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Pharmacokinetic Behavior and Biodistribution of Paclitaxel-Loaded Lipid Nanosuspension

Sung-Up Choi, Jung Min Park, Woo Sik Choi, Jaehwi Lee and Young Wook Choi†

Division of Pharmaceutical Sciences, College of Pharmacy, Chung-Ang University, Seoul 156-756, KOREA

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ABSTRACT – In this study, paclitaxel-loaded lipid nanosuspension (PxLN) was prepared and the *in vivo* profiles after intravenous administration in rats were investigated. We compared the manufacturing processes depending on the temperature: PxLN-H for a hot homogenization process and PxLN-C for solidification of lipid-drug mixtures by liquid nitrogen. Both formulations showed submicron size distribution and the similar drug loading efficiency of about 70%. *In vitro* release of PxLNs and Taxol® performed by a dialysis diffusion method showed similar pattern for PxLN-H and Taxol®, but the reduced release profile for PxLN-C. PxLN or Taxol® was intravenously administered to the rats at a dose of 5 mg/kg as paclitaxel. The drug in blood samples were assayed by the HPLC/MS/MS method. The AUC_t of PxLN-H was 3.4-fold greater than that of Taxol®. PxLN-H gave higher biodistribution in all tissues than did Taxol®. In addition, it maintained the higher drug concentration for 12 h. This lipid nanosuspension might be a promising candidate for an alternative formulation for the parenteral delivery of poorly water-soluble paclitaxel.

Key words – Paclitaxel, Lipid Nanosuspension, Pharmacokinetic Behavior, Biodistribution

Paclitaxel, an anticancer agent, has a narrow therapeutic index, so that the therapeutic response is usually associated with toxic-side effects.[1)] Furthermore, Cremophor EL, a co-solvent solubilizing paclitaxel, has caused hypersensitivity reaction, which was used in the commercial formulation of paclitaxel, Taxol®. Paclitaxel is well-known poorly water-soluble drug and the aqueous solubility is measured to be 0.5 μg/ml.[1)] It is highly hydrophobic compound having an octanol-to-water partition coefficient of over 1000. Furthermore, the addition of surfactants alone cannot increase the aqueous paclitaxel solubility.[2)] Therefore, the commercial product of paclitaxel (Taxol®) was formulated utilizing a mixture of a co-solvent and a surfactant consisting of ethanol and Cremophor® EL to increase the aqueous solubility of paclitaxel. Although it has a unique therapeutic property and sensitivity for tumors, the success of its clinical application is mainly limited by its low therapeutic index, low bioavailability, and extremely low solubility in water. Paclitaxel-loaded solid lipid nanoparticle (SLN) has been formulated[3)] employing triglyceride, lecithin and PEG_{2000} for the surface modification of steric stabilization. Although SLNs are known to be suitable as an intravenous delivery system for paclitaxel in terms of particle size,[3-5)] only a few research articles have been published with regard to *in vivo* evaluation of such delivery systems.[2)]

In the previous study, we formulated intravenously injectable lipid nanosuspensions based on solid lipid carriers without toxic excipients and evaluated the blood-concentration profiles in rats. Lipid nanosuspension is a dispersed system in which drug-loaded particles are stably suspended in an aqueous phase and was selected for the parenteral delivery system of paclitaxel. It consisted of glyceryl palmitostearate as a core material, and lecithin, poloxamer and Vitamin E TPGS as surfactants. In this study, we compared the temperatures during the manufacturing processes of a hot dispersion technique and a solidification of a lipid-drug mixture using liquid nitrogen, and we focused on the biodistribution of the formulations after intravenous administration in rats.

Materials and Methods

Materials

Paclitaxel (Genexol®, Lot. G127, Chromatographic Purity 99.53%) was obtained from Samyang Genex (Daejeon, Korea). L-a-Lecithin and Baccatin III (internal standard in LC/MS/MS measurement of paclitaxel) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Poloxamer 188 was obtained from BASF (Mount Olive, NJ, USA). Precirol® ATO 5 (glyceryl palmitostearate) and Vitamin E TPGS (d-a-tocopheryl polyethylene glycol 1000 succinate) were provided by Gattefosse (Saint-Priest Cedex, France) and Eastman (King-

†본 논문에 관한 문의는 이 저자에게로
Tel : 02)820-5609, E-mail : ywchoi@cau.ac.kr
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sport, TN, USA), respectively. All other chemicals and solvents were of reagent grade and used without further purification.

Preparation of Lipid Nanosuspension of Paclitaxel (PxLN)

PxLN was produced using a homogenization technique reported previous[6] and elsewhere.[7] Briefly, for the hot method (PxLN-H), the aqueous phase containing surfactants (poloxamer and Vitamin E TPGS) was added to the melted lipidic phase containing paclitaxel and lecithin, and the mixture was sonicated to produce coarse pre-emulsion with a texture of milky suspension. The emulsion was then passed through a microfluidizer (10,000 psi, 7 cycles, Microfluidizer® M-110S, Microfluidics, Newton, MA, USA), resulting in clear nanosuspension. The nanosuspension was cooled down under the ambient temperature and then stored in a fridge. For the cold method (PxLN-C), the melted lipid mixture was solidified in liquid nitrogen and ground to produce microparticles. The resultant was then dispersed in an aqueous surfactant solution and homogenized at the temperature between 5 and 10°C.

***In Vitro* Drug Release**

A dialysis bag (MWCO 12,000-14,000, Spectra/Por®, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) containing PxLNs was placed into 10% ethanol solution maintaining perfect sink conditions. The sample container in the shaking incubator (Jeiotech, Seoul, Korea) was maintained at 37±0.5°C and shaken at 150 rpm. Samples were withdrawn from the receptor medium at predetermined time intervals and assayed for the drug by the validated HPLC/UV as published.[6]

Animal Study

Male Sprague-Dawley rats (weighing 200-250 g, 4-6 weeks old, Hanrim Animals, Suwon, Korea) were used for this study. The animals were allowed to acclimate to the environment maintained at 21-25°C and 45-65% relative humidity in an animal care room for over 1 week before the experiment. Care and handling of animals were in accordance with the guidance of Korea Food and Drug Administration (KFDA). Rats were fasted for over 10 h before drug administration and anesthetized lightly with diethyl ether prior to operation. Femoral vein and artery were cannulated with polyethylene tube (i.d. 0.5 mm, o.d. 0.58 mm). PxLN-H, PxLN-C and Taxol® at a dose of 5 mg/kg as paclitaxel were administered by intravenous injection for 30 sec. Blood samples (200 μl) were collected from the cannulated artery at predetermined time points.

For the body distribution of Px, rats were sacrificed at times of 2, 4, 6, 8 and 12 h after iv injection. Liver, brain, lung, heart, kidney, spleen, and testis were carefully taken at the predetermined times. All organ samples were accurately weighed and homogenized in 4-volume of drug extraction solvent mixture (acetonitrile : 35 mM ammonium acetate buffer = 3:1). Paclitaxel amount in the tissue homogenates was analyzed after extraction.

Analysis of Paclitaxel in Biological Matrices

An aliquot (20 μl) of internal standard (Baccatin III 5 μg/ml in methanol) was added to each blood sample and vortexed with 2-volume of acetonitrile followed by centrifugation for 10 min at 12,000 rpm. The supernatant (400 μl) was transferred to Eppendorf tube containing 2-volume of water. The sample (20 μl) was analyzed using LC/MS/MS system with slightly modification[6] of published method.[8]

Results

Preparation and *In Vitro* Characterization of PxLN

Paclitaxel-loaded lipid nanosuspension (PxLN) was successfully prepared by the homogenization method as shown in Table I.[6] The drug content in the lipid nanosuspension and drug loading efficiency was 1.42 mg/ml and 71.2±4.3% of the initial drug amount, respectively. The 99% accumulated particle size was below 200 nm for PxLN-H, indicating that it was suitable to directly inject through iv route without causing capillary embolism.

For the preparation of PxLN-C, once the particles were formed with sizes of up to about 10 μm after manual mortar milling in liquid nitrogen. It was greatly reduced down to nanometer sizes by performing a high pressure homogenization with a microfluidizer (10,000 psi and 7 cycles) at 40°C. PxLN-C, PxLN prepared by the cold method, had mean particle size of 500-800 nm. The average zeta potential of -41.5 mV for PxLN-H was sufficient to exhibit the physical stability. In fact, the particle growth was not observed for more than 6 months when stored under a refrigerated condition. The zeta potential of PxLN-C showed similar ranges to that of PxLN-H prepared by a hot method. The appearance of the resulting

Table I–*The Effect of Preparation Methods on the Average Particle Size and Zeta potential*

Method	Particle size (nm)	Polydisperse index	Zeta potential (mV)
Hot homogenization	72.1±26.3	0.32	-41.5±4.7
Cold homogenization	683.7±179.8	0.37	-38.6±4.8

system was milky suspension, while PxLN-H was transparent suspension. Drug content in PxLN-C was comparable to PxLN-H. The difference in manufacturing processes, i.e., a hot dispersion technique and a cold dispersion technique hardly had an influence on the drug loadings. Thus, PxLN-H with good nano-particle size was surely suitable for *in vivo* studies.

In Vitro Paclitaxel Release

Prior to studying *in vitro* drug release, the measurement of an equilibrium solubility of paclitaxel (M.W. 853.9, Log P 3.5) was performed to determine a release medium in pH 7.4 phosphate buffered solution (PBS), PBS containing 0.1% Tween 80, 10% ethanolic solution, or 5~10% Diluent 12 (Cremophor® EL:ethanol, 1:1, solvent mixture used as paclitaxel solubilizer in Taxol®). An empty dialysis bag (MWCO 12,000-14,000, Spectra/Por®, Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA) and excess amount of paclitaxel bulk powder were placed in each medium. Samples were collected from the inner side of the dialysis bag after shaking for 24 h with the release rate of 150 rpm at 37±0.5°C. The paclitaxel concentration in the samples was measured by the validated HPLC.

The solubility of paclitaxel in PBS was determined to be 1.82 μg/ml. The drug solubility in PBS containing 0.1% Tween 80, 5% Diluent, 10% Diluent and 10% ethanolic solution were 2.01, 24.73, 116.86 and up to almost 200 μg/ml, respectively. Thus, 10% ethanolic solution was selected as a release medium, since it has the highest drug solubility among the medium tested.

Evaluation of *in vitro* drug release from the colloidal systems using a dialysis bag diffusion method has been frequently used by many research groups.[9,10] Therefore, the diffusion method was considered as a suitable methodology for our current study.

The drug release profile obtained from a dialysis bag diffusion technique is presented in Figure 1. Taxol® and PxLN-H demonstrated a similar release pattern, and the release rates of paclitaxel from Taxol® and PxLN-H were much faster than PxLN-C. For PxLN-C, after 12 h of the release experiment, only 12% of the initially loaded paclitaxel was released, compared to over 30% release from PxLN-H formulation.

Pharmacokinetic Behaviors and Biodistribution of Paclitaxel

After iv administration of PxLNs and Taxol® formulations, plasma concentration time profiles were shown in Figure 2. The concentration profile of PxLN-H was higher than that of Taxol®. The area under the blood concentration-time curve (AUC) of PxLN-H was 3.4-fold higher than that of Taxol®; $AUC_{0\text{-}t}$ (μg/ml·h) of PxLN-H and Taxol® was 15.03 and 4.40, respectively.

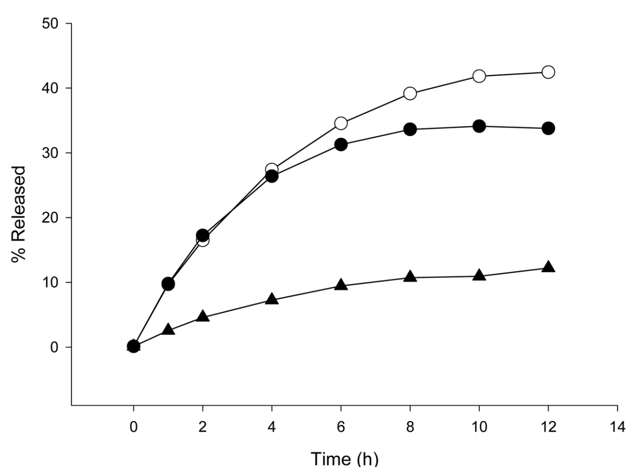

Figure 1–*In vitro* release profile of paclitaxel in 10% ethanolic solution when the dialysis bag diffusion technique was employed. Each point represents mean±S.E. of 3 experiments (○; Taxol®, ●; PxLN-H produced by hot homogenization, ▲; PxLN-C produced by cold homogenization).

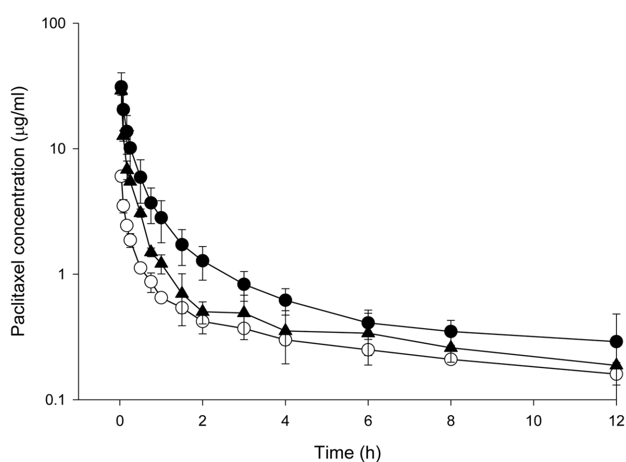

Figure 2–Blood concentration-time curve of paclitaxel after intravenous injection at a dose of 5 mg/kg as paclitaxel in rats. Each point represents mean±S.E. of 5 animals (○; Taxol®, ●; PxLN-H produced by hot homogenization, ▲; PxLN-C produced by cold homogenization).

The values of K_α in the distributive phase of paclitaxel were 5.13, 5.03, and 7.41 h for Taxol®, PxLN-H and PxLN-C, respectively. PxLN-C demonstrated faster distributive property while the other formulations showed similar values. PxLN-H showed considerably higher blood concentration at the first sampling time point (0.033 h, C*i*) than that of Taxol®. The val-

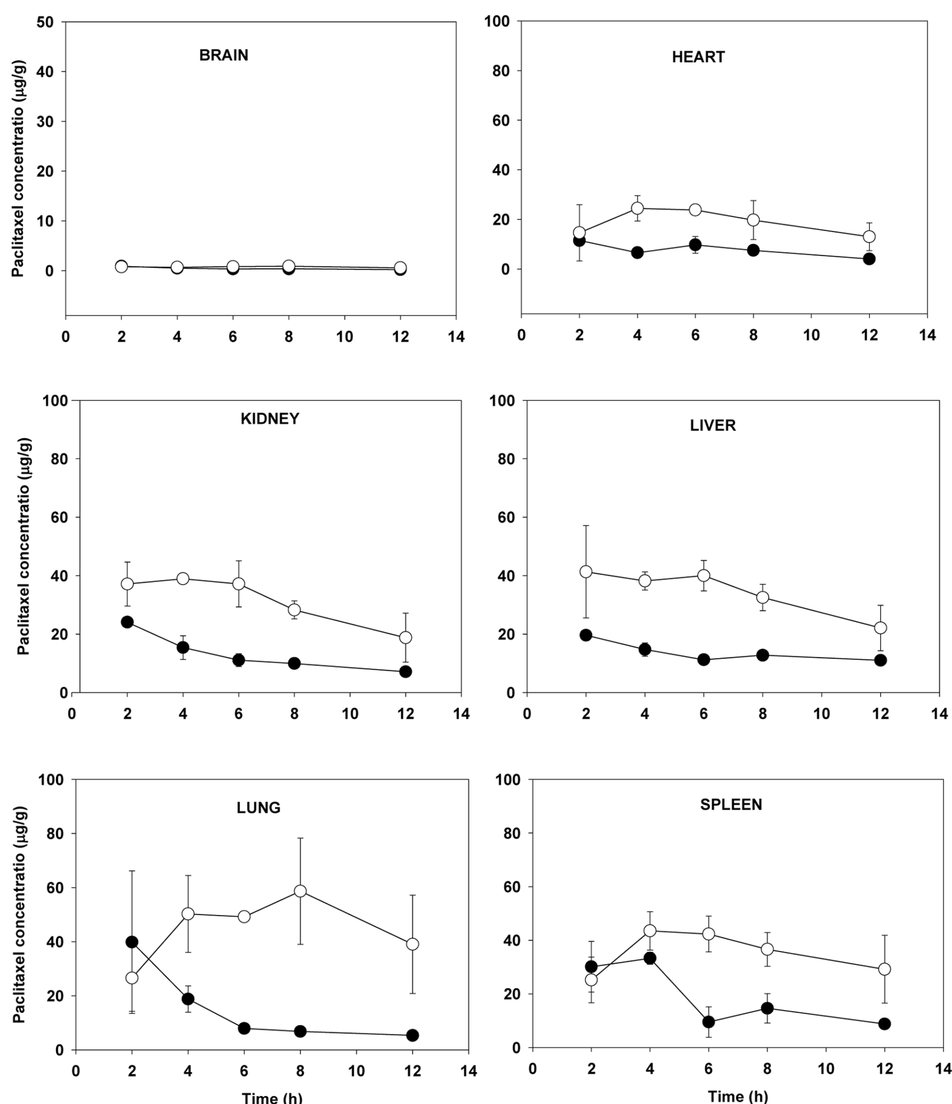

Figure 3. Paclitaxel concentration-time profiles in organs following intravenous injection of PxLN-H (○) and Taxol® (●). Each point represents mean±S.E. of 5 animals.

ues for Ci were 31.10 μg/ml and 29.13 μg/ml, and 6.03 μg/ml for PxLN-H, PxLN-C, and Taxol®, respectively. The elimination half-lives were 9.43, 12.33 and 7.24 h for Taxol®, PxLN-H and PxLN-C, respectively.[6)]

Since PxLN-H showed greater AUC than Taxol® formulation, we used PxLN-H formulations for biodistribution study rather than PxLN-C. Distributive profiles of paclitaxel in various organs after iv administration of PxLN-H and Taxol® are shown in Figure 3. Moreover, the F values (ratio of AUC_{PxLN}/$AUC_{Taxol^{®}}$) of each organs tested were presented in Figure 4. In all tested organs, the drug level in the group administered PxLN was higher compared to Taxol® group. The F was significantly increased compared to Taxol®, especially in blood and lung revealed 3-fold higher.

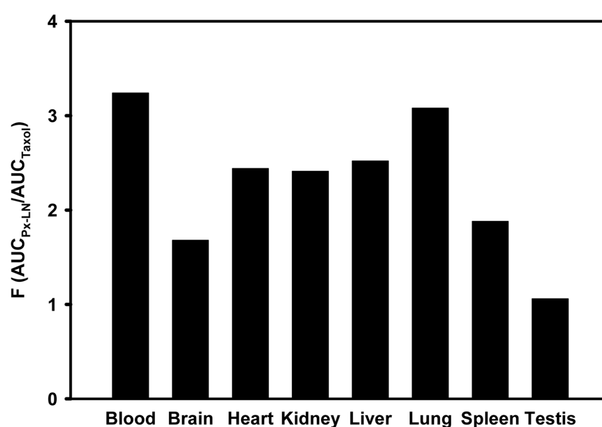

Figure 4. F values of PxLN-H compared with Taxol® after iv administration at a dose of paclitaxel 5 mg/kg.

Discussion

To develop a new parenteral delivery system for paclitaxel we formulated lipid nanosuspensions using Precirol® ATO 5 (glyceryl palmitostearate, a mixture of mono-, di-, and tri-palmitic acid (16:0) and stearic acid (18:0) esters of glycerol) as a lipid core material. Mixed triglycerides with mono- and diglycerides, such as Precirol, have been good drug carriers with respect to drug incorporation capacity due to their lower degree of crystalline order.[7,11] The mono- and diglycerides in the lipid used as a matrix material were reported to promote drug solubilization in molten state[12] as well as a solid matrix core.

Moreover, additional solubilizers can be added to further enhance drug solubility in the molten lipid for higher drug loading.[13] In the previous report,[14] it was not possible to prepare stable emulsions using pure phosphatidylcholine without co-surfactants. This might be the result of the absence of phosphatidylethanolamine existed as impurity in pure lecithin. Thus, we used a mixture of surfactants and co-surfactants, along with hydrophilic polymers in order to increase physical stability and/or drug entrapment. Greater particle size of PxLN-C was caused by the relatively low temperature compared to PxLN-H. Higher pressure and/or more passages should be considered to get smaller particles under 100 nm when using a cold method. This cold homogenization technique could be used for the temperature-sensitive drugs and peptide drugs for antigen delivery.[15]

Overall release profiles of Taxol® and PxLN-H were similar to each other, except PxLN-C. Especially, up to 4 h, the release profiles of Taxol® and PxLN-H were quite similar. Since the average particle size of PxLN-H was very small (<72.1 nm), the release property of PxLN-H was resembled to that of Taxol®, the solubilized formulation of paclitaxel. Small particle size was enabling to provide the large surface area available for paclitaxel release. Loosened lipid structures created by the preparation at higher temperature and the slower cooling step in the ambient temperature, could be another explanation to the fast release. Combination of surfactants and co-surfactants could also help drug release from the surface of lipid particles. After 4 h, paclitaxel entrapped into the core lipid began to release, however the amount was lesser than before. Since the experimental condition was 37°C and the melting point of lipid was around 55°C, it was thought to be hard or to need some time for paclitaxel to be released from the solid or supercooled lipid core.

The paclitaxel release from PxLN-C was quite slow compared to PxLN-H, due to relatively larger particle size and/or rigid and ordered structures of lipid particles since the drug-lipid mixture was solidified by liquid nitrogen when prepared. The melting point of Precirol is known as 53-57°C[16,17] and this is relatively higher than that of the release medium maintained at 37±0.5°C. Therefore, the particles were considered to have well packed-surface compared to that of PxLN-H. Low hydrophilic lipophilic balance (HLB) value of 2 could also be one of possible explanations.

Another possible explanation can be achieved from Wenk and co-worker's study.[18] They observed extremely high liposome-water coefficient of 9500. A partition enthalpy of ΔH = -25±3 kcal/mol^{-1} was calculated from the binding studies. A temperature increase of 10°C reduces the paclitaxel solubility in the lipid phase by a factor of 4 due to paclitaxel exhibits large negative binding enthalpies. When the drug was made at lower temperature, it could be well encapsulated in to the lipid because of increased solubility.

When solid lipids were used as a drug carrier material, whole blood was usually taken for determination of drug concentrations rather than plasma.[10,19] Generally, since the release of lipophilic drugs from the lipid carriers is slow, and the metabolism of the drug incorporated in the lipid due to drug metabolizing enzymes and other metabolizing components in blood is prohibited by the protective effect of the core material, it is assumed that high concentration of the drug present in solid cores can be maintained for a longer period of time and higher concentration in blood compared to solubilized formulations like Taxol®. Thus, although a disadvantage of measuring whole-blood drug levels in liposomal formulation was reported that liposome-incorporated drug cannot be distinguished from the released drug, and the released drug could consist of free-, protein-bound-, and cell-associated fractions,[20] whole blood containing the lipid particles and drug was chosen to analyze the drug. Low drug concentration in plasma due to extremely low aqueous solubility and high plasma protein binding property (88-98%) could be another limit in the HPLC analysis.

PxLN-H had a narrow particle size range in a nanometer scale and the surface of the carriers was coated with a hydrophilic polymer. Therefore, these nanometer-sized particles possessing hydrophilic surface could lead to a long circulation in the blood stream.[19]

It has been reported that the paclitaxel appeared initially in plasma is rapidly cleared in spite of extensive plasma protein binding property of paclitaxel.[1] Similar phenomenon was observed from PxLN formulations as well as Taxol®. K_α was similar to PxLN-H and Taxol®, however, PxLN-C showed faster distribution than other formulations

Although Taxol® and PxLN-H displayed overall similar blood concentration profiles and comparable $t_{1/2\alpha}$ (half-life in the distributive phase) values, it was considered that the mechanism of distribution between them were somewhat different. The $t_{1/2\alpha}$ of Taxol® was 0.135 h, probably resulting from fast distributive property of paclitaxel itself. PxLN-H had also a similar $t_{1/2\alpha}$ value of 0.138 h. It might be considered that the drug carriers, rather than the released paclitaxel, tended to distribute directly to the tissues (Figure 3, 4). As PxLN-H is very small particulated carriers, the carriers themselves were distributed to the organs due to the protection of paclitaxel by a lipid core. Thus, the relative drug amount in the blood was retained significantly for a longer period and PxLN-H was showed a higher blood concentration profile. Fetterly and Straubinger[20] showed pharmacokinetic parameters from liposomal paclitaxel in rats. They compared Taxol® formulation to liposomal paclitaxel. AUC and half-life of elimination were similar to each other. Half-life of distribution for liposomal paclitaxel, however, was approximately 10-fold greater than that of Taxol® formulation. The distribution clearance for Taxol® was more than 6-fold greater than for liposomal paclitaxel. These results suggest that the kinetics of drug transfer from the central compartment to the peripheral tissue compartment is delayed for liposomal formulation. In other words, the release of paclitaxel from liposomes is not instantaneous upon administration.

PxLN-H showed considerably higher blood concentration at the first sampling time point (0.033 h) than that of Taxol®. The distributive behavior of paclitaxel from Taxol® formulation is fast according to a previous report[1] and the Ci (the initial concentration at the first sampling point) decreased quickly after iv injection. Since the release of paclitaxel from PxLN was slow process owing to the highly lipophilic core material, it did not exhibit a fast distributive property of intact paclitaxel. The drug carriers were viscous due to the peculiar character of the lipid, so that they seemed to exist as physical associates with the blood cells. The higher initial concentration of PxLN-H was resulted from the assay of paclitaxel in the whole blood including these associated forms. It was, therefore, anticipated that they were distributed to the tissues as the associated forms and released the drug. PxLN-C was distributed rapidly compared to Taxol® and PxLN-H. It was presumably attributed to the uptake of larger particles by the RES organs.

The concentrations at the first sampling time point (0.033 h, Ci) of PxLNs showed higher blood levels than Taxol®. Yang et al.[10] have reported an analogous result that the higher blood concentration of camptothecin after iv injection of camptothecin-SLN suspension was observed compared with camptothecin-solution. They interpreted their findings by assuming that it might be due to the small size of nanoparticles or association with the lipid bilayers of red blood cells. Since we analyzed the drug in the whole blood, the paclitaxel from the drug-lipid carriers associated with blood cells could be assayed as paclitaxel.

The extended circulation of paclitaxel in the blood stream was achieved by PxLN-H. The surface coating of PxLN with a hydrophilic moiety (e.g., poloxamer) and the submicron size of PxLN seemed to contribute to the sustained release of paclitaxel. It has been reported that the inhibition of tubulin isolation was activated at the paclitaxel level of ca. 85 ng/ml.[1] The C_{12h} of Taxol® and PxLN-H was measured to be 163 ng/ml and 290 ng/ml, therefore, the times showing the concentrations below 80 ng/ml were 24 h for Taxol® and 36 h for PxLN-H, as calculated by the half-life of each formulation.[6]

Biodistribution profiles of paclitaxel in various organs after iv administration of PxLN-H and Taxol® are shown in Figure 3. In all tested organs, the drug level in the group administered PxLN-H was significantly higher compared to Taxol® group. The tissues of the reticuloendothelial system (RES: liver, spleen, and lung) comprise the major route of clearance for the particulated carriers.[20] Therefore, one would expect drug deposition in the RES tissues to be significantly greater for PxLN-H formulation than for Taxol®. We also observed high levels in all RES tissues.

The F values in liver, spleen, lung, kidney and heart were above 2 (Figure 4). Since PxLN showed 2-times higher concentration in all organs, the dose of paclitaxel in the formulation could be reduced as half as Taxol®, the commercial drug.

In spite of small particle sizes less than 100 nm, which was not generally regarded as inducing the lung capillary embolism, the lung AUC of PxLN-H was greater than that of Taxol®. When Taxol was administered iv injection, the lung showed the highest concentration among the tested organs as other publications.[1,20] Spencer et al. [1] have reported that paclitaxel was predominantly distributed to the liver, lung, spleen, pancreas, heart, muscle and kidney, but was not found in the nervous system or testis in rodents, after iv administration of Taxol®. In spite of extensive plasma protein binding of paclitaxel, the drug was rapidly distributed to those organs. Other investigators showed similar results.[20,21] Highest level in lung might be due to the lipidic particles that could interact with other components in blood and then aggregate in the narrow lung capillary, or adhesive property of lipids to tissue because of their sticky nature.

Yang et al.[10] reported biodistribution of camptothecin-SLN (CA-SLN) in mice after iv injection. Similar to our results, the lung AUC of CA-SLN was the highest among the tested organs such as lung, liver, heart, spleen, kidney, and brain. They suggested that this might be mainly due to accumulation in RES organs although the mean particle size was 196.8 nm and zeta potential value ranged -45.2~-69.3 mV. The AUC of tested tissues was increasing order; lung, liver, heart, spleen, brain, and kidney.

It might seem natural phenomenon that this sticky formulation using lipid nanosuspension should be accumulated in the RES organs. PxLN for the specific target organ such as liver, might need further surface modification to increase specificity.

Conclusions

Paclitaxel was successfully incorporated into the lipid nanoparticles with a good physical stability. PxLN formulations containing paclitaxel in their lipid core showed an increased bioavailability in rats. PxLN demonstrated a 3-fold higher AUC and a 1.3-fold longer elimination half-life compared to Taxol® after intravenous administration. A prolonged residence in various tested organs was achieved after administration of PxLN compared to Taxol®. Moreover, the low dose of PxLN formulations could be sufficient to achieve the identical therapeutic effect to that shown by Taxol®. The PxLN formulations, therefore, can be a promising candidate as an alternative carrier system for the parenteral delivery of poorly water-soluble drugs, such as paclitaxel.

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