Evaluation of a Nanoemulsion Formulation Strategy for Oral Bioavailability Enhancement of Danazol in Rats and Dogs

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ABSTRACT: The objective of this study was to determine whether nanoemulsion formulations constitute a viable strategy to improve the oral bioavailability of danazol, a compound whose poor aqueous solubility limits its oral bioavailability. Danazol-containing oil-in-water nanoemulsions (NE) with and without cosurfactants stearylamine (SA) and deoxycholic acid (DCA) were prepared and characterized. Nanoemulsion droplets size ranging from 238 to 344 nm and with surface charges of −24.8 mV (NE), −26.5 mV (NE-DCA), and +27.8 mV (NE-SA) were reproducibly obtained. Oral bioavailability of danazol in nanoemulsions was compared with other vehicles such as PEG400, 1% methylcellulose (MC) in water (1% MC), Labrafil, and a Labrafil/Tween 80 (9:1) mixture, after intragastric administration to rats and after oral administration of NE-SA, a Labrafil solution, or a Danocrine® tablet to dogs. The absolute bioavailability of danazol was 0.6% (PEG400), 1.2% (1% MC), 6.0% (Labrafil), 7.5% (Labrafil/Tween80), 8.1% (NE-DCA), 14.8% (NE), and 17.4% (NE-SA) in rats, and 0.24% (Danocrine), 6.2% (Labrafil), and 58.7% (NE-SA) in dogs. Overall, danazol bioavailability in any nanoemulsion was higher than any other formulation. Danazol bioavailability from NE and NE-SA was 1.8- to 2.2-fold higher than NE-DCA nanoemulsion and could be due to significant difference in droplet size. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3808–3815, 2013

Keywords: danazol; nanoemulsion; stearylamine; bioavailability; surfactants; absorption; particle size; hepatic clearance

INTRODUCTION

The factors that can limit the oral bioavailability of drugs, which are commonly encountered problems in the drug discovery area include solubility, dissolution rate, metabolism, and permeability. Although these factors are generally addressed in the discovery phase through structural modifications of new chemical entities, in some cases this approach can result in a significant loss of biological activity. Recent literature data show that formulation strategies can effectively enhance the drug delivery of prospective new drug molecules and present attractive complementary alternatives to the chemical modification of the drug.[1–3]

A number of candidate drug molecules selected for full-scale development suffer from poor oral bioavailability mainly because of their limited solubility in water. Improved oral bioavailability for lipophilic drugs can be achieved by using various lipid-containing formulations.[4,5] Recent developments in delivery of lipophilic drugs include lipid-based formulations,[6–9] microemulsions,[10] liposomes,[11] self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems,[12–15] as well as supersaturatable SEDDS.[16–18] Among various approaches to drug delivery, nanoemulsions of lipophilic drugs are especially attractive.[19–22] The rate of drug release from oil droplets into aqueous media is a function of a drug intrinsic solubility, an interfacial barrier, the transfer rate constant, and the droplet size.[23,24] Enhancing danazol release by dissolving it in small oil droplets with various surfactant interfacial barriers might improve its bioavailability.

A nanoemulsion is a colloidal dispersion of oil and aqueous phase stabilized by a surfactant or emulsifier and cosurfactant. Their preparation requires the input of a high amount of energy leading to the production of oil droplets in the nanometer-scale range. Nanoemulsions can be produced by high-energy emulsification (high-pressure homogenization or ultrasonication) using excipients with appropriate hydrophilic–lipophilic balance at appropriate concentration. All oil/water (O/W) nanoemulsions in the current study were prepared by ultrasonication.

The purpose of this study was to develop stable nanoemulsion formulations, which enhance oral bioavailability of Biopharmaceutics Classification System (BCS) Class II and IV compounds. Danazol, a synthetic steroid with low oral bioavailability due to its low solubility in water, slow dissolution rate, and high first pass metabolism was used as a model compound. Various techniques have been explored to improve the danazol oral exposure, such as forming a water-soluble danazol complex with hydroxypropyl-β-cyclodextrin (HPβCD),[25,26] using sodium docusate as a surfactant dispersant,[27] Labrafil solution as a lipid vehicle,[4] coprecipitation with hydroxypropyl methylcellulose phthalate,[28] as well as solutions in PEG400/Povidone or Polysorbate 80. *In vitro* and *in vivo* performance of SEDDS with various surfactants using danazol as a model drug has been extensively explored.[29–32] The water solubility of danazol is reported to be 0.59 mg/mL, and the octanol/water partition coefficient is 3.8.[33] Based on the partition coefficient, danazol is a lipophilic drug, suggesting that O/W nanoemulsion formulations would be a good approach to enhance the danazol solubility, dissolution, and bioavailability.

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Various kinds of vegetable oils are suitable for producing oil-in-water emulsions and are compatible with oral administration. Long-chain triglycerides such as corn oil, olive oil, peanut oil, sesame oil, and soybean oil, fractionated medium-chain triglyceride from coconut oil or palm seed, and oleic acid have been explored previously.[5] The solubility of danazol was estimated in various oils and surfactants. Based on visual estimation of danazol solubility in short-chain triglyceride oil (flaxseed), medium-chain triglyceride oil (palm), and long-chain triglyceride oil (soybean), it was found that solubility was not significantly different in the tested excipients. The advantage of long-chain triglycerides for danazol formulated in SEDDS has been reported by Porter and coauthors.[29,30] Long-chain lipid digestion products showed the increased solubilization in the gastrointestinal (GI) environment and enhanced danazol absorption compared to medium-chain triglycerides. The long-chain triglyceride soybean oil was chosen in this study as a lipophilic vehicle for producing O/W nanoemulsions. To stabilize O/W nanoemulsions, a surfactant must be added to water or oil prior to mixing. Nonionizable as well as ionizable surfactants are suitable in formulations for oral dosing. The nonionic surfactants compatible with oral administration are mainly based on the ethoxy group containing polymers attached to a hydrophobic end (Brij polymers, Pluronic, Cremophor, TPGS), sorbitol (Span), and macrogolglycerides (Labrafil, Labrasol, Gellucire), although there are many others. Nonionic surfactants are predominantly used in microemulsions or self-emulsifying drug delivery systems. In contrast, anionic or cationic surfactants, bearing the acidic or basic group, respectively, are attractive for preparing stable nanoemulsions. The advantage of ionic surfactants is that bearing a surface charge makes emulsions more stable due to repulsive forces.[34] An anionic phosphatidyl containing surfactant, egg lecithin, was used as a primary emulsifier for nanoemulsion preparation in the current study. For further nanoemulsion stabilization, some cosurfactants might be added as a nanoemulsion constituent. Two cosurfactants were explored in the study: the anionic cosurfactant deoxycholic acid (DCA) and the cationic cosurfactant stearylamine (SA), to impart negative and positive charge, respectively, to the nanoemulsions.

Three types of danazol O/W nanoemulsions, differing in expected net charge at intestinal pH, were prepared and orally administered to rats and one type was orally administered to dogs. The effect of the nanoemulsion's type on danazol bioavailability was evaluated and compared with that of the other vehicles such as PEG400 (danazol solution), 1% methylcellulose (MC) in water solution (danazol suspension), Labrafil, and the Labrafil/Tween 80 mixture as a surfactant solution. To determine the absolute oral bioavailability, danazol was dosed intravenously to rats and dogs in a 10% aqueous HPβCD formulation as well.

MATERIALS AND METHODS

Materials

Danazol, HPβCD, SA, DCA, PEG 400, MC, and soybean oil were obtained from Sigma-Aldrich (St. Louis, Missouri). Egg phosphatidylcholine (Lipoid® E80) was a gift sample from Lipoid (Ludwigshafen, Germany). Labrafil M2125CS was a gift sample from Gattefosse, Saint-Priest Cedex, France. Solvents were also obtained from Sigma and used without further purification.

Formulation Preparation

The intravenous formulation was prepared by dissolving the danazol powder at 1–2 mg per milliliter concentration in water containing 10% (w/v) HPβCD followed by filtration through a 0.2-μm NY (nylon) sterile syringe filter (Corning®). For nanoemulsion preparation, an aqueous phase was prepared by adding 60 mg of egg phosphatidylcholine (Lipoid® E80) to 4 mL of deionized water and stirring the mixture for 30 min. Separately, a 1-mL oil phase was prepared by mixing danazol with soybean oil at 25 mg/mL concentration. After heating the two phases separately, the oil phase was added to the aqueous solution and the mixture was sonicated at 21% amplitude and 50% duty cycle (Vibra Cell VC 505; Newtown, Connecticut) for 10 min, resulting in the formation of the nanoemulsion. To prepare cosurfactant containing nanoemulsions, NE-SA or NE-DCA, calculated amounts of either SA or DCA was added to the aqueous phase of the nanoemulsions to result in a 6-mg/mL cosurfactant final concentration. All nanoemulsions were filtered through 0.45 NY syringe filters (Corning®). The final danazol concentration was confirmed by HPLC analyses. The drug load in the rat study resulted in 2, 1.9, and 2.9 mg/mL in nanoemulsion (NE), NE-SA, and NE-DCA, respectively. The drug load in NE-SA for dog study resulted in 1.43 mg/mL nanoemulsion. Freshly prepared nanoemulsions were characterized for droplet size and surface charge. An aqueous danazol suspension in 1% (w/v) MC solution or danazol solutions in either PEG400 or Labrafil or Labrafil/Tween 80 was prepared by mixing 4 mg of danazol per one milliliter of corresponding vehicle using a magnetic stirrer at ambient temperature. All formulations were kept protected from light both during preparation and storage.

Characterization of Nanoemulsions

The mean particle size of the droplets, polydispersity index, and zeta potential were measured by dynamic light scattering and electrokinetic mobility experiments on a Malvern Zetasizer Nano ZS 90 (ZEN-3600) instrument fitted with 633-nm "red" laser, using 1-cm glass cuvette. To keep the count rate below 250 kpcs, the nanoemulsions were diluted in deionized water additionally purified by filtration through a 0.4-μm filter prior the measurements. Quantification of danazol in danazol-containing nanoemulsions was done using an high-performance liquid chromatography (HPLC) method with UV detection in an Agilent 1100 HPLC system consisting of an Agilent binary pump, an Agilent autosampler, an ALS Therm controller, an HP ColCom, and an HP diode-array detector. Data analysis was accomplished using Agilent ChemStation software. All standards were prepared from a 0.5-mg/mL danazol stock solution in DMSO followed by a serial dilution with an acetonitrile/water mixture to concentrations ranging from 6 to 70 μg/mL. The sample injection volume was 10 μL injected onto a Phenomenex Gemini C18 analytical column, 50×2 mm^2, 5 μm particle size. The aqueous part (A) of the mobile phase consisted of water with 0.1% formic acid and the organic part (B) of acetonitrile with 0.1% formic acid. The mobile phase was degassed before use. Elution was carried under gradient mode (15% B at time 0 to 90% B at 5 min) at a flow rate 0.5 mL/min with 285 nm detection wavelength. The retention time was 4.5 min.