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RESEARCH PAPER

Low-Molecular-Weight Methylcellulose-Based Thermo-reversible Gel/Pluronic Micelle Combination System for Local and Sustained Docetaxel Delivery

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ABSTRACT

Purpose To develop low-molecular-weight methylcellulose (LMw MC)-based gel/Pluronic F127 micelle combination system for local and sustained delivery of docetaxel (DTX).

Methods LMw MC and Pluronic F127 were used to formulate an injectable thermo-reversible gel/micelle combination system containing DTX. The DTX-loaded combination system was characterized and its therapeutic efficacy evaluated in a subcutaneous tumor model.

Results Mixtures of LMw MC, AS, and Pluronic F127 formed gel at ~15–40°C depending on AS concentration. The combination system released DTX for >30 days with a biphasic and sustained release pattern, and DTX stability was maintained during release. The combination system significantly enhanced anti-cancer effects of DTX and prolonged survival of the model mouse in comparison with free DTX.

Conclusions The LMw MC gel/Pluronic F127 micelle combination system constitutes a promising tool for reducing tumor size and eradicating remaining tumor cells before and after surgery.

KEY WORDS cancer therapy · docetaxel · drug delivery · gel/micelle combination system · methylcellulose

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INTRODUCTION

FDA-approved taxanes such as docetaxel (DTX) and paclitaxel (PTX) are the most widely used anti-mitotic agents for the treatment of breast, ovarian, and non-small cell lung cancer. However, Tween 80 and Cremophor EL, surfactants used to prepare the injection formulation for poorly water-soluble DTX and PTX, have been reported to cause hypersensitivity and neurotoxicity (2,3). Efforts have continued to develop surfactant-free injection vehicles to reduce the side effects of surfactants and dose-limiting toxicity of DTX and PTX in normal cells (4). A major problem of PTX is the hypersensitivity which results from the activation of complement system, occurring within the first few minutes of the drug administration. In addition, many solid tumors including breast cancers show limited sensitivity to the drugs following systemic administrations with the maximum tolerated dose (5). Local injection of chemotherapeutics could be an alternative to the conventional systemic administration for the efficient regression of primary tumors because they can be used to reduce tumor size and eradicate remaining tumor cells before and after surgery. Advantages of the local injection are high local drug accumulation in the tumor region, increased efficacy and decreased side effects, and therby reduced dose (6).

A variety of drug carriers has been developed for local anti-cancer drug delivery. For PTX, micro- or nano-particulate systems showed promising anti-cancer effects; however, high internal pressure forced out drug-loaded particles after intratumoral injection (7). A polymeric micelle formulation containing PTX without Cremophor EL, Genexol-PM, has been successfully developed and is able to increase the administration dose by 3 fold compared

to commercial injection formulation with less toxicity. Gel depot systems, on the other hand, have notably advanced the local delivery of PTX and DTX (2,8–10). Regel/PTX system (Oncogel®), an injectable gel depot system in a Phase II clinical trial, allowed local administration with sustained release of the agent over 6 weeks following a single injection (1,11). In this regard, the gel-based system has been considered as an appropriate formulation for enhancing local drug concentration at tumor site and reducing toxic effects in normal cells. The injectable gel systems based on synthetic polymers, proteins and naturally-occurring polysaccharides have been widely used to formulate thermo-reversible gels (12). Among them, methylcellulose (MC) undergoes sol-to-gel transition at temperatures higher than the body temperature; therefore, the gelation temperature of MC has to be lowered below the body temperature for *in vivo* applications. We have reported that the gelling temperature of MC could be lowered by simply adding salting-out salts, which could be used for *in vivo* application (13,14).

Despite advances in hydrogel-based drug delivery, the hydrophilic nature of the hydrogel limits its drug-entrapment capabilities to highly water-soluble drugs because lipophilic drugs tend to precipitate or aggregate in the hydrophilic polymer networks (15). Limitations of hydrogel-based drug delivery may be overcome through a combination of the polymeric micelles and the injectable hydrogel (16). An amphiphilic polymer, Pluronic F127, has been widely used to formulate micelles (17). The hydrophobic core of Pluronic micelles entraps poorly soluble drugs in aqueous solution, while the hydrophilic outer shell maintains the uniform dispersion and stability of the micelles in aqueous environments (15). The micelles are able to encapsulate and solubilize the lipophilic agent, and the gel provides a carrier for local and sustained delivery of the drugs to tumor sites. A gel/micelle combination system may therefore overcome the drawbacks of both hydrogel and polymeric micelle as a local drug carrier.

The objective of this study was to develop a MC gel/Pluronic F127 micelle combination system for local and sustained delivery of DTX. Low molecular weight (LMw) MC and Pluronic F127 were used to formulate an injectable thermo-reversible gel and encapsulate DTX, respectively. We determined the thermo-reversible rheological properties of the combination system, and tested the *in vitro* release and stability of DTX using an HPLC. In addition, an *in vivo* imaging technique was applied to demonstrate clearance of LMw MC from the injection site. Finally, the therapeutic efficacy of the gel/micelle combination system was tested in a subcutaneous tumor (B16-F10-Luc melanoma) model following a single intratumoral injection.

MATERIALS AND METHODS

Materials

LMw MC (Mw = 14,000 Da), ammonium sulfate (AS), N, N′-disuccinimidyl carbonate (DSC), 0.1% trifluroacetic acid (TFA)/acetonitrile (ACN) and 0.1% TFA/water were purchased from Sigma-Aldrich (St. Louis, MO); DTX and ethylenediamine, from Fluka (Buchs, Switzerland); Pluronic F127, from BASF Corp. (Parsippanny, NJ); the B16-F10-Luc stable cell line, from Caliper Life Sciences, Inc. (Hopkinton, MA); and male C57BL/6 and BALB/c mice, from Orient Bio Inc. (Seongnam, Gyeonggi-do, Korea). All other chemicals and solvents were of analytical grade and used without further purification.

Preparation of MC, AS and Pluronic F127 Stock Solutions

The stock solutions of MC, Pluronic F127 and AS were prepared in PBS at final concentrations of 15%, 15% and 9%, respectively. MC and Pluronic F127 were dissolved in PBS at 4°C under gentle stirring to obtain clear solutions. These stock solutions were kept at 4°C until use.

Preparation of Pluronic F127 Micelle Encapsulating DTX

DTX was dissolved in ethanol at 40 mg/ml and 10 μl of this solution was added into 60 μl PBS containing 10.5 mg Pluronic F127. The mixture of DTX and Pluronic F127 was gently mixed at 4°C until the powders were completely dissolved. The remaining small volume of ethanol was removed under reduced pressure.

Preparation of MC Gel/Pluronic F127 Combination

Stock solutions of MC or Pluronic F127 were mixed with various concentrations of AS for gel formation and the gelation temperatures were determined by a tube inverting method. To form the gel, the final concentrations of MC and Pluronic F127 were fixed at 5% (w/w) and the AS concentration was increased from 0% to 5% with 1% increments. The mixtures were incubated for 5 min under all test conditions.

Transmission Electron Microscope (TEM) Image of DTX-Loaded Micelle

Samples were prepared with aqueous solution of DTX-loaded pluronic F127 micelle. One drop of the solution was dried on a carbon grid and stained with osmium tetroxide. The morphological characteristics of DTX-loaded pluronic F127 were visualized using a TEM (LEO-912AB, LEO

electron microscopy, USA; Korea Basic Science Institute, Chuncheon, Korea).

Preparation and Characterization of DTX-Loaded Combination System

To load DTX into the MC gel, DTX-encapsulated Pluronic F127 was mixed with MC and AS stock solutions at an equal volume ratio. The final concentrations of MC, AS and Pluronic F127 were 5%, 3% and 5% as prepared by mixing the respective stock solutions. The rheological properties of the MC/AS/Pluronic F127 gel were observed using an oscillatory rheometer (Bohlin, Malvern, UK) by a cone and plate geometry at both 25°C and 37°C.

In Vitro Release of DTX from the Combination System

In the present study, the transwell system was used for *in vitro* DTX release experiment. This system has been widely used for the *in vitro* drug release from gel formulations and studies on cultured cell system. For *in vitro* release of DTX, the DTX-loaded gel was prepared as described above. In brief, 210 μl of the mixture was loaded onto the upper plate of a transwell system and incubated for 5 min at 37°C to allow gel formation. The outer wells were then filled with 1 ml of pre-warmed PBS (pH 7.4, DTX solubility in PBS: 10–20 ug/ml). The release medium was replaced with fresh medium at pre-determined time points. HPLC (1525 dual pump, Waters, Milford, MA) was performed using a C18 column (symmetry® C18, 5 um, 4.6×150 mm column, Waters, Milford, MA) with a UV-detector (Waters, Milford, MA) to measure the concentration of DTX. The column was equilibrated with 5% ACN containing 0.1% TFA, and 95% water containing 0.1% TFA. The standard curve of DTX was made with a series dilution of DTX at concentrations from 100 ug/ml to 3.12 ug/ml. Samples (100 μl) were injected and the column was eluted with a linear gradient of ACN from 5% to 70% for 30 min at a flow rate of 1 ml/min. Eluent samples were analyzed at the wavelength of 290 nm and released amount of DTX was calculated through the Breeze program (Version 3.30, Waters, Milford, MA).

In Vivo Anticancer Activities of DTX-Loaded Combination System

A mouse tumor model was prepared by injecting B16-F10-luc melanoma cells into 6-week-old male C57BL/6 mice. Prior to injection, the cells were grown to 80% confluence at 37°C with 5% CO_2, then trypsinized and suspended in PBS at a concentration of 3.0×10^7 cells/ml. The mice were anaesthetized by an intraperitoneal injection of ketamine and

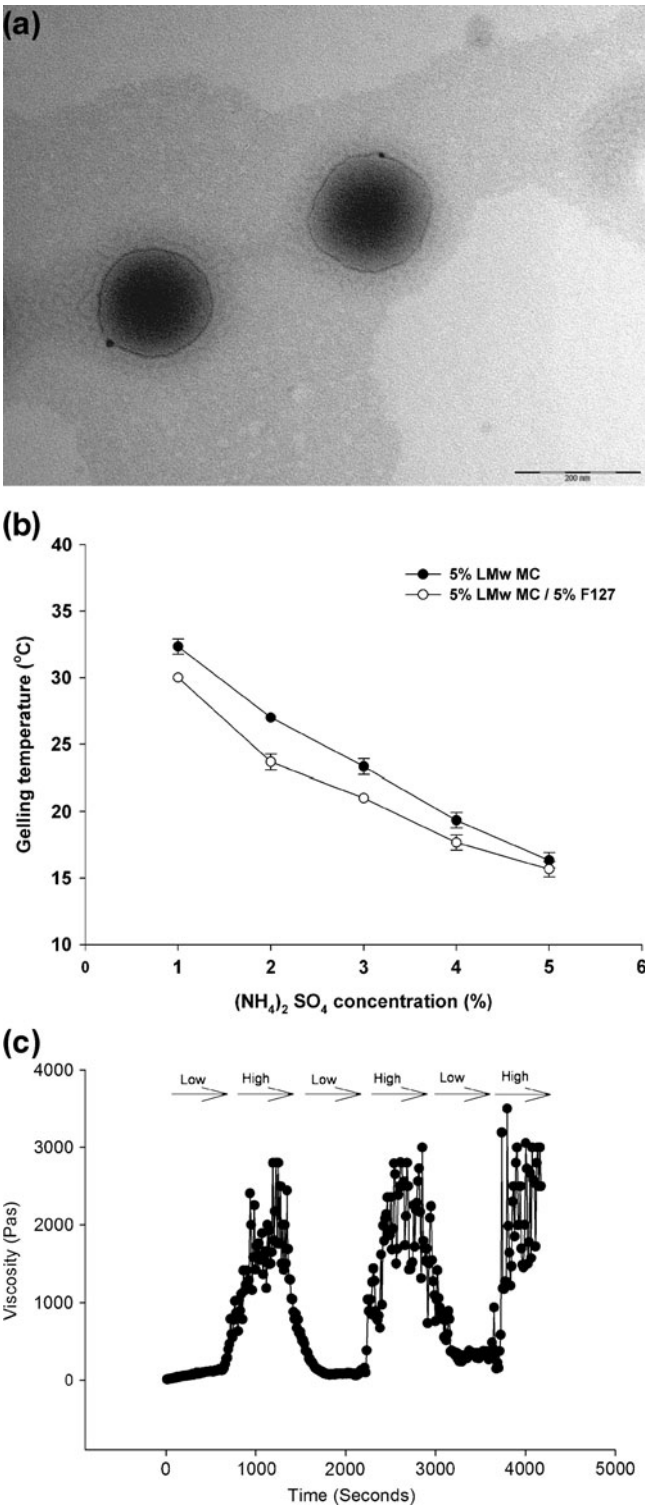


Fig. 1 Morphology of DTX loaded micelles and gelation property of hydrogel/micelle combination system. (**a**) TEM image of DTX loaded pluronic F127. (**b**) The gelation temperatures of 5% LMw MC in aqueous solution with and without Pluronic F127 micelle were measured by the tube-inverting method. The gelation temperature of 5% LMw MC decreased with increasing the AS concentration. Addition of 5% Pluronic F127 solution slightly decreased the gelation temperature. (**c**) Gel viscosity increased reversibly with increasing temperature.

xylazine (100 mg/kg), and the melanoma cells (1.5×10^6 B16-F10-luc) were subcutaneously injected into the left hind flank. When the tumor volume reached ~100 mm^3, the mice were randomly separated into seven groups. To show the anticancer effect of DTX-containing gel on the tumor, the combination systems containing 5 mg/kg, 10 mg/kg, and 30 mg/kg DTX were prepared and administered to the mice through 100 μl of intratumoral injection. Equal volumes of PBS, unloaded gel/micelle combination and free DTX (intravenous injection formulation, 0.4% Tween 80/ 0.6% ethanol/99% PBS), DTX-encapsulated pluronic F127 were injected by the same route into the mice. Tumor volume and body weight were measured for 14 days post-administration, and survival rates of the mice were observed for 21 days. The longest (a) and shortest (b) diameters of tumors were measured three times per week using vernier calipers, and tumor volume was calculated as $V=0.5ab^2$. The relative tumor volumes were expressed as percentages compared to the final tumor volume of PBS group at day 14. In addition, the tumor growth was observed by *in vivo* live imaging (IVIS 200 series, Caliper Life Sciences, Hopkinton, MA) of the B16-F10-luc cells.

Cyanine Dye 5.5 (Cy5.5) Labeling to MC

MC (2.4 g) were dissolved in 30 ml PBS and DSC (0.22 g) were dissolved in 10 ml methanol. These two solutions were gently mixed at room temperature for 48 h. After reaction, the solution was transferred into cellulose ester dialysis membrane (MWCO 3500 Da) and the dialysis bas was immersed in dialysis buffer and gently stirred for 2 days. The initial and final dialysis buffers were PBS containing 25% (v/v) methanol and pure PBS, respectively. After dialysis, MC solution was collected and ethylenediamine (103 mg) were added. This mixture was gently stirred for 24 h to react and dialyzed in PBS for 24 h. The Cy5.5 was added to this dialyzed LMw MC solution and mixed for 24 h. Finally, the Cy5.5-labled LMw MC solution was collected, dialyzed and freeze dried.

Biodistribution and Bioelimination of the MC Gel

Cy5.5-labled thermo-reversible hydrogels were subcutaneously injected into the right hind flank of BALB/c male mice and monitored at 0, 1, 2, 7 and 14 days post injection. The mice and their internal organs including liver, lung, heart, spleen and kidney were monitored by a quantitative fluorescent digital imaging (Kodak Image Station 4000MM; Eastman Kodak Company, Scientific Imaging Systems, New Haven, CT).

RESULTS & DISCUSSION

MC, a well-known derivative of cellulose, is widely used in pharmaceutical applications. While most natural polymers

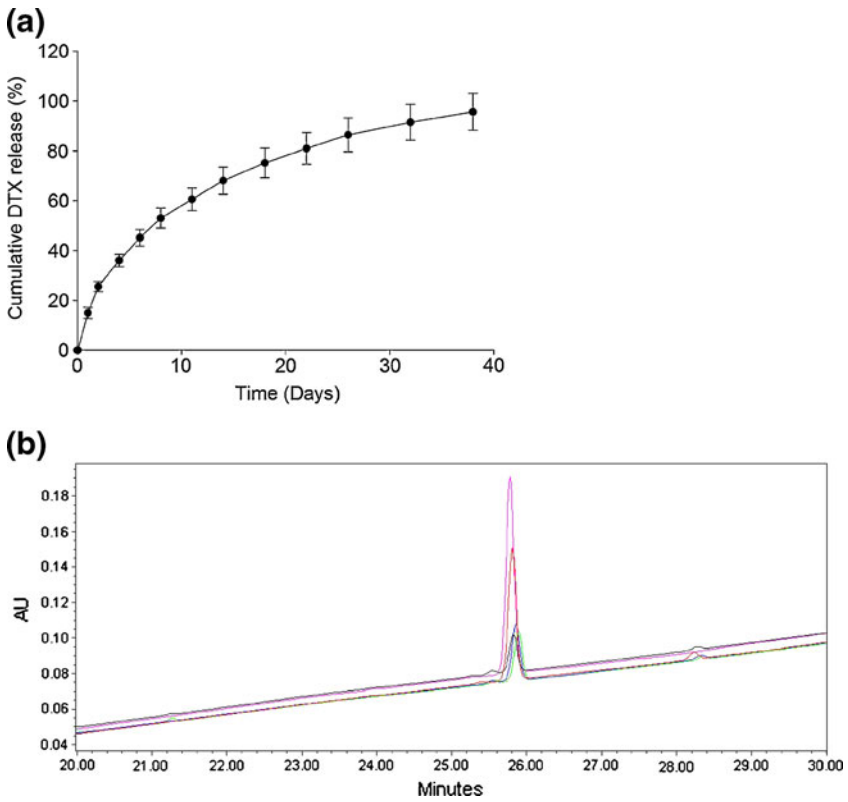


Fig. 2 *In vitro* release profile of DTX from MC gel/Pluronic F127 micelle combination system in PBS (pH = 7.4, 37°C). (**a**) The DTX was released continuously from the gel for over 30 days, with a minimal initial burst. (**b**) Stability of the released DTX assessed by HPLC (Black: 25 μg/ml DTX standard; blue: 1 day; red: 8 days; light green: 14 days; light blue: 22 days after release).

undergo thermal-gelation in cold environments, the cellulose derivatives show the reverse property, i.e., they form gels when they are warmed (18,19). Although MC shows sol-to-gel transition, its use *in vivo* as an injectable thermoreversible gel is negative because its gelation temperature (55°C) is higher than the body temperature. The injectable gel system should maintain the sol phase at room temperature and form the gel within a few minutes after injection into the body. We therefore investigated the gelation temperature of MC in the presence of various concentrations of AS as a salting-out salt, which lowers the sol-to-gel transition temperature. The AS may enhance hydrophobic interaction between polymer chains either by immobilizing nearby water molecules and thereby stabilizing hydrophobic hydration, or by increasing local order of water molecules around the polymer and thus driving hydrophobic interactions (13).

The Pluronic F127 micelle composed of a hydrophilic poly (ethylene oxide) (PEO) and a hydrophobic poly (propylene oxide) (PPO) improves the solubility and stability of lipophilic drugs such as camptothecin or DTX by encapsulating the drug into its hydrophobic core (10,20). The PEO and PPO moieties of Pluronic F127 are hydrophilic at low temperatures; however, the PPO

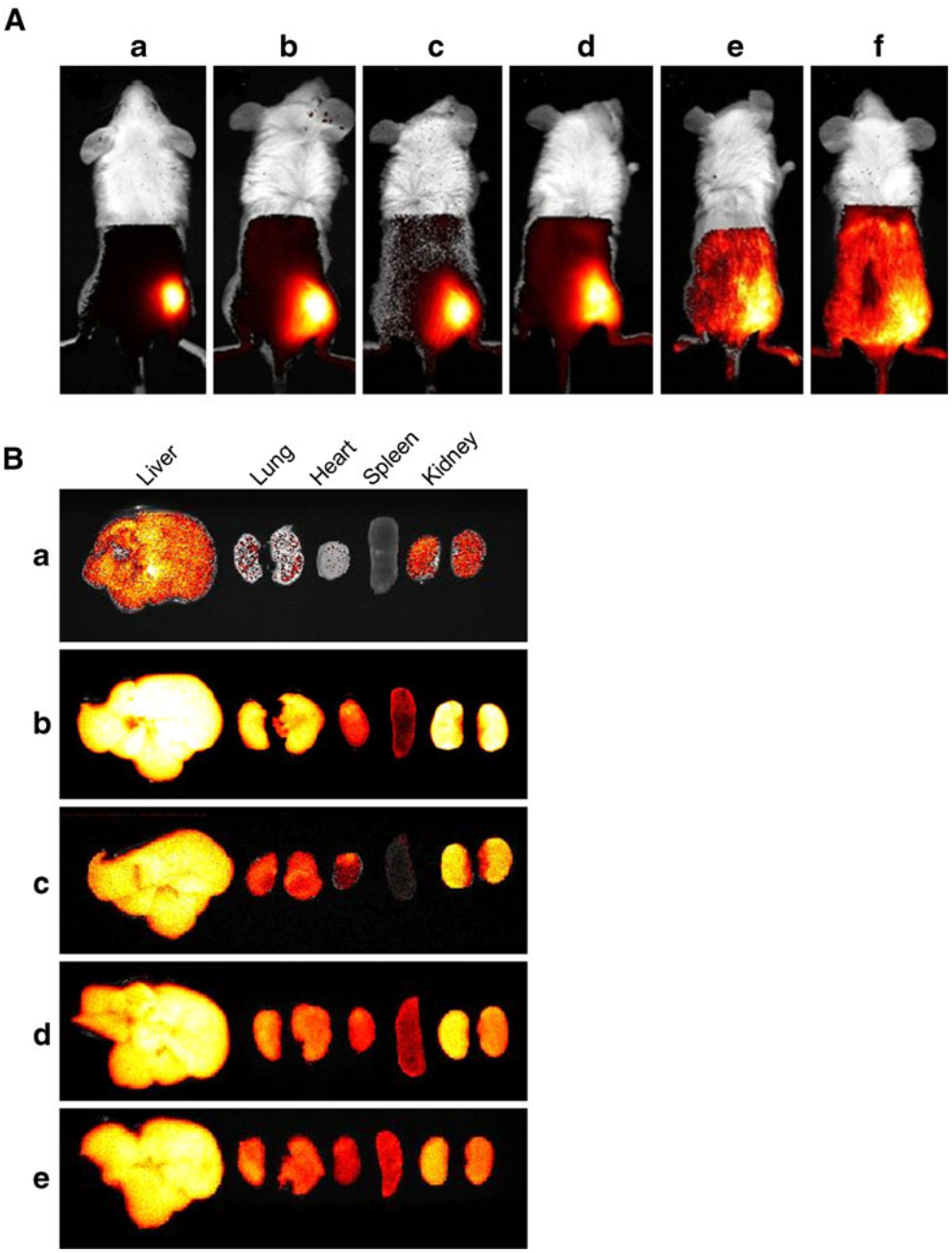


Fig. 3 Bio-distribution of hydrogel/micelle combination system. (**A**) Residence time and elimination of the MC gel visualized using non-invasive live animal imaging. (**B**) *Ex vivo* organ distribution of the MC gel. The livers, lungs, hearts, spleens and kidneys were isolated from mice after single subcutaneous injection of Cy5.5-labled MC gel, and Cy5.5 images were obtained at (**a**) 0 day, (**b**) 1 day, (**c**) 2 days, (**d**) 1 week, (**e**) 2 weeks and (**f**) 3 weeks after injection of the Cy5.5-labled gels.

becomes hydrophobic at higher temperatures, while the PEO remains hydrophilic (21). Hydrophobic interactions among the PPO groups in aqueous solution drive the micelle formation by which the Pluronic F127 micelle encapsulates lipophilic drugs (22). Pluronic F127 has been reported to solubilize and stabilize lipophilic drugs in the mixed micelle gel formed by Pluronic F127 and Tween 80 (22). The DTX-gel system formed in this way was shown to be thermo-sensitive (10). Tween 80, a nonionic surfactant polysorbate 80 composed of polyoxyethylene-20-sorbitan monooleate, is commonly used to prepare the intravenous infusion of DTX (23). However, this formulation frequently causes hypersensitivity, due in part to intrinsic toxicity of Tween 80. Various side-effects reported from the use of Tween 80 include an increase in membrane permeability, decrease in plasma osmotic pressure and plasma viscosity and changes in erythrocyte morphology (23). Another study demonstrated sustained local DTX release *in vitro* from an injectable blend of chitosan-phospholipid (2). However, despite localized drug delivery and positive anticancer effects in both studies, neither study showed a superior effect for the respective drug formulations over free DTX on tumor regression and stability of the released DTX. In the present study, the MC/AS/Pluronic F127 micelle combination system was developed to enhance the therapeutic efficacy and reduce the side effects of the local DTX delivery.

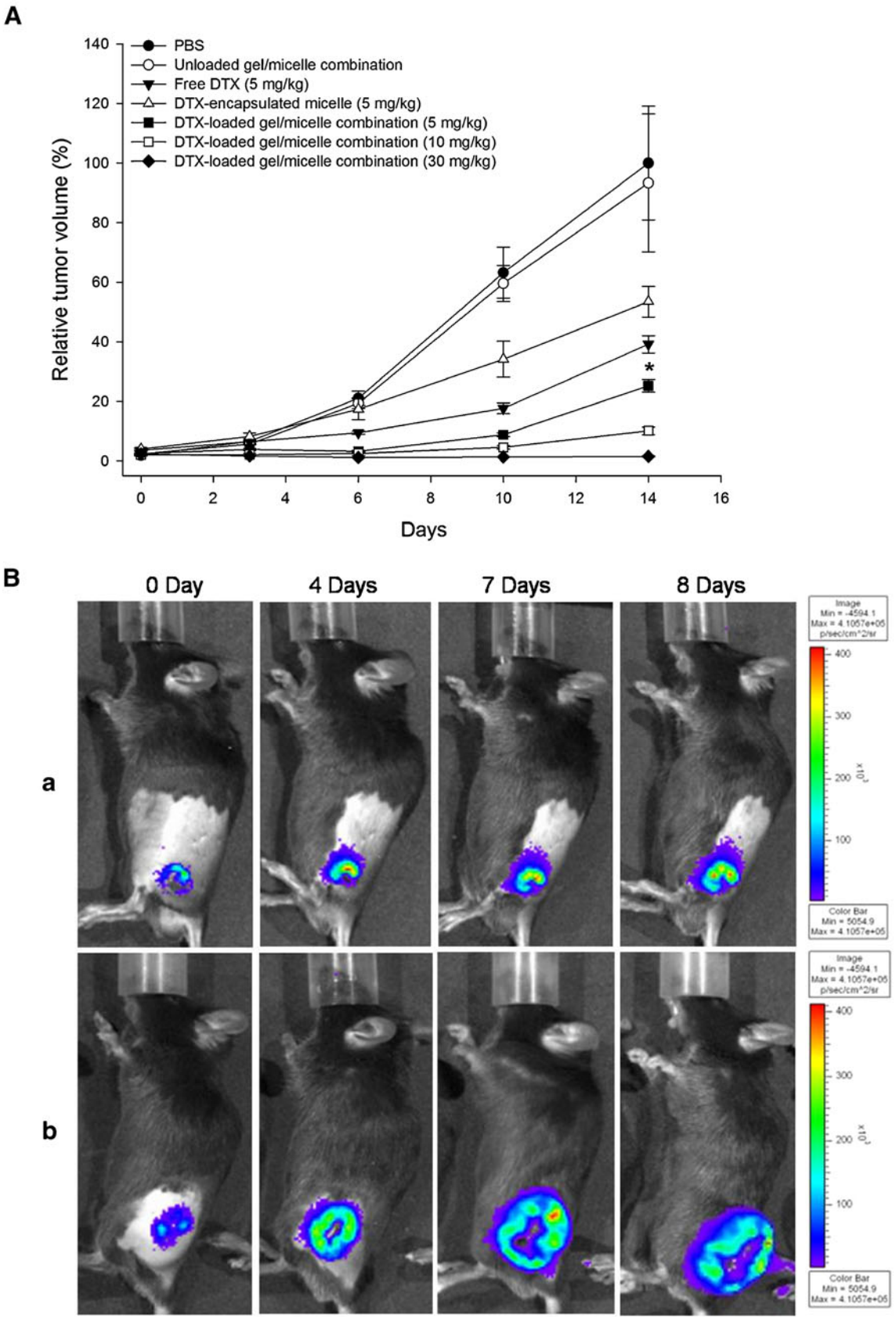
Characterization of Gel/Micelle Combination System

To formulate the DTX-loaded micelle/gel combination system, DTX was first encapsulated into the Pluronic F127 micelles and the gel was then formed by mixing the micelles containing DTX with the MC/AS. The spherical morphology of DTX-loaded micelle was confirmed by TEM (Fig. 1a). The DTX was solubilized by pluronic F127 polymer through the formation of micelle and size of micelle was approximately 180 nm in diameter. To optimize properties of the combination system, we determined gelation properties of two formulations in the predetermined temperature range using the tube inverting method. Mixtures of MC/AS and MC/AS/Pluronic F127 formed gels at temperatures between ~15°C and ~40°C depending on the AS concentration (Fig. 1b). MC gelled at ~40°C in the absence of AS and the gelation temperature gradually decreased as the AS concentration increased up to 5%. Addition of 5% Pluronic F127 to the MC solution lowered the gelation temperature slightly as compared with the formulation that was prepared without Pluronic F127. Although both MC and MC/Pluronic F127 form gels in the absence of AS, their higher gelation temperature than the body temperature, relatively long gelation times and low viscosities hinder their use for *in vivo* drug delivery. Two important considerations of an injectable thermo-reversible gel for the clinical application are a suitable viscosity to handle at room temperature and the speed of gel formation in the body. An injectable gel with low viscosity may release the drug too rapidly, whereas a highly viscous gel is difficult to inject. Gel stability and viscosities of MC/AS/Pluronic F127 mixtures in all test conditions were determined to find the optimal AS concentration. It was found that 3% AS provided an optimal gelation time at 37°C and appropriate viscosity at room temperature. Thermo-reversibility of MC/AS/Pluronic F127 combination system was confirmed by measuring viscosities at 25°C and 37°C. Viscosity patterns fluctuated markedly with initial low values at 25°C and increased significantly within minutes when the temperature was raised to 37°C (Fig. 1c). Viscosities returned to basal levels when the temperature was restored to 25°C. Thermo-reversible polymers usually showed similar pattern converting from a liquid sol to a solid gel and vice versa without losing its physicochemical properties (24). The solution state below lower critical solution temperature (LCST) and solid phase above the LCST in aqueous solution allow ease of injection and rapid gelation, while maintaining the micelle domain containing hydrophobic drugs.

Stability and *In Vitro* Release Profile of DTX from Gel/Micelle Combination System

DTX stability after release has to be guaranteed because of a positive correlation between the stability and the functionality. A validated HPLC method was developed to determine the stability of DTX by separating DTX from its degradation products (25,26). The release of DTX from the MC/AS/Pluronic F127 (denoted as gel/micelle combination system) was studied in the transwell system and the stability of released DTX was measured by the HPLC method. Figure 2a shows the biphasic and sustained cumulative release of DTX from the combination system for over 35 days, with less than 20% release in the first 24 h, approximately 50% at day 7, and a very slow release up to 95% for 38 days. The DTX stability during the release period is shown in Fig. 2b. In the stability-indicating HPLC, pure DTX typically eluted between 25.5 and 26.0 min depending on the DTX concentration in the HPLC condition. All samples of the released DTX were eluted within this time range, without other peaks that indicate DTX degradation. These results demonstrate that the DTX-loaded gel/micelle combination system can

Fig. 4 *In vivo* anticancer activity of DTX-loaded combination system. (**A**) B16-F10-luc melanoma tumor-bearing mice were treated with PBS, unloaded combination system, free DTX, DTX-encapsulated micelle and DTX-loaded combination system, and tumor volumes were measured for 2 weeks after single intratumoral injection (*$p < 0.01$ *vs.* free DTX). (**B**) Luciferase activity in B16-F10-luc melanoma tumor-bearing mice. (**a**) DTX-loaded (30 mg/kg) and (**b**) unloaded combination system.


A
PBS
Unloaded gel/micelle combination
Free DTX (5 mg/kg)
DTX-encapsulated micelle (5 mg/kg)
DTX-loaded gel/micelle combination (5 mg/kg)
DTX-loaded gel/micelle combination (10 mg/kg)
DTX-loaded gel/micelle combination (30 mg/kg)
Relative tumor volume (%)
Days
*
B
0 Day
4 Days
7 Days
8 Days
a
b
Image
Min = -4594.1
Max = 4.1057e+05
p/sec/cm^2/sr
Color Bar
Min = 5054.9
Max = 4.1057e+05

sustain the DTX release in the tumor for a long time following a single injection, without loss of therapeutic effectiveness. Kinetic of *in vitro* drug release from micelle systems is influenced by an interplay of factors, including the drug loading, micelle core construction, state of the drug in the micelle and compatibility between the drug and the core-forming block, in the kinetic behavior of the system (27). It was implied that the drug release profile was due to the continual release of the drug not only by passive diffusion, but also through polymer degradation (28–30). Human MC degradation mechanisms remain controversial because only a few human organs, such as intestine produce enzymes, which degrade MC (13). For this reason, study of DTX release *in vitro* was conducted in the absence of the enzymes for mimicking the *in vivo* environment. Therefore, the kinetic of *in vitro* DTX release in the present study was considered primarily as the outcome of passive drug diffusion from the gel/micelle combination system.

Bio-distribution of Gel/Micelle Combination System

All drug delivery systems raise concern for biodegradation and elimination of constituents. Although MC is a well known natural polymer, its clearance from the body must be confirmed before an MC-based carrier is clinically applied. Exoglucanases and endoglucanases are two main types of enzymes that degrade cellulose-derivates through hydrolysis (31,32). Endoglucanases disrupt the internal bonds of the cellulose crystal structure and exoglucanases cleave 2–4 polysaccharide units from the end of cellulose chains. Cellulose-derivatives may be degraded or not, depending on the route or site of administration, because most animals, including humans, produce these particular enzymes in the intestine (13). Peroxidases may, however, act at the C2-C3 position of cellulose to produce aldehyde cellulose, which is a degradable cellulose-derivative; and after further partial acid hydrolysis, the remaining non-biodegradable cellulose may be eliminated through the kidney. In addition, the elimination mechanisms of macromolecules may include glomerular filtration and phagocytic clearance, all of which are affected by the molecular weight, flexibility and three-dimensional structures of the macromolecules (13).

In order to confirm whether the MC gel is eliminated from the injection site, non-invasive live animal imaging technique was used to visualize the *in vivo* residence time and potential pathways for the elimination of the MC gel (33). Cy5.5-labelled MC gel was subcutaneously injected into the right hind flank of the BALB/c mouse. The C57BL/6 mice as a melanoma tumor model were not appropriate for *in vivo* live imaging because their characteristic black coat color blocks the NIR fluorescence of Cy5.5-labelled gel. Monitoring the real-time NIR fluorescence intensity in the backside of the mouse confirmed the elimination of MC gel from the injection site (Fig. 3a). A strong NIR fluorescence was detected at the injection site after 1 h and slight spreading of the fluorescence intensity was observed in the subsequent 24 h. The intensity extended continuously across the entire backside of the mouse, diminishing gradually at the injection site for 3 weeks. *Ex vivo* images of major organs, including liver, lung, spleen, kidney and heart, showed the strong intensities in the kidney and liver at all time points of observation (Fig. 3b). These results suggest that the renal route and glomerular filtration can gradually eliminate the MC gel (34).

Table I Survival Rate of the Mouse Tumor Model 3 Weeks after Administration and the 50% Survival in the Test Groups

Group	50% survival	Survival rate (3 weeks)
PBS	13	0
Unloaded gel/micelle combination	13	0
Free DTX (5 mg/kg)	11	40
DTX-encapsulated micelle (5 mg/kg)	18	40
DTX-loaded gel/micelle combination (5 mg/kg)	19	40
DTX-loaded gel/micelle combination (10 mg/kg)	–	80
DTX-loaded gel/micelle combination (30 mg/kg)	–	100

Therapeutic Effect of DTX-Loaded Gel/Micelle Combination System

The anticancer effect of the DTX-loaded gel/micelle combination system was assessed in a B16-F10-luc mouse tumor model in a dose-dependent manner following a single intratumoral injection. Figure 4a shows an increase in relative tumor volume in the test groups treated with PBS, unloaded gel/micelle combination, free DTX and

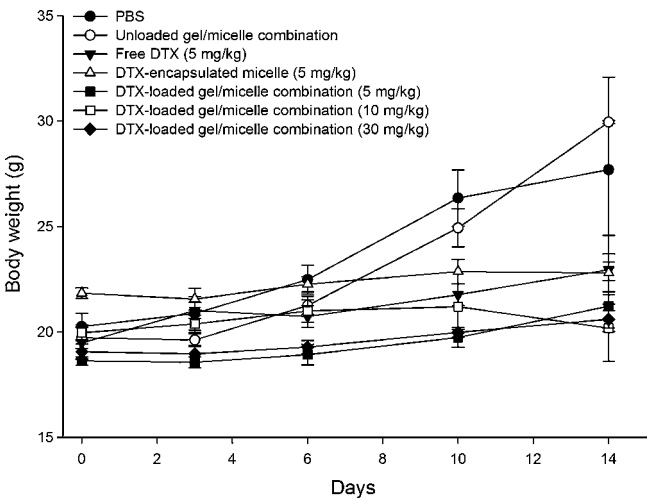


Fig. 5 Body weight changes in mouse tumor mocel injected with PBS, gel, free DTX, and DTX-loaded gels.

DTX-loaded gel/micelle combinations. DTX-loaded combination systems with different doses exhibited significant remission in tumor growth compared to PBS, unloaded gel/micelle combination, and DTX-encapsulated micelle groups. Comparing the anticancer effect of DTX-loaded gel/micelle combination system with that of free DTX showed the advantages of the gel/micelle system. The relative tumor volumes of the free DTX and the combination system (5 mg/kg) were approximately 40% and 20%, respectively, at 14 days post-administration. The improved anticancer effects shown in the combination system in comparison with the free DTX group is mainly due to the sustained release of DTX in the tumor. Although the initial available dose of DTX in the free DTX group is higher than that in the combination system when DTX is injected locally, the sustained release of DTX leads to continuous anticancer effects in cancer cells, resulting in the enhanced therapeutic efficacy. The groups treated with 10 mg/kg and 30 mg/kg DTX-loaded combination system showed negligible tumor growth. Live imaging after treatment, based on tumor luciferase expression, is shown in Fig. 4b. In mice treated with 30 mg/kg DTX-loaded gel, the regions of luciferase-expressing cells were similar in size at 0 day and 8 days after the single injection, as expected from the tumor volume results, while regions of tumor involvement in the unloaded combination system treatment group increased significantly in a time-dependent manner. High dose of DTX administration may cause severe side effects, even though DTX is administered by local intratumoral injection. The survival rate and 50% survival of the mouse tumor model after treatment were shown in Table I. Fifty percent of the mice died at 11 days after the treatment of free DTX, whereas the 50% survival in the group treated with combination system (5 mg/kg) was 20 days. The 50% survival in the group of free DTX was almost same as that in the unloaded combination system and PBS, even though the significant difference in the tumor volume between those groups was observed (Fig. 4a). These data reveal that the high lethality in mice treated with free DTX is, at least in part, due to the intrinsic toxicity of DTX. Despite the usefulness of pluronic micelle to overcome the severe side effects of DTX, it cannot be used as local injection formulation for DTX because of its low anticancer effect. On the other hand, the gel/micelle combination system exhibited the highest anticancer effects and the longest 50% survival among the 5 mg/kg DTX treatment groups. Based on these data, it is conclusive that the use of gel/micelle system advanced the anticancer effects and overcome the severe side effects of DTX associated with the dose-limiting toxicity and high probability of drug resistance. Recently, frequent low-dose DTX administration ('metronomic' chemotherapy) was recommended to provide an effective alternative to the conventional maximum tolerated dose regimens (35). Increasing evidence supports the metronomic chemotherapy as a strategy to improve the effectiveness and reduce dose-limiting side-effects of anticancer drugs (2). Therefore, the use of gel/micelle combination system constitutes a promising tool to improve the anticancer effects and increase survival rate. Body weights in the PBS- and gel-treated groups both increased rapidly with tumor growth; while other groups showed negligible changes in the body weight (Fig. 5).

CONCLUSION

LMw MC gel/Pluronic F127 micelle combination system for the local delivery of DTX was proven to be very effective and practical. The combination system achieved complete tumor remission at high dose of DTX administration without side effects and reduced the lethality that results from the DTX's toxicity in comparison with the free DTX formulation. The gel/micelle combination system provided advantages over the free DTX formulation in terms of reduced side effects and improved anticancer effects, both of which were resulted from the sustained release of DTX in the tumor region. In addition, the MC gel was observed to be gradually eliminated from the injection site through the glomerular filtration. Despite future challenges regarding pharmacokinetics of DTX and immune profiling of the combination system, the present data will promote future applications of LMw MC gel/Pluronic F127 micelle combination system as an efficient local injection vehicle for various types of hydrophobic drugs.

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