ELSEVIER

International Journal of Pharmaceutics 145 (1996) 137–143

international journal of pharmaceutics

Bioavailability of danazol–hydroxypropyl-β-cylodextrin complex by different routes of administration

Sherif I. Farag Badawy[a], Mahmoud M. Ghorab[b], Christianah Moji Adeyeye[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA 15282, USA*
[b]*Faculty of Pharmacy, Cairo University, Cairo, Egypt*

Received 19 April 1995; revised 22 September 1996; accepted 26 September 1996

Abstract

Bioavailability of danazol–hydroxypropyl-β-cyclodextrin complex was investigated in rats and dogs using different routes of administration. The complex was administered buccally in an aqueous solution form to rats in which the esophagus had been ligated to prevent swallowing of the complex, while oral administration was by gastric gavage. In dogs, the complex was administered buccally in the form of rapidly dissolving adhesive patch formulation, and orally in the form of hard gelatin capsules. Buccal absorption of the complex was evaluated in an attempt to bypass presystemic elimination. Buccal absorption of danazol in rats was slow and mean plasma concentration exhibited a plateau for 5 h. However, the extent of absorption was higher than the oral route, and bioavailability was 186% relative to oral administration. The absolute bioavailability was 26.4%, indicating incomplete absorption of the complex after buccal administration. Plasma profiles and pharmacokinetic parameters obtained in dogs were similar in the orally and buccally administered doses, suggesting that drug release from the buccal patch was not slow enough and the drug was consequently swallowed rather than absorbed across the buccal mucosa. Copyright © 1996 Elsevier Science B.V.

Keywords: Bioavailability; Danazol; Hydroxypropyl-β-cyclodextrin; Complex; Buccal route; Oral route

1. Introduction

Danazol is a synthetic steroid used in the treatment of endometriosis, hereditary angioedema, fibrocystic breast disease, and has high potential for the treatment of autoimmune diseases such as acute myeloid leukemia and HIV-associated thrombocytopenia (Dmowski, 1990). Danazol is practically insoluble in water (Bakatselou et al., 1991), is known to undergo hepatic metabolism (Davison et al., 1976), and has low oral

* Corresponding author. Tel.: +1 412 3965133; fax: +1 412 3965130; e-mail: adeyeyechri@duq3.cc.duq.edu

PII S0378-5173(96)04763-1

bioavailability (Hooper et al., 1991). Consequently, danazol is usually administered in a relatively high dose range in order to reach effective blood concentration. Recently, a soluble danazol–cyclodextrin complex has been prepared that significantly increased oral bioavailability of danazol in rats (Badawy et al., 1996). However, the oral route of administration is subject to first-pass metabolism, and oral absolute bioavailability of the complex is still low in the rats. Administration of danazol via another route that bypasses presystemic metabolism can increase the efficiency of danazol delivery to the systemic circulation. This would result in a lower dose, and might reduce side effects and increase tolerance to danazol. Administration of danazol–cyclodextrin complex through the buccal route may achieve this goal. Aside from the fact that compounds delivered through the buccal mucosa reach the systemic circulation directly (Hussain et al., 1986, 1987; Pitha et al., 1986), the buccal mucosa is also an easily accessible and convenient site for drug delivery. In addition, it has the advantages of being a robust mucosa, and has high patient acceptability and compliance (Nair and Chien, 1993).

The purpose of this study is to test the feasibility of delivering danazol in complex form with hydroxypropyl-β-cyclodextrin (HPCD), through the buccal mucosa. Buccal absorption of danazol was studied in rats using aqueous solution of the complex and compared with the oral absorption of the complex as well as intravenously (i.v.) administered danazol reported previously (Badawy et al., 1996). In dogs, the complex was administered buccally using adhesive patch formulation, and orally in hard gelatin capsules.

2. Materials and methods

2.1. Materials

Danazol was purchased from Miat Chemical Company (Milan, Italy). Hydroxypropyl-β-cyclodextrin was donated by Roquette Corporation (Gurnee, IL). All other chemicals were of analytical grades.

2.2. Methods

2.2.1. Preparation of inclusion complex

Danazol–HPCD coprecipitate (1:10 w/w) was prepared by mixing 1% w/v ethanolic solution of danazol with an equal volume of 10% ethanolic solution of HPCD and the resulting solution was stirred at ambient temperature until complete evaporation of the solvent. The resulting coprecipitate was kept in a desiccator for at least 48 h and then ground in glass mortar for size reduction. Particles passing through a sieve size of 150 μm (mesh # 100) were used for bioavailability studies.

2.2.2. Buccal dosing of rats

Five male Lewis rats (300–350 g) were fasted overnight, and dosed buccally with danazol–HPCD complex. The rats were anesthetized with pentobarbital (50 mg/kg, intraperitoneally) and the esophagus was ligated through a small incision on the neck, which was then closed with surgical staples. The trachea was also cannulated with a polyethylene cannula to allow free breathing of the animal. Rats were maintained on their abdomen with their lower jaw on the surface of the bench. An aqueous solution of the complex (approximately 100 μl) containing the calculated dose (6.75 mg danazol/kg) was applied between the cheek and the lower gum using a syringe and a blunt needle. Ligation of the esophagus prevents the dosing solution from being swallowed, allowing it to remain in the buccal cavity. Blood samples (0.35 ml) were collected into heparinized test tubes after cutting the tip of the tail at 0, 0.25, 0.5, 1, 2, 3 and 5 h after dosing. Blood samples were centrifuged immediately to obtain plasma which was stored at -20°C until analyzed by high-performance liquid chromatography (HPLC).

2.2.3. Oral dosing of rats

Another group of rats, fasted overnight, was dosed with aqueous solution of danazol–HPCD complex (13.5 mg/kg) by gavage. Blood samples (0.35 ml) were drawn through a jugular vein cannula at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 and 12 h after dose. Blood samples were treated similarly as above.

2.2.4. Intravenous dosing of rats

Danazol solution in ethanol/water/propylene glycol (1:1.5:7.5) equivalent to a dose of 6.75 mg/kg was administered i.v. to a third group of rats. The solution was administered through a jugular vein cannula followed by flushing the cannula with 0.5 ml of saline. Blood samples were drawn through the jugular vein cannula at the time intervals specified under oral dosing and treated similarly.

2.2.5. Buccal dosing of dogs

Buccal bioavailability of the complex was also determined in a group of four female beagle dogs each weighing about 10 kg. The complex was administered in a rapidly dissolving adhesive patch formulation (Hussain et al., 1988). The patches were composed of the following: complex/hydroxypropylcellulose/polycarbophil (carbopol 934)/polyethylene glycol (PEG) 400 (63.75:28.4:2.5:5.35 weight ratio). The dry powders were triturated in a mortar and after a uniform mixture was formed, the PEG 400 was added and levigated with the powder mixture. The powder was then pressed in a hydraulic press at 220°F under 25 000 psi for 7 min. From the thin films, patches were cut to an oval shape (roughly 5×3.5 cm) containing the desired dose of 3.25 mg/kg of danazol. This dose, which is approximately half of that used for the rats, was chosen to reduce bulkiness of the administered patch. Dosing was performed by placing the patch between the cheek and the lower gum after moistening the dog's mouth with water. The patches became adhesive when wet. The mouth was held closed for approximately 7 min during which the patch dissolved. Blood samples (5 ml) were collected by jugular venipuncture after 0, 0.25, 0.5, 1, 2, 4, 6 and 8 h. Plasma was separated by centrifugation and the samples were stored at −20°C until analyzed by HPLC.

2.2.6. Oral dosing of dogs

A calculated weight of the complex equivalent to a dose of 3.25 mg/kg was placed in a hard gelatin capsule and administered orally to a different group of four dogs. Blood samples were collected at the time intervals specified under buccal dosing, and treated similarly.

2.2.7. 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. A 50 μl sample was injected into a C18 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). Ultraviolet 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, Columbia, MD). Day-to-day reproducibility of the assay was tested by analyzing danazol samples of known concentrations on different days.

2.2.8. Pharmacokinetic analysis

Pharmacokinetic analysis was carried out by nonlinear regression curve-fitting. Danazol concentration–time data were analyzed according to a one-compartment open model and a two-compartment open model by nonlinear regression (PCNONLIN, SCI Software, Lexington, KY), which uses the Gauss–Newton algorithm for minimizing the sum of squared residuals. All danazol plasma profiles were found to be better represented by the two-compartment model as shown by higher correlation coefficient value, lower Akaike information criterion (AIC) and Schwartz criterion (SC) for that model. Both AIC and SC are model selection criteria functions based on residual sum of squares. If a model attains a solution with a lower weighted sum of squares of residuals than any other model, then it is considered to be a better model (Nerella et al., 1993).

Plasma concentration–time data obtained after oral or buccal administration of the complex were

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. All pharmacokinetic parameters for oral and buccal administration, including C_{max}, T_{max} and $AUC_{0-\infty}$, were obtained from the curve fitted through data points by nonlinear regression. $AUC_{0-\infty}$ was obtained from the integrated form of the corresponding model equation shown below:

$$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta} - \frac{A+B}{K_a}$$

where A and B are constants, β is the terminal phase rate constant, α is the distribution phase rate constant, and K_a is the absorption rate constant.Similarly, $AUC_{0-\infty}$ after i.v. administration was obtained using the equation:

$$AUC_{0-\infty} = \frac{A}{\alpha} + \frac{B}{\beta}$$

For concentration–time data obtained after buccal administration of the complex in rats, $AUC_{0-\infty}$ was calculated by the trapezoidal rule only, using the mean value of β obtained from i.v. data, since convergence was not obtained due to slow absorption. The ratio of mean $AUC_{0-\infty}$ value after buccal administration to that of $AUC_{0-\infty}$ after oral administration was used to determine the extent of buccal absorption (b) of the complex relative to oral absorption (o) using the equation:

$$F = \frac{AUC^b}{AUC^o} \times \frac{Dose^o}{Dose^b} \times 100\%$$

Absolute bioavailability of the complex after buccal administration in rats was also calculated using this equation:

$$F = \frac{AUC^b}{AUC^{i.v.}} \times \frac{Dose^{i.v.}}{Dose^b} \times 100\%$$

3. Results and discussion

Cyclodextrin complexes do not permeate biologic membranes and only the free drug is absorbed across the buccal mucosa. Once in solution, cyclodextrin complexes dissociate and equilibrium exists between free and complexed drug. The absorption of the free drug leads to reduction of its concentration in solution and, therefore, more drug has to dissociate from the complex to maintain this equilibrium. The dissociation of the complex is a very rapid process; the more the drug is absorbed, the more the complex dissociates until absorption of the drug is complete (Szejtli, 1988).

3.1. Accuracy and precision of assay

The HPLC assay method showed good day-to-day reproducibility; the concentration of danazol was 17.9 ± 2.3 ng/ml (accuracy = 89.5% and RSD = 12.8%) for the 20 ng/ml solution, and 414.2 ± 22.4 ng/ml (accuracy = 96.5% and RSD = 5.4%) for the 400 ng/ml solution.

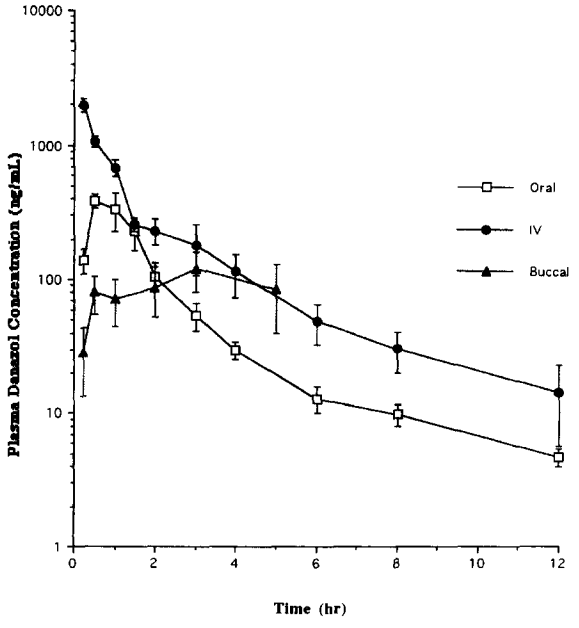

Fig. 1. Danazol concentrations in rat plasma (mean ± S.E.M.) following the administration of danazol intravenously (6.75 mg/kg, $n = 5$), and danazol–hydroxypropyl-β-cyclodextrin complex orally (13.5 mg danazol/kg, $n = 6$) and buccally (6.75 mg danazol/kg, $n = 5$).

Table 1
Pharmacokinetic data of danazol–HPCD complex in rats

	Oral (13.5 mg/kg)		Buccal (6.75 mg/kg)	
	Mean[a]	S.E.M.	Mean[b]	S.E.M.
C_{max} (ng/ml)				
Actual	453.2	77.0	174.1	38.7
Normalized[c]	33.6	5.7	25.8	5.7
T_{max} (h)	0.51	0.06	2.3	0.8
AUC (ng·h/ml): trapezoidal rule				
Actual	677.3	133.8	643.4	149.4
Normalized[c]	50.2	9.9	95.3	22. 1
AUC (ng·h/ml): nonlinear regression				
Actual	706.9	143.6	—	—
Normalized[c]	52.4	10.6	—	—

[a] $n = 6$.
[b] $n = 5$.
[c] Normalized data = actual value divided by dose.

3.2. Bioavailability in rats

Absorption of danazol after buccal administration of the complex to rats was much slower and extended over a longer period of time than after oral administration (Fig. 1, Table 1). The mean value of T_{max} was 2.3 h compared with 0.51 h after oral administration. The mean value of C_{max} was 174.1 ng/ml after buccal administration and mean plasma concentrations exhibited a plateau up to 5 h after administration. This slow absorption precluded convergence of the algorithm used for nonlinear regression and, therefore, modeling of the buccal data was not achieved. The absolute bioavailability of danazol after buccal administration was found to be 26.4%. Buccal bioavailability of danazol relative to oral administration was 186%. Although bioavailability values calculated using individual animal data would give a more accurate estimate, such calculation would require a cross-over design that was not feasible due to technical difficulties. Compounds that permeate through the buccal mucosa do not go through the hepatic portal vein. Instead, they reach the systemic circulation directly; thus, this higher bioavailability of danazol after buccal administration could be due to avoidance of hepatic first-pass metabolism. This finding supports the conclusion that the low absolute oral bioavailability of danazol in rats is due to presystemic elimination (Badawy et al., 1996). Buccal absorption of danazol, however, was not complete, which might be attributed to the slow permeation of danazol through the buccal mucosa in rats, as demonstrated by the extended absorptive phase. The buccal mucosa of rats, unlike human buccal mucosa, is keratinized (Harris and Robinson, 1992). In order to investigate the possibility that this slow absorption is due to keratinization, buccal absorption of the complex was studied in dogs, which have nonkeratinized buccal mucosa.

3.3. Bioavailability in dogs

Oral absorption of danazol in dogs was also rapid; the mean value of T_{max} was 0.52 h and the mean K_a-value was 4.2 h^{-1}. The plasma profile also followed two-compartment kinetics with a mean α-value of 2.9 h^{-1} and mean β-value of 0.29 h^{-1} (Table 2). Although a lower dose was used for oral dosing in dogs than in rats, comparable mean plasma concentrations were obtained in both cases. Plasma profiles obtained after administration of buccal patches showed close resemblance to those obtained after oral dosing (Fig. 2). The mean value of T_{max} was 0.39

Table 2
Pharmacoklnetic data of danazol–HPCD complex in dogs

	Buccal		Oral	
	Mean[a]	S.E.M.	Mean[a]	S.E.M.
α (h^{-1})	3.9	0.35	2.9	0.6
β (h^{-1})	0.28	0.05	0.29	0.06
K_a (h^{-1})	4.9	0.43	4.2	0.2
Lag time (min)	10.3	5.7	9.5	7.0
C_{max} (ng/ml)	750.4	179.2	705.8	59.1
T_{max} (h)	0.39	0.04	0.52	0.17
AUC (ng·h/ml)	1340	331	1376	200.8

[a] $n = 4$.

h and the mean K_a-value was 4.87 h^{-1}. The mean α-value was 3.9 h^{-1} and the mean β-value was 0.28 h^{-1}. The difference in the mean value of either $AUC_{0-\infty}$ or C_{max} after oral or buccal administration was not significant by the t-test.

The buccal administration of rapidly dissolving adhesive patches resulted in plasma profiles that exhibited pharmacokinetic parameters that closely resemble those obtained after oral administration. Also, the extent of absorption was not increased after administration of the patches, as shown by $AUC_{0-\infty}$ values that are very close to those obtained after oral administration (Table 2). This suggests that the complex was mostly swallowed and not absorbed through the buccal mucosa. Unlike the rat experiment, this system is not closed (the esophagus is not ligated) and the complex can be swallowed if absorption is not fast enough. Therefore, buccal absorption was not observed due to rapid drug release from the delivery system and consequent reduced contact time of the drug with the buccal mucosa.

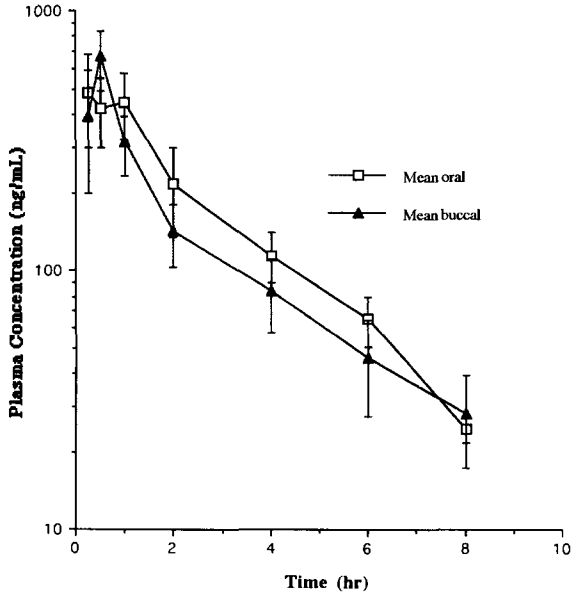

Fig. 2. Danazol concentrations in dog plasma (mean ± S.E.M., $n = 4$) after the administration of danazol–hydroxypropyl-β-cyclodextrin complex, equivalent to 3.25 mg danazol/kg, orally and buccally.

4. Conclusions

Buccal absorption of danazol across the keratinized mucosa of the rats was slow and the absorption phase was long. Administration of the complex in the form of rapidly dissolving adhesive patches in dogs did not result in buccal absorption of danazol and the complex was mostly swallowed. Clinical utilization of this route requires the development of a controlled release delivery system that will remain in contact with buccal mucosa longer to allow for adequate absorption. This investigation of the buccal administration of a water-soluble danazol complex can shed more light on the feasibility of this route for the delivery of similar drugs. Work is underway for a delivery system that will allow for adequate buccal absorption in dogs.

Acknowledgements

The authors would like to express their thanks to Dr Munir A. Hussain of The DuPont–Merck Pharmaceutical Company for the valuable discussions, and to Ms Nancy Rogers for her technical assistance. This research was partly funded by the Hunkele Dreaded Diseases grant awarded to C.M.A. from Duquesne University.

References

Badawy, S.I.F., Ghorab, M.M. and Adeyeye, C.M., Characterization and bioavailability of danazol–hydroxypropyl-β-cyclodextrin coprecipitates. *Int. J. Pharm.*, 128 (1996) 45–54.

Bakatselou, V., Oppenheim, R.C. and Dressman, J.B., Solubilization and wetting effects of bile salts on the dissolution of steroids. *Pharm. Res.*, 8 (1991) 1461–1469.

Davison, C., Banks, W. and Fritz, A., The absorption, distribution and metabolic fate of danazol in rats, monkeys and human volunteers. *Arch. Int. Pharmacodyn.*, 221 (1976) 294–310.

Dmowski, W.P., Danazol: a synthetic steroid with diverse biologic effects. *J. Reprod. Med.*, 35 (Suppl. 1) (1990) 69–74, discussion 74–75.

Harris, D. and Robinson, J.R., Drug delivery via the mucous membranes of the oral cavity. *J. Pharm. Sci.*, 81 (1992) 1–10.

Hooper, W.D., Eadie, M.J. and Dickinson, R.G., Single oral dose pharmacokinetics and comparative bioavailability of danazol in humans. *Biopharm. Drug Disp.*, 12 (1991) 577–582.

Hussain, M.A., Aungst, B.J. and Shefter, E., Buccal and oral bioavailability of nalburphine in rats. *J. Pharm. Sci.*, 75 (1986) 218–219.

Hussain, M.A., Aungst, B.J., Kearney, A. and Shefter, E., Buccal and oral bioavailability of naloxone and naltrexone in rats. *Int. J. Pharm.*, 36 (1987) 127–130.

Hussain, M.A., Aungst, B.J., Koval, C.A. and Shefter, E., Improved buccal delivery of opioid analgesics and antagonists with bitterless prodrugs. *Pharm. Res.*, 5 (1988) 615–618.

Nair, M. and Chien, Y.W., Buccal delivery of progestational steroids: I. Characterization of barrier properties and effect of penetrant hydrophilicity. *Int. J. Pharm.*, 89 (1993) 41–49.

Nerella, N.G., Block, L.H. and Noonan, P.K., The impact of lag time on the estimation of pharmacokinetic parameters. I. One-compartment open model. *Pharm. Res.*, 10 (1993) 1031–1036.

Nygard, G.A., Lovett, L.J., Erdmann, G.R. and Khalil, S.K.W., Analysis of danazol in serum using high-performance liquid chromatography. *J. Chromatogr.*, 415 (1987) 438–444.

Pitha, J., Harman, M. and Michel, M.E., Hydrophilic cyclodextrin derivatives enable effective oral administration of steroidal hormones. *J. Pharm. Sci.*, 75 (1986) 165–167.

Szejtli, J., *Cyclodextrin Technology*, Academic Publisher, Dordrecht, The Netherlands, 1988.