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# Docetaxel-loaded liposomes: preparation, pH sensitivity, pharmacokinetics, and tissue distribution*

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**Abstract:** Docetaxel (DTX), as a member of taxoid family, has been widely used in the treatment of cancers. The present study prepared pH-sensitive DTX-loaded liposomes (DTX-Lips) by thin-film dispersion method and various physico-chemical and morphological properties were examined. The pH sensitivity of in vitro DTX release and the in vivo pharmacokinetics and tissue distribution using Kunming mice were also investigated. The mean particle size and zeta potential of DTX liposomes were (277±2) nm and (−32.60±0.26) mV, respectively. Additionally, in vitro drug release study showed that the cumulative release rate was 1.3 times more at pH 5.0 than at pH 7.4, suggesting a pH-dependent release ability of DTX-Lips. Pharmacokinetic and pharmaceutical studies in comparison with Duopafei® showed that the half-time period ($t_{1/2}$) and area under the curve (AUC) of DTX-Lips in mouse plasma were 1.8 times longer and 2.6 times higher, respectively, and that DTX-Lips selectively accumulated in macrophage-rich organs such as liver and spleen. These results together suggest that the DTX-Lips could be a promising formulation for the clinical administration of DTX.

**Key words:** Docetaxel-loaded liposomes, pH sensitivity, Pharmacokinetics, Tissue distribution
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## 1 Introduction

Docetaxel (DTX), belonging to the taxanes, is a semi-synthesized anticancer drug, representing a new class of cytotoxin, with a novel mechanism of action (Gelmon, 1994; Hait *et al*., 2007). DTX exerts lethal effects on dividing cancer cells by various mechanisms, all of which result in failure of the cell to divide or subsequently to grow and develop (Hanauske *et al*., 1994; Friedenberg *et al*., 2003; Herbst and Khuri, 2003; Vassilomanolakis *et al*., 2005). Thus, DTX plays an important role in the treatment of solid tumors and has been applied in clinical trials against ovarian carcinoma, breast, lung and head/neck cancers (Bissery, 1995; Posner and Lefebvre, 2003; Yang *et al*., 2011). It has been reported that DTX is more effective and safer than paclitaxel (Grant *et al*., 2003). Unfortunately, despite these advantages, clinical use of DTX is still limited due to its low water solubility and serious adverse effects, including neutropenia, peripheral neuropathy and hypersensitivity reactions (Engels *et al*., 2005; Izquierdo *et al*., 2006; Baker *et al*., 2009). It has been reported that the toxicity is closely related to formulation (Rowinsky, 1997; Immordino *et al*., 2003). As a result, current investigations are mainly focused on developing new drug delivery systems and using nanotechnological materials, such as liposomes, vesicles, polymeric micelles, and nanospheres (Parveen *et al*., 2012). Among these various drug carrier systems, liposomes are one of the most promising nanomedicines, with several Food and Drug Administration (FDA)-approved formulations for cancer treatments (Lian and Ho, 2001;

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Torchilin, 2005; Muthu and Singh, 2009). Liposomes are promising because of their merits in improving drug solubility as well as its considerable potential for encapsulation of both lipophilic and hydrophilic drugs. Therefore, reformulating DTX by liposome will help us to make a better use of the drug in clinical trails.

Resently, high polymer modified strategy was developed to improve targeting ability (Tosi *et al*., 2010; Zhang *et al*., 2012). However, in vivo degradation of artificially synthesized polymers is not well studied and the safety of the carriers limits their clinical use. Phosphatidylethanolamine (PE) is a natural phospholipid and can change from double phase to hexagonal phase. Interestingly the phase transition of PE is triggered by the alteration of pH, and the value of enthalpy change is small during the transition (Kale and Torchilin, 2010). Moreover, it has been reported that the microenvironment of certain tumors is slightly acidic compared to healthy tissues, which enables the drug release selectively from pH-sensitive liposomes and reduces side effects (Torchilin *et al*., 1993; Zignani *et al*., 2000; Maurer *et al*., 2001; Hiraka *et al*., 2008; Yuba *et al*., 2010). Therefore, designing a novel liposome based on PE and other natural materials, such as oleic acid (OA), could be a promising way to guarantee the safety of carries and enhance the delivery efficiency of bioactive drugs into cells (Ducat *et al*., 2010; Li *et al*., 2010; Sánchez *et al*., 2011).

In this paper, a novel pH-sensitive DTX-Lips based on PE and OA were prepared by thin-film depression technique. Furthermore, the pharmacokinetics and tissue distribution of DTX-loaded liposomes (DTX-Lips) and Duopafei®, a commercially available DTX formulation containing a high concentration of nonionic surfactant tween 80, in mouse plasma and tissue samples after a single intravenous (i.v.) administrtion were studied. This study provides important evidence for the further evaluation and clinical development of pH-sensitive liposome encapsulated DTX formulation.

## 2 Materials and methods

### 2.1 Materials

DTX (bulk drug) and Duopafei® (batch 1070172TA) were purchased from Qilu Pharmaceutical Co., Ltd. (Jinan, China). DTX (batch 10100666-201002, purity ≥98.0%) and paclitaxel (batch 100382-200301, purity ≥98.0%) were obtained from the National Institutes for Food and Drug Control. PE was purchased from AMRESCO (Solon, OH, USA). Cholesterol (CHOL), OA, and methanol (batch S93191) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (batch 1105290) was from TEDIA (Fairfield, OH, USA). Ultrapure water was prepared by Milli-Q purification system (Millipore Corp., Bedford, MA, USA).

### 2.2 HPLC operating conditions

Agilent 1260 serious system (Agilent Technologies, Santa Clara, CA, USA) consisting of pump, degrasser, autosampler, diode array detector, and temperature controller was used for high performance liquid chromatography (HPLC) analyses. The autosampler was used to inject 20 μl aliquots of the processed samples on a Zorbax-SB $C_{18}$ column ((4.6×150) mm, 5 μm, Agilent Technologies). The mobile phase was acetonitrile-water (55:45, v/v). The flow rate was 1 ml/min and the ultraviolet (UV) absorbance of the sample was measured at the wavelength 229 nm. Data collection and processing were performed on Agilent 1260 Chemistation.

### 2.3 Preparation of DTX-Lips

Both thin-film dispersion method and diethyl ether injection method were investigated using entrapment efficiency (EE) as the assessment index to select the best preparing process. The EE was calculated from the ratio of the drug amount contained in the liposome to the total charged drug amount. The formation of EE is shown as follows:

$$\mathrm{EE}=\frac{\text{Weight of drug in liposome}}{\text{Weight of feeding drug}}\times 100\%$$

The thin-film was formed by dissolving PE/CHOL/OA/DTX (3:2:3:1, w/w) in chloroform and the solvent was removed by vacuum at 30 °C. The desired film was then hydrated in phosphate buffered saline (PBS). In diethyl ether injecting method, the mixture consisting of PE/CHOL/OA/DTX (3:2:3:1, w/w) dissolved in diethyl ether was injected into PBS dropwise.

DTX encapsulated in liposomes was quantified by pretreatment using methanol, sonication, and filtration followed by HPLC analysis. Briefly, the suspension of DTX-Lips was filtered by a Millipore filter, and then methanol was added to break the emulsion. Subsequently, the suspension was sonicated and centrifuged for 5 min at 12000 r/min. The supernatant was subjected to HPLC analysis as described above. Single-factor analysis and orthogonal design were carried out to optimize the prescription.

### 2.4 Characterization of DTX-Lips

The morphology of DTX-Lips was examined by transmission electron microscope (TEM) (JEM-1200EX, JEOL, Tokyo, Japan). The average particle size and particle size distribution were measured by the light dynamic scattering (DLS) method using Delsa™ Nano Submicron Particle Size Analyzer (A53878, Beckman Coulter Inc., Indianapolis, IN, USA). The Zeta potential was measured by the laser doppler anemometry (LDA) on zeta Plus Zeta Potential Analyzer (Brookheaven Instruments Corporation).

### 2.5 In vitro release of DTX-Lips

The drug release from the liposome was evaluated by a dialysis bag method using buffers with various pH values (pH 7.4, 5.0, 4.0, and 3.4). The dialysis bags, with loaded free DTX or liposomes containing DTX, were immersed in buffers (pH 7.4, 5.0, 4.0, and 3.4) containing 0.5% Tween 80 (dissolution medium) and were stirred (50 r/min) at 37 °C. At different time intervals up to 72 h, the sample was taken out and the fresh PBS was replaced correspondingly. The drug content in the sample was analyzed by HPLC.

### 2.6 Pharmacokinetics and tissue distribution

#### 2.6.1 Plasma and tissue samples preparation

A volume of 100 μl of plasma or tissue samples and 250 μl methanol were added to a centrifuge tube. To each tube, 50 μl of paclitaxel solution was added. The mixture was vortex-mixed for 3 min and centrifuged at 13000 r/min for 10 min. The supernatant was used for analysis.

#### 2.6.2 Pharmaceutical and tissue distribution study

Kunming mice were randomly divided into three groups (40 mice per group). Group 1 was treated with DTX-Lips. The dosage was 16 mg/kg via the tail vein, while Group 2 was given the same dosage of Duopafei®. Group 3 was donated to the blank blood and tissue sample. Blood samples (1 ml) were obtained from removing the eyeball of the mice and were added into heparinized centrifuge tubes just before dosing (0 h) and after 0.083, 0.25, 0.5, 1, 2, 4, 6, and 8 h, the samples were then centrifuged at 4000 r/min for 10 min to separate the plasma. At the same time, various tissues (the heart, liver, spleen, lung, and kidney) were rapidly removed, washed with 0.9% (9 g/L) saline solution, then wiped with filter paper, weighed and homogenized with 0.9% saline (1 ml per tissue, the lung was cut and weighted for about 0.2 g). All blood samples and tissue homogenates were stored at the −20 °C before testing. Animal experiments were approved by the Shandong University animal ethical experimentation committee.

#### 2.6.3 Pharmacokinetic analysis and statistics

Pharmacokinetic parameters of DTX in mice plasma and tissue were calculated by a statistical moment algorithm—Drug and Statistics by DAS program. Further pharmacokinetics analysis was performed to calculate the area under the curve (AUC), the elimination half-lives ($t_{1/2}$), apparent volume of distribution ($V$), and the clearance rate (CL).

## 3 Results and discussion

### 3.1 HPLC analysis of DTX

A typical chromatograph of DTX is shown in Fig. 1. The accuracy and precision of the method is described in Table 1. Drug recoveries of 40, 50,

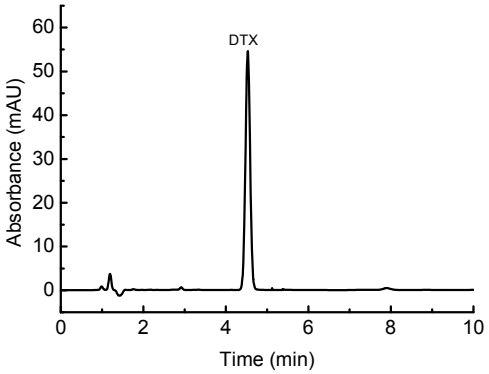


**Fig. 1 Typical chromatograph of DTX**

**Table 1 Accuracy and precision of HPLC analysis method**

| Added concentration (μg/ml) | Mean±SD | | Accuracy (%) | | RSD (%) | |
|---|---|---|---|---|---|---|
| | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day |
| 5 | 4.09±0.0108 | 5.22±0.0312 | 97.6 | 100 | 1.70 | 6.48 |
| 30 | 30.4±0.451 | 32.3±0.0507 | 101.0 | 108 | 3.16 | 2.35 |
| 50 | 49.9±0.453 | 50.5±1.82 | 99.8 | 101 | 1.48 | 6.12 |

$n$=5, SD: standard deviation; RSD: relative standard deviation

and 60 mg were 104.6%, 99.5%, and 93.0%, respectively (data not shown). These suggest that the method is reliable to determine DTX in the samples obtained by in vitro experiments.

### 3.2 Preparation of DTX-Lips

Average EE values of DTX in DTX-Lips prepared by thin-film dispersion method was higher (36.4%) than that by diethyl ether injecting method (28.7%) (data not shown). Moreover, chloroform, which was used in thin-film dispersion method to increase the dissolving ability of drugs, can be removed under vacuum. Based on these two aspects, the thin-film dispersion method was chosen for the preparation of DTX-Lips.

To optimize the prescription, DTX-Lips were prepared by various conditions and the EE values obtained were statistically analyzed. The scheme and results of the orthogonal design are shown in Tables 2 and 3. The important factors that have impact on EE, obtained from single-factor research, are the time of hydration mixing ($A$), the temperature of hydration mixing ($B$), the ratio of PE to CHOL ($C$), and the ratio of PE to OA ($D$). $R$ represents range, the greater the $R$ value indicates the deeper influence of a factor. $K$ represents the mean value of different levels; the higher of the mean refers to the higher encapsulation. Thus, the sequence of factors is: $B>C>A>D$, and the sequence of levels is: $A_3>A_2>A_1$, $B_1>B_3>B_2$, $C_2>C_1>C_3$, $D_3>D_2>D_1$. The best prescription is $B_1C_2A_3D_3$, which is PE/CHOL/OA (6:2:3, w/w). The fixed amount of DTX of 10 mg was used in all studies, and the temperature is 60 °C, the time for hydration is 0.5 h. Three different batches of DTX-Lips were prepared using the best prescription and the average EE is (82.3±4.24)% (data not shown). Compared with previous EE ((88±11)% and (90±0.36)%, respectively) (Immordino *et al*., 2003; Zhao *et al*., 2009), the present EE is good, suggesting the formulation and preparation process is practical.

**Table 2 Levels of experimental factors**

| Level | Factor | | | |
|---|---|---|---|---|
| | $A$[a] (°C) | $B$[b] (h) | $C$[c] | $D$[d] |
| 1 | 40 | 0.5 | 6:1 | 6:1 |
| 2 | 50 | 1 | 6:2 | 6:2 |
| 3 | 60 | 2 | 6:3 | 6:3 |

[a] The time of hydration mixing; [b] The temperature of hydration mixing; [c] The ratio of PE to CHOL; [d] The ratio of PE to OA

**Table 3 Scheme and results of the orthogonal design**

| No. | Factor | | | | EE (%) |
|---|---|---|---|---|---|
| | $A$[a] | $B$[b] | $C$[c] | $D$[d] | |
| 1 | 1 | 1 | 1 | 1 | 76.9 |
| 2 | 1 | 2 | 2 | 2 | 61.0 |
| 3 | 1 | 3 | 3 | 3 | 46.5 |
| 4 | 2 | 1 | 2 | 3 | 97.7 |
| 5 | 2 | 2 | 3 | 1 | 37.2 |
| 6 | 2 | 3 | 1 | 2 | 72.3 |
| 7 | 3 | 1 | 3 | 2 | 85.4 |
| 8 | 3 | 2 | 1 | 3 | 79.7 |
| 9 | 3 | 3 | 2 | 1 | 85.2 |
| $K_1$ | 0.611 | 0.863 | 0.760 | 0.661 | |
| $K_2$ | 0.691 | 0.593 | 0.813 | 0.729 | |
| $K_3$ | 0.835 | 0.680 | 0.564 | 0.746 | |
| $R$ | 0.224 | 0.270 | 0.249 | 0.085 | |

[a] The time of hydration mixing; [b] The temperature of hydration mixing; [c] The ratio of PE to CHOL; [d] The ratio of PE to OA

Negatively charged lipids such as OA distributed among PE molecules provide electrostatic repulsions which decrease PE intermolecular interactions, thus preventing interbilayer interactions under physiological conditions (Connor and Huang, 1985; Straubinger *et al*., 1985; Chu *et al*., 1990). The protonation of OA in the acidic endosomal compartment neutralizes their negative charges, and the vesicles become destabilized as the PE component reverts to hexagonal II (HII) phase. Additionally, OA from pH-sensitive liposomes will be redistributed between liposome and endosome membranes during the process of liposome capture by the cell. The loss of

OA may lead to the structural destruction of pH-sensitive liposomes, and at the same time, the entrapped drug will be transferred into cytoplasm (Philippot *et al*., 1985; Torchilin *et al*., 1992). Both PE and OA are natural materials, which could make the present DTX-Lips safer than liposomes reported by previous studies (Ducat *et al*., 2010; Sánchez *et al*., 2011).

### 3.3 Characterization of DTX-Lips

The particle size distribution of DTX-Lips was shown in Fig. 2. DTX-Lips prepared in this study were a milky white suspension, well-distributed, and well-shaped. Particle size and zeta-potential of liposomes were (277±2) nm and (−32.60±0.26) mV, respectively. Fig. 3a shows a typical TEM image of the liposomes at pH 7.4. It presents a similar spherical morphology of the self-assembled liposomes. In contrast, as shown in Fig. 3b, the liposomes which were stable in the physiological environment were broken under pH 5.0.

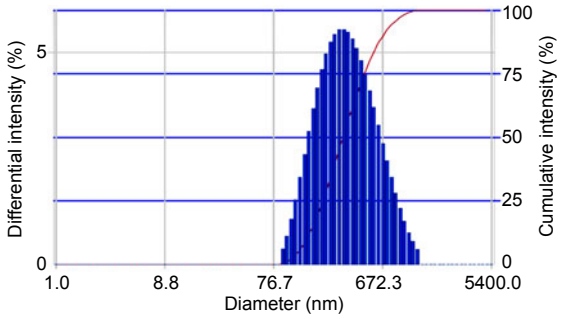


**Fig. 2 Particle size distribution of DTX-Lips**
The particle size was (277±2) nm

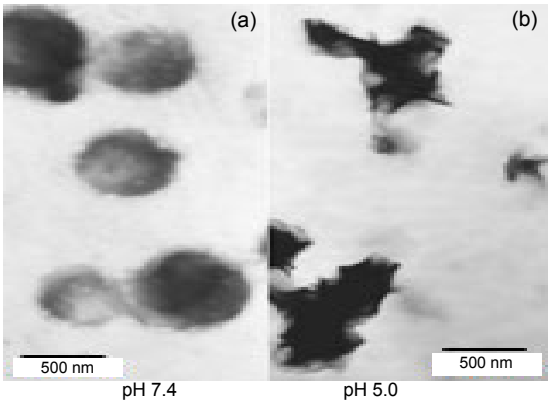


**Fig. 3 Morphological images of liposomes by TEM under different pH conditions**
(a) At pH 7.4, the liposomes were spherical; (b) At pH 5.0, the loposomes were broken

When the pH-sensitive liposome retaining DTX was internalized in the endosomes, the liposome would be rapidly exposed to the acidic environments of endosomes and become unstable, resulting in the rearrangement of structure as described above. These changes in structure are thought to allow the release of drugs at low pH (endosome), hence to increase the cellular uptake of drugs. Based on the facts that the acidity around cancer cells is higher than that of normal cells, we predict that the pH-sensitive liposomes would target tumors and decrease side effects.

### 3.4 In vitro release of DTX-Lips

The cumulative release of free drugs and the drugs from DTX-Lips was shown in Fig. 4. Within 8 h, the release of free drug is up to 80%, while the released drug from the liposome is approximately 20%. Since the control release by DTX-Lips was observed up to 72 h (Fig. 5), the property would

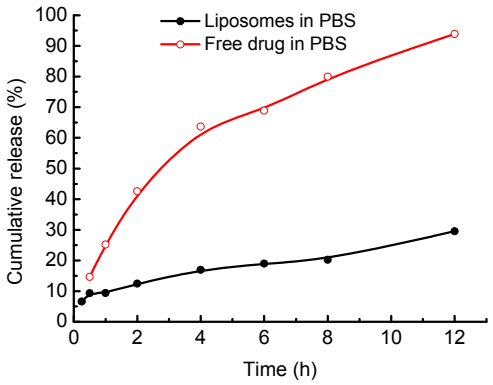


**Fig. 4 Drug release of DTX-Lips and free drug at pH 7.4**
The cumulative release of free drug was 4 times higher than that of DTX-Lips within 8 h

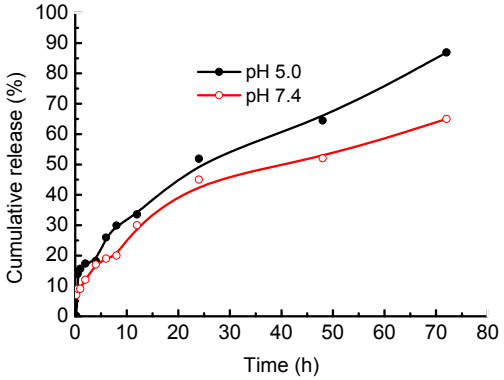


**Fig. 5 Drug release of DTX-Lips at different pH**
The control release of DTX-Lips was up to 72 h, and shows the acid-trigged character

increase retention time of DTX in blood circulation and enhance the drug effects. Furthermore, the acid-trigged character of liposomes was partly revealed from the in vitro study as shown in Fig. 5. After 72 h, the cumulative release percentage reached 86.9% at pH 5.0, while the percentage at pH 7.4 was only 64.6%, suggesting that the DTX-Lips possess pH sensitivity.

### 3.5 HPLC analysis of DTX in plasma and tissue samples

The typical equations for the standard curves are listed in Table 4. The linearity of this method was good to determine DTX in plasma and various tissues in mice. The assay method offered the limits of quantification of 20.0 ng/ml in mouse plasma and 25.0 ng/g in tissue homogenate samples. It can be seen from Table 5 that the accuracy of plasma and tissue samples ranged from 91.2% to 107%. Thus, the analysis method was thought to be accurate and precise to determine DTX in the samples obtained by in vivo experiments. The extraction recoveries of docetaxel in plasma, heart, liver, spleen, lung, and kidney were within the range of 74.1%–83.9%, 84.2%–87.3%, 86.3%–96.9%, 84.8%–87.6%, 85.5%–97.1%, and 70.0%–89.4%, respectively (data not shown).

### 3.6 Pharmacokinetics study

The mean plasma drug concentrations at various time points after i.v. administration in mice are shown in Fig. 6, and the main pharmacokinetic parameters are summarized in Table 6. The mean peak plasma concentration of DTX-Lips reached (0.93±0.33) μg/ml for Duopafei®, compared to (1.49±0.07) μg/ml for DTX-Lips. Injection of DTX-Lips gave a higher DTX concentration in plasma at each time point than

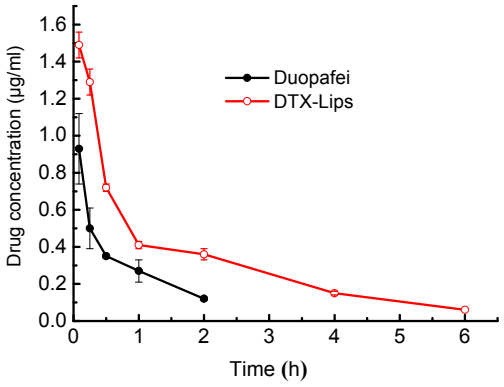


**Fig. 6 Mean drug plasma concentration-time course after i.v. administration of DTX-Lips and Duopafei® in mice (*n*=5)**

**Table 4 Linear ranges, standard curves, and correlation coefficients of DTX in different biosamples[a]**

| Biosample | Linear range (μg/ml or μg/g)[b] | Standard curve | Correlation coefficient *r* |
|---|---|---|---|
| Plasma | 0.20–39.92 | $R$=0.5163$c$+0.0158 | 0.9998 |
| Heart | 3.61–720.72 | $R$=0.4908$c$+0.0093 | 0.9995 |
| Liver | 1.51–301.82 | $R$=0.4900$c$+0.0196 | 0.9995 |
| Spleen | 3.58–716.20 | $R$=0.5229$c$−0.0171 | 0.9996 |
| Lung | 2.61–522.87 | $R$=0.5222$c$+0.0024 | 0.9996 |
| Kidney | 1.35–270.45 | $R$=0.5081$c$–0.0081 | 0.9994 |

[a] The values are arithmetic means, $n$=3; [b] The unit of drug concentration in plasma: μg/ml; The unit of drug concentration in tissue: μg/g. $R$: the ratio of docetaxel to paclitaxel; $c$: the concentration of docetaxel

**Table 5 Intra- and inter-day accuracy and precision of DTX in mice plasma and liver homogenates[a]**

| Sample | Concentration (μg/ml or μg/g)[b] | Mean±SD (μg/ml or μg/g) | | Accuracy (%) | | RSD (%) | |
|---|---|---|---|---|---|---|---|
| | | Intra-day | Inter-day | Intra-day | Inter-day | Intra-day | Inter-day |
| Plasma | 0.399 | 0.390±0.0101 | 0.400±0.0352 | 97.6 | 100 | 1.75 | 7.48 |
| | 2.00 | 2.14±0.0789 | 2.15±0.0517 | 107 | 108 | 3.24 | 2.35 |
| | 29.9 | 30.4±0.453 | 30.2±1.82 | 101 | 101 | 1.48 | 6.02 |
| Liver | 0.0846 | 0.410±0.0273 | 0.420±0.0372 | 102 | 106 | 6.03 | 6.37 |
| | 0.423 | 2.12±0.101 | 2.17±0.0881 | 106 | 109 | 4.69 | 3.52 |
| | 6.35 | 27.3±1.32 | 27.4±0.631 | 91.2 | 91.5 | 1.32 | 2.31 |

[a] The values are arithmetic means, $n$=3; [b] The unit of drug concentration in plasma: μg/ml; The unit of drug concentration in tissue: μg/g. The concentrations of intra-day and inter-day are expressed as mean±SD

that of Duopafei® (Fig. 6), suggesting that the protection by the lipid bilayer membranes lead to the slow drug release and slow clearance of DTX-Lips. The AUC increased from 0.80 mg/(L·h) for Duopafei® to 2.14 mg/(L·h) for DTX-Lips, which is consistent with the decrease of the clearance from 19.9 L/(h·kg) to 7.47 L/(h·kg) (Table 6). The half-lives of DTX in plasma for Duopafei® and DTX-Lips were 0.85 and 1.54 h, respectively (Table 6). The evidence above demonstrates that DTX-Lips are more effective than Duopafei® for its merit of continuous and stable drug release.

### 3.7 Tissue distribution study

The tissue distribution of DTX after i.v. administration of DTX-Lips and Duopafei® at different time points was shown in Fig. 7. Pharmacokinetic parameters (area under the curve of drug concentration (AUC) and mean residence time (MRT)) and targeting parameters (*Re* and *Te*) are given in Table 7. *Re* is a good indicator for the relative efficiency of two delivery systems to one tissue. All of the *Re* values exceeded 1, especially in the liver and spleen (4.76 and 6.35, respectively) (Table 7). MRT of DTX-Lips were greater than that of Duopafei®, especially in plasma and lung which were 1.7-fold and 1.3-fold increased, this was mainly attributed to the sustained releases of DTX from liposomes. Ratio of *Te* indicates liposome targeting efficiency compared

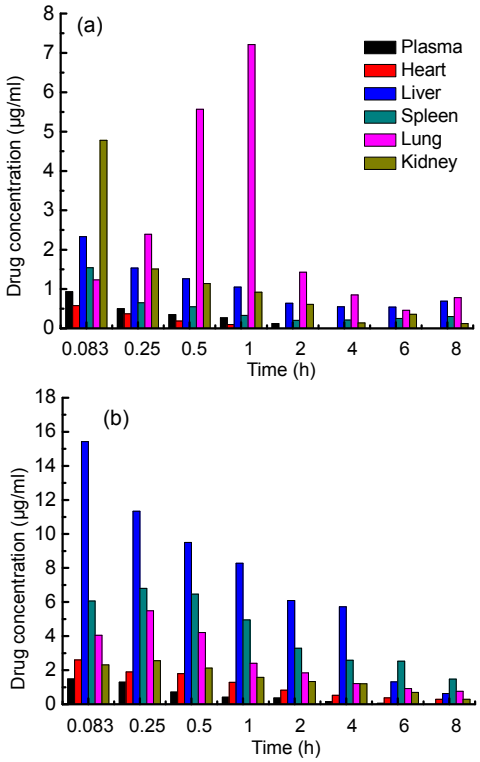


**Fig. 7 Distribution of DTX in mouse organs at various time points after i.v. administration**

(a) Duopafei®; (b) DTX-Lips. Data show average values from five experiments. The unit of drug concentration: μg/ml for plasma and μg/g for organs. The concentration of DTX-Lips was higher than that of Duopafei® in different organs, and DTX-Lips were selectively accumulated in macrophage-rich organs such as the liver and spleen

**Table 6 Comparation of plasma pharmacokinetic parameters of Duopafei® and DTX-Lips after i.v. administration in mice**

| Group | $t_{1/2}$ (h) | $AUC_{(0-\infty)}$ (mg/(L·h)) | $MRT_{(0-\infty)}$ (h) | $C_{max}$ (μg/ml) | CL (L/(h·kg)) | $V$ (L/kg) |
|---|---|---|---|---|---|---|
| Duopafei® | 0.850 | 0.804 | 1.12 | 0.93 | 19.90 | 24.4 |
| DTX-Lips | 1.540 | 2.140 | 1.99 | 1.49 | 7.47 | 16.5 |

$n$=5. $t_{1/2}$: half-time period; AUC: area under the curve of drug concentration; MRT: mean residence time; $C_{max}$: maximum concentration of drug; CL: clearance rate; $V$: apparent volume of distribution of drug

**Table 7 Target evaluations of Duopafei® and DTX lipsomes after i.v. administration in mice**

| Tissue | AUC mg/(L·h) | | MRT (h) | | *Re* | *Te* | | Ratio of *Te* |
|---|---|---|---|---|---|---|---|---|
| | Duopafei® | DTX lipsomes | Duopafei® | DTX lipsomes | | Duopafei® | DTX-Lips | |
| Heart | 1.12 | 5.01 | 1.02 | 4.78 | 4.18 | 0.034 | 0.045 | 1.32 |
| Liver | 8.46 | 40.3 | 11.2 | 2.78 | 4.76 | 0.238 | 0.359 | 1.51 |
| Spleen | 5.73 | 36.4 | 12.7 | 6.86 | 6.35 | 0.161 | 0.324 | 2.01 |
| Lung | 14.7 | 16.4 | 4.17 | 5.47 | 1.12 | 0.413 | 0.146 | 0.353 |
| Kidney | 4.69 | 12.1 | 3.01 | 4.24 | 2.58 | 0.132 | 0.108 | 0.817 |
| Plasma | 0.804 | 2.14 | 1.12 | 1.99 | 2.66 | 0.023 | 0.019 | 0.844 |

$n$=5. AUC: area under the curve of drug concentration; MRT: mean residence time; *Te*: the relative effeciency of two delivery systems to one tissue; *Re*: the selectivity of a delivery system to one tissue

with Duopafei®. As shown in Table 7, DTX-Lips gave higher concentrations in liver and spleen compared to Duopafei®, suggesting that the liposomal formulation causes the high distribution in these two organs and that the uptake of DTX-Lips mainly occurred in the reticuloendothelial system (RES)-rich organs. Pharmacokinetics study and tissue distribution experiment in mice indicated that the AUC and MRT were significantly increased when DTX was encapsulated in liposomes, especially the uptake in liver and spleen were increased in a great extent. These results demonstrated that the liposome carrier altered the tissue distribution pattern of DTX in mice significantly in comparison with Duopafei®. All these properties may help to increase the therapeutic efficacy and decrease the toxicity and side effect.

## 4 Conclusions

Novel DTX-loaded liposomes have been prepared and their physico-chemical properties were investigated. DTX-Lips showed a pH-dependent release behavior, which would be favorable for selectivity against tumor cells. Compared with Duopafei®, DTX-Lips gave a prolonged residence time of the drug in mice and an improved efficiency to RES organs such as the liver and spleen but not to non-RES organs, which might potentially contribute to decrease the risk of toxicity. The present study investigated one composition of pH-sensitive liposomes and its pharmacokinetics in healthy mice. In our further work, various compositions of pH-sensitive liposomes will be investigated to compare their pharmacokinetic and pharmaceutical characteristics such as in vitro cytotoxicity and in vivo antitumor effects and to develop a reformulation of DTX which will be feasible and promising for clinical applications.

## References

Baker, J., Ajani, J., Scotté, F., Winther, D., Martin, M., Aapro, M.S., von Minckwitz, G., 2009. Docetaxel-related side effects and their management. *Eur. J. Oncol. Nurs.*, **13**(1):49-59. [doi:10.1016/j.ejon.2008.10.003]

Bissery, M.C., 1995. Preclinical pharmacology of docetaxel. *Eur. J. Cancer*, **31**(S4):S1-S6. [doi:10.1016/0959-8049(95)00357-O]

Chu, C.J., Dijkstra, J., Lai, M.Z., Hong, K., Szoka, F.C., 1990. Efficiency of cytoplasmic delivery by pH-sensitive liposomes to cells in culture. *Pharm. Res.*, **7**(8):824-834. [doi:10.1023/A:1015908831507]

Connor, J., Huang, L., 1985. Efficient cytoplasmic delivery of a fluorescent dye by pH-sensitive immunoliposomes. *J. Cell Biol.*, **101**(2):582-589. [doi:10.1083/jcb.101.2.582]

Ducat, E., Deprez, J., Peulen, O., Evrard, B., Piel, G., 2010. Cellular uptake of long-circulating pH sensitive liposomes: evaluation of the liposome and its encapsulated material penetration in cancer cells. *Drug Discov. Today*, **15**(23-24):1083. [doi:10.1016/j.drudis.2010.09.362]

Engels, F.K., Sparreboom, A., Mathot, R.A., Verweij, J., 2005. Potential for improvement of docetaxel-based chemotherapy: a pharmacological review. *Br. J. Cancer*, **93**(2):173-177. [doi:10.1038/sj.bjc.6602698]

Friedenberg, W.R., Graham, D., Greipp, P., Blood, E., Winston, R.D., 2003. The treatment of multiple myeloma with docetaxel (an ECOG study). *Leuk. Res.*, **27**(8):751-754. [doi:10.1016/S0145-2126(02)00344-2]

Gelmon, K., 1994. The taxoids: paclitaxel and docetaxel. *Lancet*, **344**(8932):1267-1272. [doi:10.1016/S0140-6736(94)90754-4]

Grant, D.S., Williams, T.L., Zahaczewsky, M., Dicker, A.P., 2003. Comparison of antiangiogenic activities using paclitaxel (taxol) and docetaxel (taxotere). *Int. J. Cancer*, **104**(1):121-129. [doi:10.1002/ijc.10907]

Hait, W.N., Rublin, E., Alli, E., Goodin, S., 2007. Tubulin targeting agents. *Update on Cancer Therapeutics.*, **2**(1):1-18. [doi:10.1016/j.uct.2006.10.001].

Hanauske, A.R., Depenbrock, H., Shirvani, D., Rastetter, J., 1994. Effects of the microtubule-disturbing agents docetaxel (Taxotere®), vinblastine and vincristine on epidermal growth factor-receptor binding of human breast cancer cell lines in vitro. *Eur. J. Cancer*, **30**(11):1688-1694. [doi:10.1016/0959-8049(94)00338-6]

Herbst, R.S., Khuri, F.R., 2003. Mode of action of docetaxel-a basis for combination with novel anticancer agents. *Cancer Treat. Rev.*, **29**(5):407-415. [doi:10.1016/S0305-7372(03)00097-5]

Hiraka, K., Kanehisa, M., Tamai, M., Asayama, S., Nagaoka, S., Oyaizu, K., Yuasa, M., Kawakami, H., 2008. Preparation of pH-sensitive liposomes retaining SOD mimic and their anticancer effect. *Colloids Surf. B Biointerfaces*, **67**(1):54-58. [doi:10.1016/j.colsurfb.2008.07.014]

Immordino, M.L., Brusa, P., Arpicco, S., Stella, B., Dosio, F., Catte, L., 2003. Preparation, characterization, cytotoxicity and pharmacokinetics of liposomes containing docetaxel. *J. Control. Release*, **91**(3):417-429. [doi:10.1016/S0168-3659(03)00271-2]

Izquierdo, M.A., García, M., Pontón, J.L., Martínez, M., Valentí, V., Navarro, M., Gil, M., Cardenal, F., Mesía, R., Pérez, X., Salazar, R., Germà-Lluch, J.R., 2006. A phase I clinical and pharmacokinetic study of paclitaxel and docetaxel given in combination in patients with solid tumours. *Eur. J. Cancer*, **42**(12):1789-1796. [doi:10.1016/j.ejca.2005.10.031]

Kale, A.A., Torchilin, V.P., 2010. Environment-responsive multifunctional liposomes. *Methods Mol. Biol.*, **605**:

213-242. [doi:10.1007/978-1-60327-360-2_15]

Li, J., Yu, H., Li, S., Wang, G.J., 2010. Enhanced distribution and extended elimination of glycyrrhetinic acid in mice liver by mPEG-PLA modified (mPEGylated) liposome. *J. Pharm. Biomed. Anal.*, **51**(5):1147-1153. [doi:10.1016/j.jpba.2009.11.005]

Lian, T., Ho, R.J., 2001. Trends and developments in liposome drug delivery systems. *J. Pharm. Sci.*, **90**(6):667-680. [doi:10.1002/jps.1023]

Maurer, N., Fenske, D.B., Cullis, P.R., 2001. Developments in liposomal drug delivery Systems. *Expert. Opin. Biol. Ther.*, **1**(6):923-947. [doi:10.1517/14712598.1.6.923]

Muthu, M.S., Singh, S., 2009. Targeted nanomedicines: effective treatment modalities for cancer, AIDS and brain disorders. *Nanomedicine*, **4**(1):105-118. [doi:10.2217/17435889.4.1.105]

Parveen, S., Misra, R., Sahoo, S.K., 2012. Nanoparticles: a boon to drug delivery, therapeutics, diagnostics and imaging. *Nanomedicine*, **8**(2):147-166. [doi:10.1016/j.nano.2011.05.016]

Philippot, J.R., Mutaftschiev, S., Liautard. J.P., 1985. Extemporaneous preparation of large unilamellar liposomes. *Biochim. Biophys. Acta.*, **821**(1):79-84. [doi:10.1016/0005-2736(85)90156-7]

Posner, M.R, Lefebvre, J.L., 2003. Docetaxel induction therapy in locally advanced squamous cell carcinoma of the head and neck. *Br. J. Cancer*, **88**(1):11-17. [doi:10.1038/sj.bjc.6600685]

Rowinsky, E.K., 1997. The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu. Rev. Med.*, **48**:353-374. [doi:10.1146/annurev.med.48.1.353].

Sánchez, M., Aranda, F.J., Teruel, J.A., Ortiz, A., 2011. New pH-sensitive liposomes containing phosphatidylethanolamine and a bacterial dirhamnolipid. *Chem. Phys. Lipids*, **164**(1):16-23. [doi:10.1016/j.chemphyslip.2010.09.008]

Straubinger, R.M., Düzgünes, N., Papahadjopoulos, D., 1985. pH-sensitive liposomes mediate cytoplasmic delivery of encapsulated macromolecules, *FEBS Lett.*, **179**(1):148-154. [doi:10.1016/0014-5793(85)80210-6]

Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. *Nat. Rev. Drug Discov.*, **4**(2):145-160. [doi:10.1038/nrd1632]

Torchilin, V.P., Zhou, F., Huang, L., 1993. pH-sensitive liposomes. *J. Liposome Res.*, **3**(2):201-255. [doi:10.3109/08982109309148213]

Torchilin, V.P., Lukyanov, A.N., Klibanov, A.L., Omelyanenko, V.G., 1992. Interaction between oleic acid-containing pH-sensitive and plain liposomes. Fluorescent spectroscopy studies. *FEBS Lett.*, **305**(3):185-188. [doi:10.1016/0014-5793(92)80663-2]

Tosi, G., Vergoni, A.V., Ruozi, B., Bondioli, L., Badiali, L., Rivasi, F., Costantino, L., Forni, F., Vandelli, M.A., 2010. Sialic acid and glycopeptides conjugated PLGA nanoparticles for central nervous system targeting: in vivo pharmacological evidence and biodistribution. *J. Control. Release*, **145**(1):49-57. [doi:10.1016/j.jconrel.2010.03.008]

Vassilomanolakis, M., Koumakis, G., Barbounis, V., Demiri, M., Panopoulos, C., Chrissohoou, M., Apostolikas, N., Efremidis, A.P., 2005. First-line chemotherapy with docetaxel and cisplatin in metastatic breast cancer. *Breast*, **14**(2):136-141. [doi:10.1016/j.breast.2004.08.017]

Yang, F., Jin, C., Jiang, Y., Li, J., Di, Y., Ni, Q., Fu, D., 2011. Liposome based delivery systems in pancreatic cancer treatment: from bench to bedside. *Cancer Treat. Rev.*, **37**(8):633-642. [doi:10.1016/j.ctrv.2011.01.006]

Yuba, E., Kojima, C., Harada, A., Tana, Watarai, S., Kono., K., 2010. pH-sensitive fusogenic polymer-modified liposomes as a carrierof antigenic proteins for activation of cellular immunity. *Biomaterials*, **31**(5):943-951. [doi:10.1016/j.biomaterials.2009.10.006]

Zhang, P., Ling, G., Pan, X., Sun, J., Zhang, T., Pu, X., Yin, S., He, Z., 2012. Novel nanostructured lipid-dextran sulfate hybrid carriers overcome tumor multidrug resistance of mitoxantrone hydrochloride. *Nanomedicine*, **8**(2):185-193. [doi:10.1016/j.nano.2011.06.007]

Zhao, L., Wei, Y.M., Zhong, X.D., Liang, Y., Zhang, X.M., Li, W., Li, B.B., Wang, Y., Yu, Y., 2009. PK and tissue distribution of docetaxel in rabbits after i.v. administration of liposomal and injectable formulations. *J. Pharm. Biomed. Anal.*, **49**(4):989-996. [doi:10.1016/j.jpba.2009.01.016]

Zignani, M., Drummond, D.C., Meyer, O., Hong, K., Leroux, J.C., 2000. In vitro characterization of a novel polymeric-based pH-sensitive liposome system. *Biochim. Biophs. Acta*, **1463**(2):383-394. [doi:10.1016/S0005-2736(99)00234-5]