International Journal of Pharmaceutics, 64 (1990) 61–66
Elsevier

IJP 02166

Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats

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(Received 2 March 1990)
(Accepted 14 April 1990)

Key words: Nasal absorption; Absorption enhancement; 17-β-Estradiol; Progesterone; Dimethyl-β-cyclodextrin; Rats

Summary

The nasal absorption of progesterone was studied in male Wistar rats, using dimethyl-β-cyclodextrin (DMβCD) as an absorption enhancer. DMβCD solubilizes steroids which are poorly water soluble by formation of inclusion complexes. Drug preparations with a molar ratio of steroid to DMβCD of 1:2 were used in these experiments. The systemic bioavailabilities of progesterone with DMβCD, and a progesterone suspension were compared. DMβCD increased the bioavailability of progesterone from 18 ± 13% for the suspension to 58 ± 16% for the inclusion complex. The nasal absorption of simultaneously administered estradiol and progesterone with DMβCD as absorption enhancer was also studied, in order to investigate the potential use of this combination in the treatment of postmenopausal disorders. The bioavailabilities of estradiol and progesterone were high, 59 ± 16 and 67 ± 23%, respectively. They were not significantly different from those achieved after separate administration. The combined preparation had only a mild effect on the ciliary beat frequency of human nasal adenoid tissue in vitro.

Introduction

Estrogen substitution therapy is often used in the treatment of postmenopausal disorders (Young and Goldzieher, 1987). Orally administered estrogens, however, are extensively metabolized in the gastrointestinal tract and liver. The large breakdown of estrogens is believed to result in complicating side effects (Lievertz, 1987). Routes of administration which circumvent first-pass metabolism are therefore attractive alternatives for the pharmacotherapy with estrogens. Nasal administration can be such an alternative, since the nasal mucosa has proven to be a potential site of absorption for several steroid hormones (Rigg et al., 1977; Öhman et al., 1978; Steege et al., 1986; Chien et al., 1988; Bawarshi-Nassar et al., 1989). In earlier experiments we studied the nasal absorption of estradiol using dimethyl-β-cyclodextrin (DMβCD) as an absorption enhancer, and demonstrated a bioavailability of approx. 65% in rats (Hermens et al., 1990). This preparation did not effect nasal ciliary activity, which is an important prerequisite for nasal drug delivery (Hermens and Merkus, 1987).

Cyclodextrins are biocompatible compounds, which are able to form inclusion complexes with

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drugs (Uekama and Otagiri, 1987). By this mechanism, DMβCD forms aqueous solutions of poorly water-soluble steroids (Uekama et al., 1982), and thus increases the concentration of steroid that can be applied to the absorption site.

Long-term application of estrogens exclusively is considered to be a risk at the incidence of endometrial abnormalities. Oral progestogens in addition to estrogens are known to prevent these endometrial abnormalities (Whitehead et al., 1987). However, synthetic progestogens are associated with unwanted metabolic side-effects (Whitehead et al., 1987; Young and Goldzieher, 1987). Absorption of the natural progesterone is highly variable due to stomach content and strong first-pass metabolism (Maxson and Hargrove, 1985). It may therefore be advantageous to administer both estradiol and progesterone by the nasal route.

The aim of the present study was to investigate the nasal absorption of progesterone, and of estradiol and progesterone together in rats, using DMβCD as absorption promoter.

Experimental

Materials

Progesterone and 17-β-estradiol were obtained from Bufa Chemie (Castricum, The Netherlands), and Sigma (St. Louis, MO, U.S.A.), respectively. Dimethyl-β-cyclodextrin and Hypnorm^R were from Janssen (Beerse, Belgium). All other chemicals were of reagent or analytical grade.

Drug preparation

The administered progesterone-DMβCD as well as the estradiol-progesterone-DMβCD solutions were prepared by dissolving the steroids together with DMβCD, in a molar ratio of steroid to DMβCD of 1 : 2, in 96% (v/v) ethanol to form inclusion complexes. The ethanol was then evaporated at 50 °C under a mild stream of nitrogen. The residue was redissolved in 0.9% (w/v) saline, containing 0.01% (w/v) benzalkonium chloride. The final concentration of progesterone in the progesterone-DMβCD preparation was 10 mg/ml, containing 9% (w/v) DMβCD. The combined progesterone-estradiol preparation had a progesterone and estradiol concentration of 10 and 0.5 mg/ml, respectively, the DMβCD concentration being 9% (w/v).

A progesterone suspension was prepared by suspending 500 mg progesterone in 50 ml of 0.9% (w/v) saline with 0.01% (w/v) benzalkonium chloride, using a micronizer. The suspension was stabilized by adding 20 μl of 2% (v/v) Tween 80.

Nasal absorption studies

The nasal absorption studies were performed according to procedures described earlier (Fisher et al., 1985; Deurloo et al., 1989). Briefly, male Wistar rats weighing approx. 200 g were anaesthetized with an initial intramuscular injection of Hypnorm^R (1.0 ml/kg body weight), and additional injections of 0.5 ml/kg every 1.5–2 h. A small incision was made in the neck, and the trachea was canulated with teflon tubing. This prevented the rat from breathing through the nose, and thus facilitated nasal application. The oesophagus was tied up to prevent peroral absorption. A femoral artery was canulated to take blood samples. Intravenous (i.v.) administration was done through a canulated femoral vein. In the case of i.v. administration, the trachea canula was omitted. During the experiment animals were kept lying on their back on a thermostated rug. Rectal temperature was monitored and maintained between 37 and 38 °C. The nasal preparations (20 μl, corresponding to 200 μg progesterone and 10 μg estradiol) were administered unilaterally through the nares using PVC tubing connected to a microliter syringe. Intranasal administration of placebo (0.9% saline) and i.v. administration of the drug-DMβCD complexes were performed in order to determine absolute bioavailabilities. For i.v. administration, the drug preparations were diluted with saline, and 80 μl (corresponding to 100 μg progesterone and 5 μg estradiol) were slowly injected. Blood samples of 0.3 ml were taken at regular time intervals. The samples were centrifuged within 2 h to obtain serum, and subsequently stored at −20 °C until analysis.

Analytical procedures

Serum levels of progesterone and estradiol were measured by radioimmunoassay using Coat a

Count^R kits from DPC Laboratorium Service Benelux (Apeldoorn, The Netherlands). The detection limits of the assays were 0.05 and 0.01 ng/ml for progesterone and 17-β-estradiol, respectively.

Data analysis

The areas under the individual serum concentration vs time curves (AUC) up to the last data point at 2 h were calculated using the linear trapezoidal method. Nasal bioavailabilities were calculated using mean AUC values. AUCs were corrected for basal levels of steroid by subtraction of the mean blank AUC.

The Wilcoxon rank test was used for statistical evaluation of the results. Differences were assigned to be significant for values of $p < 0.05$.

The estradiol data from the combined progesterone/estradiol preparation were compared with data from a previously reported study in which estradiol was given intranasally as a DMβCD-complex to rats (Hermens et al., 1990).

Ciliary beat frequency measurements

Nasal ciliary beat frequency was measured on human adenoid tissue with a photoelectric registration device, as described earlier (Van de Donk et al., 1980). The drug solutions, as administered intranasally, were diluted five times in a Locke-Ringer (LR) solution. The experiments were performed at 30 °C. Ciliary beat frequency was measured every 10 min during a 60 min period. Control experiments were performed in pure LR solution. Data were calculated as the relative frequency of the ciliary beat frequency at the start of the experiment, the latter being 100%.

Results

Basal serum concentrations of endogenous progesterone and the serum concentration-time profiles of progesterone after i.v. administration are shown in Fig. 1. The basal levels of progesterone fluctuated between 2 and 5 ng/ml. Serum concentrations after i.v. administration declined rapidly, with an initial half-life of approx. 10 min. Basal levels were reached within 60–120 min.

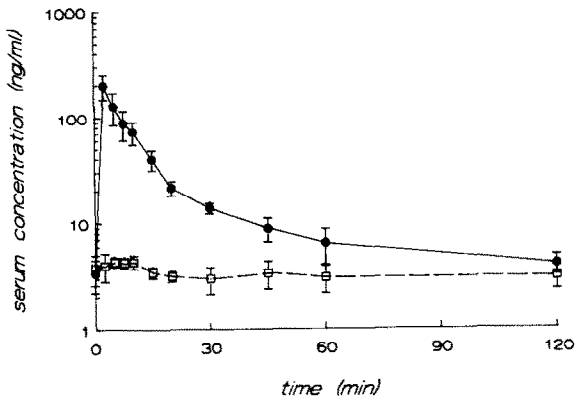

Fig. 1. Mean serum concentrations of progesterone in rats following intravenous administration. (□) 0.9% saline; (●) 100 μg progesterone with DMβCD.

Nasal administration of both the progesterone suspension and the progesterone-DMβCD complex resulted in elevated progesterone serum levels for over 60 min (Fig. 2). In both cases maximum serum levels were reached at 15 min. The peak concentrations, however, were much higher and persisted for a longer period of time for the progesterone-DMβCD preparation. The absolute bioavailability as determined for the nasally administered DMβCD complex was 58%. This was about 3-times higher than the bioavailability of the progesterone suspension (Table 1).

Serum concentration-time profiles of progesterone and estradiol were quite similar when

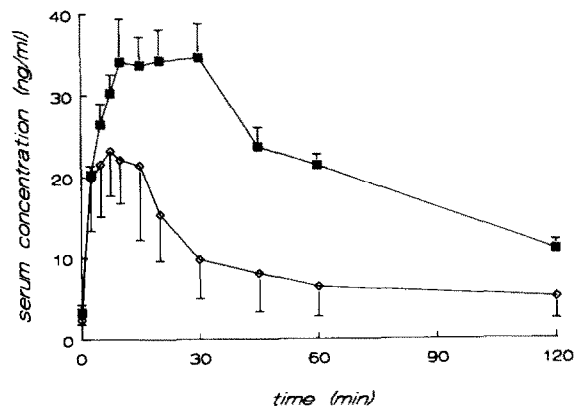

Fig. 2. Mean serum concentrations of progesterone following nasal administration of (■) 200 μg progesterone with DMβCD; (◇) 200 μg progesterone suspension without DMβCD.

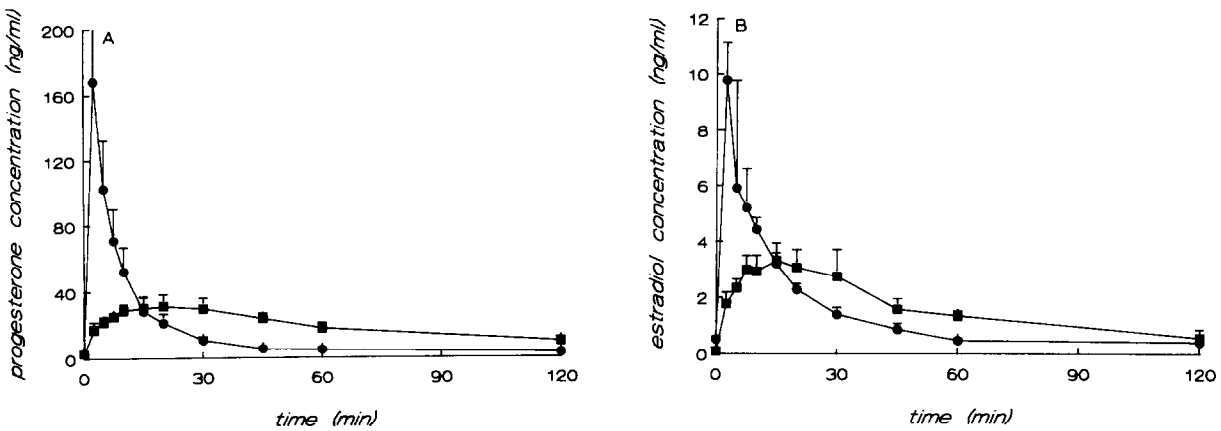

Fig. 3. Mean serum concentrations of (A) progesterone, and (B) estradiol, following administration of a progesterone-estradiol-DMβCD preparation. (●) Intravenous administration of 100 μg progesterone and 5 μg estradiol with DMβCD; (■) nasal administration of 200 μg progesterone and 10 μg estradiol with DMβCD.

administered together in one DMβCD formulation as compared to their separate administrations, both for i.v. and intranasal administration. Fig. 3A and B shows serum concentration-time curves for progesterone and 17 β-estradiol, following administration of the combined steroid preparation. For the estradiol-progesterone-DMβCD formulations absolute bioavailabilities after nasal instillation were found to be 59 and 67% for estradiol and progesterone, respectively (Table 2). These values were not significantly different from those obtained with separate DMβCD preparations of estradiol and progesterone.

The effects of the used DMβCD preparations, diluted five times with LR, on the ciliary beat

TABLE 1

AUC values and bioavailabilities of progesterone (P) after i.v. and intranasal (i.n.) administration of a P-DMβCD formulation, a P-suspension and placebo in rats

Dose (μg)	Route	Preparation	AUC (ng ml min^{-1})	Bioavailability (%)
100	i.v.	P-DMβCD	2334 ± 508	100 ± 37
0	i.n.	placebo	388 ± 83	0
200	i.n.	P-DMβCD	2664 ± 121 [a]	58 ± 16
200	i.n.	P susp	1106 ± 483 [a]	18 ± 13

[a] Values are significantly different from each other.
Data are expressed as means ± S.D. The number of rats used was 3 for the palcebo, and 4 for all other groups. AUC denotes the area under the concentration time curve up to 2 h.

TABLE 2

Nasal bioavailability of progesterone (P) and estradiol (E2) in rats using separate (P-DMβCD and E2-DMβCD) and combined (P-E2-DMβCD) cyclodextrin formulations

Preparation		Bioavailability (%)
Progesterone	P-DMβCD	58 ± 16
	P-E2-DMβCD	67 ± 23
Estradiol	E2-DMβCD	67 ± 16 [a]
	P-E2-DMβCD	59 ± 16

[a] Data from Hermens et al., (1990).
Data are expressed as means ± S.D. The number of rats used was 6 for the estradiol-DMβCD, and 4 for the other groups.

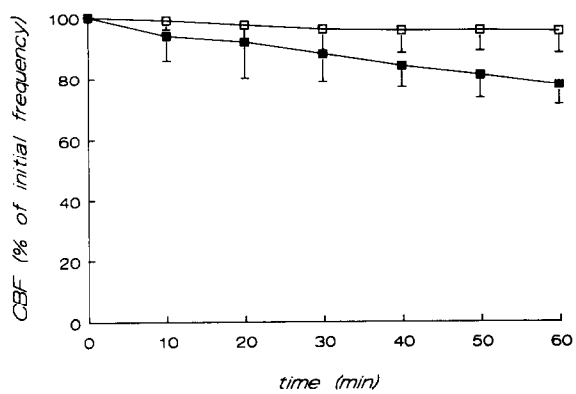

Fig. 4. Time vs CBF plot of human adenoid tissue in (■) progesterone-DMβCD, and (□) blank LR solutions.

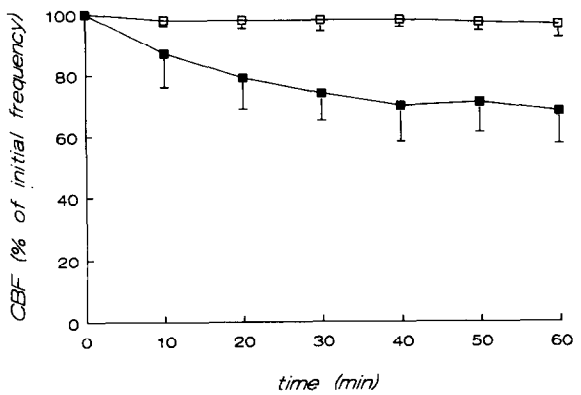

Fig. 5. Time vs CBF plot of human adenoid tissue in (■) progesterone estradiol-DMβCD solutions, and (□) blank LR solutions.

frequencies of human adenoid tissue are shown in Figs 4 and 5. For both preparations the ciliary beat frequency decreased to 70 or 80% of their initial value. The ciliary beat frequency in control experiments remained between 90 and 100% throughout the experiment.

Discussion

The nasal absorption of progesterone appears to be markedly enhanced by the addition of DMβCD. The increase in bioavailability from about 20% for the suspension to about 60% for the DMβCD complex is the same as that previously found for estradiol (Hermens et al., 1990). The increased solubility of the steroids by formation of inclusion complexes with DMβCD is an important factor in the mechanism of absorption enhancement. However, evidence is available that cyclodextrins and in particular DMβD can extract lipids from the gastrointestinal mucosa, possibly leading to facilitated oral drug absorption (Uekama and Otagiri, 1987). Such a mechanism might also be implicated with nasal drug transport.

The amount of DMβCD in the previously described estradiol formulation (Hermens et al., 1990) was 10-times lower than that in the present estradiol-progesterone-DMβCD formulation. Apparently, these different amounts do not have any influence on the estradiol bioavailability. It is assumed that only the dissociated drug is available for absorption. Consequently, the total absorption is dependent on the equilibrium between free and complexed drug (Frömming, 1987; Uekama, 1987). The equilibrium between free steroid and steroid-DMβCD is expected to be approximately the same with the combined and separate preparations. The stability constants for the progesterone-DMβCD and the estradiol-DMβCD complexes are in the same order of magnitude (Uekama and Otagiri, 1987; Hermens et al., 1990). Moreover, the molar ratio steroid : DMβCD used in the combined preparation was similar (1 : 2) to those in the separate estradiol and progesterone preparations.

The effects of DMβCD on biological membranes are likely to be concentration dependent. The fact that the much higher concentrations of DMβCD in the combined preparation do not seem to influence the estradiol bioavailability may indicate that possible effects of DMβCD on the nasal epithelial membranes play only a minor role in the mechanism of absorption enhancement.

The present study shows that DMβCD is a potent absorption enhancer for nasally administered progesterone. Moreover, it has been demonstrated that simultaneous delivery of progesterone with estradiol using DMβCD inclusion preparations does not effect the nasal bioavailability of the single steroid hormones. The drug preparations used in the rat experiments were diluted five times in LR solution before ciliary beat frequency measurements. Although this dilution is somewhat arbitrary, it mimics the in vivo situation where the preparation will be diluted by the mucus layer after nasasl instillation. The progesterone-DMβCD and the estradiol-progesterone-DMβCD formulation were found to inhibit the nasal ciliary movement to some extent. This inhibition is probably due to the concentration of DMβCD in the preparation. Unpublished results from our laboratory on ciliary beat frequency measurements with the same concentrations of DMβCD but without estradiol and progesterone were in the same range as those reported in this study. The effects on ciliary beat frequency should not be disregarded. The possibilities of adjusting the concentration of

DMβCD, and the use of other cyclodextrins in the nasal formulations are presently under investigation. Nevertheless, when compared to other absorption enhancing agents used in nasal delivery (Hermens and Merkus, 1987), the effects of the present nasal preparations with DMβCD on the ciliary beat frequency are very mild.

In conclusion, the increased bioavailability of estradiol and progesterone following nasal instillation of the DMβCD formulations, together with the observed minor ciliotoxicity demonstrate the potential of DMβCD as an absorption enhancer for nasally administered steroid hormones. In addition, the present study indicates that simultaneous nasal delivery of estradiol and progesterone has promising prospects for pharmacotherapy with these hormones, for instance, in postmenopausal disorders.

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