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RESEARCH PAPER

Computed Tomography-Guided Screening of Surfactant Effect on Blood Circulation Time of Emulsions: Application to the Design of an Emulsion Formulation for Paclitaxel

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ABSTRACT

Purpose In an effort to apply the imaging techniques currently used in disease diagnosis for monitoring the pharmacokinetics and biodisposition of particulate drug carriers, we sought to use computed tomography (CT) scanning methodology to investigate the impact of surfactant on the blood residence time of emulsions.

Methods We prepared the iodinated oil Lipiodol emulsions with different compositions of surfactants and investigated the impact of surfactant on the blood residence time of emulsions by CT scanning.

Results The blood circulation time of emulsions was prolonged by including Tween 80 or DSPE-PEG (polyethylene glycol 2000) in emulsions. Tween 80 was less effective than DSPE-PEG in terms of prolongation effect, but the blood circulating time of emulsions was prolonged in a Tween 80 content-dependent manner. As a proof-of-concept demonstration of the usefulness of CT-guided screening in the process of formulating drugs that need to be loaded in emulsions, paclitaxel was loaded in emulsions prepared with 87 or 65% Tween 80–containing surfactant mixtures. A pharmacokinetics study showed that paclitaxel loaded in 87% Tween 80 emulsions circulated longer in the bloodstream compared to those in 65% Tween 80 emulsions, as predicted by CT imaging.

Conclusions CT-visible, Lipiodol emulsions enabled the simple evaluation of surfactant composition effects on the biodisposition of emulsions.

KEY WORDS lipiodol · emulsion · pharmacokinetics · computed tomography · paclitaxel

INTRODUCTION

Oil-in-water (o/w) emulsions, in which the inner oil core is dispersed in aqueous media that is stabilized by surfactants, are attractive parenteral dosage forms for lipophilic drugs because of their high solubilization capacity (1). They also provide additional advantages, including reduced irritation or toxicity of the incorporated drugs, relative ease of manufacture on an industrial scale, and the possibility of sustained drug release (2,3). Furthermore, production of emulsions in the nanoscale range (50 to 200 nm), which has been enabled by recent advances in nanotechnology (4), allows filter sterilization of the final product, adding to the value of o/w emulsions as a parenteral formulation.

Several studies have shown that the blood circulation time and tissue distribution of drugs loaded in emulsions are quite different from those of free drugs (5–7) and are also very dependent on droplet size, surface charge, and surfactant composition of the emulsion (8,9). These latter findings suggest that formulation variables play a critical role in determining the *in vivo* biodisposition of drug-loaded emulsions (10). In this regard, a few studies have directly addressed the impact of surfactant composition on the *in vivo* disposition of emulsions by investigating the *in vivo* disposition of emulsions following intravenous dosing of emulsions labeled with radioisotopes. For example, inclusion of amphiphilic poly(ethylene glycol) (PEG) derivatives in a surfactant mixture was found to prolong the blood circulation time of resultant emulsions, probably by

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sterically reducing emulsion interactions with mononuclear phagocytic system (MPS) cells (11,12). The potency of the prolongation effect appeared to be related to PEG derivative density and chain length (13–15). Considering that the *in vivo* biodisposition of emulsions is a critical factor in determining the performance of the loaded drug, understanding the relationship between surfactant composition and the biodisposition of emulsions would be important in the formulation process in cases where an emulsion formulation is considered a promising dosage form for a new drug. Therefore, developing a methodology for conveniently evaluating the *in vivo* disposition of the emulsion without the problems associated with the use of radioisotope would be advantageous.

Recently, there have been attempts to apply imaging techniques that are currently used in the early diagnosis of diseases for monitoring the pharmacokinetics, biodisposition, and drug-delivery efficiency of particulate drug carriers (16,17). The imaging modalities frequently used for this purpose include magnetic resonance imaging (MRI), positron emission tomography (PET), and computed tomography (CT) (18–20). These imaging technique-based biodisposition studies have been carried out by incorporating the contrast agents themselves or as conjugates linked to components that constitute the carrier (e.g., phospholipids). These techniques allow the real-time, noninvasive imaging and quantitation of particulate carriers without the need to collect blood or tissue samples for later analysis. Applying these modalities to monitor the disposition of emulsions can provide an improved solution for establishing the surfactant composition–biodisposition relationship.

CT is among the most convenient imaging/diagnostic tools in clinical use today in terms of availability, efficiency, cost, and speed (21). In addition, its resultant image data, which represent attenuation values of tissue, are highly quantifiable. As a method that measures X-ray absorption, CT requires the use of contrast agents containing high-atomic number elements to increase image contrast. Currently used CT contrast agents are predominantly based on small, hydrophilic compounds containing iodine. Therefore, loading hydrophilic iodine compounds in particulate carriers is required if CT technology is to be applied to monitor the *in vivo* disposition of carriers. The difficulty in loading these compounds in particulate carriers at a concentration high enough to obtain CT images with satisfactory image contrast, however, hinders the application of CT technology for this purpose.

Recently, we and others demonstrated that iodinated oil can be loaded into emulsions and liposomes, enabling the acquisition of CT images with good resolution (22–24). The CT signal density in the bloodstream provided by these oils is high enough to allow these oils to serve as blood pool contrast agents, and the signal duration is much longer than that of hydrophilic iodine compounds. In particular, it has been shown that Lipiodol, an iodinated ethyl ester of poppy seed oil, could be used as an inner oil core of emulsions to yield loading iodine concentrations high enough to obtain CT images of various organs with high contrast enhancement. This finding prompted us to investigate whether Lipiodol-containing emulsions could be used in conjunction with CT imaging techniques as a screen to evaluate the surfactant effect on the *in vivo* disposition of emulsions.

In the present study, we prepared Lipiodol-emulsions with different compositions of surfactants and investigated the impact of surfactant on the blood residence time of emulsions by real-time analysis of CT images. A comparison of the time-dependent changes in the CT signal intensity after administration of emulsions prepared with different surfactant compositions revealed that the blood residence times of emulsions were quite dependent on surfactant composition, particularly the content of Tween 80, a short PEG derivative. As a proof-of-concept demonstration of the usefulness of CT-guided screening in the drug formulation process, we loaded paclitaxel, a widely used anticancer drug with poor water solubility, in two different emulsion formulations whose PK difference was predicted from the CT data. Here, we report that the differences in paclitaxel pharmacokinetics between formulations accorded with predictions made based on CT data.

MATERIALS AND METHODS

Materials

1, 2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) was purchased from Avanti Polar Lipid Inc. (Alabaster, AL, USA). N-(Carbonyl-methoxypolyethyleneglycol 2000)-1, 2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) was purchased from NOF corporation (Shibuya-ku, Tokyo, Japan). Sorbitan trioleate (Span 85) and polyoxyethylenesorbitan monooleate (Tween 80) were bought from Sigma-Aldrich (St. Louis, MO, USA). Paclitaxel was obtained from LC laboratories (Boston, MA, USA) and Taxol® was obtained from Bristol-Myers Squibb (New York, NY, USA). Lipiodol was purchased from Guerbet (Aulnay-Sous-Bois, France). Hexyl 4-hydroxybenzoate and was obtained from TCI (Tokyo, Japan). Acetonitrile, *tert*-butyl methyl ether, methanol and distilled water were HPLC grade. All other materials were of reagent grade and used without further purification.

Preparation of Emulsions

o/w emulsions were prepared by a homogenization method. Briefly, total 149 μmole of surfactant mixture and 200 μl Lipiodol were dissolved in ethanol. In case of paclitaxel-loaded emulsions, 2.2 mg paclitaxel was also dissolved in above mixture. Ethanol was removed from the mixture under slightly reduced pressure until a pre-emulsion concentrate was

obtained. 0.8 ml of saline was then added dropwise to the pre-emulsion concentrate with vortexing. The mixture was sonicated at 37°C for 30 min by using a bath sonicator until crude emulsions were obtained. These emulsions were put in an ice bath and homogenized at 11,500 rpm by using a homogenizer (IKA-Werke, Stufen, Germany) for 10 min, followed by standing for 5 min at room temperature. This process was repeated three times to get a homogeneous emulsion. In case of paclitaxel-loaded emulsions, to remove the unentrapped/precipitated paclitaxel from paclitaxel-loaded emulsions, the emulsion dispersions were immediately filtered through a 0.8 μm membrane filter. Emulsions were stored at 4°C until use.

Physicochemical Characterization of Emulsions

The mean particle size and polydispersity index of emulsion dispersions were determined by dynamic light-scattering method using fiber-optics particle analyzer (FPAR-1000, Otsuka Electronics, Japan) as described in earlier studies (22). Prior to determination, dispersions were diluted 1 to 500 with filtered saline. The polydispersity index is a measure of the uniformity of the particle size distribution in a system studied (25). Zeta potential, the electrical potential at the shear plane of the emulsion droplet, was measured using the electrophoretic light-scattering spectrophotometer (Delsa Nano Series, Beckman Coulter, USA). Prior to measurement, emulsion dispersions were diluted until analytical measurement range in deionized water. Viscosity of emulsions was determined by using the cone-plate rheometer (LVDV-II+Pro, Brookfield, USA) without dilution. The viscosity of emulsions previously kept at 25°C was afterwards measured at 25±2°C with a constant shear gradient of 45.0 s^{-1}. The CPE-40 spindle was used. The results were expressed in centipoises (cP).

In case of paclitaxel-loaded emulsions, the concentration of paclitaxel loaded in emulsions was determined by HPLC analysis. Briefly, 100 μL of paclitaxel-loaded emulsions were rapid frozen at −70°C and dried in a freeze dryer (FDU-1200, EYELA, Tokyo, Japan) overnight. The freeze-dried samples were dissolved in methanol and then filtered through a 0.2 μm membrane filter for HPLC injection. Nanospace SI-2 HPLC system (Shiseido, Japan) equipped with a mobile phase delivery pump (Model 3201) and a UV-visible detector (Model 3002) was used. The mobile phase was 65:35 (v/v) mixtures of acetonitrile and distilled water containing 0.2% acetic acid (pH 3.5). The injection volume was 20 μl and the retention time of paclitaxel was approximately 4.7 min at UV wavelength of 230 nm, when the column oven temperature was retained at 55°C and flow rate was maintained at 1.0 mL/min through a C 18 reverse-phase column (10 μm, 250×4.6 mm, Phenomenx, USA). Elevation of column temperature to 55°C did not cause any significant changes in the signal intensity of paclitaxel standard solution compared to room temperature.

The stability of emulsions were evaluated by monitoring the changes of mean particle size and the paclitaxel content remaining loaded in emulsions during storage at 4°C.

Animal Study on CT Imaging

All protocols used in the present study were reviewed and approved by the Experimental Animal Ethical Committee of Yonsei University according to the Guide for the Care and Use of Laboratory Animals. Female Crj/Bgi–SD rats at 9 weeks of age, weighing 200–250 g (Charles River Japan Inc., Japan), were used in all experiments. Rats were housed in a plastic cage (2–3 per cage) with wood chip bedding, allowed access to food and water *ad libitum*, and were maintained on a 12 h dark: 12 h light (7:30 to 19:30) cycle in a temperature (23±2°C)- and humidity (55±10%)-controlled room. Rats were anesthetized with a mixture of Zoletil® (Virbac, Carros, France) (50 mg/kg) and xylazine (10 mg/kg) administered by intraperitoneal injection. Then a tail vein was catheterized for a manual injection. Emulsions were immersed in a 36.5°C water bath for 30 min before injection. After the precontrast CT scan was performed, 1.5 mL of iodized oil emulsion was manually bolus injected with a 5 cc syringe via the venous catheter into tail vein. Postcontrast CT scans were performed at 1, 5, 15, 30, 45, 60, 90, 120, 180 min and 240 min after injection. Quantitative analysis was performed by locating regions-of-interest (ROI) in the liver, spleen, kidney, aorta, and inferior vena cava (IVC). Mean attenuation (in Hounsfield units, HU) was determined at each time point. The section numbers and locations of the ROI within a section were adjusted for minor variations in anatomic configuration of the rats from one measurement time point to another.

CT Image Acquisition and Analysis

At designated time points, CT images were obtained with a commercial 64-channel multi-slice CT (Discovery CT750 HD; GE Medical Systems, Milwaukee, WI) using the following parameters: rotation time 0.5 s, 120 kV, 190 mAs, and beam collimation 0.625 mm. A reconstruction section thickness of 1 mm and an interval of 1 mm were used to interpret axial images.

Imaging data from the CT scans were transferred to a PACS workstation (Centricity, GE Medical Systems, Milwaukee, WI) for quantitative evaluation. To assess the enhancement achieved with the contrast agents (Lipiodol in emulsions), the CT signal intensity was expressed in HU. All image measurements were obtained by the same experienced CT radiologist.

Pharmacokinetic Study

The protocols for the animal studies were reviewed and approved by the Experimental Animal Ethical Committee of Ajou University (Suwon, Korea) according to the Guide for the Care and Use of Laboratory Animals. Healthy male Sprague–Dawley rats (age of 8 weeks and weighing 240–260 g) were purchased from Koatech Company (Gyeonggi-do, Korea). The procedures used for housing and handling of the rats were similar to those reported previously (26,27).

The procedures used for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) of each rat were conducted in a similar manner to previously reported methods (27,28). The rats were not restrained in the present study.

Taxol and paclitaxel-loaded emulsions were diluted in saline to make concentration of 1.5 mg/mL as paclitaxel. Taxol and paclitaxel-loaded emulsions were slowly administered intravenously via jugular vein at the paclitaxel dose of 5 mg/kg over 1 min in the Taxol group (n=8) and paclitaxel-loaded emulsion group rats (n=7–8). Blood samples (~0.22 mL, each) were collected via the carotid artery at 0 (control), 1 (end of the infusion), 5, 15, 30, 60, 90, 120, 180, 240 and 360 min after the start of the infusion of the drug. Each blood sample was centrifuged immediately and 100 μL of plasma sample was stored at −70°C until being used for the HPLC analysis of paclitaxel.

Plasma Paclitaxel Concentration Analysis

Concentrations of paclitaxel in the plasma samples were determined by a slight modification of a reported HPLC method (29). In brief, 10 μL of methanol containing 100 μg/mL of hexyl 4-hydroxybenzoate as an internal standard was added to 70 μL of a biological sample and extracted with 0.8 mL of tert-butyl methyl ether. After vortex-mixing and centrifugation (3,000 rpm for 10 min), the organic layer was transferred into a clean Eppendorf tube and evaporated under a gentle stream of nitrogen gas at 50°C. The residue was reconstituted in 50 μL of the mobile phase and 30 μL was injected directly onto a reversed-phase HPLC column (Cadenza CD-C18; 150 mm, length×4.6 mm, i.d.; particle size, 3 μm;Imtakt Corp., Japan). Nanospace SI-2 HPLC system (Shiseido, Japan) equipped with a mobile phase delivery pump (Model 3201) and a UV-visible detector (Model 3002) was used. The mobile phases, acetonitrile:methanol:10 mM ammonium acetate buffer (pH 5.0) at a ratio of 48.5:16.5:35 (v/v/v), was run at a flow-rate of 0.5 mL/min, and the column eluent was monitored using an ultraviolet detector at 227 nm at 35°C. The retention times of paclitaxel and hexyl 4-hydroxybenzoate (internal standard) was 10.7 and 18.9 min, respectively. The quantitation limit of paclitaxel in rat plasma was 0.01 μg/mL.

Pharmacokinetic Analysis

Standard methods were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA, USA): the total area under the plasma concentration-time curve from time zero to infinity (AUC) (30), the time-averaged total body clearance (CL), the terminal half-life, the first moment of AUC (AUMC), the mean residence time (MRT) and the apparent volume of distribution at steady state (V_{ss}).

Statistical Analysis

Statistically significant differences between values obtained under different experimental conditions were determined using two-tailed unpaired Student's t-tests. Statistically significant differences among the three means for the unpaired data were determined using a Duncan's multiple range test of SPSS *posteriori* ANOVA program.

RESULTS

Effect of Including PEG Derivatives on the Blood Circulating Time of Emulsions Screened by CT Imaging

Several studies have shown that inclusion of amphiphilic PEG derivatives as emulsifiers prolongs the circulation time of emulsions by reducing the affinity of the emulsion droplets for the MPS system. The prolongation effect of Tween 80, a PEG derivative with a shorter PEG chain length, was found to be less potent than that of DSPE-PEG, a PEG derivative with a longer chain length (13). Researchers found that inclusion of a small amount of DSPE-PEG significantly extended the blood circulation time of Tween 80 emulsions (14), in contrast to the slight extension reported by Yoshizawa *et al.* (15). Considering that differences in other formulation factors might account for the differences between these studies, as part of our exploration of the usefulness of CT imaging technology, we investigated whether the prolongation effect of PEG derivatives is affected by surfactant composition of emulsions.

We first prepared emulsions containing Span 85 as a main surfactant. Span 85, a surfactant that does not contain a PEG chain, was chosen to exclude the major surfactant effect that can confound evaluations of the blood circulating time of emulsions in which small amounts of Tween 80 (shorter PEG derivative) or DSPE-PEG (longer PEG derivative) are included as a co-surfactant. The mean particle size of

emulsions prepared with a 90:10 molar ratio of Span 85:DMPC as a surfactant mixture was larger than 600 nm, whereas the mean particle size of emulsions prepared with a 90:10 molar ratio of Span 85:Tween 80 or Span 85:DSPE-PEG mixtures was reduced by more than two fold (Fig. 1a). Increasing Tween 80 content to 35% of surfactant mixture further decreased the Span 85:Tween 80 emulsion size to 212 nm. A 10 mol% content of DSPE-PEG was chosen based on earlier studies showing that a prolongation effect DSPE-PEG was achieved at 5–10 mol% through steric inhibition of emulsion interactions with MPS cells (13).

To determine whether inclusion of Tween 80 or DSPE-PEG resulted in longer CT images of blood pools, we intravenously injected rats with one of three different Span 85 emulsions—90:10 Span 85:DMPC, 90:10 Span 85:DSPE-PEG and 63:35 Span 85:Tween 80. 63:35 Span 85:Tween 80 emulsion was chosen instead of 90:10 Span 85:Tween 80 emulsion because it was difficult to expect that Tween 80 exerts prolongation effect as potent as DSPE-PEG at the same content. It was also considered that using Span 85:Tween 80 emulsions with mean droplet size similar or smaller compared to 90:10 Span 85:DSPE-PEG emulsions would be advantageous to minimize the size effect in interpreting the surfactant composition effect on the blood clearance of emulsions. The contrast enhancement characteristics of each emulsion in the aorta and IVC are illustrated in the time-enhancement curves shown in Fig. 1b. In the case of Span 85:DMPC emulsions, only a small enhancement (30–40 HU difference) was detected 5 min after injection; this difference further decreased to near baseline by 15 min post-injection, suggesting the very rapid blood clearance of these emulsions. In contrast, high aorta and IVC enhancement (>150 HU difference) was observed immediately after administering Span 85:DSPE-PEG or Span 85:Tween 80 emulsions. For both emulsions, CT values in the aorta and IVC subsequently declined gradually over time, but the duration over which a ΔHU of more than 30 (clinically relevant ΔHU) was maintained was 3-fold longer for Span 85:DSPE-PEG emulsions than for Span 85:Tween 80 emulsions (120 min for Span 85:DSPE-PEG emulsions *vs.* 40 min for Span 85:Tween 80 emulsions). These data demonstrate that inclusion of DSPE-PEG or Tween 80 as a co-surfactant in Span 85-emulsions effectively extended the

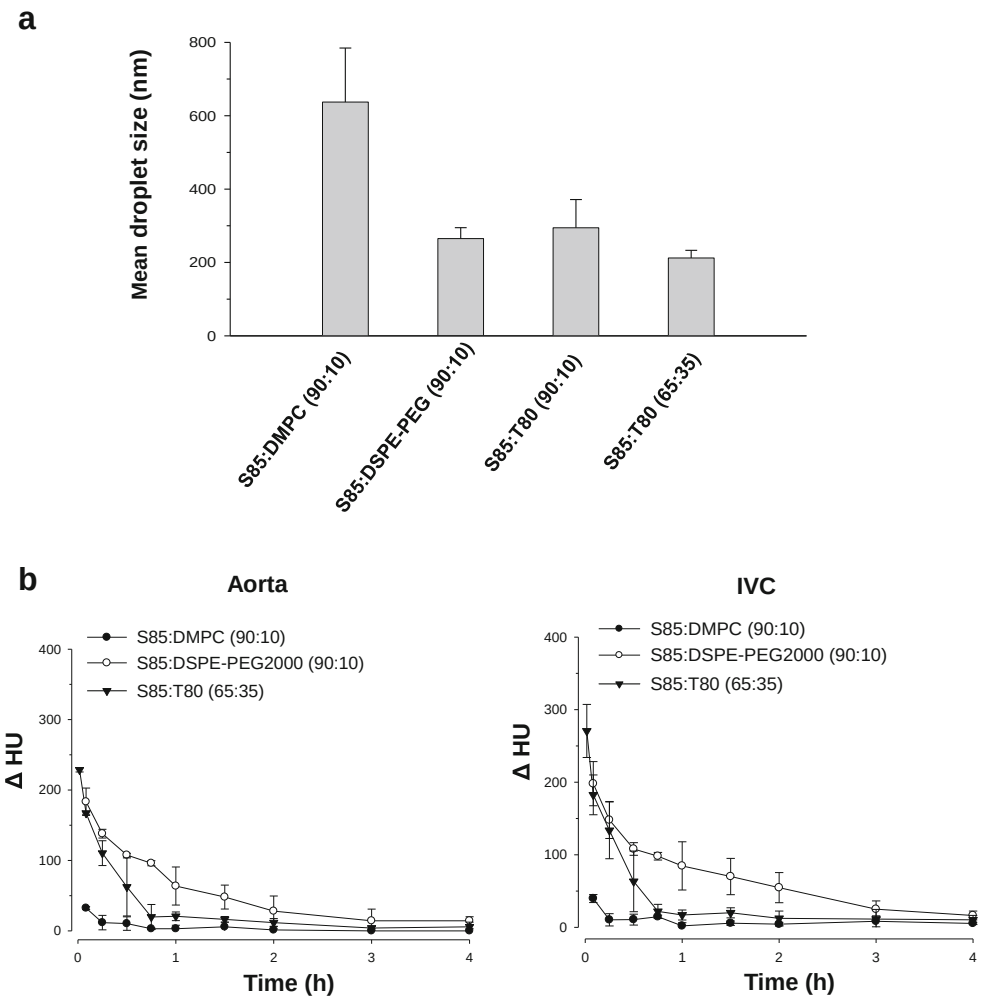

Fig. 1 Effect of including PEG derivatives on the (**a**) mean particle size and (**b**) contrast enhancement curve as a function of time in the aorta and IVC. Lipiodol-containing emulsions were prepared with a surfactant mixture containing Span 85, as a main surfactant, and DMPC, Tween 80 or DSPE-PEG2000 as a co-surfactant. The mean droplet size of emulsions was measured as described in the text. The contrast enhancement curve was obtained based on CT images acquired after intravenous administration of 1.5 ml of emulsions via the rat tail vein. *Each point* represents the mean ± S.D. ($n = 3 \sim 4$). The increase in HU for each time point is the difference between the dynamic value and the initial value.

blood circulation time of emulsions and further show that DSPE-PEG was more effective than Tween 80 in terms of the prolongation effect, probably owing to the more potent inhibition of interactions between emulsion droplets and MPS cells. Because droplet size was greatly reduced by incorporation of either co-surfactant, our data do not exclude the possibility that the prolongation effect is partly attributable to a decrease in particle size caused by inclusion of Tween 80 or DSPE-PEG. The fact that inclusion of 10 mol% of DSPE-PEG more effectively increased the blood residence time of emulsions than 35 mol% of Tween 80, even though the reduction in droplet size by the former was less than that by the latter, however, suggests that the prolongation effect is not wholly due to the reduction in size.

CT Assessment of the Tween 80 Content Effect on the Blood Circulating Time of Emulsions

Larger emulsion droplets (200~250 nm) are known to be cleared faster from the blood circulation than smaller droplets (2,31). Considering that producing droplets smaller than 200 nm is required to minimize the impact of droplet size on the blood circulation time of emulsions, we prepared emulsions using Tween 80 and Span 85 at various molar ratios. Emulsions containing a 78:9:13 mixture of Tween 80:DSPE-PEG:Span 85 as a surfactant were also prepared to investigate whether co-inclusion of Tween 80 and DSPE-PEG more effectively extended the blood circulation time of emulsions. As shown in Table I, increasing Tween 80 content steadily decreased the mean droplet size of emulsions. In Tween 80:Span 85 emulsions in which Tween 80 was used as the main surfactant, the droplet size in all emulsions was less than 200 nm. Droplet sizes of emulsions obtained with a 78:9:13 mixture of Tween 80:DSPE-PEG:Span 85 were similar to those of emulsions obtained with a 87:13 Tween 80:Span 85 mixture. The polydispersity index of all Tween 80–based emulsions was less than 0.3, suggesting a homogeneous distribution suitable for intravenous injection.

Emulsions with four different surfactant compositions were injected into rats, and the time-contrast enhancement curves of these emulsions in various organs were compared (Fig. 2). The contrast enhancement in the aorta and IVC, as judged by ΔHU, was high 5 min post-administration, regardless of formulation. The decrease in contrast enhancement following this initial high depended on Tween 80/Span 85 content; it was most rapid for emulsions containing 35% Tween 80 and decreased successively for emulsions containing 65% and 87% Tween 80 (Fig. 2a and b). Specifically, a ΔHU greater than 30 was maintained for 30, 120 and >240 min after injection of emulsions with 35%, 65% and 87% Tween 80 content, respectively. At 240 min post-injection, ΔHU was still greater than 100 with 87% Tween 80–containing emulsions, but had approached basal levels by 90 and 180 min for emulsions containing 35% and 65% Tween 80, respectively. These data indicate that the blood circulating time of emulsions was correlated with the Tween 80 content in emulsions.

Table I Mean Droplet Size and Polydispersity Index of Emulsions Prepared with Various Surfactant Compositions. Data are Presented as Mean ± SD ($n=4$)

Surfactant Mixture (mol%)	Mean droplet size (nm)	Polydispersity Index
T80:S85 (35:65)	212 ± 21	0.294 ± 0.011
T80:S85 (60:40)	182 ± 65	0.242 ± 0.050
T80:S85 (65:35)	151 ± 28	0.232 ± 0.045
T80:S85 (87:13)	142 ± 25	0.258 ± 0.017
T80:DSPE-PEG:S85 (78:9:13)	130 ± 25	0.269 ± 0.029

The time-dependent contrast enhancement in liver, spleen, and kidney was also compared among three formulations with different Tween 80 content. In liver and spleen, the ΔHU increase was greatest in rats receiving 35% Tween 80–containing emulsions, and successively decreased in emulsions containing 65% and 87% Tween 80. The maximal increase in HU in liver was 3.4- and 2.3-fold higher in rats receiving emulsions formulated with Tween 80:Span 85 ratios of 35:65 or 65:35, respectively, compared to those receiving 87:13 Tween 80:Span 85; the corresponding fold-differences in HU for spleen were 7.3 and 3.5. In addition, the HU increase in these organs was the most immediate after injection of 35% Tween 80–containing emulsions and was progressively slower with emulsions containing 65% and 87% Tween 80. Contrast enhancement was maintained at greater than 200 (liver) and 350 (spleen) for up to 240 min post-injection in rats receiving emulsions with Tween 80:Span 85 ratios of 35:65 or 65:35, whereas it remained low throughout the observation period, never reaching a ΔHU greater than 80 (liver) and 110 (spleen) with 87% Tween 80–containing emulsions (Fig. 2c and d). All of these emulsions slightly increased renal contrast enhancement at 5 min post-injection, but the extent and duration of ΔHU varied somewhat among formulations (Fig. 2e). The maximal ΔHU achieved for Tween 80-containing emulsions followed a rank order of 87% (~72 ΔHU) >65% (~55 ΔHU) >35% (~37 ΔHU) Tween 80 content. These data suggest that the renal clearance of emulsions increased with increasing Tween 80 content, probably owing to the increased surface hydrophilicity provided by Tween 80.

The time-enhancement curves obtained in rats receiving 87:13 Tween 80:Span 85 emulsions or 78:9:13 Tween 80:DSPE-PEG:Span 85 emulsions were very similar in the aorta, IVC, liver, spleen, and kidney (Fig. 2a–e). These results indicate that the *in vivo* disposition of these two emulsion formulations was very similar, despite differences in composition, suggesting that substitution of 9% Tween 80 with 9%

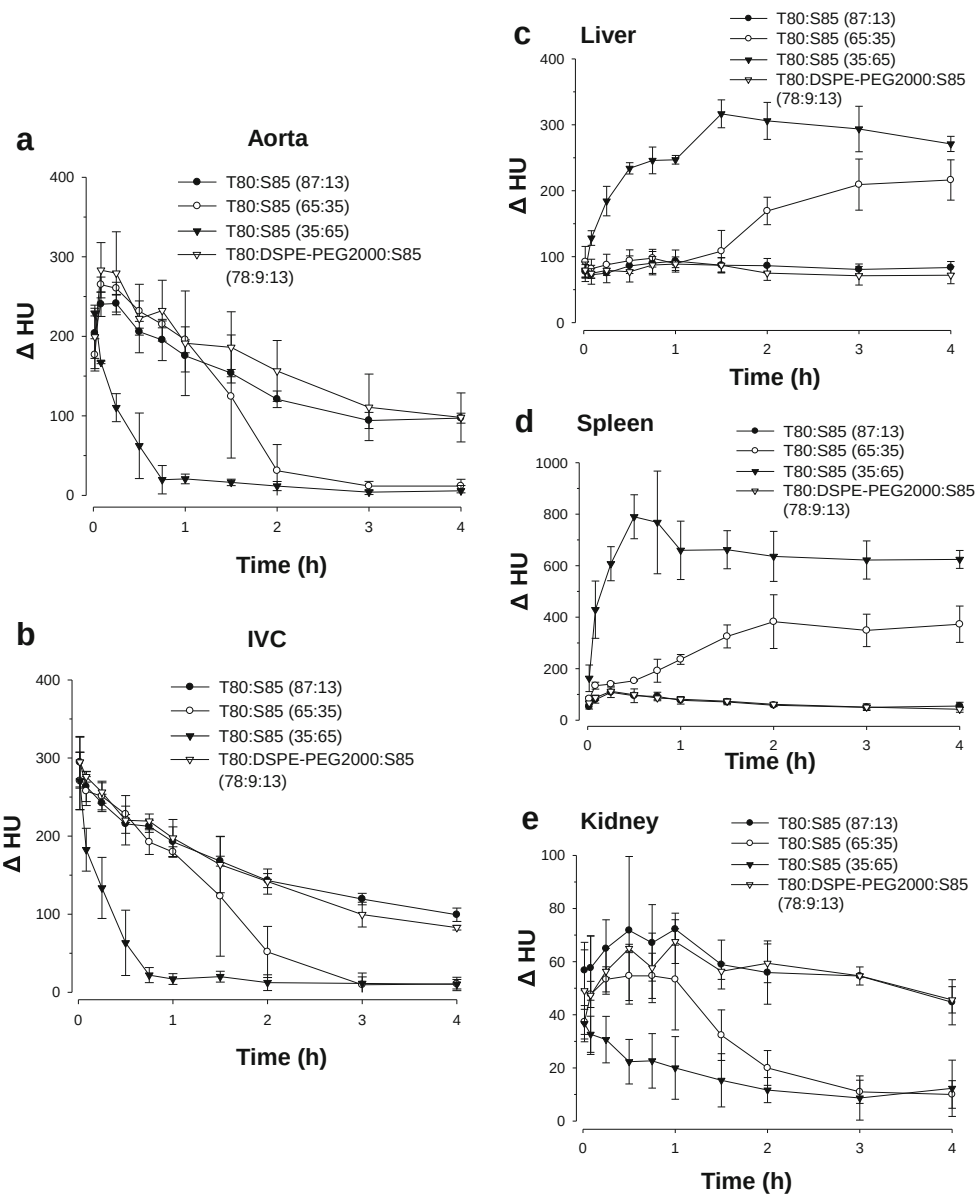

Fig. 2 Effect of Tween 80 content in the surfactant mixture used to prepare emulsions on the contrast enhancement curve as a function of time in the (**a**) aorta, (**b**) IVC, (**c**) liver, (**d**) spleen, and (**e**) kidney. Lipiodol-containing emulsions were prepared with varying Tween 80 content as described in the figure legends, and the contrast enhancement curve was obtained as described in Fig. 1. *Each point* represents the mean ± S.D. ($n=4$).

DSPE-PEG did not further extend the blood circulation time of emulsions. The *in vivo* disposition of emulsions with higher DSPE-PEG content could not be investigated because the significant increase in the viscosity of resultant emulsions hindered injection.

Representative CT images of various organs following intravenous dosing of emulsions are presented in Fig. 3. At 30 min post-injection, opacification in the aorta and IVC was stronger with 65:35 or 87:13 Tween 80:Span 85 emulsions and 78:9:13 Tween 80:DSPE-PEG:Span 85 emulsions than with 35:65 Tween 80:Span 85 emulsions. In contrast, opacification of the liver and spleen was strongest with 35:65 Tween 80:Span 85 emulsions, less strong with 65:35 Tween 80:Span 85 emulsions, and weakest with 87:13 Tween 80:Span 85 or 78:9:13 Tween 80:DSPE-PEG:Span 85 emulsions.

Preparation and Characterization of Paclitaxel Emulsions with Varying Tween 80 Content

CT imaging studies revealed that the blood circulation time and disposition of reticuloendothelial system (RES)-rich organs is very dependent on the Tween 80 content of emulsions. We next explored the possibility that the data obtained by CT-guided screening could be used in the formulation process of a drug that requires loading in emulsions. Paclitaxel was chosen as a model drug, since it needs particulate carriers, such as emulsions, liposomes or micelles, for parenteral administration owing to its poor aqueous solubility.

When paclitaxel was loaded in emulsions with varying molar ratios of Tween 80:Span 85, the concentration of paclitaxel loaded in emulsions tended to increase with

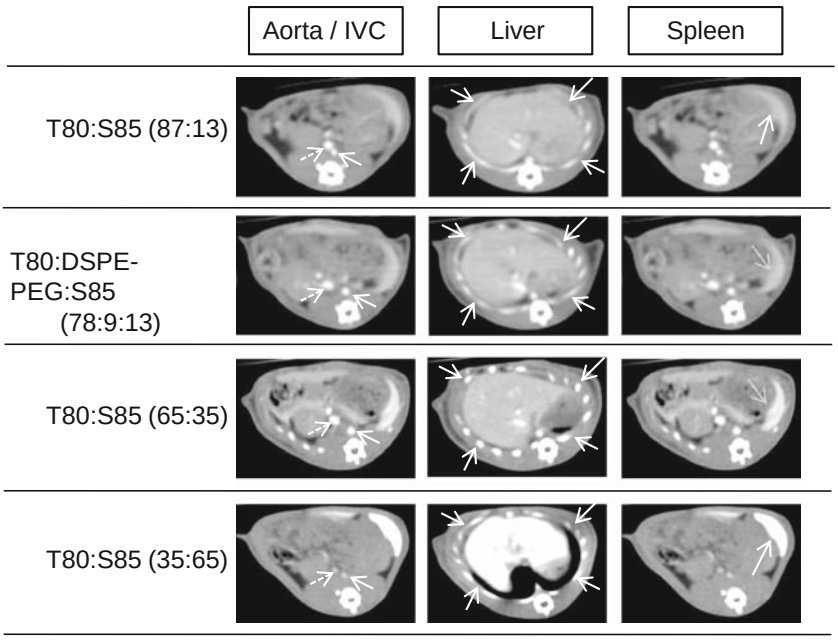

Fig. 3 Representative CT images of the aorta, IVC, spleen, and liver 30 min after injecting emulsions with varying Tween 80 content. *Each arrow* represents aorta (*straight line*), IVC (*broken line*), liver, or spleen.

increasing Tween 80 content of emulsions. The paclitaxel concentration loaded in emulsions containing 10% Tween 80 in the surfactant mixture was as low as 0.30±0.12 mg/ml, but increased 5.8-fold with an increase in Tween 80 content to 35% (Table II). The concentration of loaded paclitaxel reached a maximum (~2.0 mg/ml) in emulsions containing more than 50% Tween 80. Substitution of 9% DSPE-PEG for Tween 80 only slightly increased the concentration of loaded paclitaxel (1.1-fold). The paclitaxel loading concentration obtained in this study is comparable to that reported in an earlier study using tricaproin emulsions stabilized with Tween 80:phosphatidylcholine (32). Paclitaxel loading efficiency was greater than 85% in emulsion formulations containing more than 50% Tween 80, indicating that these emulsions offer sufficient solubilization capacity to provide a potential paclitaxel dosage form.

The droplet sizes of paclitaxel-loaded emulsions tended to decrease with increasing Tween 80 content, but remained within the range of 100 to 230 nm (Table II). Regardless of formulation, paclitaxel loading caused no significant increase in droplet size. The polydispersity index of all paclitaxel-loaded emulsions was also low (≤0.250), similar to those of empty emulsions. These data suggest that paclitaxel loading does not destabilize Lipiodol emulsions. The zeta potential of paclitaxel-loaded emulsions—the electrical potential at the shear plane of the emulsion droplets—was nearly neutral or weakly negative, except in the case of emulsions incorporating DSPE-PEG. Among emulsions with varying Tween 80 content, there was a tendency toward reduced zeta potential with increasing Tween 80 content, consistent with previous studies (33). Probably the higher Tween 80 content increases the adsorption of negatively charged ions present in the emulsion dispersion media, increasing the negativity of zeta potential values. In the case of Tween 80:Span 85 micelles, the zeta-potential was more highly negative, a property that is probably related to the much smaller droplet size of these particles. Because of the negatively charged property of DSPE-PEG (34), the zeta-potential of Tween 80:DSPE-PEG:Span 85

Table II Paclitaxel Loading Concentration, Mean Droplet Size, Polydispersity Index, Zeta-Potential and Viscosity of Lipiodol Emulsions Prepared with Varying Surfactant Composition. Data are Presented as Mean ± SD ($n=4$)

Surfactant composition (mol%)	Loaded paclitaxel (mg/mL)	Droplet size (nm)	Polydispersity Index	Zeta-potential (mV)	Viscosity (cP)
T80:S85 (10:90)	0.30±0.12	–	–	–	–
T80:S85 (35:65)	1.74±0.36	224±36	0.209±0.001	−10.6±1.7	3.4±1.1
T80:S85 (60:40)	1.94±0.13	145±25	0.188±0.061	−5.7±1.4	4.4±0.3
T80:S85 (65:35)	1.99±0.11	159±33	0.203±0.045	−3.1±6.2	4.6±0.2
T80:S85 (87:13)	1.88±0.19	125±49	0.231±0.051	−0.1±6.2	6.1±0.1
T80:DSPE-PEG:S85 (78:9:13)	2.10±0.13	109±6	0.222±0.011	−28.2±5.9	11.6±0.1
T80:S85 (87:13) micelle	1.87±0.01	32±14	0.234±0.106	−22.0±3.4	2.5±0.2

emulsion was also more highly negative. The viscosity of all paclitaxel-loaded emulsions was less than 12 cP, values much lower than the reported limit of viscosity as a criterion of injectability (250 cP) (35). Taken together, these data indicate that emulsions containing more than 35% Tween 80 could offer suitable paclitaxel loading concentration, droplet size, zeta potential, and viscosity to serve as intravenous paclitaxel dosage forms.

Stability of Paclitaxel Emulsions with Differing Tween 80 Content

Several studies have reported that the use of nanoparticles as a paclitaxel dosage form is hampered by time-dependent release/precipitation of paclitaxel (36,37). Therefore, we tested the stability of paclitaxel-loaded Lipiodol-emulsions by measuring time-dependent changes in mean droplet size and concentration of loaded paclitaxel. Storage of paclitaxel-loaded formulations at 4°C caused a time-dependent precipitation of paclitaxel depending on formulations (Fig. 4a): Among emulsions with differing Tween 80 content, those containing 35% Tween 80 showed the most rapid decrease, losing more than 60% of their initial loaded paclitaxel concentration through precipitation after only 4 weeks at 4°C. Emulsions containing 65% or 87% Tween 80 lost~15% of their initial loaded concentration of paclitaxel after 12-week storage but the loss was not statistically significant ($p>0.05$). Compared to emulsions containing 87:13 Tween 80:Span 85, those with a 78:9:13 mixture of Tween 80:DSPE-PEG:Span 85 exhibited slightly greater paclitaxel precipitation, but still retained more than 80% of the loaded paclitaxel after 12-week storage. Collectively, these results indicate that the presence of Lipiodol in emulsions and the inclusion of ≥65% Tween 80 in the surfactant mixture were beneficial in retarding the precipitation of paclitaxel from formulations.

Measurements of droplet sizes of various paclitaxel formulations during storage revealed that the sizes of micelles increased by approximately 3-fold after 12 weeks, suggesting that the precipitation of paclitaxel from micelles was caused by fusion of micelles (Fig. 4b). An increase in the mean droplet size also occurred in emulsions obtained with surfactants containing 35% Tween 80, but the increase was much less (1.4-fold after 12 weeks). The size of 78:9:13 Tween 80:DSPE-PEG:Span 85 emulsions increased 1.6-fold during the initial 4-weeks, but remained relatively unchanged thereafter. In emulsions containing 65% or 87% Tween 80 in the surfactant mixture, there was no significant increase in droplet size during 12-week storage. Taken together with the paclitaxel precipitation data, these findings demonstrate that inclusion of more than 65% Tween 80 in the surfactant mixture contributes to the stabilization of paclitaxel-loaded emulsions during storage.

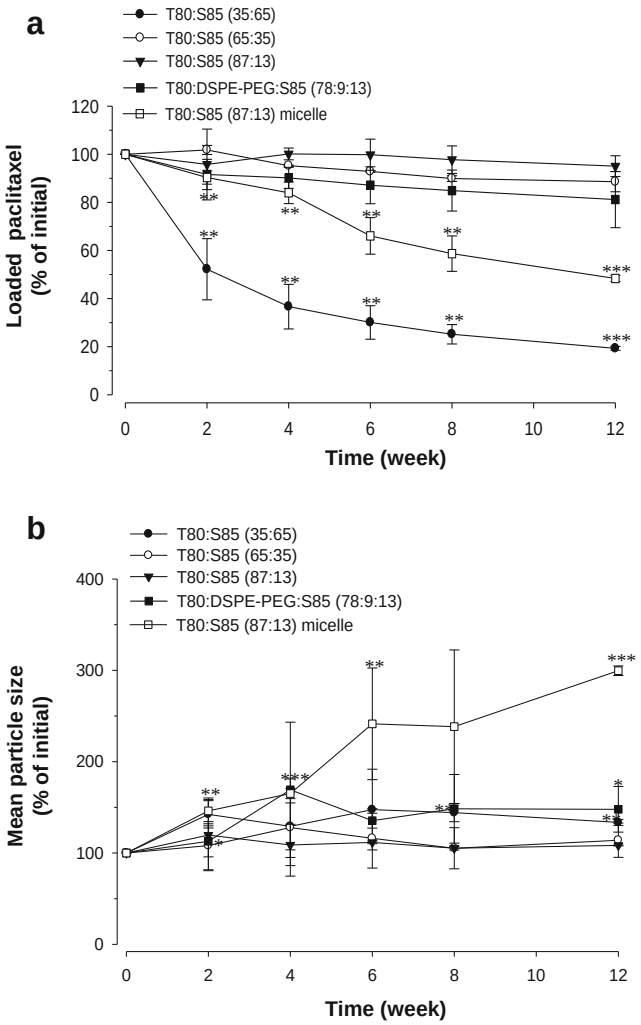

Fig. 4 Emulsion stability assessed as changes in (**a**) concentration of loaded paclitaxel and (**b**) mean droplet size of emulsions. The paclitaxel concentration remaining in emulsions during storage at 4°C was determined by HPLC analysis, and changes in droplet size were determined using a dynamic light-scattering method. Data are presented as means ± S.D. ($n=3$). Significant differences are indicated by *asterisks*: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$, compared with the initial concentration of loaded paclitaxel (**a**) and the initial mean droplet size (**b**) of each formulation.

Pharmacokinetics of Paclitaxel Loaded in Emulsions

In many cases in which drugs are administered as emulsion-loaded forms, a longer circulation in the bloodstream is likely to give the drug a greater opportunity to reach its therapeutic target organs. However, in some cases, such as targeting to specific organs, rapid clearance of emulsions from the blood can be beneficial. Accordingly, we next explored whether the CT-predicted difference in the blood circulation time of emulsions with different surfactant composition could be used to formulate two different emulsions—one with a long and one with a short blood circulation time—as carriers for paclitaxel. Two different emulsions stabilized with 87:13 or 65:35 surfactant mixture of Tween 80:Span 85 were chosen for this

purpose. These formulations were selected because their mean droplet size, zeta potential, stability and paclitaxel loading concentration are similar, but they exhibit different blood circulation time, as shown in CT imaging studies.

Figure 5 shows the plasma concentration-time curves of paclitaxel after intravenous administration of the two emulsion formulations and Taxol®. The mean plasma concentration of paclitaxel after injection of 87:13 Tween 80:Span 85 emulsions was higher than that after injection of 65:35 Tween 80:Span 85 emulsions: at 2 and 4 h post-injection, paclitaxel plasma concentration was 2.4- and 3.0-fold higher with 87:13 Tween 80:Span 85 emulsions. These observations suggest that 87:13 Tween 80:Span 85 emulsions were removed more slowly from the systemic circulation. The overall paclitaxel plasma concentration profile produced with 87:13 Tween 80:Span 85 emulsions was similar to that of Taxol®. Considering that paclitaxel solubilized by Cremophor EL (in case of Taxol®) would be present as mainly micelle forms after administration (38), differences in droplet sizes and drug release characteristics would exist between micelles and emulsions, rendering it difficult to explain why 87:13 Tween 80:Span 85 emulsions could not provide longer circulation of paclitaxel compared to Taxol®.

The pharmacokinetic parameters of paclitaxel obtained by administration of the two emulsion formulations or Taxol are summarized in Table III. The total plasma concentration of paclitaxel was significantly higher with 87:13 Tween 80:Span 85 emulsions than with 65:35 Tween 80:Span 85 emulsions. In particular, the area under the curve (AUC) of the drug obtained following administration of the 87:13 emulsion was about 1.8-fold greater than that of the 65:35 emulsion. In addition, the 87:13 emulsion exhibited a significantly higher mean residence time (MRT; 2.2-fold), longer terminal half-life ($T_{1/2}$; 2.1-fold), and lower clearance (1.8-fold) compared with the 65:35 emulsion. The apparent volume of distribution (Vss) of the drug did not differ significantly between the 87:13 and 65:35 emulsion groups. A comparison of the pharmacokinetic parameters between the 87:13 emulsion and the commercial product Taxol® showed no statistical difference in any pharmacokinetic parameter. The value for AUC for Taxol® obtained here is similar to values reported previously (39). The 65:35 emulsion exhibited a significantly lower AUC (1.7-fold) and higher clearance (1.7-fold) compared to Taxol®, and showed a trend toward a shorter MRT (1.7-fold) and $T_{1/2}$ (1.5-fold) that failed to reach statistical significance. Taken together, these data indicate that paclitaxel loaded in emulsions with surfactant mixture containing 87% Tween 80 circulated in the blood stream for a longer period compared to emulsions containing 65% Tween 80, as predicted by CT imaging.

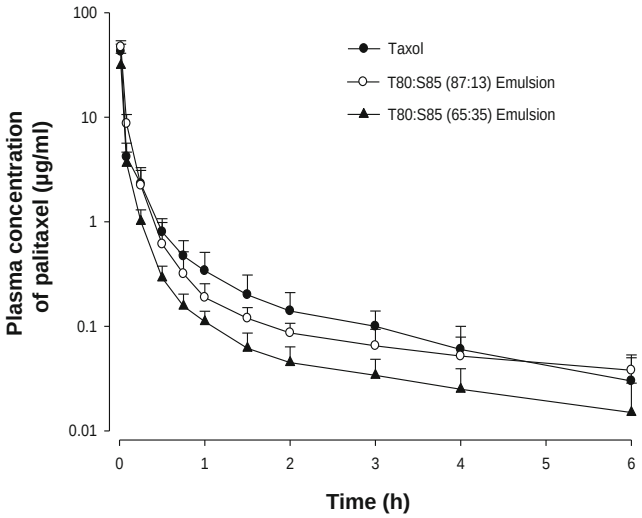

Fig. 5 The plasma concentration-time profiles of paclitaxel in rats after intravenous administration of Taxol® or paclitaxel-containing emulsions at a paclitaxel dose-equivalent of 5 mg/kg. *Each point* represents the mean ± S.D ($n=7\sim8$).

DISCUSSION

The *in vivo* fate of nanoparticulate drug carriers is one of the key factors affecting the performance of loaded drug since the pharmacokinetics and tissue distribution of loaded drugs are quite different from those of free drug. In the present study, we explored the possibility of using CT, the most frequently used diagnostic imaging modality, as a platform technology for the noninvasive, convenient, and quantitative evaluation of the biodisposition of o/w emulsions. This was possible because Lipiodol, an iodinated oil with a high iodine content, could serve as the inner oil phase constituting emulsions, thereby allowing emulsions to possess a high iodine payload (96 mg I/ml), which is required to enhance the contrast of CT images. The biodisposition of Lipiodol emulsions was visualized by CT at designated time points after administration, and a quantitative analysis of CT images revealed that the blood residence time of emulsions was very dependent on the surfactant composition used to stabilize emulsions, particularly the density and type of surfactant containing a PEG chain.

Our first finding obtained in the CT imaging study was that the blood circulation time of emulsions was extended by the inclusion of PEG derivatives, either DSPE-PEG or Tween 80. Of these two derivatives, DSPE-PEG, with a longer PEG chain, was more effective in extending the blood circulation time of emulsions compared to Tween 80, with a shorter PEG chain. This difference in prolongation was observed despite the larger droplet size and lower PEG derivative content of Span 85:DSPE-PEG emulsions compared with Span 85:Tween 80 emulsions. Therefore, it seems likely that the differences in blood circulation time are attributable to the nature of surface coating materials, particularly the length of the amphiphilic PEG chain, which is involved in inducing steric stabilization (40). Consistent with our data, Liu and

Table III Pharmacokinetic Parameters of Paclitaxel After Intravenous Administration of Taxol or Paclitaxel-Containing Emulsions at a Dose of 5 mg/kg as Paclitaxel in Rats (Mean ± SD, One-Way ANOVA, Tukey's Multiple Comparison)

	Taxol ($n=8$)	87:13 Emulsion (T80:S85 = 87:13) ($n=8$)	65:35 Emulsion (T80:S85 = 65:35) ($n=7$)
Body weight (g)	250 ± 20	250 ± 20	250 ± 20
$T_{1/2}$ (min)[a]	117.0 ± 43.4	168.0 ± 66.3	80.0 ± 40.5
$AUC_{0-\infty}$ (ug•min/mL)[b]	278.0 ± 63.2	291.0 ± 51.7	164 ± 44.8
MRT (min)[a]	34.1 ± 11.7	42.0 ± 21.3	19.5 ± 4.5
CL (mL/min/kg)[c]	18.9 ± 4.6	18.0 ± 3.3	32.7 ± 9.5
Vss (mL/kg)	624 ± 180	755 ± 461	716 ± 792

The data were expressed as mean ± standard deviation (SD)

$T_{1/2}$, terminal half-life time; AUC, total area under the plasma concentration–time curve from time zero to infinity; CL, time-averaged total body clearance; MRT, mean residence time; V_{ss}, apparent volume of distribution at steady state

[a] 87:13 Emulsion group was significantly different ($P<0.05$) from 65:35 Emulsion group

[b] 65:35 Emulsion group was significantly different from Control ($P<0.05$) and 87:13 Emulsion group ($P<0.01$)

[c] 87:13 Emulsion group was significantly different from Control and 65:35 Emulsion group ($P<0.001$)

Liu demonstrated that inclusion of a PEG derivative with a longer PEG chain was more effective in extending the blood circulation times of emulsions (13).

Our CT imaging study also demonstrated that the blood circulation time of emulsions, judged based on the duration of contrast enhancement in the aorta and IVC, was correlated with the Tween 80 content on the emulsion surface. Furthermore, CT scanning following injection of 35:65 Tween 80: Span 85 emulsions showed the most rapid and evident enhancement in liver and spleen throughout the observation period, while that of 87:13 Tween 80:Span 85 emulsions showed the slowest and least evident enhancement, indicating that the emulsion uptake in RES-rich organs was inversely correlated with the Tween 80 content of the emulsions. Probably Tween 80 inclusion on the emulsion surface contributed to sterically inhibiting the binding of MPS cells with emulsion particles, thereby reducing the rapid clearance of emulsions from the blood through uptake by circulating monocytes and macrophages in RES-rich organs. In addition, we found that the additional inclusion of DSPE-PEG was ineffective in further prolonging the blood circulation of emulsions with a high Tween 80 content (≥87%). Probably 87% Tween 80 content on the emulsion surface is already enough to provide steric stabilization of emulsions. Our data suggest that the effectiveness of amphiphilic PEG derivatives in extending the blood circulation time of emulsions may also be affected by the amount and type of other surfactant constituents of the emulsions and this may account for the differences found in earlier studies.

During their circulation in the bloodstream, emulsion-loaded drugs are released from emulsions and thus circulate as free or loaded forms. Therefore, the pharmacokinetics of drugs does not directly correspond with those of emulsions. However, in cases where other formulation parameters apart from the surfactant composition are similar between two different emulsions, the drugs loaded in longer-circulating emulsions are expected to circulate longer than those in shorter-circulating emulsions. With this in mind, we used the relationship between the surfactant composition and the blood circulation time of emulsions established by CT imaging study to design and optimize an emulsion formulation for paclitaxel. Among emulsions with varying composition of Tween 80 and Span 85, two emulsion formulations with higher (87%) or lower (65%) Tween 80 content were chosen as a paclitaxel carrier. These two emulsions exhibited similar mean droplet size, loaded paclitaxel concentration, zeta potential and stability (Table II and Fig. 4), but the pharmacokinetics of paclitaxel following intravenous injection into rats was significantly different between them. The mean residence time, terminal half-life and AUC of paclitaxel were increased 2.2-, 2.1- and 1.8-fold, respectively, with 87:13 Tween 80:Span 85 emulsions. This shows that a longer blood circulation time of paclitaxel could be achieved by selecting the surfactant composition used to constitute emulsions based on CT imaging data. Thus, the CT-guided screening of the surfactant effect presented in the current study may be a simple and convenient technology for facilitating the emulsion formulation process.

There are potential limitations to the use of CT-based data for the emulsion formulation process. When the solubility/release characteristics of drugs require replacing Lipiodol with different oils, the difference in oil may affect the biodistribution of the emulsions. However, the oil phase of o/w emulsions exists inside of emulsions and thus does not directly interact with monocytes or macrophages. The effect of oil type on the biodisposition of emulsions would primarily be indirect, including effects on droplet size and emulsion stability. Moreover, obtaining CT data using emulsions obtained with the desired oil mixture together with Lipiodol may help to overcome these limitations.

Cancer nanotheranostics, which combines diagnostic and therapeutic modalities into a single nano-sized carrier, is a recently emerging technology. This combination allows physicians to monitor the carrier biodistribution and target site localization in real time. In this regard, the CT-visible, Lipiodol emulsion optimized in the current study may also be a promising carrier for theranostic purposes. The blood circulation time of paclitaxel-loaded Lipiodol emulsions containing 87:13 Tween 80:Span 85 was long and similar to that of the commercial product, Taxol®. Moreover, we have found that the anticancer activity of paclitaxel as Taxol® or as the optimized emulsion formulation described here is also similar in terms of the IC_{50} of paclitaxel (concentration required to inhibit the cellular proliferation by 50%) in human lung cancer cells (unpublished data). Furthermore, the droplet size of 87:13 Tween 80:Span 85 emulsions was approximately 120 nm, and the surface charges of these emulsion droplets were almost neutral. Given these physicochemical properties, the Lipiodol emulsions optimized in this study would be expected to exert an adequate enhanced permeability and retention (EPR) effect, considering that approximately 100-nm nanoparticles with a weak negative charge are optimal in this context (41). Lastly, the fact that the release/precipitation of paclitaxel loaded in emulsions was greatly retarded compared to those loaded in micelles with the same surfactant composition also demonstrate that Lipiodol plays a role in stabilizing paclitaxel-loaded nanoparticle formulations. Therefore, it would be of interest to investigate whether the Lipiodol emulsions described in this study would provide a useful theranostic tool for effective cancer diagnosis and treatment.

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