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Relative bioavailability of danazol in dogs from liquid-filled hard gelatin capsules

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Abstract

Danazol was dissolved in non-aqueous mixtures containing either polyethylene glycol 400 or polysorbate 80, and filled into hard gelatin capsules at 50 mg concentrations. The bioavailability of these formulations was compared with commercial danazol capsules in a two-way crossover study using young female beagle dogs. Both formulations showed greater oral bioavailability when compared with either the 100 or 200 mg commercial brand of danazol. The bioavailability of the polyethylene glycol 400 and polysorbate 80 formulations was enhanced 3.7 and 15.8 times, respectively, when compared at the 100 mg dose level.

Keywords: Danazol; Bioavailability; Liquid-filled capsules; Solubility; Dog

1. Introduction

Danazol is a synthetic androgen that inhibits the output of pituitary gonadotropins. The drug is neither estrogenic nor progestational, and it depresses the output of both follicle stimulating hormone and luteinizing hormone. It is used in treatment of endometriosis, where the recommended therapeutic dose is 600–800 mg/day in two divided doses (Fedele et al., 1993; Wheeler et al., 1993). In the treatment of fibrocystic breast disease, the dose ranges from 100 to 400 mg/day

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in two divided doses. At a starting dose of 200 mg, two to three times a day, it is used in the management of migraine (Lichten et al., 1991) and hemophilia (Saidi et al., 1986). These high doses of danazol in humans are required because the bioavailability of commercial danazol (50, 100 and 200 mg capsules) is only 30%. The bioavailability, in dogs, of a conventional suspension of danazol is only $5.1 \pm 1.9\%$ (Liversidge and Cundy, 1995). These observations have been linked to its poor water solubility (0.59 μg/ml) and higher than optimum partition coefficient (log P of 3.8 by the shake flask method). Recent publications have suggested that the bioavailability of danazol can be enhanced by size-reducing the drug into nanometer particles or by forming water-soluble drug and hydroxypropyl-β-cyclodextrin complexes (Liversidge and Cundy, 1995). The surface treatment of danazol with the surfactant docusate sodium, is another technique that improves the dissolution of danazol (Brown et al., 1998). The use of enteric hydroxypropyl methylcellulose phthalate coprecipitates has been suggested as another viable option (Kondo et al., 1994).

This paper examines a couple of enhanced solubility systems that have the ability to promote the intestinal absorption of danazol. Polyethylene glycol 400 (polyethylene glycol) is a popular solvent employed in soft gelatin capsule technology, that has been used extensively to formulate poorly water-soluble drugs (Shelley, 1996). Some of the drugs that have benefited from this technology are temazepam and digoxin (Jones et al., 1988). Polysorbate 80 (polysorbate), on the other hand, is a surfactant that promotes the oral absorption of hydrophobic drugs by increasing wetting, dissolution and the formation of microemulsions, in vivo.

2. Materials

Danazol was obtained from Schering AG (Germany). The commercial brand of danazol, Danocrine®, 100 and 200 mg, was obtained from Sterling Winthrop-Breon; polyethylene glycol 400, sentry grade, was from Union Carbide (Danbury, CT); Povidone K-30 (PVP) is from BASF (Parsippany, NJ); and Polysorbate 80 is from ICI (Wilmington, DE). The hard gelatin capsules were purchased from Capsugel (Greenwood, SC).

3. Procedure

3.1. Solubility of danazol

The saturated solubility of danazol was determined in water, ethanol, glycerin, propylene glycol, polysorbate 80 and polyethylene glycol 400 by shaking an excess of drug in 5 ml of pure solvent for at least 24 h at 25°C. The solubility was also determined in polyethylene glycol containing 10, 20 and 30% w/w of PVP. The mixtures were then filtered and the filtrate assayed by UV spectrophotometry ($\lambda_{max} = 286$ nm) for drug content.

3.2. Formulation

Two formulations of danazol were prepared in non-aqueous solvent mixtures (Table 1). Danazol was added to polyethylene glycol (S.G. 1.12) containing dissolved PVP or to polysorbate (S.G. 1.08) and stirred to achieve complete dissolution. Warming both preparations to 40°C facilitated the dissolution of drug. These mixtures were then cooled to ambient conditions and filled into size-000 hard gelatin capsules (capsule volume 1.37 cm^3) to allow for a dose of 50 mg per capsule.

Table 1
Liquid-filled hard-shell formulations of danazol

Ingredients	PEG formulation (mg)[a]	Polysorbate formulation (mg)
Danazol	50	50
Polyethylene glycol 400, NF[b]	1000	–
Povidone, USP[c]	300	–
Polysorbate 80, NF[b]	–	1350

[a] PEG, polyethylene glycol.
[b] NF, national formulary.
[c] USP, US Pharmacopoeia.

3.3. Assay

The drug content in the capsules was determined by their dissolution in a 70% isopropyl alcohol–water mixture and assay by UV–VIS spectrophotometry at a λ_{max} of 286 nm. The same solvent was used to evaluate the dissolution of the capsules (USP method II). The plasma samples obtained from the in vivo study were assayed by a reverse-phase high performance liquid chromatography method, similar to that described by Nygard et al. (1987).

3.4. Bioavailability in dogs

Six healthy young adult female beagle dogs, body weight 9.3–11.4 kg, were used as test subjects. In a randomized two-way crossover study, one 200 mg Danocrine®, two 50 mg polyethylene glycol or two 50 mg polysorbate capsules were used per test animal. In this plan, four animals received each formulation. All dogs were fasted for approximately 20 h prior to dosing and for 3 h post-dosing. Water was provided ad libitum. Immediately following dosing, the animals were gavaged with 60 ml of water. Blood samples (5–8 ml) were collected by a fore-leg venipuncture into heparinized tubes shortly prior to dosing and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, and 24 h post-dose. Samples were centrifuged within 1 h of collection and the plasma portion frozen until assayed. A week of washout was allowed between the two dosing periods. As a follow-up to this design, only four dogs were then dosed with one 100 mg Danocrine® capsule and plasma collected as before.

3.5. Data analysis

The plasma concentration of danazol (ng/ml) was plotted against time (h) and the WIN-NONLIN computer software (Pharsight Corporation, Mountain View, CA) was used to calculate the relevant pharmacokinetic parameters. The area under the plasma level curve (AUC_{0-24}) was computed for each treatment and the relative bioavailability of each 100 mg test dose was compared with that of the Danocrine® 100 mg capsule.

4. Results and discussion

4.1. Solubility of danazol

The saturated solubility (25°C) of danazol in water, ethanol, glycerin, propylene glycol, polyethylene glycol 400 and polysorbate 80 was found to be approximately 0.00058, 26.5, 0.1, 10.8, 33.4 and 40.0 mg/ml, respectively. The solubility of danazol in polyethylene glycol containing 10, 20 and 30% w/w of PVP was approximately 37, 48 and 56 mg/ml, respectively. Based on this observation, polyethylene glycol–30% PVP and polysorbate were obvious choices for formulation development.

4.2. Formulation

Both the polyethylene glycol and the polysorbate formulations were stable under ambient conditions, for the duration of the study. The parameters evaluated were assay, dissolution, clarity of capsule fill and integrity of capsule shell wall.

4.3. Bioavailability in dogs

The mean plasma levels obtained for the four oral formulations are presented in Fig. 1. Table 2

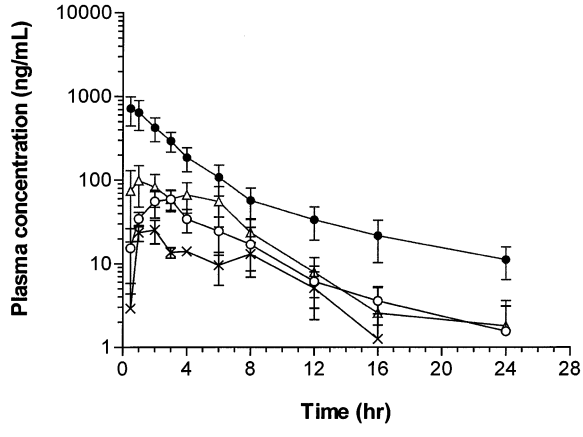

Fig. 1. Effect of formulation on the mean ± S.E. plasma concentrations of danazol following oral administration: (×) Danocrine®, 100 mg; (○) Danocrine®, 200 mg; (△) polyethylene glycol–PVP, 100 mg; and (●) polysorbate, 100 mg.

Table 2
Pharmacokinetic parameters following oral administration of danazol formulations to fasted female beagle dogs (S.D. for $n = 4$)

Formulation	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}z$ (h)	AUC_{0-24} (ng h/ml)	Relative F (%)
Danocrine®, 100 mg	25.98 (10.06)	3.00 (3.37)	2.72 (1.40)	155.25 (52.51)	–
Danocrine®, 200 mg	65.50 (29.30)	1.88 (1.03)	3.34 (2.05)	340.33 (222.52)	219.2
PEG 400–PVP, 100 mg[a]	132.75 (89.10)	1.88 (1.55)	3.39 (2.61)	570.13 (421.90)	367.2
Polysorbate 80, 100 mg	817.00 (448.86)	0.88 (0.25)	7.41 (2.33)	2460.21 (1734.81)	1584.7

[a] PEG, polyethylene glycol.

lists the calculated pharmacokinetic parameters following non-compartmental analysis of the data. The area under the curve, AUC_{0-24}, for the polysorbate formulation was significantly greater than that for the other three formulations studied ($p < 0.002$ by unpaired t-test). The bioavailability of the polyethylene glycol 400 and polysorbate 80 formulations was enhanced 3.7 and 15.8 times, respectively, when compared at the 100 mg dose level. The bioavailability of the polyethylene glycol formulation was about three times greater than for the Danocrine® 100 mg capsule, but the difference was not statistically significant. The half-life of the terminal phase ($t_{1/2}z$) for the polysorbate formulation was 7.4 h, compared with 3.3 h for the Danocrine® 200 mg and the polyethylene glycol–PVP formulation. The increase in $t_{1/2}z$ by approximately 4 h is attributed to a combination of a few factors. The increased bioavailability and tissue distribution of a highly lipophilic drug would result in a slower than expected clearance, as supported by the bi-exponential concentration vs time curve, i.e. two-compartment model. One dog receiving the Danocrine® 100 mg capsule experienced slight emesis after dosing. No other adverse effects were noted.

5. Conclusions

The complete dissolution of danazol in the polyethylene glycol–PVP complex and the polysorbate surfactant provided an increased bioavailability of drug over traditional powder-filled capsule formulations. The permeation enhancement properties of polyethylene glycol and polysorbate may be a relevant factor to be considered. We believe that in the case of danazol, the solubility enhancement property outweighs any permeation enhancement property. This study lends credence to the use of these two solvent mixtures in liquid-filled hard shells or soft-gel capsules. The addition of three parts of PVP to one part of drug provided a soluble stable complex that did not precipitate even when small amounts of water migrated from the gelatin shell wall into the drug core during process development and scale-up manufacturing of soft-gel capsules.

The absolute bioavailability of a conventional dosage form in dogs is approximately 5.1%, as previously reported. Hence, a 15.8-fold increase with the polysorbate formulation would result in an absolute bioavailability of approximately 80.6%. Based on this observation, we have recommended that a 25 mg soft-gel capsule be considered for further development. The daily intake of danazol would now be reduced by at least a factor of four. In addition, the increased $t_{1/2}z$ of the drug warrants additional investigation into the possibility of once a day dosing.

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