-cell lung cancer, ovarian cancer, and breast cancer [2, 3]. PTX is an anti-microtubule agent originally isolated from the bark of *Taxus brevifolia*, which can cause cell death by disrupting the dynamic equilibrium within the microtubule system [4, 5]. However, the poor solubility of PTX in aqueous solution greatly limits its clinical application. Taxol[®] and Abraxane[®] are two commercial formulations for PTX. Taxol is composed of 50% Cremophor EL and 50% dehydrated alcohol with 6 mg/mL PTX. How-

ever, Cremophor EL can cause serious adverse reaction such as nephrotoxicity, hypersensitivity, and neurotoxicity [6]. Abraxane is a Cremophor EL-free formulation of PTX albumin-bound particles approved by FDA in 2005. It utilizes endogenous albumin pathways to increase intratumoral concentrations of the active drugs and decreases the toxicity of Taxol-associated hypersensitivity reactions [7, 8]. However, Abraxane is restricted to the treatment of small types of cancer, such as pancreatic carcinoma, and its therapeutic index is too narrow. Therefore, the development of an active sustained-release system for PTX to increase the drug accumulation in tumor and decrease the side effects on normal tissues has become a main focus for researchers.

Among the new drug delivery systems, liposome has great potential in the delivery of drugs, genes, and imaging agents [9-11]. Liposome is a closed spherical vesicle consisting

of an aqueous core and one or more lipid bilayers of biocompatible and biodegradable lipids. Water-insoluble drugs can be encapsulated in the hydrophobic bilayers for increased solubilization, enhanced penetration, prolonged circulation, and sustained and controlled delivery, thus improving the drug efficacy and decreasing the side effects [12]. Passive and active targeting by liposomes can also be realized by enhanced permeability and retention (EPR) effect and the modification of tumor-targeting ligands to increase the accumulation of the drug in tumors. In this study, PTX-loaded cationic liposomes (PCLs) were prepared using a novel single-tailed cationic lipid, 6-lauroxyhexyl lysinate (LHLN). LHLN was designed and synthesized on the basis of 6-lauroxyhexyl ornithinate with one tail which was more efficient and had lower cytotoxicity compared to 1,2-dioleoyl-3-trimethylammonium propane [13, 14]. It has been demonstrated that LHLN exhibits lower critical micelle concentration than cetyltrimethylammonium bromide and the lipid-based formulation using LHLN has low cytotoxicity in A549, HeLa, and HepG2 cell lines [14-16].

A number of targeting ligands have been used in sustained-release drug delivery systems in recent years, including folic acid, asparagine-glycine-arginine peptide, and monoclonal antibodies [17-19]. Hyaluronic acid (HA), abundant in the extracellular matrix and synovial fluid, is a linear polysaccharide consisting of two alternating units, N-acetyl-D-glucosamine and D-glucuronic acid [20]. In addition to the advantages of non-toxicity, biocompatibility, and biodegradability, HA is a negative polysaccharide with carboxyl and hydroxyl groups, which makes it tend to be conjugated to the drug delivery system. Modification of HA in drug delivery systems can protect nano-carriers, regulate their circulation time, and improve biodistribution in vivo [21]. Therefore, HA, as a tumor-targeting ligand, has been extensively investigated. The modification of HA in drug delivery system include chemical reaction and electrostatic interaction. Chemical reaction is a complex process requiring the use of organic solvents, whereas electrostatic interaction is an easier process without the use of organic solvents. Thus, we chose electrostatic interaction for HA modification in this study.

In the present study, a novel HA-modified PCL (HA-PCL) with LHLN as a cationic lipid was developed for the tumor-targeting delivery of PTX. Through both in vivo and in vitro experiments, the clinical value of HA-modified liposomes as sustained-release carrier for PTX was evaluated, in an attempt to provide some experimental foundation for the application of HA-PCL as a drug carrier in targeted drug delivery systems.

Materials and methods

Materials

The experimental materials and their suppliers were as follows: PTX (Chenxin Pharmaceutical, China), HA (Shandong Freda Biochem, China), injectable soya lecithin (containing 95% phosphatidylcholine, pH = 5.0-7.0, Shanghai Taiwei Pharmaceutical, China), cholesterol (Shanghai Chemical Agent, China), murine malignant melanoma (B16) cells (Shandong Institute of Immunopharmacology and Immunotherapy, China), female Kunming mice (18-22 g, Medical Animal Test Center of the New Drugs Evaluation Center, Shandong University). The animal experiments were approved by the Ethics Committee of Shandong University Hospital.

Preparation of PCL and HA-PCL

PCL was prepared by the film dispersion method with slight modifications [22]. The preparation of PCL was optimized by single-factor experiments. The prescription factors, including the amount of LHLN, the mass ratio of PTX to lipids (w/w), the volume ratio of ethanol phase to aqueous phase (v/v), and the mass ratio of injectable soya lecithin to cholesterol (w/w), as well as the process factors, including the time and temperature of hydration, were investigated according to the different levels of entrapment efficiency (EE).

The HA-PCL was prepared by electrostatic interaction [21]. Briefly, 1 mL of PCL dispersion was slowly added to a HA solution (0.25 mg/mL) under vigorous stirring with the mass ratio of HA to LHLN as 0.5:1.

Determination of EE and DL (drug loading)

A desired amount of PCL or HA-PCL was dispersed in methanol and sonicated for 5 min for

completely dissolving the free drugs. After filtration, the drug content in the dispersions was measured by high-performance liquid chromatography (HPLC; pump: SPD-10AVP, Shimadzu, Japan; UV-VIS detector: LC-10AVP, Shimadzu, Japan). The HPLC parameters were as follows: Hypersil ODS column (4.6 mm × 250 mm, pore size: 5 μm), mobile phase (acetonitrile: water, 55:45, v/v), flow rate (1.0 mL/min), and measured wavelength (227 nm). The calibration curve of peak area (A) against concentration of PTX (C) was $A = 22859C + 20818$ ($r = 0.9998$) under the concentration of 5-100 μg/mL PTX. Intraday and interday variabilities were less than 1% and 2%, respectively. Mean recovery rates of each organ exceeded 95%, and relative standard deviation was lower than 1%. The EE and DL values were calculated using the following equations: $EE\% = (W_{\text{supernatant}}/W_{\text{total}}) \times 100$; $DL\% = (W_{\text{PCL}}/W) \times 100$. W_{total} was the weight of PTX in PCL before centrifugation; W_{PCL} was the weight of the PTX measured in the supernatant; W was the total weight of injectable soya lecithin, cholesterol, LHLN, and PTX.

Characterization of PCL and HA-PCL

The morphologies of PCL and HA-PCL were evaluated using a transmission electron microscope (JEM-1200EX, Japan). Particle size and zeta potential of PCL and HA-PCL were measured by photon correlation spectroscopy (PCS, Delsa™ Nano Zeta-Potential and Submicron Particle Size Analyzer, Beckman Coulter, USA). All measurements were performed at least three times at 25°C.

In vitro release studies

The release of PTX from Taxol and HA-PCL was evaluated in vitro using dialysis bag diffusion technique [23]. The cumulative release percentage of PTX was calculated based on different release amounts at different time points. The regression equation was established to explore the release behavior of PTX from Taxol and HA-PCL.

Pharmacokinetics study

The mice were injected through the tail vein with Taxol or HA-PCL with PTX at a dose of 15 mg/kg. At different time points (0.083 h, 0.25 h, 0.5 h, 0.75 h, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h), the blood samples were collected by terminal

retro-orbital bleeding and placed in heparinized microtubes. After centrifugation at 10,000 rpm for 10 min, the plasma samples were collected from the supernatant. Methyl alcohol and acetonitrile (50/50, v/v, 0.4 mL) was added into each plasma sample (0.2 mL) and mixed for 1 min by a vortex mixer to extract PTX. After centrifugation at 10,000 rpm for 10 min and filtration, 20 μL of the final solution was injected into the HPLC column to detect PTX peak area, and the concentration was calculated according to the standard curve.

The main pharmacokinetic parameters were calculated by the statistical moment method using Debris Assessment Software (version 2.0, NASA Orbital Debris Program Office, Houston, USA) [24]. The area under the plasma concentration-time profiles, distribution, elimination half-life, mean residence time, and total plasma clearance were calculated.

In vivo antitumor efficacy

The female Kunming mice (18-22 g) were injected at the right axillary space with 0.1 mL of cell suspension containing 1×10^5 B16 cells [25]. When the volume of the tumor reached 100 mm³ after one week, the mice were randomly divided into four groups of ten mice each and were injected through tail vein with normal saline (NS), Taxol, PCL, or HA-PCL at a dose of 10 mg/kg PTX (injection once per week for three weeks). After the treatment, the tumor diameter was measured with a caliper every other day. The tumor volume was calculated as length (L, the longest diameter) × width (W, shortest diameter)²/2. The body weight of the mice was also monitored to evaluate the physical conditions of the mice [25]. Six mice surviving to the end of study were randomly selected for measuring the tumor volume and the body weight. At the end of the experiment, all the mice were sacrificed by cervical dislocation. The animal experiments conformed to the ethical principles and requirements of Shandong University Hospital.

Statistical analysis

SPSS statistical software (version 22.0) was applied for statistical analysis. Measurement data are presented as mean ± standard deviation. Independent t-test was used for comparison between two groups. One-way analysis of

Table 1. Factors and their levels in the single-factor experiments

Factor	Level 1	Level 2	Level 3	Level 4	Level 5
LHLN (mg)	0.4	0.5	0.6	0.7	0.8
PTX/lipids (w/w)	1:5	1:10	1:15	1:20	
Ethanol/aqueous (v/v)	0.5:1	1:1	2:1	3:1	4:1
Soya lecithin/cholesterol (w/w)	5:1	7:1	9:1	11:1	13:1
Hydration time (min)	5	10	15	20	
Temperature (°C)	30	40	50	60	

Note: LHLN: 6-lauroxyhexyl lysinate; PTX: paclitaxel.

variance and Bonferroni post hoc test were used for multiple comparisons. Enumeration data are presented as percentage and compared by chi-square test. $P < 0.05$ indicated a statistically significant difference.

Results

Preparation and optimization of PCL

PCL was prepared by film dispersion. The optimal amount of LHLN, the mass ratio of PTX to lipids (w/w), the volume ratio of ethanol phase to aqueous phase (v/v), the mass ratio of injectable soya lecithin to cholesterol (w/w), the hydration period, and the hydration temperature were 0.6 mg, 1:10, 2:1, 9:1, 10 min, and 40°C, respectively. See **Table 1**.

Characterization of PCL and HA-PCL

The characterization results of PCL and HA-PCL are shown in **Table 2** and **Figure 1**. Intergroup differences were found in the particle size and zeta potential between PCL and HA-PCL (all $P < 0.001$). The EE and DL of PCL were also different from those of HA-PCL (EE: $P = 0.038$; DL: $P = 0.010$).

In vitro release

In the HA-PCL group, the rate of drug release was relatively fast at the initial stage and slowed down subsequently, whereas a burst release of PTX was observed in the Taxol group, in which up to 60% of total amount was released during the first 12 h. HA-PCL presented a biphasic drug release pattern in which fast drug release occurred during the first 12 h with more than 40% of total amount released, followed by a sustained release of 51% PTX in the next 48

h. The release of PTX from HA-PCL was slower than that from Taxol ($\chi^2 = 8.000$, $P = 0.005$). The release of PTX from Taxol could be expressed by the following equation: $\ln(1/(1-Q/100)) = 0.9632\ln t - 2.3540$, $r = 0.9970$. The release of PTX from HA-PCL could be expressed as: $100-Q = 45.9567e^{-0.2010t} + 54.8828e^{-0.0026t}$, $r_\alpha = 0.9972$, $r_\beta = 0.9642$. See **Figure 2**.

Pharmacokinetics

The PTX concentration-time curves of Taxol and HA-PCL are displayed in **Figure 3**. The initial PTX concentration of HA-PCL was much higher than that of Taxol during the initial 30 min. After that, the PTX concentration of HA-PCL was slightly lower than that of Taxol. As shown in **Table 3**, the total clearance rate in HA-PCL was slightly higher than that of Taxol. The K_{10} of HA-PCL was calculated to be $2.16 \pm 0.21 \text{ h}^{-1}$, which was lower than that of Taxol ($P < 0.001$). No significant differences were observed between HA-PCL and Taxol in the levels of key parameters, including area under the curve value and mean residence time.

In vivo antitumor efficacy

The tumor volume, which increased significantly in the mice treated with NS, was bigger than that in the Taxol group ($P < 0.001$). No differences were observed in the tumor volume between the Taxol and PCL group ($P > 0.05$). Furthermore, the tumor volume in the HA-PCL group was smaller than that in the Taxol group ($P < 0.001$, **Figure 4**). In the NS group, the body weight increased markedly, whereas the body weight in the PCL and HA-PCL groups was much more stable with just slight increase (**Figure 5**). In the Taxol group, one mouse died on the 3rd day, one died on the 15th day, two died on the 17th day, and six were alive throughout the experiment; in the PCL and HA-PCL groups, only one mouse died in that group on the 21st day and the rest were alive throughout the experiment.

Discussion

With the development of molecular biology techniques, there has been great progress in the research on sustained-release drug delivery systems. So far, there have been few stud-

Hyaluronic acid-modified liposome as a sustained-release carrier for paclitaxel

Table 2. Characterization of PCL and HA-PCL (n = 3)

Formulation	EE (%)	DL (%)	Size (nm)	PDI	Zeta potential (mV)
PCL	97.62±1.47	4.05±0.08	162.10±6.77	0.290±0.052	27.04±5.89
HA-PCL	88.03±5.25	3.60±0.15	239.30±6.26	0.236±0.031	-22.76±2.32
t value	3.047	4.585	14.500	1.545	13.630
P value	0.038	0.010	<0.001	0.197	<0.001

Note: PCL: paclitaxel-loaded cationic liposome; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes; EE: entrapment efficiency; DL: drug loading; PDI: polydispersion index.

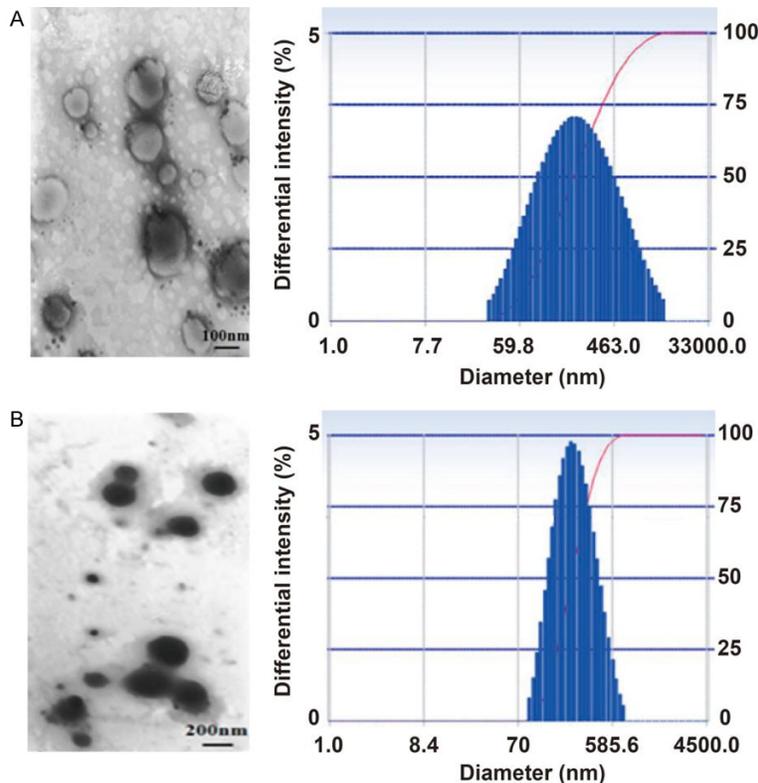


Figure 1. The morphologies and particle size distributions of PCL and HA-PCL. A: PCL; B: HA-PCL. PCL: paclitaxel-loaded cationic liposome; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes.

ies on the sustained-release ligands. In this study, HA-PCL as sustained-release delivery of PTX was evaluated in vitro and in vivo. The advantages of HA-PCL are as follows: 1) the materials of HA-PCL are biocompatible and biodegradable; 2) HA-PCL is prepared by electrostatic interaction, which is simple and avoids the use of organic solvent; 3) HA-PCL can further enhance the antitumor efficacy and reduce unspecific side effects of PTX.

For HA-PCL, the determined amount of LHLN, the mass ratio of PTX to lipids (w/w), the volume ratio of ethanol phase to aqueous phase

(v/v), the mass ratio of injectable soya lecithin to cholesterol (w/w), and the period and temperature of hydration were 0.6 mg, 1:10, 2:1, 9:1, 10 min, and 40°C, respectively. The amount of LHLN had little influence on EE. However, if the LHLN content is too high, the solution may form micelles and result in enhanced toxicity. The amount of LHLN was determined as 0.06 mg with the highest EE. The mass ratio of PTX to lipids (w/w) had great influence on EE. When the mass ratio of PTX to lipids (w/w) was 1:10 and 1:20, the EE values were both high. Taking into account the DL value, 1:10 was finally chosen. As the volume ratio of ethanol phase to aqueous phase (v/v) was 1:1, the highest EE was obtained. However, drugs and lipid could mix well to form a uniform film as the ethanol phase volume was higher than the aqueous phase volume.

Thus, 2:1 was chosen as the volume ratio of ethanol phase to aqueous phase (v/v) with little reduction in EE. Cholesterol in the liposome bilayers can increase their ordered state and the stability of liposomes [26]. The highest EE was obtained when the mass ratio of injectable soya lecithin to cholesterol (w/w) was 9:1.

The characterization results of PCL and HA-PCL indicated that the increased particle size and decreased zeta potential were due to the modification of negatively charged HA on the surface of PCL. It was observed by transmission electron microscopy (TEM) that HA was successful-

Hyaluronic acid-modified liposome as a sustained-release carrier for paclitaxel

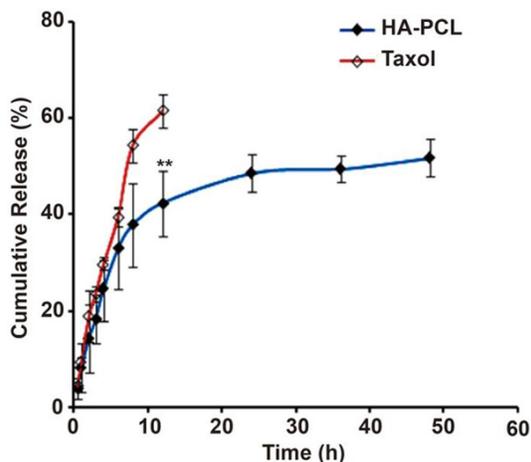


Figure 2. The release of PTX from Taxol or HA-PCL in vitro (n = 3). Compared with the drug release at 12 h in the Taxol group, **P<0.01. PTX: paclitaxel; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes.

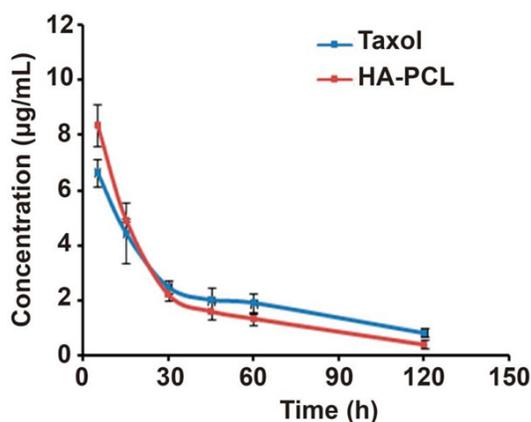


Figure 3. The mean blood concentration-time curves of PTX in the mice following intravenous administration of Taxol solution or HA-PCL (n = 3). PTX: paclitaxel; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes.

ly coated on the surface of PCL. HA with different molecular weight has different roles. HA with the molecular weight more than 2 million Da has the properties of excellent viscoelasticity, unctuousity, and inhibitory effect on inflammatory response. HA with the molecular weight between 100 w and 200 w has good unctuousity and moisturizing property as well as sustained release of drug; HA with the molecular weight between 10 kDa and 80 kDa has the tumor-targeting ability; HA with the molecular weight less than 10 kDa can promote angiogenesis and wound healing [27, 28]. To achieve the tumor-targeting effect, HA with molecular wei-

ght of 7.9 kDa was applied in the system of drug delivery in our study. The modification method of HA by electrostatic interaction was simple and controllable without introducing chemical reagents. The main factor in the process was the ratio of positive to negative charge. Thus, the mass ratio of HA to LHLN was investigated using TEM for preparing HA-PCL with good formability. Finally, the mass ratio of HA to LHLN was set at 0.5:1.

The dialysis method was used for the release study in vitro. It revealed a two-stage behavior in the HA-PCL group and one-stage behavior in the Taxol group. Differences were observed in the release of PTX between the HA-PCL and the Taxol groups. The release of PTX from Taxol followed the Weibull equation and the release of PTX from HA-PCL followed the bio-exponential equation, which aligned with the results by Parashar et al. [29]. The release profiles of PTX exhibited the presence of a sustained release phase in the HA-PCL group. The initial fast release of PTX may be caused by the high drug concentration gradient between the liposome and the medium, and the sustained release behavior mainly resulted from the erosion and degradation of the components of liposomes.

The results of pharmacokinetics study indicated that PTX concentration in HA-PCL may be induced by the passive targeting effect based on the EPR effect of this nano-sized delivery system. These results were consistent with the study by Rezaei et al. [30]. In this study, the main pharmacokinetic parameters were calculated using the DAS 2.0 software according to the concentration-time curves. It was also confirmed that the peak of PTX concentration for the HA-PCL group was owing to the passive targeting distribution. We deduced that the nano-scaled formulation of HA-PCL was characterized by the bioavailability similar to that of Taxol. Further evaluation needs to be conducted for the application of this formulation

Previous studies reported that doxorubicin is encapsulated in aqueous compartment of hyaluronan-polycaprolactone polymersomes by the method of nanoprecipitation. In comparison with PEG-PCL-DOX nanoparticles, HA-PCL polymersomes have better in vivo antitumor efficacy, resulting in wider tumor tissue necrosis in metastatic breast cancer model [31]. In this study, the antitumor efficacy was evaluated in

Hyaluronic acid-modified liposome as a sustained-release carrier for paclitaxel

Table 3. Calculation results of the main pharmacokinetic parameters (n = 3)

Parameters	Taxol	HA-PCL	t value	P value
t _{1/2α} (h)	0.10±0.08	0.11±0.02	0.210	0.844
t _{1/2β} (h)	1.24±0.57	0.71±0.27	1.455	0.219
V1 (L/kg)	0.75±0.62	0.78±0.06	0.083	0.938
CL (L/h/kg)	1.54±0.11	1.68±0.02	2.169	0.096
AUC 0-2 (mg/L*h)	4.92±0.34	4.78±0.17	0.638	0.558
AUC 0-∞ (mg/L*h)	6.53±0.46	5.95±0.08	2.152	0.098
k ₁₀ (1/h)	13.00±20.18	2.16±0.21	8.573	<0.001
k ₁₂ (1/h)	6.20±6.95	2.94±0.15	0.812	0.462
k ₂₁ (1/h)	1.79±0.47	2.63±1.09	1.226	0.288
MRT (h)	0.63±0.02	0.48±0.05	3.939	0.059
Tmax (h)	0.08±0.00	0.08±0.00		
Cmax (mg/L)	6.65±0.49	8.37±0.74	2.741	0.111

Note: MRT: mean residence time; AUC: area under the curve; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes. t_{1/2α}: half-life of distribution phases; t_{1/2β}: half-life of exterminate phases; V1: central volume; CL: clearance; k₁₀: the elimination rate constant for the central compartment; k₁₂: the transfer rate constant from the central to peripheral compartment; k₂₁: the transfer rate constant from the peripheral to central compartment; MRT: mean residence time; Cmax: peak concentration; Tmax: time to peak.

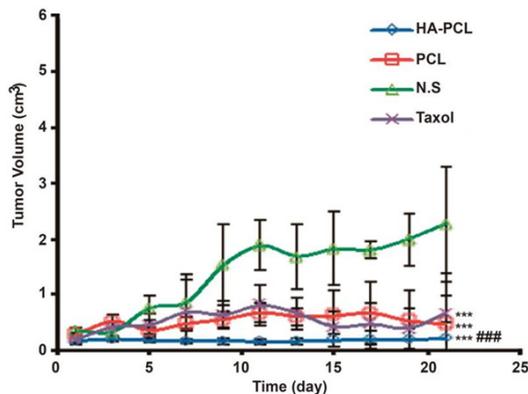


Figure 4. The variations in the tumor volume in the B16-bearing Kunming mice (n = 6). Compared with the NS group, ***P<0.001; compared with the PCL or the Taxol groups, ###P<0.001. NS: normal saline; PCL: paclitaxel-loaded cationic liposome; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes.

B16-bearing Kunming mice treated with intravenous administration of NS, Taxol, PCL with HA-PCL at a dose of 10 mg/kg. These results revealed better antitumor effect of Taxol compared with that of NS and no significant differences in antitumor efficacy between PCL and Taxol. This increased antitumor effect demonstrated that HA-PCL can be an ideal delivery system for PTX. Compared with PCL, the better antitumor effect of HA-PCL may be ascribed to

the active targeting of HA, which resulted in much higher accumulation of PTX loaded liposomes in tumor tissue and higher cellular uptake efficiency. Meanwhile, the body weight of the mice was monitored in this experiment. The body weight increased markedly in the NS group as a result of the tumor growth. There were slight increases in the body weight in the PCL and HA-PCL groups, which confirmed the low side effect induced by PCL and HA-PCL. Moreover, the numbers of dead mice in the experiment also reflected that Taxol has strong side effects. It was deduced that the side effect can be lowered markedly by the delivery of HA-PCL formulation. In light of the significantly increased antitumor effect and lowered side effect, HA-PCL can be a potential delivery system for PTX used in anti-cancer treatment.

In this study, PCL with LHLN as a cationic lipid was successfully prepared by film-dispersion method, and HA-PCL was obtained by electrostatic interaction. This HA modification method was simple and controllable without introducing chemical reagents. High EE and reasonable particle sizes of both PCL and HA-PCL were characterized. The in vitro drug release study indicated that HA-PCL has sustained release profiles. HA-PCL was calculated to have similar bioavailability compared with Taxol in the in vivo pharmacokinetic study. Furthermore, HA-PCL could achieve superior antitumor efficacy and lower side effect compared with Taxol in the B16-bearing Kunming mice model. In summary, HA-PCL, which was prepared by the film dispersion method and electrostatic interaction, can be a potential sustained-release delivery system for PTX.

However, there are still some limitations to the study. For example, no deep investigations were performed on the molecular mechanisms of HA-PCL such as leakage to the interstitium of tumor for sustained-release carriers, the long-term effect of HA-PCL, the toxic effect of HA-PCL, the effect of HA-PCL on proliferation and apoptosis of tumor cells, and the clinical effect of HA-PCL in tumor treatment. Therefore,

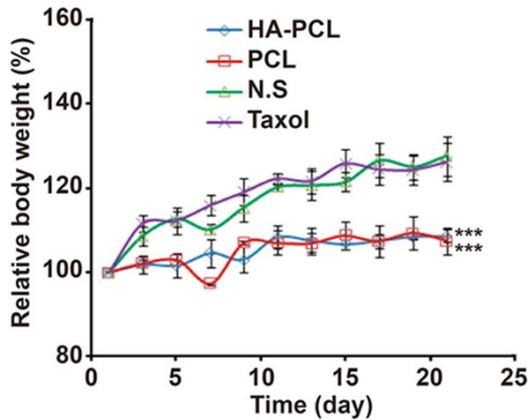


Figure 5. The body weight changes in the mice after administration of NS, Taxol, PCL or HA-PCL (n = 6). Compared with the NS or the Taxol groups, ****P<0.001. PCL: paclitaxel-loaded cationic liposome; HA-PCL: hyaluronic acid-paclitaxel-loaded cationic liposomes; NS: normal saline.

more studies need to be carried out in the future in an attempt to provide an experimental foundation for the application of HA-PCL as a sustained-release carrier.

Disclosure of conflict of interest

None.

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