Pharmaceutical Research, Vol. 7, No. 5, 1990

Report

Nasal Absorption Enhancement of 17β-Estradiol by Dimethyl-β-Cyclodextrin in Rabbits and Rats

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Received August 15, 1989; accepted November 8, 1989

A new formulation for nasal administration containing 17β-estradiol (E_2) with dimethyl-β-cyclodextrin (DMβC) as a solubilizer and absorption enhancer is described. Nasal administration of this E_2-DMβC formulation gave a significantly higher E_2 absorption than an E_2 suspension in both rabbits and rats. Relative to an intravenous injection of the E_2-DMβC formulation, absolute bioavailabilities of 94.6 and 67.2% were calculated for the nasal E_2-DMβC formulation in rabbits and rats, respectively. Differences in bioavailability may have resulted from differences in experimental animal conditions. The effects on human nasal ciliary activity of the E_2-DMβC formulation were studied with an *in vitro* method. The formulation was found to exert only a minor effect on ciliary beat frequency. Thus, nasal delivery of E_2, using a cyclodextrin inclusion formulation, may have potential for clinical application, e.g., in the therapy of postmenopausal disorders.

KEY WORDS: 17β-estradiol; dimethyl-β-cyclodextrin; nasal absorption.

INTRODUCTION

Endogenous estrogen production in women is known to decrease around menopause, and estrogen therapy is often required in the management of postmenopausal disorders (1). Oral substitution with estrogens is subject to extensive first-pass elimination and is associated with potentially harmful side effects (2). These drawbacks emphasize the need for suitable, nonoral routes to administer natural female sex hormones. This goal may be realized by nasal administration because the nasal mucosa has proven to be a potential site of absorption for various drugs (3). The bioavailability of nasally administered drugs depends on several factors, such as the solubility and dissolution rate. Estradiol (E_2), the most important estrogen in women, is a poorly water-soluble drug, thereby complicating nasal absorption. Nasal administration of a suspension of micronized E_2 in saline has been shown to elicit only a very short rise in plasma estrogen levels (4). Another study demonstrated that much higher E_2 plasma levels were reached with an E_2 solution as compared to an E_2 suspension after intranasal administration to rhesus monkeys (5). The solution was prepared by dissolving E_2 in a mixture of ethanol, propylene glycol, and water (1:1:3). Formulations containing these solvents, however, cannot be used for long-term application, because of their harmful effects on the nasal mucosa and impairment of mucus rheological properties (6,7). Recently formulations containing E_2 with 1% (w/v) polysorbate 80 as solubilizer gave bioavailabilities of 50–84% after nasal administration to rats (8).

The present study describes a nasal formulation for E_2 with dimethyl-β-cyclodextrin (DMβC) as a solubilizer and absorption enhancer. Cyclodextrins are biocompatible polymers, able to form inclusion complexes with drugs (9,10). With DMβC, stable aqueous solutions can be prepared of the poorly water-soluble steroid E_2.

In nasal drug delivery it is a prerequisite to investigate the effects of drugs and additives on nasal functioning at an early stage. The self-cleaning capacity of the nose, as effectuated by the ciliary epithelium and necessary to remove dust, allergens and bacteria, should not be influenced by nasal medication. Ciliary movement is a major factor of the mucociliary clearance in the upper airways (11). Many drugs and additives, however, inhibit nasal ciliary movement, as demonstrated *in vitro* (12).

The objective of this study was to determine the absolute bioavailability of a E_2-DMβC formulation compared to an E_2 suspension after nasal administration to rabbits and rats. Since this dosage form is meant for human application, the effects of the E_2-DMβC formulation on human ciliated epithelium were also studied.

MATERIALS AND METHODS

Chemicals

17β-estradiol (E_2) was obtained from Bufa Chemie (Castricum, The Netherlands), dimethyl-β-cyclodextrin (DMβC) from Janssen Chimica (Beerse, Belgium), and Hypnorm from Janssen Pharmaceutica (Beerse, Belgium).

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0724-8741/90/0500-0500$06.00/0

Stability Constant

Solubility measurements of the E_2-DMβC complex were carried out according to the methods of Higuchi and Conners (13). The stability constant (K_c) of the E_2-DMβC complex was calculated from S_0 (aqueous solubility, determined in fivefold) and the slope of the initial straight line of the phase solubility diagram according to the equation

$$K_c = \frac{\text{slope}}{S_0 \cdot (1 - \text{slope})} \quad (1)$$

Estradiol Formulations

The E_2-DMβC formulations were prepared by dissolving both compounds (molar ratio, 1:2) in ethanol 96% to form inclusion complexes. Then the ethanol was evaporated at 50°C under a mild nitrogen stream. The residue was dissolved in an aqueous medium to the desired concentration.

For the rabbit experiments, the residue was dissolved in a viscous solution, containing sodium chloride (0.9%), benzalkonium chloride (0.01%), sodium edetate (0.1%), and hydroxypropylmethylcellulose (2%). The final concentration of the nasal solution was 2 mg E_2/ml, and that of the intravenous solution was 1 mg E_2/ml. The formulation for the rat experiments was prepared by dissolving the residue in 0.9% saline to a final concentration of 0.5 mg E_2/ml.

The E_2 suspension formulations were prepared by suspending the micronized E_2 in the viscous solution for the rabbit experiments (2 mg E_2/ml) and in 0.9% saline for the rat experiments (0.5 mg E_2/ml). The viscous formulations for the rabbit experiments were used to prevent these formulations from being cleared too rapidly to the throat (14).

Nasal Absorption Studies in Rabbits and Rats

These studies were performed according to procedures described earlier (15,16). Five New Zealand rabbits, weighing approximately 4 kg, were given 0.2 ml of Hypnorm intravenously in an ear vein to prevent sneezing while formulations were instilled intranasally. Nasal estradiol formulations (50 μl corrsponding to 100 μg E_2) were instilled unilaterally using a microliter syringe connected with a PVC cannula. An intravenous bolus injection of estradiol (100 μl corresponding to 100 μg E_2) in the ear vein and nasal placebo (the viscous basis solution) were given to determine the absolute bioavailabilities of the nasal estradiol formulations. Venous blood samples (500 μl) were taken from an ear vein at regular time intervals. All formulations were given in a random order to each of the five rabbits. Subsequent administrations were performed after a washout period of at least 1 week.

Male Wistar rats, weighing 175–225 g, were anesthetized with Hypnorm (0.1 ml/100 g body weight) intramuscularly, and additional injections of 0.05 ml/100 g were given, usually 75 and 165 min after the first injection. In order to facilitate nasal administration and to prevent peroral absorption, the trachea was canulated and the esophagus was tied to this cannula. Animals were kept lying on the back on thermostated rugs (37°C) during the experiment. Nasal formulations (20 μl corresponding to 10 μg E_2) were instilled unilaterally through the nares 105 min after the first Hypnorm injection using PVC tubing affixed to a microliter syringe. Blood samples (300 μl) were taken from a canulated femoral artery at regular time intervals. Intravenous administration of estradiol (20 μl corresponding to 10 μg) and intranasal placebo (0.9% saline) were given to determine the absolute bioavailabilities of the nasal formulations. For intravenous administration the trachea cannula was omitted and a femoral vein was cannulated.

Analytical Procedures

Serum levels of 17β-estradiol were measured using a Coat a Count radioimmunoassay from DPC (Laboratorium Service, Apeldoorn, The Netherlands) with a sensitivity of 8 pg/ml.

Data Analysis

The areas under the individual serum concentration–time curves (0–120 min) for estradiol were calculated using the linear trapezoidal rule. Nasal bioavailabilities were calculated according to the formula $(AUC_{i.n.} - AUC_{pl.}/AUC_{i.v.} - AUC_{pl.}) \times 100\%$. In the rabbits each animal was its own control and bioavailabilities were determined for each animal. In the rat experiments bioavailabilities were calculated using the mean AUC values. For statistical evaluation of the results the one-tailed Student's t test was used. Differences were assigned to be statistically significant for values of $P < 0.05$.

Ciliary Beat Frequency Measurements

Nasal ciliary beat frequency (CBF) was measured on human adenoid tissue with a photoelectric registration device as described earlier (17). The E_2-DMβC formulation (2 mg E_2/ml) was diluted 1:5 with sterilized Locke–Ringer solution (LR). CBF was followed during 60 min ($n = 8$). The experiments were performed at 30°C. Quality of the ciliated tissue was established by control experiments in pure LR. Results are recorded as percentages of the initial frequencies (the latter being 100%) and are presented as the mean ± SD.

RESULTS

Nasal Absorption Studies

AUC values, bioavailabilities (F), and t_{max} of E_2 after intravenous and intranasal administration of the described E_2-DMβC formulations and the E_2 suspension to rabbits and rats are presented in Table I. Concentration–time curves of E_2 in rabbits and rats, respectively, are shown in Figs. 1 and 2. Nasal administration of the E_2-DMβC formulation gives a significantly higher E_2 absorption than the E_2 suspension ($P < 0.005$) in both rabbits and rats. Relative to an intravenous injection of the E_2-DMβC formulation absolute bioavailabilities of 94.6 and 67.2% can be calculated for the nasal E_2-DMβC formulation in rabbits and rats, respectively (Table I).

Stability Constant

According to Eq. (1) K_c was calculated at >50,000/mol. An exact value cannot be given, because of the impact of the

Table I. AUC Values, Bioavailabilities (F), and t_{max} of 17β-Estradiol After Intravenous (i.v.) and Intranasal (i.n.) Administration of an E_2-DMβC formulation, an E_2 Suspension, and Placebo in Rabbits and Rats[a]

	Dose	Route	Formulation	AUC (ng · min/ml)		F (%)	t_{max} (min)	(n)
Rabbits	100	i.v.	E_2-DMβC	293.9 ± 39.9		100		(5)
	0	i.n.	Placebo	48.9 ± 11.5	n.s.[b]	0		(4)
	100	i.n.	E_2-DMβC	271.8 ± 75.9	*	94.6 ± 41.6	13.8 ± 9.5	(5)
	100	i.n.	E_2-susp.	107.3 ± 27.1		25.2 ± 16.0	39.0 ± 20.1	(5)
Rats	10	i.v.	E_2-DMβC	648.2 ± 129.4		100		(6)
	0	i.n.	Placebo	25.1 ± 16.9	*	0		(6)
	10	i.n.	E_2-DMβC	443.6 ± 49.5		67.2 ± 16.1	10.8 ± 3.8	(6)
	10	i.n.	E_2-susp.	165.8 ± 19.4	*	22.6 ± 5.6	11.0 ± 2.2	(5)

[a] All values are the mean ± SD for the number of animals given in parentheses (n). AUC represents the area under the serum concentration–time curve, as determined to 2 hr.
[b] Nonsignificant.
* $P < 0.005$.

very small value of S_0 in this equation. In comparison with other steroid inclusion complexes this K_c value is large (9).

Ciliary Beat Frequency Measurements

The results of these experiments are presented in Fig. 3. The E_2-DMβC formulation exerts only a minor effect on human nasal ciliary activity.

DISCUSSION

Cyclodextrins are designated by a Greek letter to denote the number of glucose units. The cavity of the six-membered α-cyclodextrin is too small to take up most of the commonly used drugs, whereas the seven-membered β-cyclodextrin offers enough space for even relatively large molecules such as steroids. The use of β-cyclodextrin, however, is limited due to its own low aqueous solubility (1.8%, w/v) and that of its inclusion complexes. Therefore, in this study we used dimethyl-β-cyclodextrin (DMβC; solubility, 57%, w/v). Contrary to the underivatized β-cyclodextrin, DMβC has surfactant activities (9).

It is interesting to note that, despite the large stability constant of the E_2-DMβC complex as determined in this study, nasal E_2 absorption is significantly enhanced by this formulation. The solubilizing effect of DMβC is a major enhancing mechanism of steroid absorption, but other factors influencing nasal drug absorption should also be considered. The free form of DMβC after dissociation of the complex is thought to alter the lipid barrier of the absorption site, which facilitates drug absorption (9).

The absolute bioavailability in the rat (67.2%) was lower than in the rabbit model (94.6%). Thus, interspecies differences and experimental animal conditions can affect nasal drug absorption (16). For instance, in rats lying on their back it is likely that the upper part of the nasal cavity functions as the penetration barrier for the nasal formulations, which is lined with olfactory epithelium. It has been reported that rat olfactory mucosa has a marked ability to metabolize E_2, as demonstrated after intravenous injection of ^{14}C-labeled E_2 (18). The presence of a very active cytochrome P450-dependent drug metabolizing system in rodent olfactory epithelia has also been described (19). Thus metabolism in the absorption phase might explain the difference in results in bioavailability between the rabbit and the rat model, as shown in this study.

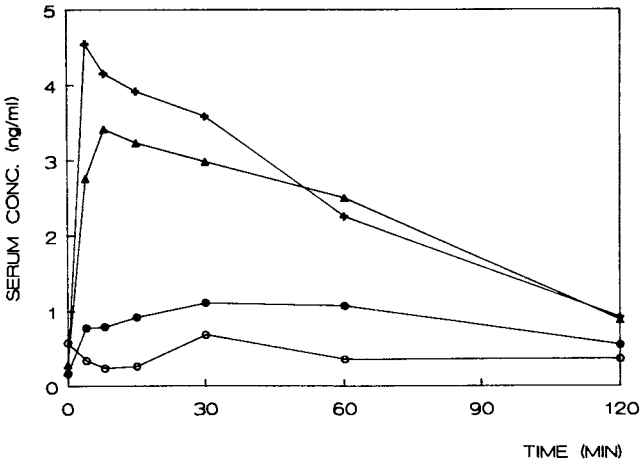

Fig. 1. Mean serum concentrations of E_2 in rabbits after administration of (+) intravenous E_2-DMβC (100 μg E_2), (▲) nasal E_2-DMβC (100 μg E_2), (●) nasal E_2 suspension (100 μg E_2), and (○) nasal placebo.

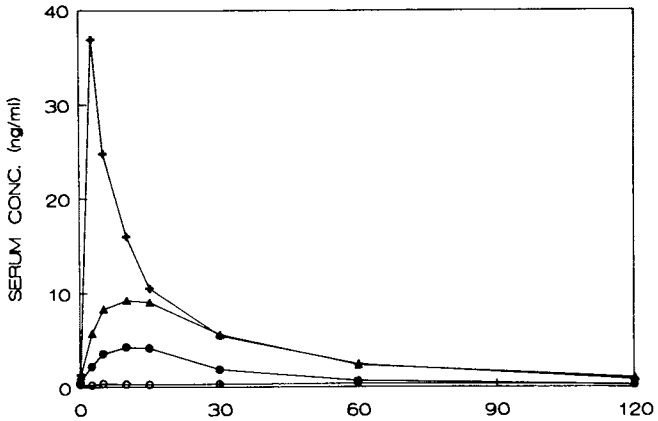

Fig. 2. Mean serum concentrations of E_2 in rats after administration of (+) intravenous E_2-DMβC (10 μg E_2), (▲) nasal E_2DMβC (10 μg E_2), (●) nasal E_2 suspension (10 μg E_2), (○) nasal placebo.

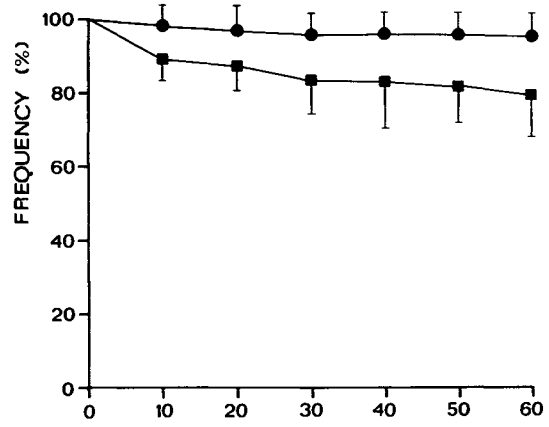

Fig. 3. Time versus frequency plot (mean ± SD) of cilia in solutions of E_2-DMβC (■) and blank (Locke–Ringer; ●).

The t_{max} values of the nasal E_2-DMβC formulations in both rabbits and rats indicate a rapid nasal absorption of E_2 (Table I). The t_{max} of the nasal E_2 suspension in rabbits, however, is markedly prolonged in comparison with the rat model. In rabbits a combination of nasal and oral absorption may occur, because of clearance by the intact mucociliary system; however, in the rat model oral absorption is not possible because the esophagus is tied up. Alternatively, the viscosity-enhancer hydroxypropylmethylcellulose gave rise to a slower absorption of E_2 than a nonviscous solution (14).

Nasal drug formulations should not disturb ciliary movement (12). This new E_2-DMβC formulation was found to exert only a minor effect on ciliary beat frequency (Fig. 3). Comparison with other drugs (12) indicates that E_2-DMβC formulations are biocompatible and very promising for chronic nasal administration of estradiol, for instance, in the pharmacotherapy of postmenopausal disorders (1).

Presently no data are available on nasal E_2 delivery by cyclodextrin inclusion complexes. With regard to other routes of administration, DMβC did not enable buccal absorption of E_2, whereas E_2 inclusion complexes with hydroxypropyl-β-cyclodextrin effectively enhanced both sublingual and buccal absorption of E_2 (20).

In conclusion, we have demonstrated that E_2 is readily released from the E_2-DMβC inclusion complex and effectively absorbed by the nasal mucosa of rabbits and rats. The *in vitro* effect of the E_2-DMβC complex on human nasal cilia appeared to be negligible. Thus, nasal delivery of E_2 using these cyclodextrin inclusion formulations may have potential for clinical application.

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