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Characterization and bioavailability of danazol-hydroxypropyl β-cyclodextrin coprecipitates

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Abstract

Danazol, a steroid with very low aqueous solubility and poor bioavailability, and its coprecipitates were characterized for solubility, dissolution rate and bioavailability. Solubility diagrams of danazol in aqueous hydroxypropyl β-cyclodextrin (HPCD) solutions and isopropanol/water solutions were constructed with concentration of HPCD solutions ranging between 0.65 and 65.0 mM. Coprecipitates of danazol and HPCD at ratios ranging from 1:1 to 1:10 were prepared by solvent evaporation method using ethanol and by freeze drying method. Solubility diagrams indicated the existence of a complex between danazol and HPCD, and showed a remarkable increase in danazol solubility. Stability constants of the complex and thermodynamic parameters of complexation were calculated in both aqueous and aqueous / organic solvents; the presence of isopropanol appeared to have negative effect on the stability of the complex. Dissolution profiles of the coprecipitates demonstrated higher dissolution rates than pure danazol. Characterization of coprecipitates by DSC and X-ray diffraction techniques showed that danazol existed almost exclusively in crystalline form in coprecipitates at low danazol to HPCD ratios; while at high ratios, danazol appeared to exist in a non-crystalline form. No distinct differences in the product characteristics could be attributed to the method of preparation. Oral bioavailability of the coprecipitate (danazol: HPCD, 1:10) and a marketed danazol were tested in Wistar rats in a two-way, randomized cross-over study. The area under the curve (AUC) of plasma concentration versus time was significantly higher ($P < 0.05$) for the complex than the commercial formulation, with mean AUC value more than two-fold higher for the complex. The bioavailability of the coprecipitate relative to the commercial formulation was calculated to be 237%. The peak plasma concentration (C_{max}) of the coprecipitate was higher and the time to reach the peak (T_{max}) was lower for the complex. The greater rate and extent of absorption of danazol from the complex makes it a formulation with the potential to decrease the oral dose of danazol. Absolute bioavailability of the coprecipitate and commercial formulation was 14.2% and 6.2%, respectively.

Keywords: Danazol; Hydroxypropyl-β-cyclodextrin; Complexation; Coprecipitate; Solubility; Bioavailability

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Danazol is an effective synthetic steroid (androgen) that is administered orally in the treatment of endometriosis and/or infertility, fibrocystic breast disease and hereditary angioedema. It has very high potential for the treatment of various autoimmune diseases such as acute myeloid leukemia, HIV associated thrombocytopenia and tropical spastic paraperesis - a progressive spastic disorder associated with T-lymphotropic virus (Dmowski, 1990; Harrington et al., 1991). It has been investigated for the use in premenopausal abnormal bleeding and metastatic breast cancer, among other indications (Zullo et al., 1991). However, due to its low aqueous solubility, first pass metabolism, and low bioavailability (Hooper et al., 1991; Bakatselou et al., 1991), danazol is usually administered in relatively high dose, ranging from 200 to 400 mg, in order to reach effective blood concentration. Because the drug suppresses ovarian steroidogenesis causing low estrogen and high androgen condition, and the fact that it is administered in high doses, many side effects such as weight gain, virilism and decrease in bone mineral content have been reported (Dodin et al., 1991). If a lower dose is used, there will be better tolerance, especially if danazol is given via other routes such as buccal or intranasal that will circumvent first-pass metabolism.

Many excipients, including cyclodextrins, have been used to increase the drug solubility. Cyclodextrins are hydrophilic, cyclic, non-reducing oligosaccharrides, composed of 6–8 glucopyranose units and have been extensively used to increase solubility of many poorly water soluble drugs (Krenn et al., 1992). Cyclodextrins have the ability to form inclusion complexes with many organic molecules, in which the guest molecule is entrapped within the cyclodextrin cavity thus resulting in an enhancement of solubility of the guest molecule. Different methods such as solvent evaporation (Pitha and Hoshino, 1992) and lyophilization (Betlach et al., 1993) have been employed for the preparation of different drug-cyclodextrin complexes in order to improve solubility of poorly water soluble drugs. Techniques such as phase solubility (Hassan et al., 1990), differential scanning calorimetry (DSC) (Amdidouche et al., 1989) and X-ray diffraction (Kitamura et al., 1990) have been used to study these complexes. These complexes usually showed higher bioavailability than the parent drug (Betlach et al., 1993). A more recently synthesized cyclodextrin derivative, 2-hydroxypropyl-β-cyclodextrin, has shown much higher solubility than parent cyclodextrins and, therefore, has higher solubilizing capabilities.

The aim of this study is to develop a new formulation by complexation of danazol with 2-hydroxypropyl β-cyclodextrin (HPCD) in an attempt to improve its solubility and consequently bioavailability. The complex was characterized by methods such as phase solubility studies, DSC and X-ray diffraction. The complexed formulation was then evaluated along with commercially available peroral danazol formulation (Danocrine®) for the bioavailability characterized by intra- and extravascular administration.

2. Materials and methods

2.1. Materials

Danazol was purchased from Miat Chemical Company, Italy. Hydroxypropyl β-cyclodextrin was donated by Roquette Corporation, Gurnee, IL. Isopropanol, ethanol and acetonitrile were all analytical grades.

2.2. Methods

2.2.1. Solubility of danazol in hydroxypropyl-β-cyclodextrin solutions

Solubility of danazol in HPCD solutions of varying concentrations was determined at 22°C, 37°C and 50°C by adding 10 ml of aqueous HPCD solution to the excess amount of danazol in a screw-capped bottle. The bottles were shaken in a thermostatically controlled water bath shaker until equilibrium was attained as demonstrated by a constant danazol content of three successive samples at 6, 12 and 24 h. Samples of 1 ml were filtered and analyzed for danazol content using HPLC. Solubility of danazol in HPCD solutions in 30% isopropanol in water was also determined at 22°C and 37°C in the same way. Aqueous-iso-

propanolic solvent was chosen because this is the pharmacopeial medium (USP XXII) for dissolution of danazol and, therefore, study of solubility of danazol-HPCD complex in this medium may be predictive of dissolution rate.

2.2.2. Preparation of danazol-HPCD coprecipitates

Coprecipitates of danazol with HPCD were prepared at different w/w ratios of 1:1, 1:1.5, 1:2, 1:3, 1:5 and 1:10 by mixing 1% w/v ethanolic solution of danazol with ethanolic solutions of HPCD of different concentrations. The resulting solution was stirred at an ambient temperature until complete evaporation of the solvent occurred. The resulting coprecipitates were kept in a desiccator for at least 48 h and then ground in glass mortar for size reduction. Particles passing through sieve size of 150 μm (mesh # 100) were kept for further study.

Lyophilized product of danazol-HPCD was prepared by mixing 50 ml of a 0.3% solution of danazol in acetonitrile with an equal volume of 0.9% aqueous HPCD solution followed by the freeze drying of the resulting solution. Organic solvent was used in this process to increase the solubility of danazol. The product obtained was found to be light cohesive cake characteristic of freeze dried products.

2.2.3. Differential scanning calorimetry (DSC) of danazol coprecipitates

Differential Scanning Calorimetry (DSC) of danazol, HPCD and danazol-HPCD products were performed in the temperature range of 25°C to 300°C using Shimadzu DSC-50 Thermal Analyzer. Samples were placed in an aluminum pan and heated at 10°C/min with an empty pan as a reference.

2.2.4. X-ray diffraction of danazol coprecipitates

X-ray diffraction patterns of danazol, HPCD and different danazol products were determined between $2\theta = 0-35°$ using a Phillips PW 3710 scanner / PW 1830 generator with a Cu kα anode at 40 kV and 40 mA.

2.2.5. Solubility of danazol-HPCD coprecipitates

Solubility of danazol from coprecipitates in water and 30% isopropanol in water was determined by adding 10 ml of water or isopropanol/water mixture to an accurately weighed amount of the coprecipitate containing 50 mg of danazol in a screw-capped bottle. The bottles were shaken at a constant temperature until equilibrium was attained. Samples of 1 ml were filtered and analyzed for danazol content.

2.2.6. Dissolution of danazol coprecipitates

An accurately weighed amount of the powdered coprecipitate having mean particle size of 110 μm, determined by image analyzer (Quantimet 500, Leica Cambridge Ltd, Cambridge, England), and containing 30 mg of danazol was subjected to dissolution study using USP Type I dissolution tester at 37°C. The powder was placed in baskets having screen size of 35 μm (VanKel Industries, Edison, NJ); the dissolution medium consisted of 900 ml of 30% isopropanol in water and stirring speed was 80 rpm. Samples were taken at different time intervals and analyzed for danazol content using HPLC.

2.2.7. Bioavailability study design

Oral dosing: Six male Wistar rats (300-350 g), fasted overnight, were dosed orally with either the coprecipitate (complex) 1:10 or Danocrine® (Sterling-Winthrop Pharmaceuticals) in a two-way randomized, crossover study. An aqueous solution of the coprecipitate or an aqueous dispersion of the content of a Danocrine® capsule containing the calculated dose (13.5 mg/kg) was administered to rats by gavage. Blood samples (0.35 ml) were drawn through the jugular vein cannula at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0 and 24.0 h after the dose. The blood samples were placed in heparinized tubes, centrifuged immediately to obtain plasma samples, and were stored at -20°C until analyzed by HPLC.

IV dosing: Danazol was also administered intravenously to another group of five male Wistar rats (300-350 g). Danazol solution in ethanol:water:propylene glycol (1:1.5:7.5) equivalent to a dose of 6.75 mg/kg was administered intravenously through the jugular vein cannula

followed by flushing the cannula with 0.5 ml of saline. Blood samples were drawn at the time intervals specified above and treated similarly.

HPLC assay method: The concentration of danazol in plasma samples was quantified by a reverse phase HPLC method using testosterone propionate (TSP) as an internal standard (Nygard et al., 1987). TSP, 0.1 ml solution in methanol (5 μg/ml), was added to 0.2 ml of plasma in screw-capped glass tubes. Hexane (4 ml) was then added and the tubes were vortexed for 40 s and centrifuged for 20 min at 3000 rpm. The organic layer was transferred to another tube and dried under a gentle stream of nitrogen at 37 °C. The samples were reconstituted in 0.1 ml of methanol and transferred to sample vials for analysis. Fifty microliters of the sample were injected into C 18 column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile:water (68:32) and the flow rate was 1 ml/min (Shimadzu Liquid Chromatography LC-10AS). UV absorbance was measured at 286 nm for danazol and 240 nm for testosterone (Shimadzu UV-VIS Detector SPD-10A). Chromatograms were recorded and analyzed using EZChrom software (Shimadzu Scientific Instruments Inc., Columbia, MD). Day to day reproducibility of the assay was tested by analyzing danazol samples of known concentrations on different days.

Pharmacokinetic analysis: Pharmacokinetic analysis of plasma concentration-time data was carried out by nonlinear regression curve-fitting (PCNONLIN, SCI Software, Lexington, KY) (Gibaldi and Perrier, 1982). The data were better described by the two-compartment open model. For IV bolus two-compartment model, the equation is as follows:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

The area under the curve ($AUC_{0-\infty}$) is obtained by integrating this equation from t = 0 to t = ∞ yielding,

$$AUC_0 - \infty = \frac{A}{\alpha} + \frac{B}{\beta} \quad (2)$$

where A and B are intercepts of the feathered and linear portions, respectively; β is the terminal phase rate constant, and α is the distribution phase rate constant.

Plasma concentration-time data obtained after the oral administration of the coprecipitate was analyzed by nonlinear regression according to a two-compartment model with first order absorption and lag time, t*, which accounts for any delay between drug administration and its systemic appearance (Nerella et al., 1993). This model is represented by the equation;

$$C = Ae^{-\alpha(t-t^*)} + Be^{-\beta(t-t^*)} - (A+B)e^{-K_a(t-t^*)} \quad (3)$$

All pharmacokinetic parameters including C_{max}, T_{max} and $AUC_{O-\infty}$ were obtained from the curve fitted through data points by nonlinear regression. Integration between t = 0 to t = ∞ yields,

$$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} - \frac{A+B}{k_a} \quad (4)$$

For the concentration-time data obtained after the oral administration of Danocrine®, $AUC_{O-\infty}$ was calculated by the trapezoidal rule only, since it was difficult to fit the curve by nonlinear regression due to the irregular profile obtained and convergence (the point where sum of squared residuals is minimum) was not achieved. $AUC_{0-\infty}$ values for the complex were also calculated by the trapezoidal rule and compared with those values obtained by nonlinear regression.

The ratio of the $AUC_{0-\infty}$ of the coprecipitate to that of $AUC_{0-\infty}$ of Danocrine was used to determine the extent of absorption of the complex relative to Danocrine®. Statistical significance for $AUC_{0-\infty}$ and C_{max} between the coprecipitate and Danocrine® was determined by using the paired *t*-test. The ratio of the $AUC_{0-\infty}$of either the coprecipitate or Danocrine to that of $AUC_{0-\infty}$ after IV administration (corrected for difference in the dose) was used to determine the absolute bioavailability of the corresponding formulation.

3. Results and discussion

3.1. Solubility studies

The solubility of danazol in water was very low and was found to be 0.61 and 0.32 mg/L at 37°C

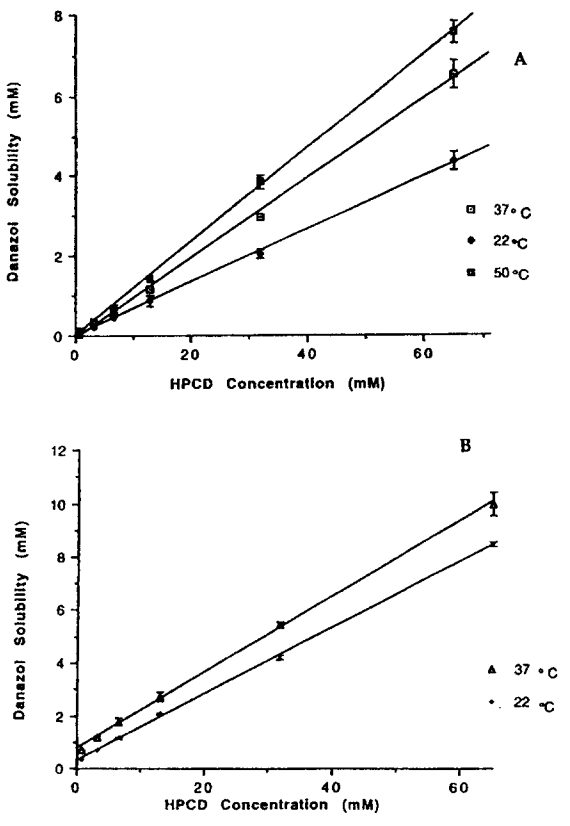

Fig. 1. Solubility of danazol (Mean ± S.D.) in (A) aqueous solutions of HPCD and (B) aqueous-isopropanolic solutions of HPCD.

and 22°C, respectively. Solubility diagrams of danazol in aqueous HPCD solutions at 22°C, 37°C and 50°C are shown in Fig. 1a. The linear A_L-type of the curve obtained in all cases suggests 1:1 complexation between danazol and HPCD (Higuchi and Connors, 1965). The stability constants (K) of the 1:1 complex were calculated (Table 1) according to the equation below (Usayapant et al., 1991),

$$[S_t] = [S_0] + \frac{K_{1:1}[S_0][L_t]}{1 + K_{1:1}[S_0]} \quad (5)$$

where $K_{1:1}$ is the stability constant of 1:1 complex; $[S_0]$ is the intrinsic solubility of danazol in the pure solvent; $[S_t]$ is the solubility of danazol in HPCD solution; and $[L_t]$ is the total concentration of ligand (HPCD). The mean values of the stability constant ($K_{1:1}$) at the different temperatures were used for calculation of different thermodynamic parameters. The enthalpy (ΔH^0) of complexation is calculated from the slope of Van't Hoff plot (Fig. 2); free energy changes (ΔF^0) and entropies (ΔS^0) of complexation between danazol and HPCD in water were also calculated using the following equations (Simonelli et al., 1976) and are shown in Table 1;

$$\Delta F^0 = -RT\ln K \quad (6)$$

$$\Delta F^0 = \Delta H^0 - T\Delta S^0 \quad (7)$$

Complexation is accompanied by a decrease in the energy of the system and enthalpy (ΔH^0) of complexation is negative. This decrease in the enthalpy of the system is brought about by the substitution of an unfavored polar-apolar interaction between the included water and the hydrophobic HPCD cavity by the more energetically favored apolar-apolar interaction between danazol and HPCD cavity (Szejtli, 1988).

Solubility diagrams of danazol in aqueous / isopropanolic solutions at 22°C and 37°C are shown in Fig. 1b. The stability constants ($K_{1:1}$) at both temperatures were calculated using equation (5), and were found to be 583 and 331 M^{-1} at 22°C and 37°C, respectively. These values of stability constants are much lower than the corresponding values in water. The decrease of stability of a cyclodextrin-drug complex due to presence of organic solvent has been previously reported (Pitha and Hoshino, 1992). This is attributed to the competition of organic solvent molecules with drug molecules for cyclodextrin cavity, thereby lowering the stability of the complex.

3.2. Thermal analysis

Differential Scanning Calorimetry thermograms of danazol coprecipitates showed endothermic peak at 224°C corresponding to fusion of crystalline danazol (Fig. 3). The enthalpy of fusion (ΔH) of danazol in coprecipitates and physical mixtures was calculated using Shimadzu TA-50 software (Shimadzu Scientific Instruments Inc., Columbia, MD). The values of ΔH of danazol fusion in the coprecipitates were found to be very

Table 1
Stability constants and thermodynamic parameters of complexation between danazol and HPCD in water

Temperature	K × 10^{-3} ± SD[a] M^{-1}	ΔH° KJ/mol	ΔF° KJ/mol	ΔS° J/mol.K
50°C	53.6 ± 2.19		-29.2	59.9
37°C	61.9 ± 3.16	-9.9	-28.4	59.8
22°C	76.6 ± 3.9		-27.6	59.9

[a] $n = 3$

close to those of the corresponding physical mixtures in ratios up to 1:3, while at 1:5 ratio, ΔH value of the physical mixture was greater than the corresponding coprecipitate. At the 1:10 ratio, the concentration of danazol was too small to be detectable even in the physical mixture and, therefore, DSC was not used for the assessment of the coprecipitate prepared at this ratio. In the freshly prepared 1:3 lyophilized product, danazol peak was not observed in the DSC thermograms; however, this peak reappeared after 30 days (Fig. 4).

3.3. X-ray difraction analysis

The powder X-ray diffraction pattern of HPCD showed no crystalline peaks indicating its amorphous nature; danazol, on the other hand, showed strong peaks in its diffractogram. X-ray diffraction of the coprecipitates showed distinct crystalline peaks of danazol up to 1:5 danazol: cyclodextrin ratio; these crystalline peaks were not observed in the diffractogram of the 1:10 coprecipitate indicating the amorphous nature of danazol in this coprecipitate (Fig. 5).

3.4. Solubility of danazol-HPCD coprecipitates

Solubility of danazol from its coprecipitates (equilibrium concentration of danazol in presence of excess undissolved coprecipitate) was superimposable with its solubility from the corresponding physical mixture up to 1:3 ratio. The amorphous form of danazol obtained in the 1:10 coprecipitate was highly soluble in water at 22°C when compared with the corresponding physical mixture. This 1:10 coprecipitate dissolved completely resulting in high concentration of danazol in solution initially, followed by decline of concentration with time. The decline in solubility of the coprecipitate with time is due to depletion of amorphous form. As a result, equilibrium of danazol in solution with the amorphous form no longer exists and danazol starts to crystallize out of solution (Fig. 6).

3.5. Dissolution studies

Dissolution profiles of pure danazol and its coprecipitates are shown in Fig. 7. The dissolution rate of danazol from the 1:10 coprecipitate is much higher than that from 1:5 coprecipitate or pure danazol. After 30 min, 87% of danazol content of the 1:10 coprecipitate was dissolved as compared to 26.8% and 16.8% from the 1:5 coprecipitate and pure danazol, respectively.

The results of solubility, thermal and X-ray studies indicate that danazol exists almost exclusively in crystalline form in coprecipitates up to 1:3 ratio. The fact that danazol exists in crys-

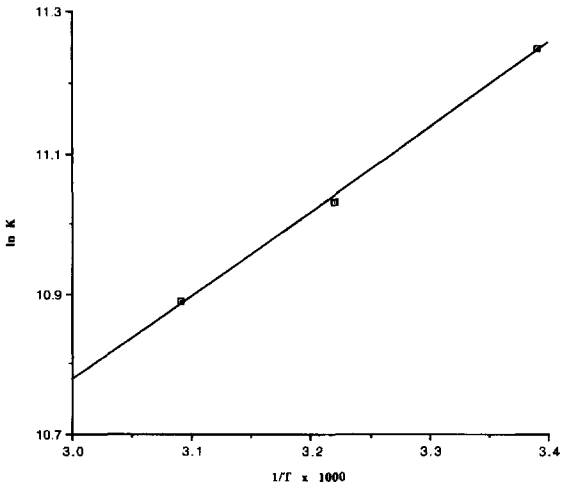

Fig. 2. Van't Hoff plot of stability constants of danazol-HPCD complex in aqueous solutions.

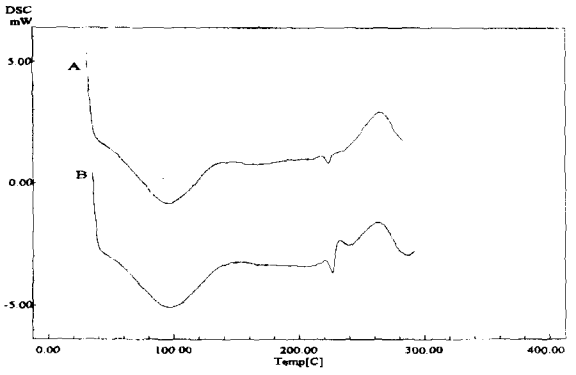

Fig. 3. Differential scanning calorimetry thermograms of (A) danazol-HPCD coprecipitate 1:5 and (B) danazol-HPCD physical mixture 1:5.

talline form and not as a complex with HPCD in the prepared coprecipitates could be due to: (1) danazol-HPCD complex is not stable to any appreciable extent in the solid state at this ratio and/or (2) the method of preparation does not favor the formation of this complex in the solid state. It can be inferred from the higher association constant of the complex in water as compared to its value in 30% isopropanol that the latter (and possibly other organic solvents) has negative effect on the stability of the complex. This observation is in agreement with the previous report (Pitha and Hoshino, 1992) that HPCD-testosterone complex did not exist in ethanolic solutions. However,

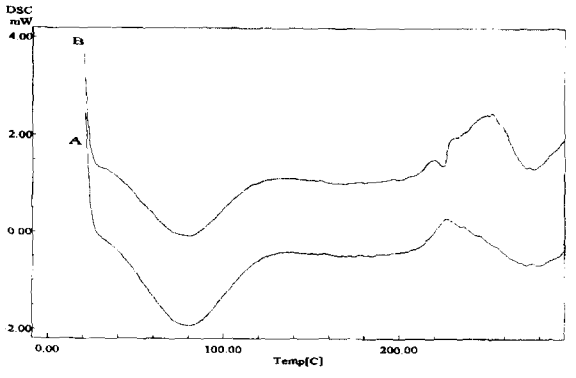

Fig. 4. Differential scanning calorimetry thermograms of (A) danazol-HPCD freeze dried product freshly prepared and (B) stored for 30 days.

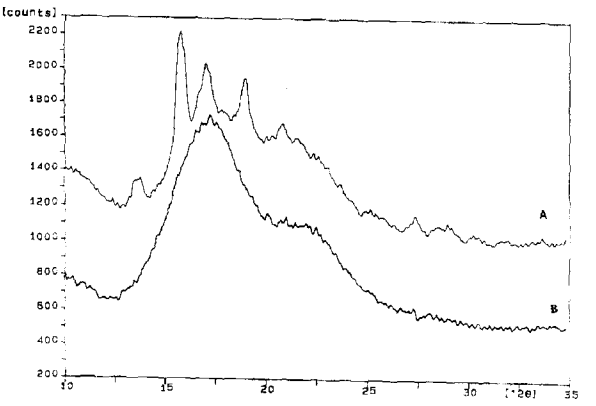

Fig. 5. X-Ray diffraction patterns of (A) danazol-HPCD physical mixture 1:10 and (B) danazol-HPCD coprecipitate 1:10 prepared by solvent evaporation.

at the end of the evaporation process HPCD-testosterone complex was obtained in the solid state since ethanol concentration was too small to compete with the steroid for HPCD complexing sites. This cannot be always true in all systems since it requires the simultaneous precipitation of both drug and complexing agent at the end of the evaporation process for successful complexation to take place. If either drug or complexing agent precipitates early in the process due to its limited

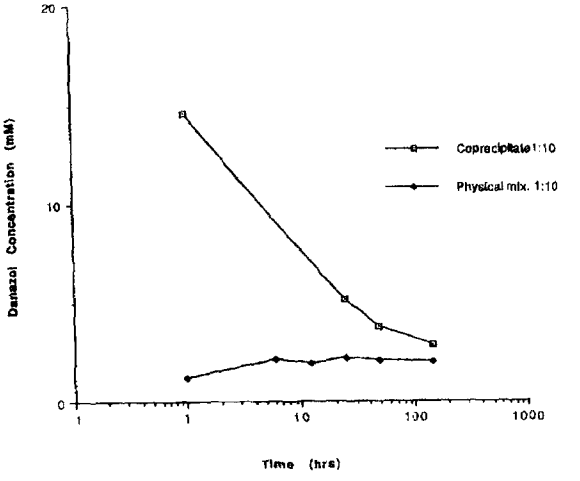

Fig. 6. Solubility of danazol from its 1:10 coprecipitate and physical mixture as a function of time at 22°C.

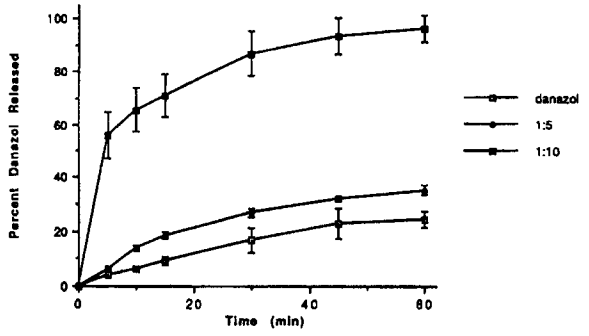

Fig. 7. Dissolution profiles of crystalline danazol and its coprecipitates. Error bars represent 1 S.D.

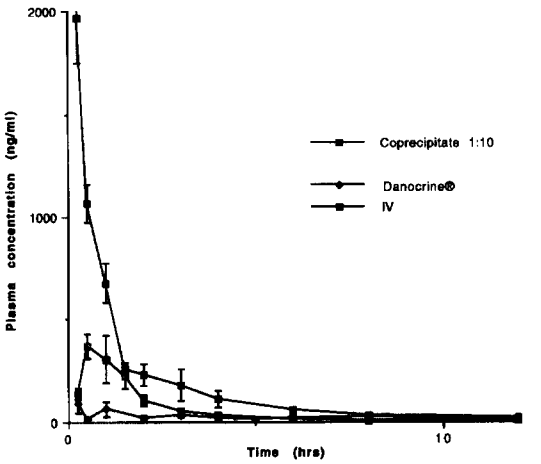

Fig. 8. Plasma danazol concentration (mean ± S.E.M., n = 5) following IV administration, Oral administration of coprecipitate and Danocrine® (mean ± S.E.M., n = 6).

solubility in the employed solvent, then no complex would be detectable in the prepared coprecipitate.

The use of lyophilization process resulted in an amorphous form of danazol in the freshly prepared 1:3 product. However, this form was unstable and reverted to the crystalline state upon storage. This is shown by the reappearance of danazol fusion peak in the DSC thermogram (Fig. 4). This indicates that the complex detected in solution is not stable to any appreciable extent in the solid state at 1:3 drug to cyclodextrin ratio and, therefore, any amorphous form obtained is not stabilized by complexation.

Using the solvent evaporation method and increasing drug to cyclodextrin ratio to 1:5 yielded coprecipitate in which danazol existed partly in a non-crystalline form as indicated by the lower ΔH of danazol fusion value and diminished X-ray diffraction peaks as compared to the corresponding physical mixture. On the other hand, danazol-HPCD coprecipitate prepared at 1:10 ratio showed no crystalline peaks in its diffractogram, indicating that danazol totally exists in an amorphous form in this coprecipitate which could be a danazol-HPCD complex. This amorphous form was found to be stable after storage for 60 days, as indicated by an X-ray diffraction pattern similar to that of the freshly prepared coprecipitate.

Table 2
Mean pharmacokinetie data of danazol in rats

	Coprecipitate		Danocrine®		IV	
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.
C_{max} (ng/ml)	453.2	77.0	127.3	33.2		
T_{max} (h)	0.51	0.06	1.41	0.52		
AUC (ng.h/ml)	677.3	133.9 (TR)	309.8	62.5	2483	443.3
	706.9	143.6 (NR)				
$\alpha(h^{-1})$	3.7	1.2			4.1	0.94
$\beta(h^{-1})$	0.38	0.07			0.39	0.11
$K_a(h^{-1})$	8.5	3.8				
Lag time (min)	13.1	0.59				

TR: Trapezoidal rule; NR: Nonlinear regression.

3.6. In vivo studies

3.6.1. Accuracy and precision of assay

The HPLC assay method showed good day to day reproducibility, and the concentration of danazol was 17.9 ± 2.3 ng/ml (accuracy = 10.5% and RSD = 12.8%) for the 20 ng/ml solution, and 414.2 ± 22.4 ng/ml (accuracy = 3.5% and RSD = 5.4%) for the 400 ng/ml solution.

3.6.2. Relative bioavailability

Danazol was better absorbed from danazol-HPCD coprecipitate than from Danocrine® (Fig. 8). The ratio of the mean $AUC_{0-\infty}$ (coprecipitate / Danocrine®) was 2.37 (relative bioavailability 237%) and the difference in $AUC_{0-\infty}$ values between the coprecipitate and Danocrine® was found to be significant ($P < 0.05$).

The more than two fold increase in the mean $AUC_{0-\infty}$ of the complex is due to increase in aqueous solubility and dissolution rate of danazol from the coprecipitate which also resulted in faster absorption as indicated by lower peak time (T_{max}) value for the coprecipitate (Table 2). Consequently, the mean peak plasma concentration (C_{max}) increased from 127.3 ng/ml for Danocrine® to 453.2 ng/ml for the coprecipitate, and this difference in C_{max} was significant ($P < 0.05$). The mean value of absorption rate constant K_a of danazol from the coprecipitate was 8.5 h^{-1} (Table 2). As referred to earlier, the slow and irregular absorption of danazol from Danocrine® precluded a precise calculation of K_a since it was not possible to fit the data by nonlinear regression. Similarly, the terminal phase rate constant (β) and the rate constant of distribution phase (α) were not obtained from Danocrine® data. The terminal phase rate constant of danazol was 0.38 h^{-1} and the rate constant of distribution phase was 3.7 h^{-1} for the coprecipitate (Table 2).

3.6.3. Absolute bioavailability

Blood concentrations of danazol were much higher following intravenous administration compared with the oral administration (Fig. 8). The mean value of $AUC_{0-\infty}$ was much lower after oral administration of danazol (either for the coprecipitate or Danocrine®) than after IV administration. The absolute bioavailability of Danocrine® and the coprecipitate was found to be 6.2% and 14.2%, respectively. The low absolute bioavailability of danazol after the oral administration is attributed to presystemic elimination of danazol (Davison et al., 1976). However, the absolute bioavailability of the coprecipitate was more than double that of Danocrine®. The mean values of terminal phase rate constant of danazol (β) and the distribution phase rate constant (α) were 0.39 h^{-1} and 4.1 h^{-1}, respectively.

4. Conclusion

Danazol was found to form a complex with 2-hydroxypropyl-β-cyclodxetrin in aqueous solution and the complexation process is accompanied by a negative enthalpy change. The addition of isopropanol to aqueous solutions of the complex resulted in pronounced decrease in the stability constant of the complex. A stable complexed form of danazol was not detectable at the low drug:HPCD w/w ratio irrespective of the method of preparation. Danazol existed in crystalline form in products up to 1:3 ratio and in partly crystalline form at 1:5 ratio. At 1:10 ratio, danazol existed almost completely in a stabilized amorphous form as confirmed by X-ray diffraction. Oral bioavailability of danazol in rats was improved by complexation with HPCD as compared with a commercial formulation (Danocrine®). Although both the complex and Danocrine® showed relatively low absolute bioavailability due to presystemic elimination of danazol, the higher rate and extent of absorption of the complex makes it a formulation that has the potential to decrease the oral dose of danazol and for delivery through other routes of administration which can further improve the bioavailability.

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