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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 co-surfactants 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 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.

Keywords

Danazol; nanoemulsion; stearylamine; bioavailability; surfactants

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 (NCEs), 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 drug¹⁻³.

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 formulations⁶⁻⁹, microemulsions¹⁰, liposomes¹¹, self-emulsifying drug delivery systems

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(SEDDS) and self-microemulsifying drug delivery systems (SMEDDS)¹²⁻¹⁵ as well as supersaturatable SEDDS¹⁶⁻¹⁸. Among various approaches to drug delivery, nanoemulsions of lipophilic drugs are especially attractive¹⁹⁻²². 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 size²³⁻²⁴. 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 co-surfactant. 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 ultra-sonication) using excipients with appropriate hydrophilic-lipophilic balance (HLB) at appropriate concentration. All oil/water (O/W) nanoemulsions in the current study were prepared by ultra-sonication.

The purpose of this study was to develop stable nanoemulsion formulations which enhance oral bioavailability of 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 (HP β CD)²⁵⁻²⁶, using sodium docusate as a surfactant dispersant²⁷, Labrafil solution as a lipid vehicle⁴, co-precipitation with hydroxypropyl methylcellulose phthalate²⁸, 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 explored²⁹⁻³². The water solubility of danazol is reported to be 0.59 mg/mL and the octanol/water partition coefficient is 3.8³³. 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.

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 previously⁵. 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), 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 CJH and co-authors^{29, 30}. Long-chain lipid digestion products showed the increased solubilization in the gastro-intestinal 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 surfactant must be added to water or oil prior to mixing. Non-ionizable as well as ionizable surfactants are suitable in formulations for oral dosing. The non-ionic surfactants compatible with oral administration are mainly based on ethoxy-group containing polymers attached to a hydrophobic end (Brij polymers, Pluronic, Cremophor, TPGS), sorbitol (Span), and macroglycerides (Labrafil, Labrasol, Gellucire), although there are many others. Non-ionic surfactants are predominantly used in microemulsions or self-emulsifying drug delivery systems. In contrast, anionic or cationic surfactants, bearing 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 forces³⁴. 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 co-surfactants might be added as a nanoemulsion constituent. Two co-surfactants were explored in the study; the anionic co-surfactant deoxycholic acid and the cationic co-surfactant stearylamine, 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 in water solution (danazol suspension), Labrafil, and 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 HP β CD formulation as well.

Materials and Methods

Materials

Danazol, hydroxypropyl- β -cyclodextrin (HP β CD), stearylamine (SA), deoxycholic acid (DCA), PEG 400, methylcellulose (MC) and soybean oil were obtained from Sigma-Aldrich (St. Louis, MO). Egg phosphatidylcholine (Lipoid® E80) was a gift sample from Lipoid (Germany). Labrafil M2125CS was a gift sample from Gattefosse. 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) HP β CD followed by filtration through a 0.2 μ m 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 minutes. Separately, a 1-mL oil phase was prepared by mixing Danazol with soybean oil at 25 milligram per milliliter 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, CT) for 10 minutes resulting in the formation of the nanoemulsion. To prepare co-surfactant containing nanoemulsions, NE-SA or NE-DCA, calculated amounts of either stearylamine or deoxycholic acid was added to the aqueous phase of the nanoemulsions to result in a 6 mg/mL co-surfactant 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 mg/mL, 1.9 mg/mL, and 2.9 mg/mL in 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) methylcellulose solution or danazol solutions in either PEG400 or Labrafil or Labrafil/Tween 80 were 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 (PI) and zeta potential were measured by dynamic light scattering (DLS) 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- μ m filter prior the

measurements. Quantification of danazol in danazol-containing nanoemulsions was done using an 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 µg/mL to 70 µg/mL. The sample injection volume was 10 µL injected onto a Phenomenex Gemini C18 analytical column, 50 × 2 mm², 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 minutes) at a flow rate 0.5 mL/min with 285 nm detection wavelength. The retention time was 4.5 minutes.

In Vivo Danazol Pharmacokinetics Studies

The danazol oral bioavailability from various formulations was estimated in a single-dose pharmacokinetic study using three animals per group. For the *in vivo* rodent studies, 8-week-old male Sprague-Dawley rats, with an average weight of 300 g, surgically fitted with jugular vein cannula were obtained from Hilltop Lab Animals, Inc (Scottsdale, Pennsylvania). The study protocol was approved by Absorption Systems LP Institutional Animal Care and Use Committee (IACUC). Animals were assigned randomly upon arrival and placed individually in cages. The acclimation period was approximately two days after arrival. Food was withheld from the animals for twelve hours prior to danazol administration and was returned four hours post dose. Water was supplied *ad libitum*. Rats were dosed orally at 10 mg/kg (10 mL/kg) either with 1% aqueous MC, PEG 400, Labrafil or Labrafil/Tween 80 (9:1) formulations. The nanoemulsions were administered (volumes of 6 mL/kg) to rats at doses of 12 mg/kg for NE, 11.3 mg/kg for NE-SA and 17.5 mg/kg for NE-DCA nanoemulsion formulations. In parallel, danazol was also dosed to rats intravenously at 0.65 mg/kg (1 mL/kg) as a 10% HPβCD in water solution. Each blood sample, ~0.35 mL, was collected at predetermined time points up to 24 hours post dosing from the jugular vein cannula and placed into a chilled polypropylene tube containing the sodium heparin as an anticoagulant. The samples were centrifuged at 3,000 × g at a temperature of 2 to 8°C, for 5 minutes. Plasma samples were stored frozen at –70°C until analysis.

For the dog studies, 1-6-year-old non-naïve male Beagle dogs (Marshall Farms) with an average weight of 10 kg were dosed either orally or intravenously. The washout period between the doses was not less than seven days. Animals were assigned randomly and placed individually per cage. The study was not blinded. The study protocol was approved by Absorption Systems LP IACUC. Food was given twice daily and water was supplied *ad libitum*. The animals were not fasted. Danazol as Danocrine tablet was administered at 20 mg/kg, as a Labrafil solution at 5 mg/kg. Danazol in nanoemulsion was dosed to dogs at 7.16 mg/kg (5 mL/kg) via a gavage tube followed with 5-mL water flush or intravenously into cephalic vein at 1.0 mg/kg dose (1.5 mL/kg) as a 10% aqueous HPβCD solution followed with a 1 mL saline flush. Each blood sample, ~1.0 mL, was collected at predetermined time points up to 24 hours from the jugular vein and placed into a chilled polypropylene tube containing the sodium heparin as an anticoagulant. The generated plasma samples were processed the same way as the rat plasma.

Sample Preparation and LC/MS/MS Analysis

Plasma samples were prepared for analysis by acetonitrile precipitation using a Tomtec Quadra 96-Model 320 liquid handling system in a 96-well plate format. A 50 µL volume of plasma sample was mixed with 150 µL of acetonitrile containing 0.1% formic acid which was pre-loaded into a Sirocco protein precipitation plate. The filtrates were collected into a

clean 2 mL/well, 96-well plate, the solvent was evaporated under nitrogen at 40°C and the samples reconstituted with 100 µL of a 20:80 methanol: water mixture. Standards were prepared in plasma containing sodium heparin as the anticoagulant. A calibration curve was prepared at concentrations of 1000, 500, 100, 50, 10, 5, 1, and 0.5 ng/mL by serial dilution. Plasma samples were analyzed for the concentration of danazol using tandem liquid chromatography - mass spectrometry with simultaneously monitoring the internal standard, pindolol. All measurements were performed on an API 4000 mass spectrometer with a Perkin Elmer autosampler in the multiple reaction monitoring mode (MRM). MRM was used with the following transitions 338.3, precursor ion, to 148.1, product ion, for danazol and 309.2 to 251.1 for pindolol. A 10-µL aliquot of sample was injected onto a reverse phase Phenomenex Polar RP, 50 × 2.1 mm id., 4µm column. An ammonium formate buffer was used for mobile phase and was prepared by adjusting the pH of 25 mM aqueous ammonium hydroxide to pH 3.5 with formic acid. The aqueous mobile phase consisted of 10% buffer and 90% water. The organic phase consisted of 10% buffer and 90% acetonitrile. Danazol was eluted with a gradient created by ramping organic mobile phase from 0% to 100% in 1 min at 1000- µL/min followed by a 1 min organic hold and 1 min column re-equilibration. Retention times ranged from 0.761 to 0.769 min. No interfering peaks were observed in blank plasma samples. Standard curves were fit with a quadratic function weighted by the concentration for both compounds. The analytical method provided good accuracy and precision for quantitation of samples between the 1 ng/mL lower-limit of quantitation (LLOQ) and the 1000 ng/mL upper-limit of quantitation (ULOQ). Recovery of all compounds from plasma was greater than 70% with no apparent concentration dependence.

Data Analysis

Noncompartmental pharmacokinetic parameters were calculated using WinNonlin 5.2.1 (Pharsight, Mountain View, CA) for individual animals, and reported as the mean ± standard deviation of the group. The highest observed oral plasma concentration and its corresponding sampling time were defined as C_{max} and T_{max} , respectively. The area under the curve from time zero to infinity ($AUC_{0-∞}$) and to the last detectable time point (AUC_{0-last}) was calculated by the linear trapezoidal method. The terminal half-life ($t_{1/2}$) was estimated by a linear regression analysis of the three or four data points of the terminal linear segment of the log plasma concentration versus time curve. Plasma clearance (CL) and volume of distribution values were calculated by standard methods. Bioavailability (%F) was calculated as the percent ratio of the mean dose-normalized AUC_{last} or $AUC_{∞}$ values for oral and intravenous dosed groups.

Statistical Analysis

Data were reported as mean ± standard deviation of means (SD). A minimum of three animals per group was used. All statistical tests were performed using Graphpad Prism (Version 4.00; Graphpad Software Inc., San Diego, CA). Significant difference between two groups was evaluated by using Student's t-test and significant differences for a multiple comparison by using ANOVA. A minimum p value of 0.05 was used as the significance level for all comparisons.

Results and Discussion

Nanoemulsion Characterization

Three types of danazol nanoemulsions were prepared and characterized for the particle size distribution and surface charge using a Malvern Zetasizer Nano ZS 90 instrument. The blank nanoemulsion droplets' diameters were in the range of 238 to 344 nm. Inclusion of danazol did not alter the size of the droplets indicating that danazol completely distributed in the oil

phase. The average diameter in danazol loaded nanoemulsions was 313 ± 18.4 for NE, 248 ± 6.2 for NE-SA and 364 ± 46.8 for NE-DCA. Nanoemulsions without any co-surfactant (soybean oil/egg lecithin/water) bearing phosphatidylcholine group had a net negative surface charge of -23.3 mV, and drug loaded droplets had a charge of -24.8 mV. The NE-SA droplets without or with danazol had net positive charges of $+26.5$ mV and $+27.8$ mV, respectively, due to positively charged stearylamine groups. The NE-DCA droplets without or with danazol had charges of -14.5 mV and -26.5 mV. The danazol loading in NE and NE-SA was measured to be 2 mg/mL while in NE-DCA was 3 mg/mL. The two nanoemulsions, NE and NE-SA were significantly different ($p < 0.01$) in droplet size when compared to the droplet size of NE-DCA. The influence of the droplet size and charge on danazol pharmacokinetics was explored in the *in-vivo* part of the study.

Pharmacokinetics of Danazol in Rats and Dogs

Following intravenous administration of danazol in 10% aqueous HP β CD formulation the pharmacokinetic profile (Figure 1) was different in rats compared to dogs. Table 1 presents the calculated pharmacokinetic parameters following a noncompartmental analysis of the danazol plasma concentration- time profile. After intravenous dosing of danazol to rats, the half-life was 0.931 ± 0.084 hr with average clearance of 4.74 ± 1.98 L/hr/kg, volume distribution at steady-state of 4.55 ± 1.77 L/kg and normalized area under the curve (AUC_{last}) of 234 ± 106 hr·kg·ng/mL/mg. Following intravenous dosing of danazol in dogs the half-life, average clearance, volume of distribution and AUC_{last} were calculated to be 4.68 ± 0.78 hr, 1.34 ± 0.10 L/hr/kg, 4.89 ± 0.62 L/kg and 737 ± 49.2 hr·kg·ng/mL/mg, respectively. The 3.2-fold higher exposure in dogs as compared with rats could be due to a slower clearance rate and longer elimination half-life in dogs than in rats. This observation is in agreement with previously reported danazol pharmacokinetic data in rats and dogs^{25, 26, 35}.

The oral exposure in rats of danazol formulated in various nanoemulsions, NE, NE-SA, and NE-DCA, was compared with alternative danazol formulations such as 1% methylcellulose in water, PEG400, Labrafil and Labrafil/ Tween 80 (9:1) mixture. The average danazol plasma concentration-time profiles following the oral administration in different vehicles to rats are shown in Figure 2A. Overall, the danazol bioavailability in any nanoemulsion formulation was higher (Figure 2B) than that in PEG400, Labrafil, or 1% MC. Table 2 presents the calculated pharmacokinetic parameters following a noncompartmental analysis of the oral plasma concentration vs time data. Danazol had oral bioavailability ranging from 0.65 to 1.2% when dosed orally to rats in either PEG400 or 1% aqueous MC formulation, the difference was not statistically significant. Even though danazol formulation in neat PEG400 resulted in a solution, precipitation in GI tract might be the reason for a low bioavailability, which is comparable with danazol bioavailability when suspended in 1% aqueous MC. At the same time when danazol was formulated in a surfactant Labrafil, it had a significantly greater bioavailability (i.e. 6.0%) compared with 0.65% in PEG400 ($p < 0.01$) and 1.17% in 1% aqueous MC ($p < 0.01$) formulations. Labrafil as a surfactant is dispersible in an aqueous milieu of GI tract eventually forming micellar solution which is able to maintain drug in a solubilized state. As it has been shown earlier by Porter *et al*³¹, the danazol bioavailability from surfactant solutions depends on dispersability and lipolysis of surfactants. Since *in vitro* evaluation was not the part of the study, adding 10% of Tween80 to Labrafil was explored *in vivo*. It was found that danazol bioavailability was not significantly different compared to neat Labrafil. As expected, danazol in NE-SA had a significantly higher bioavailability (i.e. 17.4%) compared with 6.0% in the Labrafil ($p < 0.05$) formulation. Since nanoemulsion has pre-dispersed, surfactant stabilized, well characterized oil droplets in water, it should disperse in GI tract rapidly keeping danazol in oil droplets, while Labrafil (as a highly digestible lipid) formulation when introduced into

the aqueous media might slowly disperse forming droplets with a bigger size and a large degree of distribution, which could be a target for a fast lipolysis with drug release and eventual precipitation.

Among all nanoemulsion formulations, the danazol bioavailability was found to be the highest in NE-SA ($17.4 \pm 6.41\%$) followed by the NE ($14.8 \pm 7.77\%$) and NE-DCA ($8.1 \pm 1.79\%$), but the difference between NE and NE-SA was not statistically significant. Although the particles sizes of NE-SA (248 ± 6.2) and NE-DCA (364 ± 46.8) droplets differed significantly and the droplets charge were $+27.8$ mV and -26.5 mV, for NE-SA and NE-DCA, respectively, it is evident that the charge of droplet surface, + vs –, or the interfacial barrier, did not play an important role in danazol bioavailability. In addition, it appears that a droplet size in the 250 nm to 400 nm range should be low enough to improve bioavailability in general but it is not sufficiently wide to distinguish between the nanoemulsions prepared here. At the same time, there was a 2.1-fold ($p < 0.01$) increase in the danazol half-life observed in NE-SA (3.27 h) compared to NE-DCA and other formulations. The change in danazol half-life is most likely due to a slower absorption, especially in the case of NE-SA which is explained by an increase in T_{max} .

The NE-SA formulation, which gave the best danazol exposure in rats, was further tested in fed beagle dogs. The mean plasma levels of danazol following the oral administration of the Danocrine® tablet, Labrafil solution and nanoemulsion in dogs are shown in Figure 3. The orally administered NE-SA significantly increased the danazol maximum concentration (Table 3). The C_{max} of 631 ± 306 ng/mL was reached during about 1 to 2 hours post dosing. The dose-normalized AUC_{last} and half-life, respectively, for danazol in the NE-SA formulation, 425 ± 145 hr·kg·ng/mL/mg and 4.44 ± 1.13 h, were significantly higher than those for the Labrafil solution, 69.3 ± 16.8 hr·kg·ng/mL/mg and 1.59 ± 0.06 h, and the Danocrine® tablet, 2.67 ± 1.82 hr·kg·ng/mL/mg and 1.09 ± 0.29 h. The oral bioavailability, $58.7 \pm 22.3\%$, was significantly higher ($p < 0.001$) than those of $0.24 \pm 0.16\%$ and $6.20 \pm 1.50\%$ in the Danocrine® tablet and Labrafil solution, respectively. It is likely that the nanoemulsion's droplet size was the major factor responsible for the increased danazol bioavailability. The increase in danazol half-life observed in rats after oral administration of a NE-SA nanoemulsion was noted in dogs as well, where danazol half-life in NE-SA (4.4 h) exhibited a 2.6-fold ($p < 0.01$) increase compared to Labrafil (1.6 h) with approximately the same T_{max} . NE-SA formulation performs the best of all other formulations in enhancing the oral bioavailability of danazol in dogs after fed administration. Lower than expected bioavailability from Labrafil formulation in dogs in fed state could be due to more dilution of the formulation which leads to precipitation after dosing. Since the exposure from Labrafil formulation appears low in fed dogs it seems likely that the formulation and food contributed in enhancing the oral bioavailability of danazol from NE-SA. Exposure from Danocrine® was negligible and could be due to the formulation was poorly wetted and never solubilized after oral administration.

Conclusion

The prerequisite to achieve an enhancement of the oral bioavailability for a BCS II compound is that it is finely dispersed in the intestinal liquid and that there is no aggregation. The low danazol bioavailability from 1% methylcellulose in water suspension (rats) or Danocrine tablets (dogs) suggests that danazol is not finely dispersed in the intestinal liquid and/or that there is aggregation when these formulations are administered. The present study demonstrated that the use of nanoemulsions as vehicles for oral danazol administration resulted in a significant enhancement of the danazol oral bioavailability in rats and dogs in comparison with other formulations. Three danazol containing nanoemulsions were prepared with an average droplet sizes between 250 nm and 360 nm

and various surface charges, -24.8 mV (NE), -26.5 mV (NE-DCA), $+27.8$ mV (NE-SA). 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. The absolute danazol bioavailabilities from the three nanoemulsions were not significantly different, suggesting that the droplet charge did not play a significant role and the droplet size was low enough to overcome any dissolution rate barrier to the oral bioavailability of the compound. The significantly ($p < 0.05$) higher exposure from NE-SA was observed in dogs ($58.7 \pm 22.3\%$) compared to rats ($17.4 \pm 6.41\%$), could be due to differences in the hepatic extraction of danazol and also partly due to food effect. In dogs, danazol clearance ($CL = 1.34 \pm 0.10$) is 72% of hepatic blood flow ($Q_H = 1.86$ L/hr/kg) while in rats it is ($CL = 4.74 \pm 1.98$) higher than hepatic blood flow ($Q_H = 3.30$ L/hr/kg). It is noteworthy that intravenous exposure was significantly ($p < 0.05$) higher in dogs compared than in rats, an observation that could be due to differences in the danazol hepatic extraction. Based on the present results using danazol as a model compound, nanoemulsion formulations appear to represent an attractive alternative that should be considered when there is a need to enhance the oral bioavailability of BCS Class II and possibly also Class IV compounds.

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Figure 1. Average Plasma Concentration (ng/mL) of Danazol vs Time Following Intravenous Administration to (•) Rats (0.65 mg/kg); and () Dogs (1 mg/kg) in aqueous 10%HP β CD. The data are mean $\pm$ SD of the results from 3 animals.

Figure 2. Average Plasma Concentration (ng/mL) of Danazol vs Time Following Oral Administration to Rats A) 10 mg/kg from PEG400 (●), 1%MC (○), Labrafil (△), and Labrafil + Tween 80 (9:1) (□); B) 12 mg/kg from NE (●), 11.3 mg/kg from NE-SA (○) and 17.5 mg/kg from NE-DCA (△). The data are mean $\pm$ SD of the results from 3 animals.

Figure 3.

Average Plasma Concentration (ng/mL) of Danazol vs Time Following Oral Administration to Dogs at 20 mg/kg in Danocrine Tablet (●), 5 mg/kg in Labrafil Solution (○) and 7.16 mg/kg in NE-SA (△). The data are mean $\pm$ SD of the results from 3 animals.

Table 1

Danazol Pharmacokinetic Parameters (Mean $\pm$ SD; n = 3) Following Intravenous Administration in 10% HP β CD/ water Formulation to Rats and Dogs.

Pharmacokinetic parameters (Mean $\pm$ SD)		
Species	Rat	Dog
Dose (mg/kg)	0.65	1.0
C ₀ (ng/mL)	301 $\pm$ 111	533 $\pm$ 59.0
t _{1/2} (hr)	0.931 $\pm$ 0.084	4.68 $\pm$ 0.78
MRT _{last} (hr)	0.849 $\pm$ 0.148	3.23 $\pm$ 0.43
CL (L/hr/kg)	4.74 $\pm$ 1.98	1.34 $\pm$ 0.10
V _{ss} (L/kg)	4.55 $\pm$ 1.77	4.89 $\pm$ 0.62
AUC _{last} (hr-ng/mL)	152 $\pm$ 69.0	737 $\pm$ 49.2
AUC (hr-ng/mL)	155 $\pm$ 67.2	749 $\pm$ 56.4
Dose Normalized Values		
AUC _{last} (hr-k _g -ng/mL/mg)	234 $\pm$ 106	737 $\pm$ 49.2
AUC (hr-k _g -ng/mL/mg)	238 $\pm$ 103	749 $\pm$ 56.4

Dose normalized values calculated by dividing the AUC parameter by the nominal dose of danazol

Danazol Pharmacokinetic Parameters (Mean $\pm$ SD; n = 3) Following Oral Administration to Male Sprague-Dawley Rats from Different Vehicles and Nanoemulsion Formulations.

Table 2

PK Parameters	PEG400	1% MC	Labrafil	Labrafil/Tween 80 (9:1)	NE	NE-SA	NE-DCA
C_{max} (ng/mL)	4.35 $\pm$ 0.894	11.3 $\pm$ 8.49	43.2 $\pm$ 11.0	59.8 $\pm$ 25.9	106 $\pm$ 74.3	75.3 $\pm$ 22.6	73.0 $\pm$ 32.1
t_{max} (hr)	1.00 $\pm$ 0.00	4.17 $\pm$ 3.18	2.33 $\pm$ 0.577	2.67 $\pm$ 0.577	1.67 $\pm$ 0.577	3.33 $\pm$ 1.15	1.17 $\pm$ 0.76
$t_{1/2}$ (hr)	ND	ND	1.40 $\pm$ 0.178	1.24 $\pm$ 0.152	3.43 $\pm$ 1.96	3.27 $\pm$ 0.484 ^a	1.54 $\pm$ 0.28
AUC_{last} (hr·ng/mL)	15.2 $\pm$ 5.31	27.3 $\pm$ 9.85	140 $\pm$ 30.3	177 $\pm$ 63.2	416 $\pm$ 218	461 $\pm$ 170	330 $\pm$ 73.1
$AUC_{last}/Dose$ (hr·kg·ng /mL/mg)	1.52 $\pm$ 0.531	2.73 $\pm$ 0.985	14.0 $\pm$ 3.03	17.7 $\pm$ 6.32	34.6 $\pm$ 18.2	40.8 $\pm$ 15.0	18.9 $\pm$ 4.20
% F	0.649 $\pm$ 0.227	1.17 $\pm$ 0.420	5.97 $\pm$ 1.29 ^b	7.54 $\pm$ 2.70 ^c	14.8 $\pm$ 7.77	17.4 $\pm$ 6.41 ^d	8.09 $\pm$ 1.79

^a $p < 0.01$, NE-SA vs NE-DCA;

^b $p < 0.05$, Labrafil vs PEG400 and 1% MC;

^c $p < 0.01$, Labrafil/Tween 80 vs PEG400 and 1% MC;

^d $p < 0.05$, NE-SA vs Labrafil and Labrafil/Tween 80; ND: Not determined; %F determined by dividing the individual dose normalized oral AUC_{last} values by the average dose normalized IV AUC_{last} value

Table 3

Danazol Pharmacokinetic Parameters (Mean $\pm$ SD; n = 3) Following Oral Administration from Tablet, Solution and Nanoemulsion to Dogs.

Pharmacokinetic parameters (Mean $\pm$ SD)			
Formulation	Danocrine	Labrafil	NE-SA
Dose (mg/kg)	20	5.0	7.16
Cmax (ng/mL)	27.7 $\pm$ 13.7	127 $\pm$ 30.4	631 $\pm$ 306
tmax (hr)	0.88 $\pm$ 0.25	1.00 $\pm$ 0.71	1.33 $\pm$ 0.58
t1/2 (hr)	1.09 $\pm$ 0.29	1.59 $\pm$ 0.06	4.44 $\pm$ 1.13 ^a
AUClast (hr-ng/mL)	53.4 $\pm$ 36.4	346 $\pm$ 83.9	3041 $\pm$ 1041
AUC (hr-ng/mL)	54.2 $\pm$ 36.5	409 $\pm$ 31.1	3092 $\pm$ 1027
Dose Normalized Values			
AUClast (hr-k-g-ng/mL/mg)	2.67 $\pm$ 1.82	69.3 $\pm$ 16.8	425 $\pm$ 145
AUC (hr-k-g-ng/mL/mg)	2.71 $\pm$ 1.82	81.8 $\pm$ 6.22	432 $\pm$ 143
Bioavailability (%F)	0.24 $\pm$ 0.16	6.20 $\pm$ 1.50	58.7 $\pm$ 22.3 ^b

^a $p < 0.01$,

^b $p < 0.001$ NE-SA vs Labrafil and Danocrine; Dose normalized by dividing the AUC parameter by the nominal dose of danazol; %F determined by dividing the individual dose normalized oral AUC values by the average dose normalized IV AUC value