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Research Article

Efficacy, Pharmacokinetics, and Biodistribution of Thermosensitive Chitosan/β-Glycerophosphate Hydrogel Loaded with Docetaxel

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Abstract. Docetaxel (DTX) is a widely used anticancer drug for various solid tumors. However, its poor solubility in water and lack of specification are two limitations for clinical use. The aim of the study was to develop a thermosensitive chitosan/β-glycerophosphate (C/GP) hydrogel loaded with DTX for intratumoral delivery. The *in vitro* release profiles, *in vivo* antitumor efficacy, pharmacokinetics, and biodistribution of DTX-loaded C/GP hydrogel (DTX-C/GP) were evaluated. The results of *in vitro* release study demonstrated that DTX-C/GP had the property of controlled delivery for a reasonable time span of 3 weeks and the release period was substantially affected by initial DTX strength. The antitumor efficacy of DTX-C/GP was observed at 20 mg/kg in H22 tumor-bearing mice. It was found that the tumor volume was definitely minimized by intratumoral injection of DTX-C/GP. Compared with saline group, the tumor inhibition rate of blank gel, intravenous DTX solution, intratumoral DTX solution, and DTX-C/GP was 2.3%, 29.8%, 41.9%, and 58.1%, respectively. Further, the *in vivo* pharmacokinetic characteristics of DTX-C/GP correlated well with the *in vitro* release. DTX-C/GP significantly prolonged the DTX retention and maintained a high DTX concentration in tumor. The amount of DTX distributed to the normal tissues was minimized so that the toxicity was effectively reduced. In conclusion, DTX-C/GP demonstrated controlled release and significant efficacy and exhibited potential for further clinical development.

KEY WORDS: antitumor efficacy; biodistribution; chitosan/β-glycerophosphate; docetaxel; pharmacokinetics.

INTRODUCTION

Cancer cells can aggressively grow by dividing without normal limitations, invade adjacent tissues, and lead to metastasis. According to the World Health Organization, cancer is a serious threat to human health around the world (1). The main approaches of cancer treatment are surgery, radiotherapy, and chemotherapy (1), and chemotherapy still remains the most commonly used.

Docetaxel, a mitotic inhibitor (2), is among the most active cytotoxic agents in clinical use in oncology and is widely prescribed for several kinds of malignancies such as breast, prostate, and non-small cell lung cancers (3). Despite its clinical activity, DTX has several drawbacks including poor solubility, short half-life time, nonspecific distribution throughout the body (4), and emergence of drug resistance. About 75% of DTX is rapidly eliminated by hepatobiliary extraction in humans, with similar metabolic pathways in all the species (5). Moreover, the solvent polysorbate 80 has been associated with a number of toxicities like acute hypersensitivity reactions and the alterations in docetaxel pharmacokinetic profiles (6). Due to these disadvantages, sustained drug delivery system such as chitosan-based gels combined with targeting intratumoral (i.t.) injection exhibits improved efficacy of DTX (7).

Intratumoral delivery may target chemotherapeutic agents directly to tumor and reduce the exposure at normal tissues to avoid toxicity and improve efficacy. Intratumoral injectable formulations are basically designed as liposomes (8), nanoparticles (9), or *in situ* gel systems (10). But there are several limitations of liposomes and nanoparticles, such as limited solubilized capacity or a complex preparation procedure.

In situ gel systems can go sol–gel transition after local administration under physiological conditions (11) which makes them increasingly important in drug delivery (12). Several hydrogels for localized delivery of DTX have been reported such as pluronic F127-based reversal thermal gels (13) and poly-(D,L-lactic acid-co-glycolic acid) (PLGA)–polyethylene glycol (PEG)–PLGA hydrogel (14). Nevertheless, the application of pluronic in drug delivery system is restricted, partly ascribed to its high toxicity and nonbiodegradability (15). Although the PLGA–PEG–PLGA hydrogel system showed improved anticancer effect and lower toxicity, the relatively high concentration of copolymer (20 wt%) might cause inflammatory reactions during the degradation process.

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Meanwhile, the organic solvent used to dissolve DTX could lead to neurotoxicity and liver damage.

Chitosan, a natural copolymer produced by the deacetylation of chitin (16), is particularly attractive for pharmaceutical application because of its high solubilized capacity, biodegradability, and desired safety profile (17,18). Based on excellent gel forming property, numerous chitosan-based hydrogels have been developed. It is reported that a chitosan/PEG hydrogel performed well-controlled release of the model drug and showed potential for local delivery (19). The alginate–chitosan–PLGA gel successfully delivered an immunomodulating peptide to treat experimental autoimmune encephalomyelitis in a mouse model (20). Another widely reported chitosan-based gel was obtained by simply mixing chitosan with β-glycerophosphate (β-GP) solution (21). The chitosan/β-glycerophosphate (C/GP) hydrogel has been applied as a sustained delivery system for both hydrophobic and hydrophilic drugs such as paclitaxel (18) and insulin (22). The paclitaxel-loaded C/GP hydrogel eliminated the toxicity of the solvent, Cremophor ® EL, and improved the efficacy.

In the present study, we set out to develop a C/GP thermosensitive hydrogel loaded with DTX for i.t. delivery to enhance therapeutic efficacy and alleviate system toxicity. For this purpose, the C/GP hydrogel was prepared and *in vitro* release of DTX-C/GP hydrogel was investigated. In addition, the antitumor efficacy, the pharmacokinetics, and the distributions of DTX-C/GP hydrogel were evaluated in H22 tumor-bearing mice.

MATERIALS AND METHODS

Materials

Medical grade chitosan (MW ~200,000 with deacetylation degree of 91%) was obtained from Qingdao Jinke Biomedical Co., Ltd. (Qingdao, China). β-glycerophosphate was from Sigma-Aldrich Chemical Co. (St. Louis, MO). Docetaxel was obtained from Shanghai Sunve Pharmaceutical Co., Ltd. (Shanghai, China). Paclitaxel (the internal standard) was purchased from the National Institutes for Food and Drug (Shanghai, China). Other chemicals were reagent grade.

Mice and Cell Culture

Male ICR mice, 18–22 g, were obtained from the Animal Center of China Pharmaceutical University. Murine H22 hepatoma cells were sourced from the China Institute of Cell Biology. The H22 cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum in air containing 5% CO_2 at 37°C.

Preparation of the C/GP Solution

Chitosan powders (90 mg) were dissolved in 4 mL of 0.1 M acetic acid and gently stirred for 12 h to make a homogeneous solution. Five milligrams or 20 mg of DTX was added to the chitosan solution and stirred for another 4 h. β-GP (450 mg) was dissolved in 1 mL of deionized water to get a β-GP solution. Both solutions were stored in an ice bath for 10 min. Afterwards, the two mixed solutions were uniformly stirred for 10 min by dropwisely adding β-GP. The final formulation was composed of 1.8% (*w*/*w*) chitosan, 9% (*w*/*w*) β-GP, and 1 or 4 mg/mL DTX.

Inverted tube test was used to characterize the sol–gel process (23). A sol phase or a gel phase is defined by flow or not flow of the solution in a tilted test tube. Firstly, 1 mL of DTX-C/GP solutions was added to 2-mL vials. The vials were then maintained in an incubator at 37°C and tilted every minute. When the gel remained static, the time was recorded as sol–gel transition time.

In Vitro Release Experiments

In the *in vitro* experiment, 0.1 mL of DTX-C/GP solutions was added to tubes and placed in a 37°C incubator for 2 h. Then, 1-mL release medium (phosphate-buffered solution containing 0.1% polysorbate 80, pH 6.8) was added into the tubes. Polysorbate 80 was used to increase the solubility of DTX. The tubes were maintained in an incubator at 37°C with a shaking rate of 50 rpm. At predetermined times, all the release medium was obtained and stored at −20°C until analysis and the tubes were replenished with 1 mL fresh buffer prewarmed at 37°C.

DTX concentration was determined by a reverse-phase HPLC system which consisted of Shimadzu LC-10AD pump, SPD-10A UV detector, and a HYPERSIL ODS2 column (24). The wavelength of detection used for docetaxel was 232 nm. The analysis was performed using acetonitrile/water (50/50, *v*/*v*) as mobile phase and the flow rate of 1.0 mL/min with injection volume of 20 μL per sample.

The calibration curve with eight points was established for DTX ranged from 1 to 100 μg/mL and the lowest limit of quantification was 1 μg/mL. In this method of validation, the inter-day and intra-day precisions ranged from 0.96% to 1.75% and 1.57% to 2.46%, respectively.

The release data of DTX in the first stage were applied with a semiempirical model called power law (25,26):

$$Q = kt^n (Q < 0.6)$$

where Q is the cumulative released amount of DTX at time t, k is a rate constant, and the time exponent, n, is related to the release mechanism.

If the data are well fitted in the power law equation, then for a cylindrical polymer matrix, n approaching to 0.45 and 0.89 refers to diffusion-controlled drug release and gel erosion-controlled release, respectively.

In Vivo Antitumor Activity of DTX-C/GP Hydrogel

After being cultured *in vitro*, the H22 hepatoma cells at logarithmic growth phase were harvested and inoculated intraperitoneally to ICR mice. Seven days later, the H22 cells with ascites were harvested from the mice and diluted to a concentration of 1.0×10^7/mL with sterilized saline. To establish the tumor-bearing mouse model, 0.2 mL of the diluted H22 cells was subcutaneously injected into the mouse at the lower right axilla (27). Then, the tumor size was measured every 2 days by determining two perpendicular dimensions with a vernier caliper. The volume of each tumor

was calculated by the formula width^2×length/2. When the tumors reached about 200~300 mm^3, the mice were randomly allocated to five groups (n=8) and the day was called "day 0". On day 0, each group of mice was treated i.t. with saline (control group), blank gel, DTX solution (20 mg/kg), DTX-C/GP hydrogel (20 mg/kg), and i.v. with DTX solution (20 mg/kg), respectively. Tumor size was measured every 3 days in the period of 21 days. Besides the use in comparing the final tumor volume of control group with those of treated groups, the tumor growth rate and the tumor volume doubling time (DT) were also used to evaluate the efficacy. Here, the DT was calculated with the following equation (28):

$$\mathrm{DT} = T \times \log 2/(\log\ \mathrm{VF} - \log\ \mathrm{VI}).$$

where VF and VI are the final tumor volume and the initial tumor volume, and T is 21 here.

Then, after 21 days, the mice were sacrificed with tumors removed and weighed, and the tumor inhibition rate (R) was calculated by the following equation:

$$R = (1 - \text{mean tumor weight of treated group}/\text{mean tumor weight of control group}) \times 100\%.$$

Throughout the animal studies, the body weight of mice following treatment was recorded every 3 days and changes at the injection site were observed to assess the toxicity.

Plasma Pharmacokinetic and Distribution Experiment

The H22 model mice were divided into two groups and received either i.t. injection of DTX solution (polysorbate 80/ethanol/PBS, 5:5:90, *v*/*v*/*v*) (29) or DTX-C/GP hydrogel solution at 20 mg/kg. At 0.5, 1, 2, 4, 8, 12, 24, and 48 h, the blood, liver, spleen, lung, heart, kidney, and tumor of mice in DTX solution group were collected. At 1, 3, 5, 7, 10, 15, and 21 days, the blood and the tissues of mice treated with DTX-C/GP hydrogel were sampled. Plasma was obtained by centrifugation at 4,000 rpm for 10 min and tissues were blotted with paper towel after perfusion by physiological saline (0.9% sodium chloride), weighed, and stored at −20°C until analysis. Tissue samples were homogenized in saline to prepare 100 mg/mL tissue sample. Aliquots of the homogenate (100 μL) or plasma (50 μL) were precipitated with a triple volume of acetonitrile containing 33.3 μg/L paclitaxel (the internal standard). The samples were vortex mixed for 30 s and centrifuged at 16,000 rpm for 10 min. An aliquot of 5 μL of the supernatants was injected into the LC-MS/MS system for the determination of DTX.

Concentrations of DTX in the homogenate or plasma were determined by LC-MS/MS using a published method (30). Chromatographic separation was acquired by reverse-phase chromatography with the gradient elution on a BDS HYPERSIL C18 column (5 μm, 2.1×50 mm, Thermo Scientific). The mobile phase consisted of a mixture of phase A (0.1% formic acid and 0.3 mM sodium acetate) and phase B (methanol). MS/MS studies were performed with a Thermo Scientific TSQ Quantum MS/MS system equipped with ESI source in positive ion mode. Quantification was achieved in selective reaction monitoring by monitoring the transition of 830.3→549.0 for DTX and m/z 876.0→307.8 for paclitaxel (the internal standard).

The calibration curve was constructed by plotting the peak area ratios of DTX to internal standard *versus* nominal concentration. The correlation coefficient (r^2) was better than 0.99 over the range of 1–1,000 ng/mL with the lower limit of quantification of 1 ng/mL. The RSDs of inter-day and intra-day precision were within 15% of the three QC levels (2, 50, 800 ng/mL), and the derivation of accuracy was restrained from 85% to 115%. The recoveries of DTX from plasma and tissue homogenate were greater than 85% (less than 115%). There was no significant matrix effect observed.

The calculation of pharmacokinetic parameters was performed by a non-compartmental analysis using WinNonlin computer program (version 4.0, Pharsight Corporation). Maximum concentration (C_{max}) and the time to reach C_{max} (T_{max}) were determined by the concentration–time profiles. The area under the concentration–time curves (AUC) was calculated by the linear trapezoidal rule from zero to the final sampling time (AUC_{0-t}).

Statistical Analysis

Values were expressed as mean ± standard deviation (SD). Data were analyzed by one-way analysis of variance. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSIONS

Preparation of the C/GP Solution

The thermosensitivity of C/GP solution at 37°C was investigated using inverted test tube method (Fig. 1). The color changed from transparent to opaque following gelation. The characteristics of the C/GP solution prepared with varying formulations were shown in Table I. C/GP solution composed of 1.4–2.2% (*w*/*v*) chitosan and 6–12% (*w*/*v*) β-GP was gelled at 37°C with different gelation times. The increase of chitosan and β-GP concentrations could both reduce gelation time which might be related to the increase of intermolecular interactions and entanglements (31,32). The pH values of different formulations were around 6.9 in the tumor pH range. The gelation time was also reduced by the increasing pH values. As we know, with the neutralizing action of the phosphate groups in β-GP, the pH values increased with the increasing amount of β-GP.

In Vitro Release Experiments

In vitro release experiments were performed in PBS (pH 6.8) at 37°C to investigate the release characteristics of DTX-C/GP hydrogel. Figure 2 presents the cumulative amount of DTX released from the hydrogel as a function of time. The release rate was reduced with the increase of the drug loading (see Fig. 2). Besides, the duration of the release of DTX was proportionate to the DTX content. The initial burst effects for 1 mg/mL loaded gel and 4 mg/mL loaded gel were 22.06% and 17.56%, demonstrating that the higher the concentration of DTX in the hydrogels was, the lower the initial burst effect became. After 21 days, the total release of the 1 mg/mL

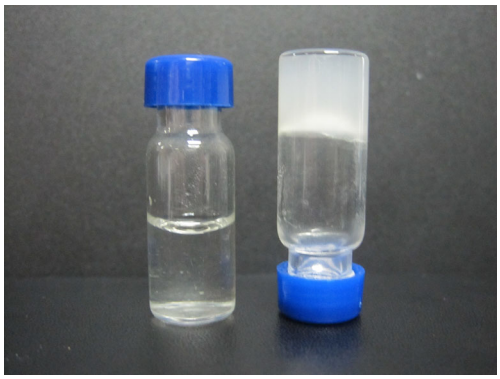

Fig. 1. The C/GP formulation at room temperature (*left*) and at 37°C (*right*)

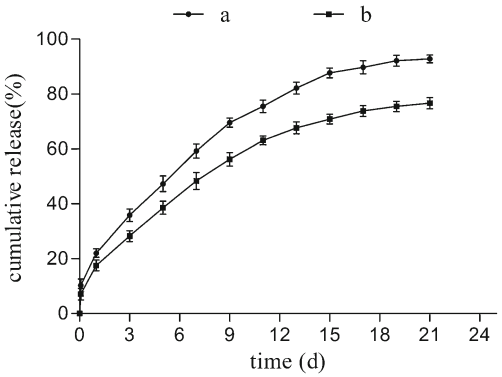

Fig. 2. *In vitro* release profiles of DTX-C/GP with varying drug loadings. *a* 1 mg/mL; *b* 4 mg/mL. *Each point* represents the mean ± SD (n=3)

loaded gel was 92.85% while the one of the 4 mg/mL loaded gel was only 76.68%. The increase of viscoelasticity might contribute to the different release characteristics (14). For gelling system, the drug release patterns may be affected by interactions between drug and gel such as hydrophobic or hydrogen bonding interactions (33). Among which, the strong hydrophobic interactions could probably result in the release disparity. Besides, it has been reported that the release of paclitaxel from C/GP gel was also concentration dependent. Hereby, the release efficacy of high drug loading hydrogel was reduced by comparing to low drug loading one (18).

In order to elucidate the mechanism of DTX release, the release data of DTX from the hydrogel were analyzed by power law. The *in vitro* release mechanism of other drugs from C/GP gel has been reported in previous papers (34). Theoretically, the drug release mainly depends on diffusion in the first stage with the gel swelling (35). In this study, the release profiles were well fitted in the power law equation (1 mg/mL gel, r^2=0.983; 4 mg/mL gel, r^2=0.989). The estimated values of n for 1 mg/mL gel and 4 mg/mL gel were 0.427 and 0.456, both quite close to 0.45, suggesting that the two formulations shared similar release mechanism of diffusion-controlled release.

In Vivo Anticancer Activity

To determine the effects of blank gel, DTX solution, and DTX-C/GP on tumor growth in mice, a mouse tumor-bearing model was constructed and different formulations were administered intravenously or intratumorally. Figure 3 showed the changes of mean relative tumor volume through the study after treatment of varying DTX formulations to H22 tumor-bearing mice. The mean relative tumor volume is the ratio of the measured tumor volume at the allocated times to the initial one on "day 0." As shown in Fig. 3, there was no tumor inhibition effect in the blank gel group, which was consistent with the saline group because the saline and the blank gel treated tumors grew to 14.9±3.1 and 14.7±3.9 times of their original size after 21 days of observation. Tumor growth was significantly inhibited in DTX-C/GP group compared with other groups. The difference between the blank gel group and the DTX-C/GP group was statistically significant (p<0.01). Comparing the tumor growth of DTX-C/GP with that of i.v. DTX (p<0.05) showed the advantages of DTX-C/GP. The improved anticancer effects of DTX-C/GP might be mainly due to the sustained release of DTX in the tumor.

To learn more about the tumor growth inhibition effect, tumor growth rate and DT were calculated, respectively, listed in Table II. It should be noted that the DTX-C/GP was efficacious in inhibiting the tumor growth as it had the lowest tumor growth rate and the longest DT. However, there was no significant difference between the groups administered with i.v. and i.t. DTX solution (p=0.561). Intratumoral DTX might not significantly prolong the DTX retention in the tumor.

Table I. Characteristics of C/Gp Hydrogel

	Solution		Hydrogel	
Sample	Chitosan (% *w/v*)	GP (% *w/v*)	pH	Gelation time (min)
1	1.4	6	6.75	>120
2	1.4	9	6.83	61
3	1.4	12	6.96	23
4	1.8	6	6.81	27
5	1.8	9	6.88	12
6	1.8	12	6.97	5
7	2.2	6	6.83	9
8	2.2	9	6.91	3
9	2.2	12	6.99	2

GP glycerophosphate

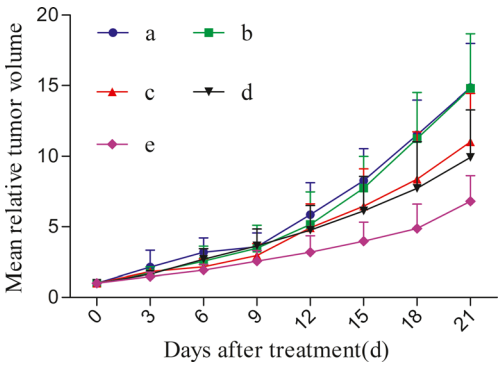

Fig. 3. *In vivo* antitumor efficacy in H22 tumor-bearing mice of different formulations. *a* saline; *b* blank C/GP hydrogel; *c* intravenous docetaxel (DTX) solution (20 mg/kg); *d* intratumoral docetaxel solution(20 mg/kg); *e* intratumoral DTX-C/GP (20 mg/kg). *Each point* represents the mean ± SD (n=8)

Table II. Tumor Growth Rate and Tumor Volume Doubling Times Following Treatments with Varying Formulations in the H22 Tumor-Bearing Mice (mean ± SD)

Treatment	Tumor growth rate (mm^3/day)	Tumor volume doubling time (days)
i.t. Saline	270.5±64.8	5.5±0.5
i.t. Blank gel	171.84±18.3	5.5±0.7
i.v. DTX solution	142.8±26.6[a]	6.3±1.0
i.t. DTX solution	121.1±32.0[a]	6.6±1.1
i.t. DTX-C/GP	82.0±30.1[*, **, ***]	7.8±1.1[*, **, ***]

*$p<0.05$, *versus* saline at the same time point, **$p<0.05$, *versus* i.t. blank gel at the same time point, ***$p<0.05$, *versus* i.v. DTX at the same time point

Compared with the saline group, the tumor inhibition rate with blank gel, i.v. DTX solution, i.t. DTX solution, and DTX-C/GP was 2.3%, 29.8%, 41.9%, and 58.1%, respectively. There were significant differences between blank gel and DTX-C/GP ($p<0.001$), i.v. DTX solution and DTX-C/GP ($p<0.001$), and i.t. DTX solution and DTX-C/GP ($p<0.05$). These results indicated that DTX-C/GP treatment had a remarkable *in vivo* anticancer activity.

The changes in body weight were measured as a function of time to observe the *in vivo* toxicity. Since chitosan is a natural polysaccharide of nontoxicity and biodegradable ability, the C/GP hydrogels are expected to be safe *in vivo*. As shown in Fig. 4, the body weight in the DTX-C/GP group kept increasing steadily and remained within the normal range. This is an important indication of the low toxicity of the treatment and shows that DTX-C/GP can be safely injected.

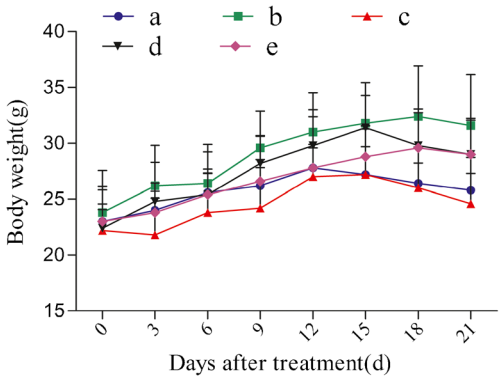

Fig. 4. Body weight changes of H22 tumor-bearing mice after administration of varying formulations. *a* Saline; *b* blank C/GP hydrogel; *c* intravenous docetaxel (DTX) solution (20 mg/kg); *d* intratumoral docetaxel solution (20 mg/kg); *e* intratumoral DTX-C/GP(20 mg/kg). *Each point* represents the mean ± SD ($n=8$)

Besides, no deaths, visible toxicity, and obvious necrosis at the injection sites were observed in the DTX-C/GP group. These results indicate that the slow release of DTX from the hydrogel is very effective against the tumor model with little toxicity.

Five mice from i.v. DTX solution group showed severe underactivity from day 9 to day 14 and two of them died before the end of the study. These toxicities might be caused by the drug itself or the vehicle (polysorbate 80). As we know, polysorbate 80 could induce unpredictable hypersensitivity reactions and cumulative fluid retention. However, no similar toxicity was observed to be superficially associated with polysorbate 80 in our study. And as the maximum tolerated dose of DTX for mice was reported to be 20–50 mg/kg, the deaths

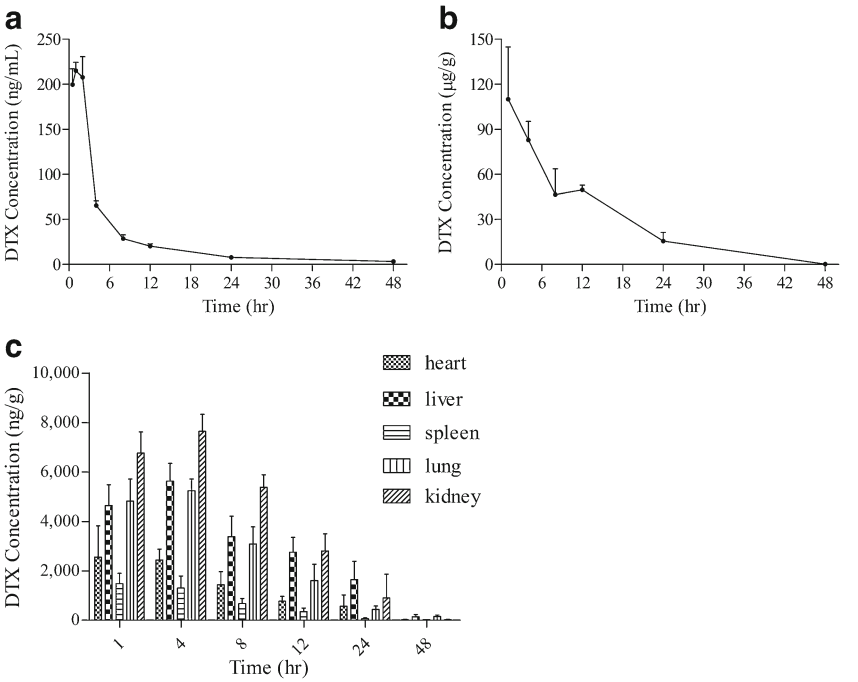

Fig. 5. Concentration–time profiles of DTX following i.t. administration of DTX solution at 20 mg/kg to H22 tumor-bearing mice in plasma (**a**), tumor (**b**), and tissues (**c**), respectively. DTX concentrations were determined by LC-MS/MS assay. *Each point* represents the mean ± SD ($n=4$)

Table III. Distribution Parameters of DTX After i.t. Administration of 20 mg/kg DTX as Solution or C/GP Hydrogel in Tumor-Bearing Mice

	AUC (day μg/g tissue)		Distribution index[a]	
Tissue	Solution	Hydrogel	Solution	Hydrogel
Tumor	64.824	587.263	1	1
Heart	1.505	1.551	0.023	0.003
Liver	3.996	0.872	0.062	0.001
Spleen	0.605	1.113	0.009	0.002
Lung	2.625	1.229	0.040	0.002
Kidney	4.199	3.649	0.065	0.006

AUC area under the concentration–time curves, *DI* distribution index
[a] Calculated as the AUC of normal tissue/AUC of tumor tissue ratio and indicates the relative level of drug distribution with respect to tumor tissue

in i.v. DTX group were, at least, partly due to the intrinsic toxicity of DTX. Comparing to i.v. DTX group, DTX-C/GP reduced the toxicity of DTX because no death was observed in DTX-C/GP group. Nevertheless, the body weight changes of these two groups showed no significant statistical difference (p=0.123). We recognize that our safety study has several limitations and further studies will focus on comparing the survival time of different groups.

Pharmacokinetic Studies in H22 Tumor-Bearing Mice

The concentration of DTX was detected by LC-MS/MS analysis and the method was validated. Following i.t. administration of 20 mg/kg of DTX solution in H22 tumor-bearing mice, the plasma and tumor concentration–time profiles of DTX are shown in Fig. 5a, b. The plasma C_{max} of DTX was 215.08 ng/mL at 1 h. Then, the concentrations dropped rapidly during the first 8 h after DTX solution administration and fell below the detection limit after 2 days. The mean residence time (MRT) of DTX in plasma was approximately 9 h. This phenomenon might be due to the increased interstitial fluid pressure and high blood perfusion which provided easy access for DTX to the blood circulation (36). Therefore, the concentration of DTX in the tumor decreased sharply and could not be detected only after 2 days.

DTX concentrations in heart, spleen, liver, lung, and kidney were also measured after i.t. administration of DTX solution (Fig. 5c). The results demonstrated that DTX was absorbed rapidly and distributed widely into the five organs which had large blood flows, and the drug concentration was the highest in kidney, followed by lung, liver, heart, and spleen. DTX levels in tissues saw a gradual decrease from 4 to 48 h after the administration. A parameter called distribution index (DI) (ratios of AUC of normal tissue to tumor tissue) is shown in Table III. The results showed that the drug distributions in normal tissues were minimal relative to tumor.

As seen in Fig. 6a, b, the plasma and tumor pharmacokinetic characteristics of DTX from DTX-C/GP were quite distinct from the solution. The C_{max} of DTX in the plasma was 22.77 ng/mL at 1 day. DTX concentration of the gel was maintained above the detection limit until 21 days which was consistent with the sustained release nature of the DTX-C/GP. The pharmacokinetic parameters indicated that the hydrogel could be effectively used as a drug carrier for sustained release as DTX-C/GP had a long MRT of 9 days and a AUC_{plasma} of 219.43 μg/L day. What is more, the tumor DTX level had a slow elimination for more than 21 days which has probably resulted from the slow dissolution of DTX from the gel. By calculating the AUC_{tumor}/AUC_{plasma} ratios of the two formulations, we could see that DTX-C/GP had a higher targeting efficiency to the tumor than DTX solution (2,727.87 *versus* 1,256.67), and the results explained the higher inhibition effect to a certain extent.

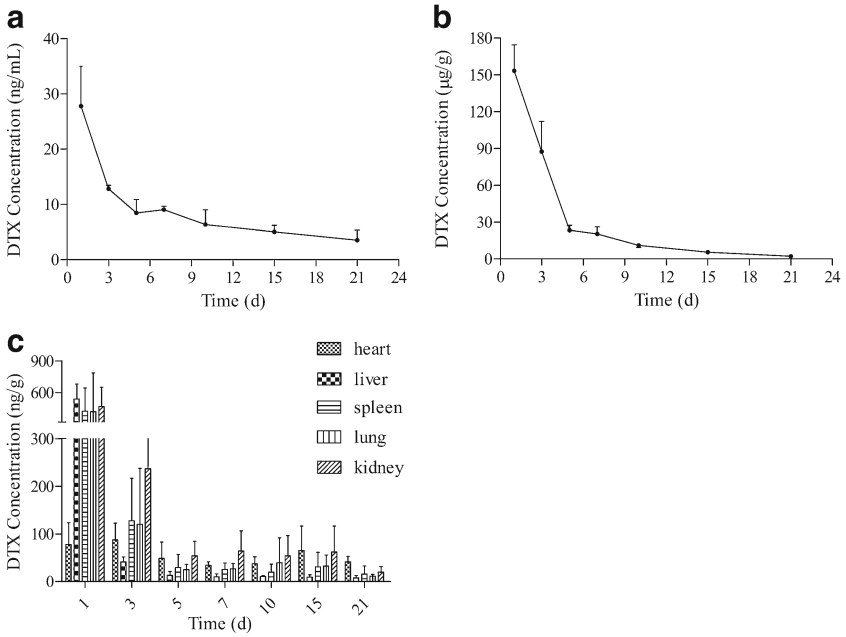

Fig. 6. Concentration–time profiles of DTX following i.t. administration of DTX-C/GP at 20 mg/kg to H22 tumor-bearing mice in plasma (**a**), tumor (**b**), and tissues (**c**), respectively. DTX concentrations were determined by LC-MS/MS assay. *Each point* represents the mean ± SD (n=4)

The tissue distribution of DTX at the allocated times after i.t. administration of DTX-loaded gel is shown in Fig. 6c. DTX concentrations in these tissues maintained at relative low levels ranging from 2 to 600 ng/g. After 1 day, the concentrations in these organs for DTX in a descending order were liver > kidney > spleen > lung > heart. However, after 3 days, the corresponding order was kidney > spleen > lung > heart > liver and stayed the same till the end of the experiment. Low liver concentrations demonstrated that DTX-C/GP could alleviate the liver damage from DTX.

In general, increasing the concentration of antitumor drugs in the tumors and prolonging the retention time are two main approaches to improve the efficacy in chemotherapy (37). In our study, DTX-C/GP produced higher AUC_{tumor}, longer MRT, and lower DI in normal tissues which agreed to the better antitumor effect of DTX-C/GP with no observed toxicity.

CONCLUSIONS

In summary, we developed a polysorbate 80-free C/GP thermosensitive gel formulation for DTX and provided proof of its preclinical efficacy in treating solid tumors. *In vitro* release studies and the pharmacokinetic test demonstrated sustained delivery for 3 weeks. DTX-C/GP significantly prolonged the DTX retention time in tumor and achieved a high value of AUC_{tumor}/AUC_{plasma} while the amount of DTX distributed to the normal tissues was reduced. Consequently, i.t. administration of DTX-C/GP is a potential effective targeting treatment strategy for solid tumor. Admittedly, further study is still needed to investigate the antitumor effect of DTX-C/GP against other solid tumors such as breast and lung cancer.

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