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Enhanced stability of nano-emulsified paclitaxel

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ABSTRACT

The main goal of this work was to develop an optimal self-microemulsifying paclitaxel prepared with PLGA and solubilizer such as tetraglycol, Cremophor ELP, and Labrasol. The prepared PTx-loaded SMES showed the size of the range of 80-130 nm by dynamic light scattering and a spherical shape by atomic force microscopy. In experiment of storage stability in deionized water (DW) or blood condition, PTx-loaded SMES showed good stability in DW and comparable stability in blood condition at 37˚C for 7 days. In addition, PTx-loaded SMES showed a significant inhibitory effect on B16F10 melanoma proliferation. In conclusion, we confirmed that the formulations tried in this study could be used as administration form for animal trials of PTx.

Keywords: Self-Microemulsifying; Paclitaxel; Stability; Anti-Tumor Activity

1. INTRODUCTION

Paclitaxel (PTx), a major anticancer drug isolated from the bark of Taxus brevifolia, has significant activity in clinical trials against a variety of tumors such as breast cancer, advanced ovarian carcinoma, lung cancer, head and neck carcinoma [1,2]. PTx is a hydrophobic drug with poor aqueous solubility. To enhance its solubility and allow parenteral administration, PTx is currently formulated with solution of Cremophor® EL and ethanol as dosage-form of PTx (Taxol®) for clinical application [3,4].

Recently, numerous investigations have been focused on the development of various PTx delivery systems such as liposomes, emulsions, micelles, microspheres, and polymeric nanoparticles [5-9]. Among these, self-microemulsifying systems (SMES) may be a promising way to load PTx in delivery system because it provides high concentration of PTx in the aqueous media system [10,11]. SMES are isotropic mixtures of oil, a surfactant, and possibly one or more hydrophilic solvents or cosurfactants, which form fine oil-in-water emulsions when exposed to aqueous media under condition of gentle agitation [12].

At present, many studies have highlighted the development of PTx-loaded SMES with optimal condition for blood circulation in effective concentration [13]. However, the major obstacle that limits the use of SMES is due to the physical and/or to the chemical instability by absorption of biological compounds such as protein during drug circulation time in blood after intravenous administration [14].

To improve stability of SMES, various formulations of SMES is needed that can extend PTx circulation time in blood. Our understanding is that the formulations of SMES could examine under the blood condition. Thus, the aim of this study was to examine various formulations of PTx-loaded SMES to increase systemic clearance through extending of the circulation time of PTx.

2. MATERIALS AND METHODS

2.1. Materials

Poly(d,l-lactide-co-glycolide) (PLGA, molecular weight, 8000, 20,000 and 90,000 g/mole) were purchased from Boerhinger Ingelheim (Ingelheim, Germany). Paclitaxel was purchased from Samyang Genex Co. (Seoul, Korea). Caprylocaproyl macrogol-8 glyceride (Labrasol®) was obtained from Gattefosse (Westwood, NJ, USA). Cremophor ELP was purchased from BASF (Germany). Tetraglycol was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade. Deionized water (DW) was prepared by a Milli-Q purification system from Millipore (Molsheim, France).

2.2. Preparation of PTx-Loaded SMES

A series of SMES was prepared in each of the various formulas with various ratios of PLGA, paclitaxel, solu-

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bilizer, surfactant, and cosurfactant. Briefly, PLGA and PTx were dissolved, respectively, by solubilizer such as tetraglycol in glass vials. Surfactant and cosurfactant were accurately weighed into glass vials. Then the components were mixed by gentle stirring and vortex mixing until PTx had perfectly dissolved. The mixture was stored at room temperature until used. Before using, PTx-loaded SMES was formed by contacting to aqueous phase of the prepared mixture.

2.3. Size Analysis of PTx-Loaded SMES

For particle size analysis, formulation (50 µl) of PTx-loaded SMES was diluted with DW to 50 ml in a volumetric flask and gently mixed by inverting the flask. The droplet sizes of resultant emulsions were determined by dynamic light scattering (DLS, ELS-8000, Photal, Japan) at room temperature. The droplet size was individually measured for three PTx-loaded SMES samples and then calculated as average value. For atomic force microscopy (AFM), one drop of PTx-loaded SMES was transferred onto silicone wafer which washed with MeOH. The wafer was quickly placed in liquid nitrogen, followed by the freeze-drying for 2 days. AFM measurements were carried out in the tapping mode with a Nanoscope IV instrument (Digital Instruments Inc.).

2.4. Stability of PTx-Loaded SMES

PTx-loaded SMES was prepared with DW or a solution of 0.9% NaCl and 5% bovine serum albumin (Sigma, Germany) and individually placed in 10 ml tube. The tube was constantly shaken at 100 rpm and 37˚C for 7 days. At the set time, the droplet size was individually measured for three PTx-loaded SMES samples and then calculated as average value.

2.5. Cell Culture and Cytotoxicity Tests

B16F10 melanoma cell line was obtained by KCLB (Korea Cell Line Bank) and cultured in MEM (Minimum Essential Medium, Gibco BRL, USA) supplemented with 10% fetal bovine serum (Gibco BRL, USA) The cells were seeded into 75 cm^2 flasks, cultured and changed medium every 2 days. For cytotoxicity tests, B16F10 cell suspension (2×10^4 cells/well) was seed in a 48-well plate. The cells were incubated overnight to allow for cell attachment. Ptx (1 µg) of SMES solution was added to each well for 7 days. Cell viability was determined by using water-soluble enzyme substrate MTT which was converted to purple water-insoluble product formazan accumulated in the cytoplasm of viable cells. Cell viability of each well performed individually and then calculated as average value. In brief, 100 µl of PBS solution of the MTT tetrazolium substrate (5 mg/ml) was added after 1, 4 and 7 days. After incubation for 4 h at 37˚C, the resulting purple formazan precipitate was solubilized by the addition of 1 ml of DMSO and shaken for 30 min. An aliquot from each well (100 µl) was transferred to 96-well plates and then read using a plate reader of an ELISA (E-max, Molecular Device, USA). The optical density of each well determined at 590 nm.

3. RESULTS AND DISCUSSION

3.1. Preparation of PTx-Loaded SMES

PLGA of different molecular weights used to compare the stability of the self-microemulsifying PTx. To prepare self-microemulsifying PTx consisting of mixtures of Ptx, oil, a surfactant and PLGA, the formulation is summarized in **Table 1**. PLGA and PTx were dissolved by solubilizers which were a mixture of tetraglycol, Cremophor ELP, and Labrasol.

Firstly, the prepared PTx-loaded SMES were observed visually. As shown in **Figure 1(a)**, PTx-loaded SMES (F1-0 - F10-0) showed the emulsion solution from transparent to semitransparent according to various formulations. The droplet size for all formulations of PTx-loaded SMES was found in the range of 80 - 130 nm. The droplet size distribution is comparatively narrow for all formulations.

The morphology of PTx-loaded SMES was measured by AFM as shown in **Figure 2**. PTx-loaded SMES (F10) showed the spherical shape with smooth surface. A comparatively uniform droplet size of PTx-loaded SMES was also observed at AFM, indicating no aggregation or adhesion among SMES.

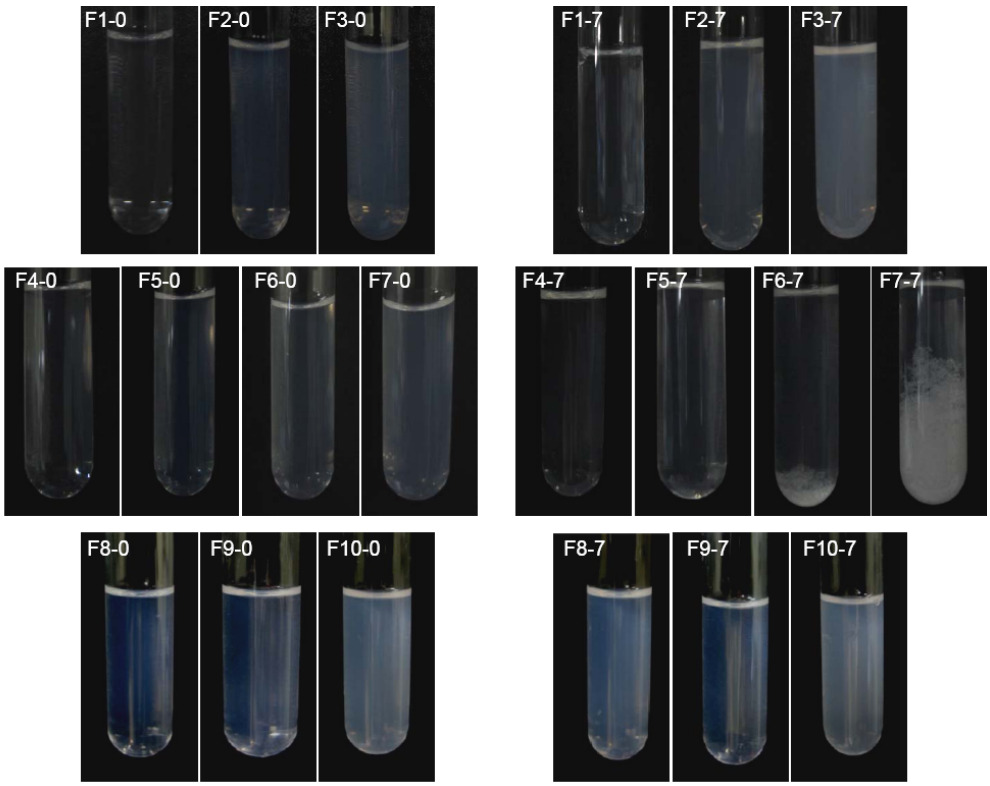
3.2. Stability of PTx-Loaded SMES

The storage stability of PTx-loaded SMES is important to maintain the therapeutic concentration of PTx. Therefore, PTx-loaded SMES was firstly prepared with DW or a solution of 0.9% NaCl and 5% bovine serum albumin. The prepared PTx-loaded SMES was constantly shaken at 100 rpm and 37˚C for 7 days to examine the storage stability. After 4 and 7 days, there is no change of particle size for F1-F5 as shown in **Figure 1(b)**, indicating the storage stability of PTx-loaded SMES in DW. Meanwhile, F6 and F7 with PTx content < 0.05 wt showed the precipitation of PTx. Hence we investigated the SMES with PTx content of 0.003 wt according to different molecular weights of PLGA (F8-F10). F8, F9 and F10 in DW showed no change of particle size at 37˚C for 4 and 7 days (**Table 2**).

Next, PTx-loaded SMES was prepared with a solution of 0.9% NaCl and 5% bovine serum albumin as a model blood condition. The haze color of PTx-loaded SMES is deeply changed according to increasing incubation time (**Figure 3**). The particle size of F8 and F10 increased to

Table 1. Formulation for the preparation of PTx-loaded SMES.

Composition (g, w/w)	Formulation									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Drug (Paclitaxel)		0.001		0.001	0.003	0.05	0.01		0.003	
Solubilizer (Tetraglycol)		0.5			0.5				0.5	
PLGA 8 kg/mol	0.005	0.01	0.015		0.005			0.005		
PLGA 20 kg/mol									0.005	
PLGA 90 kg/mol										0.005
Cosurfactant (Labrasol)		0.14			0.14				0.14	
Surfactant (Cremophor ELP)		0.16			0.16				0.16	



(a) (b)

Figure 1. Pictures (a) before and (b) after incubation at 37˚C for 7 days of PTx-loaded SMES prepared in DW with different formulations F1-F10.

Table 2. The changes of particle size by incubation for 0 - 7 days at 37˚C of PTx-loaded SMES prepared in DW and blood condition with different formulations F8-F10.

Condition		In DW			In blood condition [a]		
Formulation		F8	F9	F10	F8	F9	F10
Particle size (nm)[b]	Initial	80 ± 1	81 ± 1	128 ± 3	1483 ± 219	70 ± 1	162 ± 4
	4 days	84 ± 3	85 ± 3	129 ± 4	1920 ± 518	301 ± 152	1399 ± 791
	7 days	80 ± 2	104 ± 2	124 ± 1	1970 ± 709	773 ± 288	1005 ± 140

[a]A solution of 0.9% NaCl and 5% bovine serum albumin; [b]The mean and standard deviation of particle size for each formulation was calculated by individual measurement of three formulations.

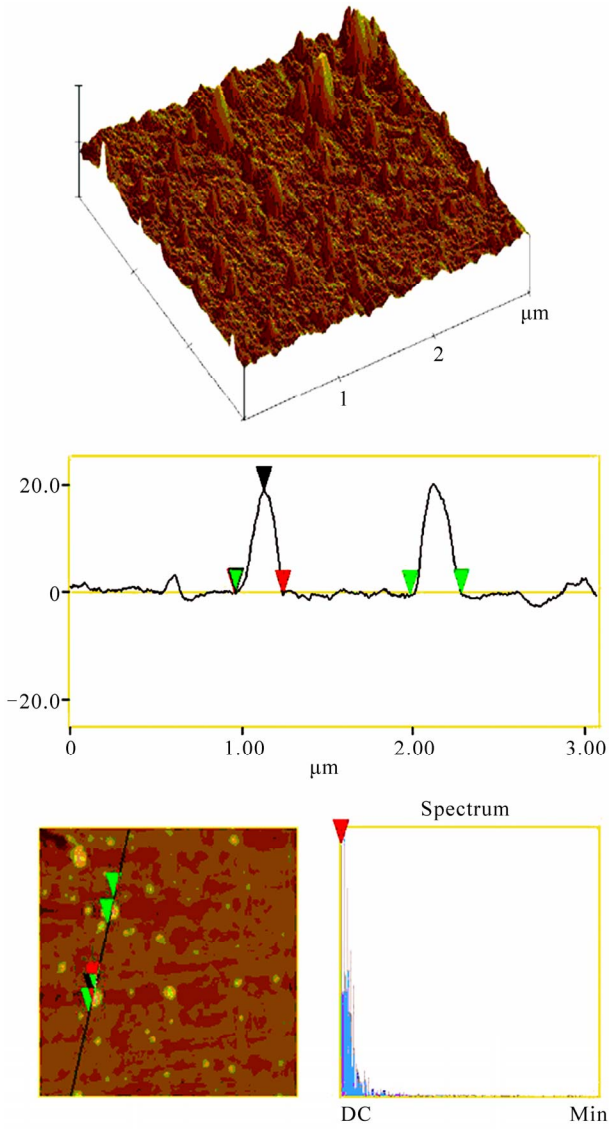


Figure 2. AFM image of PTx-loaded SMES (F10).

1000 - 2000 nm after incubation, while that of formulation F9 increased to 800 nm (**Table 2**). Even though these formulations showed the increasing in particle size, there is no precipitation under even blood condition. This study does not provide sufficient data to allow acomplete description of the stability to be proposed, however, it indicated that the stability of PTx-loaded SMES depended on the molecular weights of PLGA and concentration of PTx.

3.3. Anti-Tumor Activity of PTx-Loaded SMES

The formulations F8-F10 were examined for their antiproliferative activities against B16F10 melanoma cell line (**Figure 4**). The population of B16F10 cells shapely increased as a function of culture time after addition of saline (control). The PTx-loaded SMES (F8-F10) exerted a significant inhibitory effect on cell proliferation. The cell viability was approximately 40% and 20% at 4 days and 7 days, respectively. This indicated that PTx-loaded SMES (F8-F10) displayed marked inhibition of B16F10 cell proliferation.

3.4. Preparation of PTx-Loaded SMES

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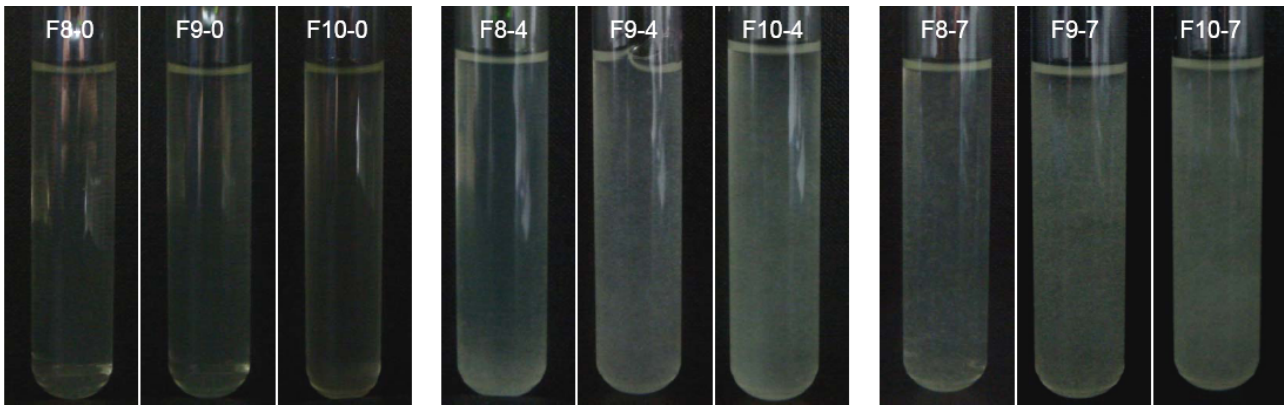

Figure 3. Pictures (a) before and after incubation for (b) 4 days and (c) 7 days at 37˚C of PTx-loaded SMES prepared in 5% BSA with different formulations F8-F10.

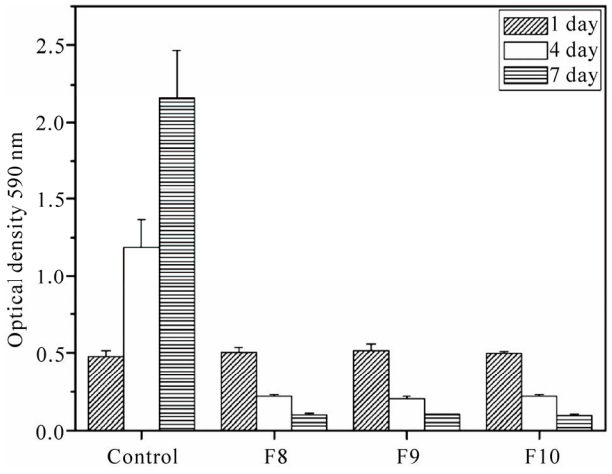

Figure 4. *In vitro* cytotoxicity of B16F10 melanoma cell against PTx-loaded SMES of formulations F8, F9 and F10 for 1, 4 and 7 days. The cells grown on a culture plate without PTx-loaded SMES were used as the control.

The droplet size distribution is comparatively narrow for all formulations.

The morphology of PTx-loaded SMES was measured by AFM as shown in **Figure 2**. PTx-loaded SMES (F10) showed the spherical shape with smooth surface. A comparatively uniform droplet size of PTx-loaded SMES was also observed at AFM, indicating no aggregation or adhesion among SMES.

4. CONCLUSIONS

We prepared the PTx-loaded SMES with different formulations to examine the storage stability. The prepared PTx-loaded SMES showed a spherical shape with ranging of 100 nm. We found the formulation of the PTx-loaded SMES with stability for 7 days. The formulation in this work could be used as administration form for animal trials. Thus, further research on the animal model using PTx-loaded SMES prepared in this work is now in progress.

5. ACKNOWLEDGEMENTS

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