**ABSTRACT SUMMARY**

Oral administration of docetaxel is still limited because of low bioavailability. In this study, we designed an efficient docetaxel-loaded liposome for oral administration. To improve the solubility and decrease toxicities, lyophilized Eudragit-coated liposomes were introduced as delivery system. The developed liposomal docetaxel formulation resulted in higher oral bioavailability compared to that of docetaxel suspension in rats. Docetaxel-loaded oral liposome would be useful for the therapy of many malignancies with further studies.

**INTRODUCTION**

Docetaxel (DTX) represents one of the most important chemotherapeutic agents with high potency against many solid tumors. However, docetaxel is insoluble in water which created difficulties in developing suitable injectable formulations. Currently, the most widely used form of docetaxel is a mixture of polysorbate 80 and dehydrated alcohol. This formulation leads to adverse drug reactions, hypersensitivity due to the solvent system. Therefore, development of alternative formulations is required to make the chemotherapeutics to achieve adequate antitumor effect and reduce side effects.

Oral delivery is the most convenient and safe route for drug administration. However, many drugs remain poorly available due to their instability in the gastrointestinal tract and low permeability in the intestinal mucosa. Among various drug delivery systems, liposomes are considered as an ideal alternative for the oral delivery of insoluble compounds. Oral liposomes may provide increased solubility and protection from the hostile environment in GI tract. Most significantly, the similarity between lipid bilayers and biomembranes and the relatively small size facilitate the oral absorption.

In this study, a pharmaceutically applicable oral liposomal formulation was developed to improve patient compliance, convenience and reduce toxicities. Various lipid compositions, coating material and cryoprotectant used in lyophilization were selected. Then characteristics of the delivery system for particle size, zeta potential and encapsulation efficiency were evaluated. The morphology examination, release test and pharmacokinetic studies were also performed to confirm their potential as oral drug delivery system.

**EXPERIMENTAL METHODS**

Liposomes were prepared by lipid film hydration method with 38.95mM EPC and 1mg DTX. To prepare Eudragit-coated liposomes, Eudragit L100 and Eudragit S100 were combined by a ratio of 4:1.

Trehalose and mannitol were used as cryoprotectant in lyophilization because they protect particle integrity and good appearance. Trehalose 20% (v/w) and mannitol 10% (v/w) were added as cryoprotectants to equal volume of Eudragit-coated liposome. The liposome samples in a vial were first frozen at -80°C for 3 days (Deep Freezer, Operon, Korea) and then lyophilized using a freeze-dryer (Operon, Korea) for 24 hours under vacuum.

Through TEM photographs(EF-TEM, EM 912 Ω, Carl Zeiss, Germany), DTX-loaded liposomes before coating, before lyophilization and after rehydration.

In vitro release of uncoated liposomes and Eudragit-coated liposomes were conducted in each of the different pH media. Solution 1 (Dissolve 2.0 g of sodium chloride in 7.0 mL of hydrochloric acid and water and fill water to 1000 mL, pH = 1.2) and Solution 2 (A mixture of phosphate buffer solution of pH 6.8 and water) were used to simulate the pH conditions of the stomach and small intestine, respectively, according to the Korean Pharmacopoeia IX (Dissolution Test).

The plasma DTX concentration was evaluated in pharmacokinetic studies. Free DTX formulated in Tween 80 and Eudragit-coated lyophilized liposomal formulation were compared after peroral administration.

**RESULTS AND DISCUSSION**

Liposomes composed of EPC showed smaller size and improved encapsulation efficiency than SPC. The mean diameter of DTX-Lipo was about 60 nm and zeta potential was about 20 mV because stearylamine having positive charge was used to prepare for coating.

Two types of Eudragit have pH-dependent solubility and mucoadhesive property, which can protect the entrapped drug from the acidic environment in the stomach and improve the oral absorption. By measuring the particle size(116nm), 0.5% concentration of Eudragit combination was used in all further studies.

The stability problems can be overcome by lyophilization with proper cryoprotectant and reconstitution immediately before use. As cryoprotectant, 20% trehalose and 10% mannitol were selected for further experiments based on the average size(205nm) and encapsulation efficiency(31.9%). The structure of respective formulations was shown from TEM images. The discrete particle and spherical structure are clearly visible (Figure 1).

The in vitro release study revealed the pH-dependent solubility of Eudragit that can induce the drug to release in small intestine to colon (Figure 2). Over a time period representing average gastric emptying time 17% of the drug was released from uncoated liposomes, which was in
contrast to the 10% of drug released from Eudragit-coated liposomes over the same time period. No significant difference between the formulations was observed at pH 6.8.

The concentration of docetaxel after i.v. administration at a dose of 10 mg/kg was decreased rapidly in 120 min. Free drug formulated in Tween 80 and liposomal formulation were administered peroral at dose of 20 and 10 mg/kg, respectively (Figure 3). After oral administration, the Cmax and tmax at a dose of 20 mg/kg docetaxel suspension were 0.011 µg/mL and 110 min, respectively. The corresponding values for the dose of 10 mg/kg liposomal docetaxel were 0.010 µg/mL and 90 min, respectively. The values of t1/2 for each formulation were 566 and 818 min. On the other hand, AUC values were not significantly different despite smaller dose. Thus, the developed liposomal docetaxel formulation resulted in a threefold higher oral bioavailability in rats compared to that of oral docetaxel suspension.

Figure 1. TEM images of various liposomal formulations: (A) liposomes containing docetaxel, (B) Eudragit-coated DTX liposomes and (C) lyophilized Eudragit-coated DTX liposomes.

Figure 2. Drug release profiles of uncoated liposome and Eudragit-coated liposome at pH 1.2 (A) and pH 6.8 (B). Drug release over 2 h, which corresponds to the average gastric emptying times, at pH 1.2 is additionally highlighted in an inserted box.

Figure 3. Docetaxel concentration in rat plasma over time after (A) oral suspension administration at a dose of 20 mg/kg and (B) oral liposome administration at a dose of 10 mg/kg.

CONCLUSION
Lyophilized Eudragit-coated liposomal docetaxel can transverse intestinal membrane and exert anti-cancer effects. Moreover, the severe side effects can be reduced because polysorbate 80 is not used in the formulation. DTX encapsulated in this oral liposomal delivery system can be promising therapeutics for many solid tumors and improve the patient convenience and quality of life.

REFERENCES

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