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Intranasal absorption of estropipate in the dog

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Summary

The utility of nasal route for administration of estropipate has been studied in dogs. Plasma levels of estrone (E1) and estradiol (E2) in male dogs were determined after intranasal spray, oral administration and intravenous injection of estropipate. Nasal spray of estropipate resulted in significant higher mean plasma peak concentrations and area under curve of estrone (two-fold higher) and estradiol (four-fold higher) compared with the other two routes. A rise in E2/E1 plasma concentration ratio near or above unity was observed beginning 15 min to 1 h after intranasal application. In vitro studies have observed a rapid enzymatic conversion of estropipate to estrone and estradiol in nasal tissue. Estrone and estradiol were interconverted in the nose, and the rate of conversion from estrone to estradiol was 2.5 times faster than that from estradiol to estrone. The presence of desulfatase, reductase and some oxidase in nasal tissue was demonstrated. This study has shown that the intranasal administration of estropipate may deliver a mixture of estrone, estradiol, estrone sulfate and other metabolite(s) across the nasal mucosa into systemic blood, and can be an effective delivery method for estrogen replacement therapy.

Introduction

Recent years have brought efforts to develop dosage forms that will improve the administration of estrogens for menopausal syndrome. Among the three known primary estrogens, estrone, estradiol and estriol, estradiol has a more potent action on the endometrium than either estrone or estriol (Shapiro and Forbes, 1978). Previous work (Longcope, et al., 1985) has shown that estrone sulfate is the major circulating estrogen after either intravenous injection or oral administration of estradiol to human. It has been suggested that estriol has a selective action on the vagina (Klopper, 1980), but is less active on the endometrium.

Estrogens are commonly administrated orally as sulfate esters or conjugates. These are physiologically inactive and must be metabolized to active estrogenic products. Estropipate, piperazine estrone sulfate, is identical in nature to estrone sulfate with a small amount of piperazine as a stabilizer.

Because of the first pass metabolism, oral administration of estropipate experiences the same problems as other hormones, and thus high doses are needed (Nichols et al., 1984). With oral dosage forms, the hydrolysis of estropipate occurs via

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esterases present in the intestine, or acidity present in the stomach. Non-oral administration of estradiol or estrone by vaginal cream, transdermal, intranasal, or sublingual routes can obtain the desired plasma level with significantly lower doses (Burnier et al., 1981; Utian, 1987; Bawarshi-Nassar et al., 1989); however, administration of estrone conjugates by the vaginal route has resulted in low estradiol or estrone plasma levels (Huge and Jaszmans, 1983; Mandel et al., 1983).

Nasal enzyme activities have been investigated by a number of researchers (Shorn and Hochstrasser, 1979; Dahl, 1982; Kay et al., 1986). It has been established that the release of drug from the prodrugs involves an action of enzyme systems located in the delivery site (Schoenwald and Chien, 1988; Harboe, et al., 1989). Ideas have been pursued of making prodrugs for improving the taste, stability, solubility (Hussain et al., 1988), or altering the lipophilic and hydrophilic properties (Rominger and Hitzenberger, 1980). The work presented in this paper shows strong evidence that enzymes present in the nasal tissue of the dog are capable of rapidly converting estropipate to the parent compound, estrone. The concept of utilizing nasal enzyme activities for prodrug delivery may deserve further attention.

Nasal sprays or drops have increasingly become an acceptable delivery route for pharmaceutical products. In addition to the well-established nasal product line of antihistamines and decongestants, this route of administration has also been adapted to deliver peptides and other more complex molecules for systemic effects. Some more recent examples are nasal delivery of LHRH analogs, such as buserelin (Rajfer et al., 1986), napharelin (Chan et al., 1988), which has just obtained approval for marketing in Canada and the U.S.A. The developmental work on other LHRH analogs, various hormones, and insulin has being very actively pursued by several pharmaceutical and biotech companies.

Materials and Methods

Materials

Estradiol, estrone and estriol were purchased from Sigma, estropipate was obtained from Abbott Laboratories, and the nasal spray bottles were provided by the 3M Co.

Solution preparations

Estropipate solutions used for in vivo studies were prepared by dissolving the compound in saline solution at room temperature, and filtering through a 0.2 μm Whatman paper. A solution concentration of 500 μg/ml was used for oral and intravenous administrations, and 2.5 mg/ml was used for the nasal spray. The concentration and stability of estropipate in solutions were examined by HPLC during the study.

In vivo studies

Male dogs, average weight 8–10 kg, were used in the studies. Usual dietary intake was maintained. A volume of 0.1 ml estropipate solution (2.5 mg/ml) was sprayed into each nostril for nasal administration using the 3M designed bottle. The volume of solution applied for oral gavage administration and intravenous injection was 1 ml (500 μg/ml).

A single dose was applied to six dogs in a three-way three-period cross-over design. A 1 week resting period was used to clear residues from the previous dosing. The total dose was 500 μg (1.15 μmol) per dog for all three routes.

Blood samples were taken through jugular vein and collected into heparinized tubes, before dosing and at 2, 5, 15, 30 and 60 min in the first hour, and then every hour for another 3 h. Plasma samples were immediately separated and stored frozen at -70°C. Three sets of controlled plasma samples, containing 1 μg/ml of estrone sulfate, 0.5 ng/ml estrone and 0.5 ng/ml estradiol, respectively, were processed and assayed along with the study samples. The assay results of controlled samples showed that E1, E2 and ES were stable in plasma before assay. Serum concentrations of estrone and estradiol were measured by radioimmunoassays which were based on the competitive binding of radioactive and nonradioactive antigens for a fixed number of binding sites on the antibody. Estradiol was extracted from plasma with alkaline ether and estrone was extracted with hexane and ethyl acetate.

Data analysis

Area under plasma concentration-time curve, AUC, was calculated using the trapezoidal rule. C_{max} was defined as the maximum observed plasma concentration and T_{max} the time corresponding to C_{max}. The statistical analysis system (SAS) was used to analyze these data.

In vitro studies

Nasal tissue for these studies was obtained by surgical removal from dogs immediately after euthanasia. Euthanasia of dog was performed by intravenous injection of Nembutal (Abbott Labs.) at a dose level of 80 mg/kg. The samples were immediately stored at 2–8°C and were homogenized by hand in cold 0.1 M Hepes buffer (Sigma). The samples were used within 30 min after surgery.

Non-enzymatic chemical conversion

The nasal tissues were preheated in 0.1 M Hepes buffer at 80°C for 30 min to eliminate enzyme activity. Estropipate, estrone or estradiol (500 μg, 1.15 μmol) was incubated with 2 g of preheated nasal tissue for 1 h. The solution samples were withdrawn and estrone sulfate was immediately separated from estradiol, estrone and estriol by extraction with ethyl acetate. ES, E1, E2 and estriol were measured by HPLC.

Conversion of estropipate to E1 and E2

500 μg of estropipate was incubated at 37°C with 2 g of freshly removed and homogenized nasal tissue which had been diluted with 10 ml Hepes buffer before addition of the compound. The solution samples were withdrawn periodically. Estrone sulfate was immediately separated from estrone, estradiol and estriol by extraction with ethyl acetate. ES, E1, E2 and estriol were determined by HPLC.

Interconversion of E1 and E2

An amount of 310 μg (1.15 μmol) E1 or E2 was incubated with 2 g of freshly homogenized nasal tissue at 37°C for 2 h. The solution samples were withdrawn periodically. Estrone and estradiol were extracted from solution immediately with ethyl acetate and E1 and E2 were assayed by HPLC.

Results and Discussion

In vivo study

Figs 1–3 plot the plasma concentrations of estrone (E1) and estradiol (E2) vs time following nasal, oral and IV administrations of 500 μg of estropipate. Table 1 summarizes the plasma peak concentration, peak time and area under curve of E1 and E2 by each of the different routes. The results showed that the plasma peak concentration and area under curve were higher, by approximately two-fold in estrone and four-fold in estradiol, by nasal spray compared to oral and IV routes. Extended plasma concentrations (shown in Fig. 3) and higher T_{max} value (shown in Table 1) of E2 were observed after nasal administration compared to the other two routes.

Fig. 4 compares the changes of plasma E2/E1 ratio vs time after administration of estropipate by three routes. A rising plasma E2/E1 ratio, approaching 1.0, was observed 1 h after application of nasal spray. The increasing plasma E2/E1 ratio (shown in Fig. 4) and extended E2 plasma concentrations (shown in Fig. 3) after nasal administration indicate that more E2 was converted from E1 in nasal tissue with time and continuously transported into the blood stream. These in vivo observations following nasal sprays can be supported by the findings from in vitro experiments. Fig. 5 shows that the E2/E1 ratio increasing with time after incubation of estropipate and estrone with nasal tissue. A kinetic pattern of in vitro conversion from estrone to estradiol in nasal tissue is shown in Fig. 6.

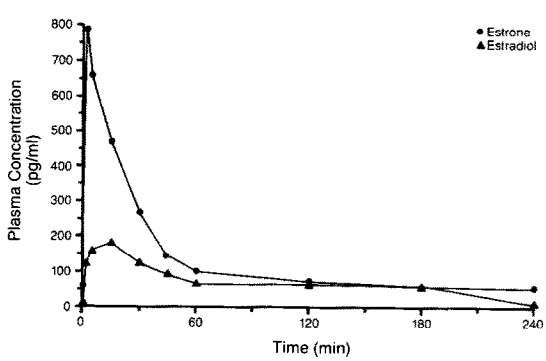

Fig. 1. The changes of plasma E1 and E2 following intranasal administration of 500 μg estropipate.

TABLE 1

The plasma peak concentration, peak time and area under curve of estrone and estradiol after intravenous, oral and nasal administrations of 500 μg estropipate in dogs (N = 6)

	C_{max} (pg/ml)	T_{max} (min)	AUC (0–4 h) (h pg ml^{-1})
Estrone (E1)			
IV mean	318	2.8	339
SD	284	1.5	257
Oral mean	231	4.5	268
SD	214	1.2	194
Nasal mean	784	4.0	522
SD	495	1.6	253
* Signif. dif.	nasal > oral = i.v.	nasal = oral > i.v.	nasal > oral = i.v.
Estradiol (E2)			
IV mean	120	7.7	114
SD	47	5.9	88
Oral mean	53	8.6	66
SD	26	5.9	40
Nasal mean	180	13.3	278
SD	80	4.1	193
* Signif. dif.	nasal > i.v. > oral	nasal > oral = i.v.	nasal > oral = i.v.

* Signif. dif.: statistically significant difference, $p < 0.05$.

In vitro studies

The conversions of estropipate to estrone, and estrone to estradiol or estradiol to estrone, were not observed in the heat pretreated nasal tissues. It could be concluded that the non-enzymatic species present in nasal tissue caused no changes to either estropipate or E1 and E2.

About 80% of estropipate was immediately converted to estrone and estradiol or other unknown metabolites within 2 min after incubation of estropipate with nasal tissue at 37°C. In this experiment, it was observed that estropipate was converted to E1 and subsequently to E2. Fig. 5 shows the ratio of E2/E1 increasing with time.

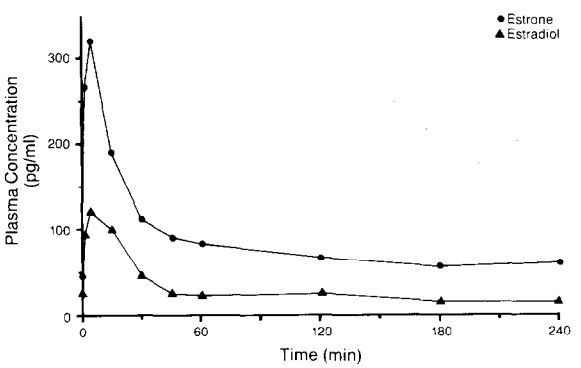

Fig. 2. The changes of plasma E1 and E2 following intravenous administration of 500 μg estropipate.

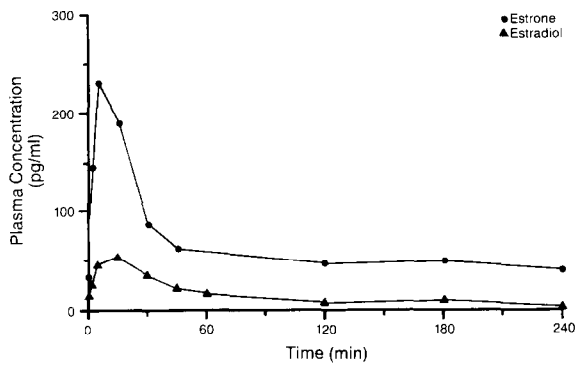

Fig. 3. The changes of plasma E1 and E2 following oral administration of 500 μg estropipate.

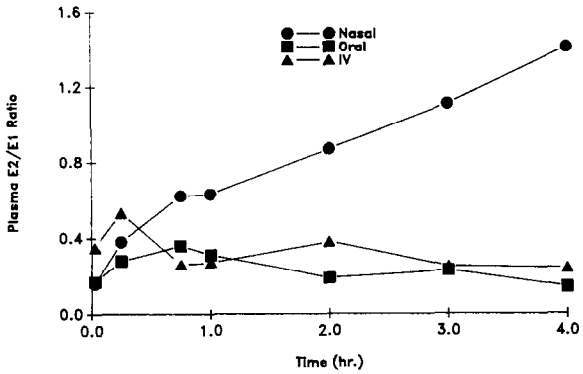

Fig. 4. Comparison of plasma E2/E1 ratio after administration of 500 μg estropipate by three different routes.

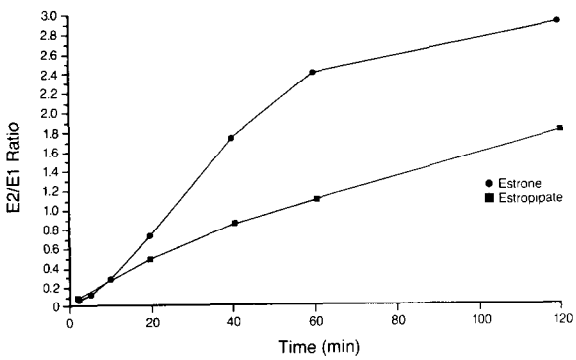

Fig. 5. The increase of E2/E1 ratio with time after incubation of 500 μg estropipate and 310 μg estrone in 2 g of nasal tissue at 37 °C.

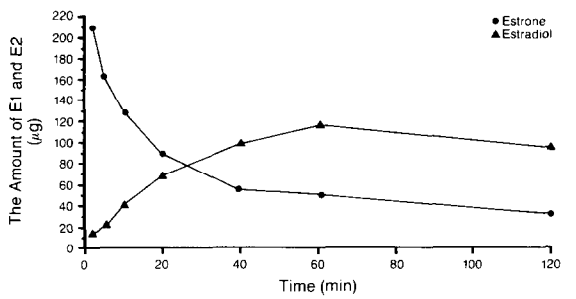

Fig. 6. The conversion rate of estrone to estradiol after incubation of 310 μg estrone in 2 g of nasal tissue at 37 °C.

Estriol was not detected in these experiments. The evidence from this experiment is that desulfatase and reductase are present in dog's nose.

Figs 6 and 7 show the interconversion patterns of estrone and estradiol in nasal tissue. These

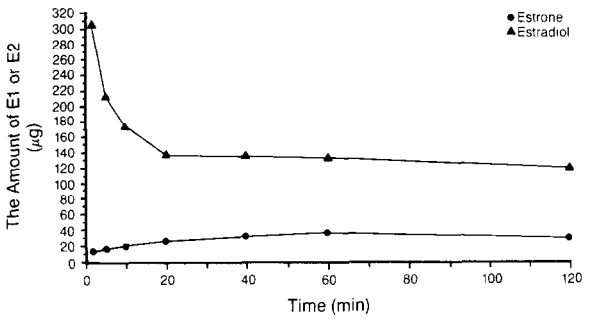

Fig. 7. The conversion rate of estradiol to estrone after incubation of 310 μg estradiol in 2 g of nasal tissue at 37 °C.

experiments demonstrated that estrone and estradiol are interconvertable in the nasal tissue. The conversion of E2 to E1 indicated the presence of oxidases in nose. The rate of conversion from E1 to E2 (6.4×10^{-3} μmol/min per g tissue) was about 2.5 times faster than that from E2 to E1 (2.8×10^{-3} μmol/min per g tissue). After equilibrium, the E2/E1 ratio remained between 3 and 4. However, as shown in Figs 6 and 7, approx. 50% of E1 or E2 was converted to unidentified metabolite(s) in the nasal tissue.

No conversion of estrone to estrone sulfate was observed in any of these experiments. It suggested that sulfatase activity is not present in the nose.

Conclusions

The present study demonstrated that rapid enzymatic conversion of estropipate to estrone and estradiol occurred in nasal tissue. Intranasal administration of estropipate may actually deliver a combination of E1, E2 and ES through nasal mucosa into systemic circulation. The lipophilic character in converted estrone and estradiol is advantageous for nasal membrane diffusion and penetration, which could play an important role to the high plasma concentrations of E2 and E1 observed in the in vivo study. The rate of uptake and the bioavailability of organic steroids has been shown to be a function of their lipophilic and/or hydrophilic character (Gibson and Olanoff, 1987). Those molecules with the highest lipophilicity have shown the greatest nasal absorption.

This study strongly suggests that intranasal delivery of estropipate can be an effective route to administer estrogen supplements. Determination of the difference between dog and man with respect to nasal enzymatic activity and membrane permeability will allow a more definitive assessment of the dosage form. Doses required by nasal administration could be significantly lower than oral route.

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