Nanoemulsion Formulations for Improved Oral Delivery of Poorly Soluble Drugs

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ABSTRACT

The objective of this study was to develop nanoemulsion formulations for enhancement of oral bioavailability of paclitaxel, a model hydrophobic drug and P-glycoprotein substrate. The oil-in-water (o/w) nanoemulsions were made with pine nut oil as the internal oil phase, egg lecithin as the primary emulsifier, and water as the external phase. Stearylamine and deoxycholic acid were used to impart positive and negative charge to the emulsions, respectively. The formulated nanoemulsions had a particle size range of 90-120 nm and zeta potential ranging from +34 mV to -56 mV. Following oral administration to C57BL/6 mice, a significantly higher concentration of paclitaxel was observed in the systemic circulation when administered in the nanoemulsion relative to control aqueous solution. The results of this study suggest that nanoemulsions are promising novel formulations that can enhance the oral bioavailability of poorly soluble drugs.

Keywords: oral absorption, Nanoemulsions, P-glycoprotein, hydrophobic drugs, paclitaxel.

INTRODUCTION

Nanoemulsions are defined as heterogeneous (i.e., oil-in-water) liquid carrier systems, where the internal phase is in the nanometer size range. Oil-in-water nanoemulsions are stabilized by addition of amphiphilic molecules (surfactants) that adsorb at the interface, thus lowering the interfacial tension. We have developed unique nanoemulsion systems by using edible oils rich in essential polyunsaturated fatty acids (PUFA).

Although oral administration is the commonly preferred route for administration of pharmaceuticals, the oral bioavailability of paclitaxel is extremely low in animals and humans. As a result, there are no marketed oral paclitaxel products. Besides low aqueous solubility, recent preclinical studies in mice have shown that the low oral bioavailability of paclitaxel is also due to the adverse effects of the multidrug efflux pump, P-glycoprotein (P-gp) that is abundantly present on the enterocyte membranes in the gastrointestinal (GI) tract. Improved oral uptake of paclitaxel has been made possible in mice and humans by co-administration of oral cyclosporin A (CsA), an inhibitor of P-gp and cytochrome P-450 (CYP) 3A4-mediated drug metabolism. In humans, co-administration of CsA resulted in a seven-fold increase in systemic exposure of paclitaxel, and plasma concentrations increased from negligible to therapeutic levels. However, this approach will not be feasible in the clinic as the co-administration of P-gp-inhibiting immunosuppressants (such as CsA) with paclitaxel to cancer patients who are already immunodeficient as a result of chemotherapy will have catastrophic consequences.

Based on the need to enhance oral bioavailability of hydrophobic drugs such as paclitaxel, we report here the development of a prototype nanoemulsion formulation. The *in vivo* biodistribution studies of the paclitaxel nanoemulsion formulations, with or without charge inducing agents (deoxycholic acid or stearylamine), were conducted in mice and compared with the performance of the commercial paclitaxel formulation administered orally.

MATERIALS AND METHODS

Materials

Paclitaxel solution for injection ONXOL™ [6 mg/mL solution in 527 mg of polyoxyl 35 castor oil NF, 2 mg anhydrous citric acid, and 49.7% (v/v) dehydrated alcohol, USP] was obtained from Ivax Pharmaceuticals, Inc. (Miami, FL). Tritiated [^{3}H]-paclitaxel with an activity of 250 µCi in 250 µl ethyl alcohol was purchased from Moravek Biochemicals (Brea, CA). Pine nut oil was purchased from Siberian Tiger Naturals Inc. (Cabot, VT). Egg phosphatidylcholine (Lipoid® E80) was provided as a gift sample by Lipoid GMBH (Ludwigshfaen, Germany). The Lipoid® E80, according to manufacturer specifications, comprised about 80% phosphatidylcholine, 8% phosphatidylethanolamine, 3.6% non-polar lipids, and about 2% sphingomylein. Deoxycholic acid and stearylamine were purchased from Sigma Chemicals (St. Louis, MO). Deionized distilled water (Barnsted/Thermolyne, Dubuque, IA) was used exclusively for the preparation of all aqueous solutions.

Preparation of Drug-Containing Nanoemulsions

A 20% oil-in-water nanoemulsion containing 420 µg/mL of paclitaxel was prepared by first dispersing an appropriate quantity of paclitaxel solution for injection in pine nut oil with stirring and gentle heating to 60-70°C. An appropriate volume of 3% aqueous solution of Lipoid® E80 was warmed to 70°C, combined with the oil solution of paclitaxel, and sonicated

with a probe type sonicator (Sonics and Materials Inc., Vibra Cell VC 505, Newtown, CT) for 10 minutes at 21% amplitude and 50% duty cycle. The resulting dispersion was a uniform and milk-white color. The nanoemulsions were filtered using 0.45 µm membrane filter. Nanoemulsion formulations were also formulated using charge inducing co-surfactants such as deoxycholic acid (anionic) and stearylamine (cationic). In those formulations, the ratio of Lipoid® E80 to co-surfactants was maintained at 2:1 (w/w).

Characterization of the Nanoemulsions

Particle size analysis: Nanoemulsion formulations were analyzed for particle size and size distribution by the light scattering method using 90Plus® particle size analyzer (Brookhaven Instruments, Holtsville, NY). The nanoemulsions were diluted suitably with deionized distilled water so as to obtain the average count rate of 50-500 kcps. Particle size analysis was carried out at a scattering angle of 90° and a temperature of 25°C.

Measurements of surface charge: Zeta potential (ξ) measurements of the nanoemulsion formulations were performed using the ZetaPALS® instrument (Brookhaven Corporation, Holtsville, NY). The nanoemulsions were diluted with deionized distilled water and zeta potential values were measured at the default parameters of the dielectric constant, refractive index, and viscosity of water, and were calculated based on the electrophoretic mobility. The pH of the final diluted samples ranged from 6 to 6.4.

Oral Absorption and Biodistribution Study

The absorption and biodistribution study of paclitaxel nanoemulsions following oral administration was conducted in 24-hour fasted female C57BL/6 mice (Charles River Laboratories Cambridge, MA) of 10 weeks of age (~30 grams). The experimental protocol involving usage of radioactive material in animals was approved by the Institutional Animal Care and Use Committee and the Office of Environmental Health and Safety at Northeastern University.

A parallel study design was used with the following four groups:

(1) Control group (commercial paclitaxel solution for injection diluted with saline);
(2) Standard nanoemulsion group (paclitaxel nanoemulsions with Lipoid® E80);
(3) Cationic stearylamine-containing nanoemulsion (paclitaxel nanoemulsions with Lipod®E80 and stearylamine);
(4) Anionic deoxycholic acid-containing nanoemulsion group (paclitaxel nanoemulsions with Lipod®E80 and deoxycholic acid).

Tritiated [^{3}H]-paclitaxel-loaded nanoemulsions, containing 2.5 µCi of labeled paclitaxel to 1050 µg of unlabeled paclitaxel, were formulated so as to contain approximately 1 µCi of radioactive dose per 1 mL of the final control and test formulations. The nanoemulsion formulations were administrated orally to conscious mice in a 1 mL volume. Blood and tissue distribution was studied at 1, 6, 12, 24 and 48 hr. Each treatment group consisted of 20 animals with n = 4 for each time point.

Measurement of paclitaxel absorption and disposition

The animals receiving control and test formulations were sacrificed after fixed time-points by cervical dislocation and the stomach, rest of the GI tract (R-GIT, which included esophagus, intestine and rectum), liver, lungs, kidney and blood (by sino-arbital vein puncture) were collected. Blood was used as such and for the tissues (stomach, R-GIT, liver, lungs and kidney), a 10% (w/v) homogenate was prepared in water and 1.0 ml each was added to a scintillation vial. All tissues and fluids (blood) were digested with Scintigest® fluid (Fisher Scientific, Pittsburgh, PA) using 1 mL of the Scintigest® and incubating for 2 hours at 50°C, and decolorized with hydrogen peroxide using 200 µl of 30% solution and incubated for 30 minutes at 50°C. Upon decolorization of the samples, 10 mL of the scintillation cocktail (ScintiSafe® Econo 1, Fisher Scientific) was added and the sample was allowed to quench for 4 hours in the dark before measuring the radioactivity with a liquid scintillation analyzer (TriCarb 1600TR, Packard Instrument Co., CT). The counts-per-minute were converted into µCi using appropriate calibration curves.

Pharmacokinetic data analysis

Non-compartmental pharmacokinetic analysis was performed for all of the formulations. The area under the drug concentration (i.e., % radioactivity recovered per gram of tissue) versus time curve from zero to 48 hours ($AUC_{0\text{-}48h}$) was calculated using the trapezoidal rule. The maximal plasma concentration of drug (C_{max}) and the time to reach maximum plasma concentration (T_{max}) were directly obtained from plasma data. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA). Results are considered significant at 95% confidence interval (i.e., $p<0.05$) and were expressed as mean ± S.D.

RESULTS AND DISCUSSION

Characterization of the Nanoemulsions

The results of particle size analysis of paclitaxel loaded nanoemulsion formulations are depicted in Table 1. Deoxycholic acid-containing nanoemulsions had the smallest mean particle size (~90.6 nm) followed by the standard

nanoemulsions (100.2 nm), and the stearylamine-containing nanoemulsions (119.0 nm). The transmission electron microscopic image of nanoemulsion droplets indicated that emulsion droplets were spherical and in the size ranges of 100 ± 25 nm(Figure 1).

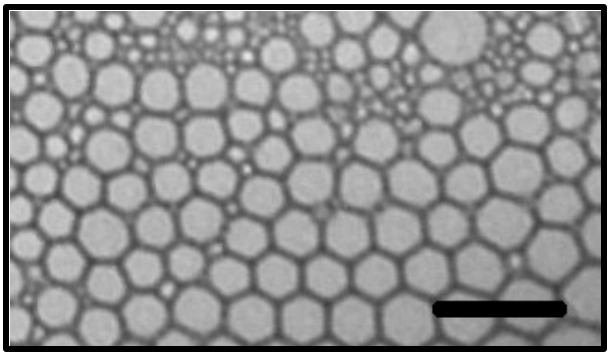

Figure 1: Transmission electron microscopic image of paclitaxel loaded standard nanoemulsion (scale bar represents 250 nm).

The zeta potential values of the nanoemulsion formulations are depicted in Table 1. The standard nanoemulsions and those containing deoxycholic acid formulations had a negative zeta potential values, -29.56 mv and -56.7 mV respectively, whereas the stearylamine-containing nanoemulsions had a positive zeta potential values (+34.7 mV). The electrical surface charge of the nanoemulsion droplets is produced by the ionization of the components forming the interfacial film.

The present formulation is stabilized by egg phosphatidylcholine (Lipoid® E80) as the principal emulsifier. Lipoid E-80 is a mixture of phospholipids from egg yolk sources, and its major component is phosphatidylcholine which is zwitterionic and neutral over a wide pH range. The minor components of the lipid are phosphatidylserine, phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI), sphingomyelin, cholesterol, and lysophosphatidylcholine. Phosphatidylserine, PA, PG, and PI are negatively charged at pH 7.0. Along with free fatty acids, these anionic fractions are probably responsible for the negative zeta potential of the standard nanoemulsion (Table 1).

Table 1: Particle size and zeta potential values of paclitaxel-loaded nanoemulsion formulations

Composition	Particle Size (nm)	Zeta Potential (mV)
1. NE-Std.	100.2 ± 9.0	-29.6 ± 8.9
2. NE-SA	119.0 ± 9.8	+34.7 ± 6.4
3. NE-DCA	90.6 ± 9.4	-56.0 ± 8.0

NE-std: paclitaxel loaded nanoemulsions with egg phosphatidylcholine (Lipoid® E80; 3% w/v) as emulsifier; NE-SA: paclitaxel loaded nanoemulsions with egg phosphatidylcholine (Lipoid® E80; 2% w/v) and stearylamine (1% w/v) as emulsifier; NE-DCA: paclitaxel loaded nanoemulsions with egg phosphatidylcholine (Lipoid® E80; 2% w/v) and deoxycholic acid (1% w/v) as emulsifier.

The negatively-charged emulsion formulation of paclitaxel was based on the use of deoxycholic acid as a co-surfactant in the formulation. The presence of deoxycholic acid was important for prolonged emulsion stability owing to its contribution to the elevated negative zeta potential of the emulsion. Unionized free cholic acids are known to be poor surface active agents. Nevertheless, it was preferred to prepare emulsions with deoxycholic acid instead of sodium deoxycholate as a result of better localization of the free acid at the interface of the oil / water emulsion owing to it high lipid solubility. Since the p*Ka* of deoxycholic acid is 6.5 and at the pH of the formulation (adjusted to pH 7.0), we expect that the majority of the deoxycholic acid molecules remained in the ionized state compared to unionized fraction. The ionized fraction of the acid was probably localized in the oil /water interface of the emulsions without being excluded from the surface regions of the oil droplets. This observation is supported by the increase in negative zeta potential value of the emulsion with incorporation of deoxycholic acid in the formulation. Deoxycholic acid was also used in the nanoemulsion to potentially facilitate the oral absorption of the nanoemulsion droplets through the bile acid transporter mechanism.

Positively-charged nanoemulsions were formulated using stearylamine, a cationic lipid, as a co-surfactant. Stearylamine confers an overall positive charge to the droplets over a wide pH range. The positive surface potential value of the droplets of the emulsion depends mainly upon the extent of the ionization of stearylamine at the oil /water interface. Positively-charged nanoemulsions were formulated with an aim of improved interaction of positively charged nanoemulsions with the negatively charged mucosal surface cells. It was speculated that such enhanced interaction would result is enhanced absorption of the encapsulated lipophillic drug.

Incorporation of paclitaxel did not alter the zeta potential values of the emulsion droplets as the formulations prepared without paclitaxel had similar zeta potential values. This can be explained by the lack of ionizable functional group in the paclitaxel molecule.

Oral Absorption and Biodistribution Study

The whole blood concentration-time profile of tritiated [^{3}H] paclitaxel in C57BL/6 mice following oral administration of nanoemulsions is shown in Table 2. The results of the pharmacokinetic studies indicated that encapsulation of paclitaxel in nanoemulsions did enhance the oral bioavailability of paclitaxel significantly. The enhanced oral bioavailability, as measured by the area-under-the curve (AUC), of paclitaxel in nanoemulsions might be attributed to the solubilization of the drug in the oil droplets and/or to the presence of surfactants at the oil–water interface. Enhanced absorption of paclitaxel can also be attributed to the protection of drug from chemical as well as enzymatic degradation. Improved oral bioavailability of various hydrophobic drugs in O/W type of emulsions has been reported in the literature. For example, the whole blood concentration–time curve (AUC) of

the O/W emulsion formulation of tacrolimus (O/W group) was significantly higher ($p<0.01$) than the commercially available formulation (T group). Enhanced oral absorption of vitamin A, vitamin D, and insulin has also been reported with use of emulsion formulations.

The enhancement in oral bioavailability of paclitaxel was highest with the deoxycholic acid-containing nanoemulsions, followed by the stearylamine-containing nanoemulsions and standard nanoemulsion formulations (Table 2). The enhancement in AUC of deoxycholic acid-containing nanoemulsions was nearly five-times when compared to the control aqueous paclitaxel solution and was more than two-times when compared with the standard nanoemulsion formulation. This formulation also exhibited highest paclitaxel plasma concentration (C_{max} = 3.72 %/g), followed by stearylamine- nanoemulsions (C_{max} = 1.10 %/g), the standard nanoemulsions (C_{max} = 0.51 %/g), and the aqueous paclitaxel solution (C_{max} = 0.40 %/g).

Table 2: Summary of plasma pharmacokinetic parameters of ^{3}H-paclitaxel nanoemulsion formulations after oral administration in female C57BL/6 mice.

Composition	$AUC_{0\text{-}48\ h}$ (% hr/g)	C_{max} (%/g)	T_{max} (hr)
1. Control sol.	6.8 ± 1.0	0.4 ± 0.1	1
2. NE-Std	15.9 ± 3.1[a]	0.5 ± 0.1	6
2. NE-SA	20.6 ± 3.1[a]	1.1 ± 0.4	6
3. NE-DCA	33.5 ± 3.1[b]	3.7 ± 0.5	6

[a] Significant at $p < 0.05$ as compared to the control

[b] Significant at $p < 0.01$ as compared to the control and the standard nanoemulsion formulation. Legends are; Control Sol.: Aqueous solution of paclitaxel; NE-std, NE-SA and NE-DCA are standard nanoemulsions, nanoemulsions with Stearylamine and deoxycholic acid respectively.

Stearylamine-containing nanoemulsions were formulated so as to enhance the interaction of the positively-charged nanoemulsions with the negatively-charged mucosal cells and thus expecting higher absorption and bioavailability. However, as described earlier, the maximum enhancement in bioavailability of paclitaxel was observed with deoxycholic acid-nanoemulsions. This might be attributed to the nature of deoxycholic acid. Deoxycholic acid is a naturally occurring bile acid and because of its surface active properties, it is also used as an absorption enhancer. It has been shown to enhance the oral absorption of poorly soluble drugs from the GI tract. Bile salts can decrease duodenal and jejunum brush-border membrane vesicle integrity and increase membrane fluidity which might increase the absorption of paclitaxel in the gut. Moreover, deoxycholic acid is known to be a substrate for the P-glycoprotein (P-gp) efflux pump. As the poor oral bioavailability of the paclitaxel is attributed to its affinity for P-gp, inclusion of another P-gp substrate (deoxycholic acid) in the formulation is expected to improve its bioavailability. The results of pharmacokinetic studies also indicated the difference in T_{max} for the control solution of paclitaxel and its nanoemulsion formulations. The T_{max} for the control aqueous solution was 1 hour, whereas for nanoemulsion formulation, it was 6 hours. This difference might be attributed either to the delayed absorption of the drug in the jejunum and ileum from the O/W nanoemulsions or to the differences in rate of drug diffusion to the membrane. In the case of emulsion, drug has to diffuse across oil/water interface, whereas in the case of solution, drug has to diffuse across micelle/water interface.

The results of tissue distribution studies indicated that the major fraction of the drug remained in the stomach and other parts of the GI tract following oral administration. The presence of paclitaxel in the liver, kidney and lungs indicate the systemic effect of the absorbed drug. The detection of the drug in the liver indicates the possibility of first pass effect on the orally absorbed drug.

CONCLUSIONS

The results of these studies indicated the potential of O/W type of nanoemulsion formulations for enhancing the oral bioavailability hydrophobic P-gp, substrates such as paclitaxel. Deoxycholic acid-containing nanoemulsions were more potent in enhancing the oral bioavailability of paclitaxel. The results suggest that nanoemulsions are promising novel formulations that can enhance the oral bioavailability of hydrophobic drugs. In addition, one could use O/W nanoemulsions as the formulation of choice for screening and evaluation of experimental drug candidates that have poor water solubility. In such cases, nanoemulsions can be easily formulated thus avoiding other time consuming and costly formulation studies.

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