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Preparation and Stability Evaluation of Docetaxel-Loaded Oral Liposome

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ABSTRACT – Docetaxel-loaded liposomes were prepared by emulsion-solvent evaporation method, then coated with chitosan at room temperature and lyophilized. This system was designed in order to improve solubility and stability of docetaxel in the GI tract for oral drug delivery. The solubilizing effect of some frequently used solubilizers and/or liposome was determined. Among the results docetaxel-loaded liposomes prepared with 0.5% TPGS as a solubilizer showed 100-fold higher solubility than docetaxel. In a stability test, mean particle size of different liposome formulations was measured by a particle size analyzer in simulated gastric fluid (SGF) and in simulated intestinal fluid (SIF). The particle size of uncoated liposomes was significantly increased compared with that of chitosan-coated liposomes in SGF, however, there was no significant difference between coated and uncoated liposome in SIF. It is evident that chitosan-coated liposomes were more stable in GI conditions. The release characteristics of docetaxel-loaded liposomes were also investigated in three buffer solutions (pH 1.2, 4.0, 6.8). Docetaxel release did not occur in pH 1.2 for 4 hrs. However, in pH 4.0 and 6.8 conditions, docetaxel was gradually released over 24 hrs as a sustained release. It seems that aggregation and precipitation of particles by electrostatic interaction might protect docetaxel from being released. In Conclusion, the results from this study show that the chitosan-coated liposomes may be useful in enhancing solubility and GI stability of docetaxel.

Key words – Docetaxel, Oral dosage form, Liposome, Chitosan-coated liposome

Liposomes are one of the most widely used drug delivery systems. They are spherical vesicles, consisting of one or more lipid bilayers, which can encapsulate hydrophobic molecules in the bilayer membrane or hydrophilic compounds in the aqueous internal cavity. Therefore liposomes have attracted considerable attention as potential vehicles for drug delivery to selected cells or tissues in vivo.[1-3] Many studies have shown that a wide variety of therapeutic and diagnostic agents can be incorporated into liposomes. Today, particulate systems, such as liposomes, were studied as a potential oral dosage form for such peptide or protein drugs.[4-5] However, for the successful introduction of oral liposomal formulations in drug therapies, much attention must be paid to their stability, due to liposomes' tendency to degrade or aggregate and fuse, which leads to leakage of entrapped drug during storage or after administration.[6]

Docetaxel (TAXOTERE™) is an anticancer agent approved for the treatment of locally advanced or metastatic breast cancer, non-small-cell lung cancer, hormone refractory prostate cancer, and advanced gastric cancer.[7] Currently, parenteral formulations are available for its clinical use while oral administration is still limited because of its low oral bioavailability.[8-9] It is well known that the low oral bioavailability of docetaxel is mainly due to cytochrome P450 (CYP) activity and drug transporters, such as P-gp in gut wall and liver.

Chitosan is a derivative of natural amino polysaccharide obtained by deacetylation of chitin. Due to its biocompatible, biodegradable and mucoadhesive nature, chitosan has been widely used in providing controlled release and improving bioavailability of many drugs.[10] Especially, chitosan molecules have shown to be effective in protecting peptide/protein drug substances from enzymatic degradation in GI tract.

In the present study, we investigated the possibility of docetaxel-loaded liposomal formulations as an oral drug delivery system. Liposomes were prepared by emulsion-solvent evaporation method. To overcome instability of liposomes in GI conditions, chitosan, as a stabilizer, was coated on liposome surface, then physico-chemical properties of liposomes were estimated.

Materials and Methods

Materials

Decetaxel was purchased from Techwell Biopharmaceutical Co., Ltd. (China). Hydrogenated soybean phosphatidylcholine (HSPC), 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphatidylcholine (DPPC), 1,2-distearoyl-sn-glycero-3-phosphatidylcholine (DSPC), 1,2-

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distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), and 1,2-distearoyl-sn-glycero-3-phospho-(1'-rac-glycerol) sodium salt (DSPG-Na) were purchased from Lipoid GmbH (Germany). Chitosan was kindly provided from by Biosyntech Inc. (Canada). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was obtatined from Cognis Korea Co., Ltd (Korea). Chloroform and ethanol were HPLC grade and supplied by Fisher Scientific Korea Ltd. (Korea). All other chemicals were reagent grade and used without further purification.

Solubilization of Docetaxel

The solubility of docetaxel was determined to evaluate the solubilizing effect of frequently used solubilizers. An excess amount of docetaxel was added into each of the 10 mL of aqueous solutions containing 0.5% (w/v) Tween80, Cremophor EL, Poloxamer188, PEG200 and TPGS, respectively. Resulting mixture solutions were vigorously vortexed for 30 min and then allowed to stand at room temperature for 7 days. The mixtures were centrifuged at 10,000 rpm for 10 min and the supernatants were analyzed using HPLC.

Preparation of Liposomes

Liposomes were prepared by modified emulsion-solvent evaporation method. First, an oil in water (o/w) emulsion was prepared by homogenizing 60 mL of immiscible solvent including docetaxel, phospholipids and cholesterol as the dispersed phase with 90 mL of aqueous solution containing a solubilizer as the continuous phase. Second, the organic immiscible solvent (chloroform) was removed from o/w emulsion by evaporating under reduced pressure. As a result, the transparent docetaxel-loaded liposome suspension was obtained.

Chitosan Coating

Docetaxel-loaded liposomes were coated with chitosan. Chitosans (MW 5,000~1,000,000) were dissolved in acetate buffer (pH 4.0) and phosphate buffer (pH 5.0 and 6.0) solution, respectively, then was mixed with an equal volume of liposome suspension at room temperature for 1 hour.

Freeze Drying

After liposome preparation, sucrose as a cryoprotectant added to uncoated or chitosan-coated liposome suspension. Each uncoated or chitosan-coated liposome suspension containing ryoprotectant was frozen at -70°C for 24 hours and then was lyophilized to remove water and residual solvent to afford dry liposome powders.

Characterization of Liposomes

Morphological characteristics of liposomes were examined by transmission electron microscopic (TEM) image. Samples for TEM were prepared at room temperature by conventional negative staining methods using 0.3% phosphotungstic acid buffer (pH 6.0). The particle size and zeta potential of liposomes were measured by an electrophoretic light scattering spectrophotometer (ELS-Z, OTSUKA, Japan). Docetaxel concentration was measured by HPLC after dissolving liposomes in ethanol. Encapsulation efficacy (EE) was calculated by below equation:

$$\text{Encapsulation efficacy}(\%) = \frac{\text{Conc. of docetaxel after filtration}}{\text{Conc. of docetaxel before filtration}} \times 100$$

Stability of Liposomes in GI Conditions

To evaluate the stability of liposomes in GI conditions, docetaxel-loaded liposomes were mixed with simulated gastric fluid (SGF, pH 1.2) or simulated intestinal fluid (SIF, pH 6.8), and the mixtures were incubated on an orbital shaker at 37°C. After 2 hrs, mean particle size of liposomes was measured.

Stability of Liposomes on Storage

To estimate the stability of liposomes on storage, freeze dried powders of liposomes were put in a stability chamber under accelerated conditions (40°C, 75%RH) for 12 weeks. Physicochemical properties, such as particle size, zeta potential and docetaxel content, were determined at designated times. The release profiles of both uncoated and chitosan-coated liposomes were also determined at pH 1.2, 4.0 and 6.8, respectively. A bag of dialysis membrane (MWCO 3,500) filled with 50 mL of liposome suspensions was incubated in a pre-warmed dissolution vessel containing 450 mL of pH 1.2, pH 4.0 and pH 6.8 buffer, respectively. At a planned time, 1 mL of sample was taken and analyzed by HPLC.

Results and Discussion

Solubilization of Docetaxel

Solubility is one of the most important parameters to achieve a desired concentration of drug in systemic circulation. Oral bioavailability can be improved significantly by solubilization study especially in poorly water soluble drugs like docetaxel which has low solubility of 8.0±0.9 μg/mL in water. In this study, the solubilizing effects of some frequently used surfactants were determined. The results were summarized in

Table I–*Solubility of docetaxel combined with various solubilizers in water and liposome suspension*

	Components	Solubility (μg/mL)
DW	Docetaxel	8.0±0.9
	Docetaxel+Tween80 0.5%	131.0±11
	Docetaxel+Cremophor 0.5%	44.6±3.2
	Docetaxel+Poloxamer188 0.5%	37.4±2.9
	Docetaxel+PEG200 0.5%	61.7±5.0
	Docetaxel+TPGS 0.1%	37.2±4.1
	Docetaxel+TPGS 0.5%	127.1±8.9
	Docetaxel+TPGS 1.0%	165.7±9.4
	Docetaxel+TPGS 5.0%	321.6±20.6
Liposome	Docetaxel	44.4±16.4
	Docetaxel+TPGS0.5%	800.4±40.1

Each value represents the Mean±S.D. (n=3)

Table I. Improvement of solubility was observed in formulations containing any solubilizer used. The solubility of docetaxel was increased significantly, especially, in two formulations containing 0.5% of Tween 80 or TPGS, to the extent of 131.0±0.9 and 127.1±8.9 μg/mL, respectively. Liposome technique gave 5 times more solubility. A combination of liposome technique and 0.5% TPGS showed synergistic effect on the solubility of docetaxel up to 100-fold. Some literatures stated that TPGS was able to increase the encapsulation efficacy of drug in liposome and improve the stability of liposome structure.[11-12] Nevertheless, additional study is needed to find the cause of the significant change of docetaxel solubility.

Characterization of Liposomes

The size of liposomes was increased 2~5 times larger than the initial size of about 100 nm by increasing the chitosan concentration from 0.05~0.5%. Transmission electron micrographs of docetaxel-loaded liposomes obtained from conventional negative staining method showed that uncoated liposomes were spherical particles while chitosan-coated liposomes were surrounded with chitosan layers on their surface(Figure 1).

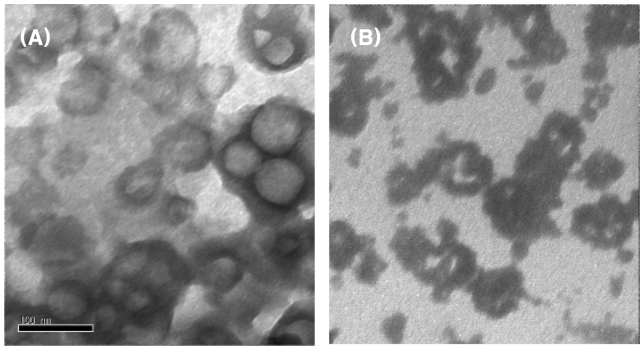

Figure 1–Transmission electron micrographs of plain liposomes(A) and chitosan-coated liposomes(B) in aqueous medium at X 100K magnification.

Regardless of the composition of phospholipids and cholesterols, high EEs in the range of 85~100% were obtained in all formulations (Table II). Even though it is known that cholesterol liposomes show better stability, the drug release rate can be reduced significantly.[10] Therefore, the molar ratio of phospholipid/cholesterol was fixed at 9 : 1.

In order to improve the stability of liposomes in GI, the liposomes were coated with chitosan. After chitosan coating, physicochemical properties of liposomes were measured to optimize coating conditions and ratio. The particle sizes of chitosan-coated liposomes in each formulation were increased by increasing the concentration of polymer solution. The measured zeta potentials of uncoated liposomes were -33 to -40 mV, while those of chitosan-coated liposomes were 34 to 37 mV, suggesting the presence of coated layer on the surface of lipo-

Table II–*Composition of docetaxel-loaded liposomal formulations and their entrapment efficiency*

Components (mmol)	F1	F2	F3	F4	F5	F6	F7
Docetaxel	10	10	10	10	10	10	10
HSPC	60					60	60
DMPC		60					
DPPC			60				
DSPC				60			
DSPE					60		
DSPG	30	30	30	30	30	30	30
Cholesterol	10	10	10	10	10	30	50
Entrapment Efficacy (%)[a]	99.4±1.1	92.1±2.1	91.1±1.1	91.6±1.8	88.5±2.3	92.4±2.0	91.6±1.4

[a]Each value represents the Mean±S.D. (n=3)

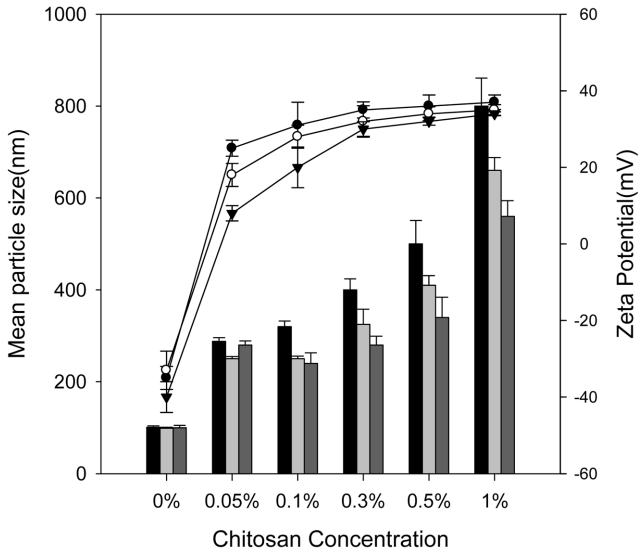

Figure 2—Physical properties of chitosan-coated liposomes in various acidic condition. Chitosan-coated liposomes were prepared by mixing docetaxel-loaded liposome (F1 formulation) and various concentration of chitosan solution. Bar chart shows mean particle size (left y axis; ■:pH 4, ■:pH 5, ■:pH 6) and line chart represents zeta potential(right y axis; ▼:pH 4, ○:pH 5, ●:pH 6). Each point represents the Mean±S.D. (n=3).

somes. Since chitosan carries highly positive charge, the adsorption of chitosan on the negatively charged surface of liposomes increased the density of positive charge and caused the reversal of the zeta potential. Also there was no change in the zeta potential over 0.3% concentration, which can be explained by the saturation phenomenon of all the available negative charges (Figure 2).[4] These positively charged chitosan molecules can strongly interact with negatively charged mucosal surface through electrostatic force. Therefore the residence time of drugs may be prolonged and thus, the bioavailability would be increased after an oral administration.

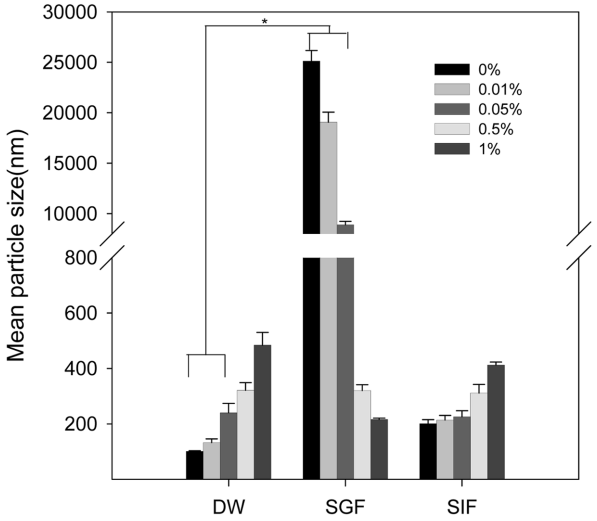

Figure 3—Stability of docetaxel-loaded liposomes prepared with different coating ratio of chitosan in water (DW), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF). Docetaxel-loaded liposomes (F1 formulation) were prepared without TPGS addition. *: $P<0.01$

Stability of Liposomes in GI Conditions

In order to evaluate the stability of liposomes in GI, particle sizes of liposomes were measured in DW, SGF and SIF. Diameters of liposomes were hundreds nanometer in DW. However, the mean diameters of low coated or uncoated liposomes were significantly increased to microns in SGF. On the other hand, there was no significant change between coated and uncoated liposomes in SIF (Figure 3).

Stability of Liposomes on Storage

Docetaxel-loaded liposomes (F1 formulation) and their chitosan-coated liposomes were taken, and their physicochemical stability was evaluated after freeze drying at accelerated conditions (40°C, 75%RH) for up to 12 weeks (Table III). Initially, the mean diameters of both uncoated and chitosan-coated lipo-

Table III—*Stability of freeze-dried liposome powder containing docetaxal in accelarated storage condition(40°C, 75%RH)*

	Time (weeks)	suspension	Freeze-Dried powder					
			Initial	1	2	4	8	12
Uncoated liposome	Contents (%)	100	102.0±1.9	99.5±1.6	101.5±1.4	100.4±1.0	98.8±2.1	99.6±2.0
	Particle size (nm)	101.1±2.3	367.5±26.4	337.4±32.1	351.2±11.7	370.5±15.4	349.4±14.5	389.4±16.6
	Zeta potential (mV)	-37.3±4.2	-21.4±1.6	-16.5±2.6	-19.4±1.8	-22.0±1.5	-20.4±0.5	-19.9±3.0
Coated liposome	Contents (%)	100	101.2±1.7	102.1±1.8	101.4±2.0	98.8±1.6	99.4±1.9	100.1±0.9
	Particle size (nm)	341.4±44.0	604.6±25.4	594.4±31.0	602.8±25.7	600.1±15.4	610.0±37.6	614.4±42.6
	Zeta potential (mV)	32.5±3.4	25.2±4.1	27.4±2.1	24.7±3.4	27.4±2.5	25.0±5.1	26.1±3.7

Particle size and zeta potential were measured after suspending 1g of dried powder into 10 mL of water.
Each value represents the Mean±S.D. (n=3)

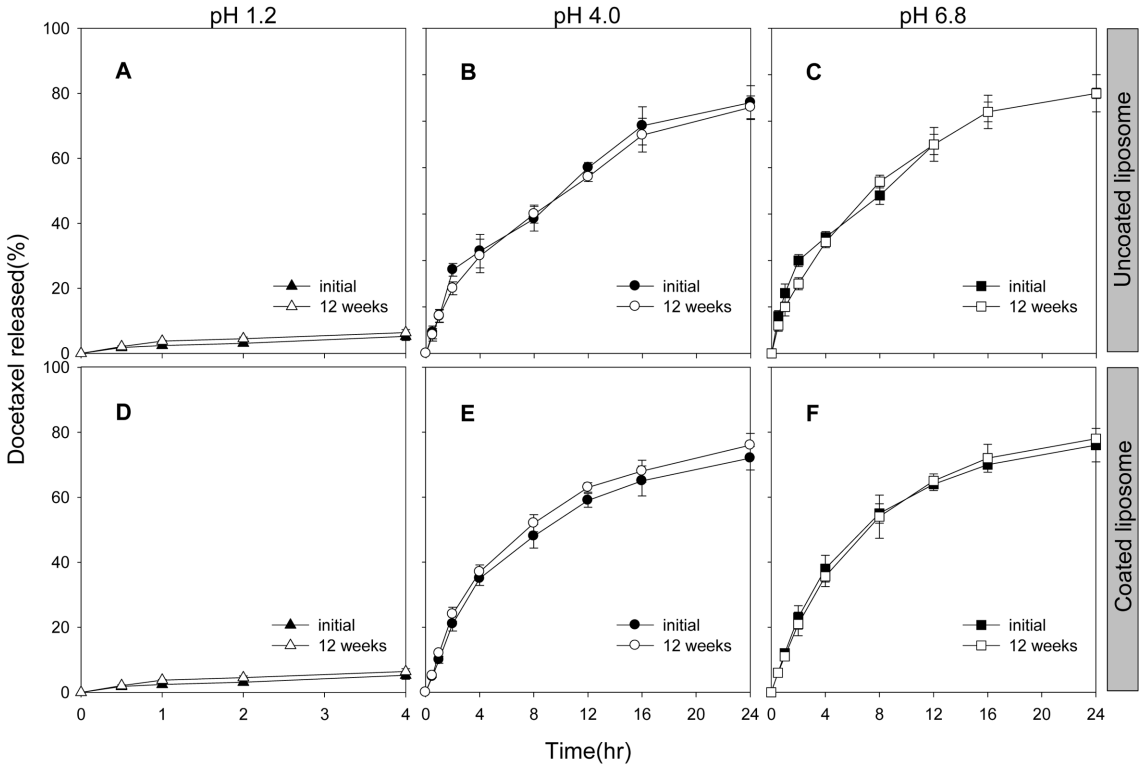

Figure 4–Dissolution profiles of uncoated and chitosan-coated liposomes containing docetaxel at 37°C. Upper windows(A~C) show dissolution characteristics of uncoated liposomes and lower windows(D~F) appear dissolution characteristics of chtosan coated liposomes in different gastrointestinal conditions during the storage stability test(accelerated condition, 40°C 75%RH) for 12 weeks. Regardless chitosan coaing, all formulations have contained 0.5% TPGS.
Each point represents the Mean±S.D. (n=3).

some suspension were 101.1±2.3 nm and 341.4±44.0 nm and the zeta potentials of them were -37.3±4.2 mV and 32.5±3.4 mV, respectively. Even though these properties were slightly changed upon reconstitution in water for characterization, we considered liposomes were stable since no significant changes in EE were observed during the course of stability study. At 12 weeks, the mean diameters and zeta potential of uncoated liposomes were 389.4±16.6 nm and -19.9±3.0 mV, respectively, and that of chitosan-coated liposomes were 614.4±42.6 nm and 26.1±3.7 mV, respectively.

The release of docetaxel-loaded liposomes did not occur at pH 1.2. It seems that aggregation and precipitation of particles by electrostatic interaction protects docetaxel from being released. However, docetaxel showed a sustained release without burst effect over 24 hrs at pH 4.0 and 6.8 (Figure 4). Interestingly, the release of docetaxel from liposomes was observed for the liposomes at pH 1.2 and also as the pH was increased. This result is useful in that the low stability of docetaxel liposomes in a stomach does not necessarily limit the availability of this liposome system as an oral delivery system.

Conclusion

We investigated the possibility of docetaxel-loaded liposomes as an oral dosage form by determining their physicochemical properties. The manufacturing process of liposomes for large scale production was established by emulsion-solvent evaporation method and the optimized formulation for high encapsulation efficacy was obtained. The liposomal formulation together with 0.5% TPGS solubilizer increased the solubility of docetaxel 100 fold higher than docetaxel alone (up to 800.4±40.1 μg/mL). Chitosan coating was proved to be useful in improving the stability of liposomes in SGF and SIF. Moreover, the freeze drying method was effective in maintaining stability on storage. We believe that this research can provide useful methods and tools for solubilization of poorly water soluble substances and the stabilization of unstable drugs in GI.

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