International Journal of Pharmaceutics, 76 (1991) 177–182

ADONIS 037851739100345 8

IJP 02554

Oral bioavailability of 17β-estradiol and various ester prodrugs in the rat

Kenneth B. Lokind [1], Finn Hjort Lorenzen [1] and Hans Bundgaard [2]

[1] *Novo Nordisk A / S, Department of Pharmaceutics, Bagsværd (Denmark) and* [2] *The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, Copenhagen (Denmark)*

(Received 13 May 1991)
(Accepted 6 June 1991)

Key words: 17β-Estradiol; Prodrug; Oral bioavailability; First-pass metabolism

Summary

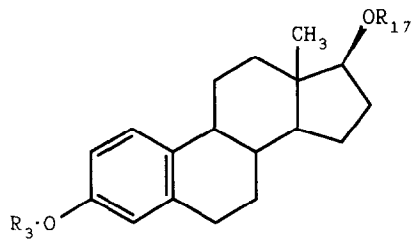
The oral bioavailability of 17β-estradiol is only about 10% in humans due to extensive first-pass metabolism. The feasibility of depressing this metabolism and hence improving the systemic bioavailability by the prodrug approach was examined using a rat model. The prodrugs studied included the 17-acetate, 17-valerate, 17-cypionate, 3-benzoate, 3-acetylsalicylate and 3-anthranilate esters of 17β-estradiol. Following oral administration to rats the systemic bioavailability of 17β-estradiol was found to be 4.3% whereas the bioavailability observed after administration of the esters ranged from 1.0 to 7.6%. This finding suggests that the esters are unable to protect the parent drug against first-pass metabolism as assessed in the rat. The poor bioavailability observed with the 3-acetylsalicylate and 3-anthranilate esters contrasts greatly with previously reported findings with these ester prodrugs in dogs.

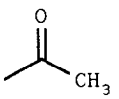
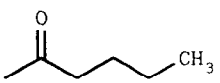
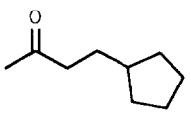
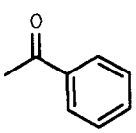
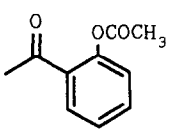
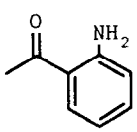
Introduction

Estrogen substitution therapy is often used in the treatment of postmenopausal disorders. The most common oral dosage forms are micronized 17β-estradiol and conjugated and esterified estrogen mixtures (Sitruk-Ware, 1990). However, the systemic bioavailability of 17β-estradiol, the primary estrogenic hormone produced by the human ovary, is only about 10% following peroral administration because of extensive first-pass metabolism of the drug in the gastrointestinal tract and liver (Longcope et al., 1985). The primary routes of this metabolism are oxidation of the 17-hydroxyl group to give estrone and conjugation of the 3-phenolic group in 17β-estradiol as well as in estrone to sulfates and glucuronides (Diczfalusy et al., 1961; Fishman et al., 1969; Yen et al., 1975; Longcope et al., 1985). These metabolites have been implicated as causing changes in hepatic function and to contribute to the undesirable side effects of chronic estradiol therapy (Powers et al., 1985; Lievertz, 1987; Balfour and Heel, 1990).

One approach to circumventing the high first-pass metabolism of 17β-estradiol is to administer the drug by a non-oral route such as transdermally (Balfour and Heel, 1990) or by the nasal route (Bawarshi-Nassar et al., 1989; Hermens et al., 1990; Schipper et al., 1990). Another ap-

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.



Number	Name	R3	R17
I	β-Estradiol	H	H
II	Estradiol-17-acetate	H	
III	Estradiol-17-valerate	H	
IV	Estradiol-17-cypionate	H	
V	Estradiol-3-benzoate		H
VI	Estradiol-3-acetylsalicylate		H
VII	Estradiol-3-anthranilate		H

Scheme 1.

proach is to administer the drug orally in the form of a prodrug which can depress the metabolism in the gastrointestinal tract and liver and, following passage of the site of first-pass metabolism, be cleaved to release the active drug moiety. This prodrug approach has recently been studied by Hussain et al. (1988). These authors reported that esters formed with acetylsalicylic acid and anthranilic acid at the 3-phenolic hydroxy group of 17β-estradiol respectively resulted in 17- and 5-fold higher bioavailability of 17β-estradiol following oral administration to dogs than after administration of the parent drug.

The aim of the present study was to explore the potential utility of the prodrug concept to depress the oral first-pass metabolism of 17β-estradiol and hence improve its bioavailability. Using a rat model, we have examined the oral bioavailability characteristics of six ester derivatives of 17β-estradiol formed either at the 3-phenolic group or at the 17-hydroxy group and compared the bioavailability with that of the parent drug. The compounds studied are shown in Scheme 1.

Materials and Methods

Materials

β-Estradiol and the esters **II–V** were purchased from Sigma Chemical Co., St. Louis, U.S.A. The 3-acetylsalicylate (**VI**) and 3-anthranilate (**VII**) esters of β-estradiol were prepared as described by Hussain et al. (1988). Their melting points and ^{1}H-NMR spectra were identical to those reported. Chremophor ^{R} EL was obtained from BASF, Germany and polyethylene glycol (PEG 400) from E. Merck, Darmstadt, Germany.

Preparation of dosing solutions

For the oral administration, 0.5 ml of solutions of β-estradiol and its esters in ethanol-Chremophor^R EL-water (1:1:8 v/v) was given. The solutions contained an amount of β-estradiol corresponding to a dose of 800 pmol/g rat or 40 μg/rat or the equivalent amount (on a molar basis) of β-estradiol ester. The solutions of β-estradiol given intravenously (0.5 ml) were made in PEG 400-isotonic saline (1:9 v/v) and contained an amount of the drug corresponding to a dose of 100 pmol/g rat or 5 μg/rat.

Bioavailability studies in rats

Female Wistar rats, weighing 190–210 g at the start of the study, were acclimatized for 14 days and kept in standard cages. They were fasted for 20 h prior to drug administration. The oral dosing was performed by means of a steel probe with a length of 6 cm, whereas the i.v. dosing was carried out by injection through the tail vein. Before blood sampling the animals were anesthetized with carbon dioxide-oxygen (80 and 20%). Blood samples of 2 ml were taken from the ophthalmic venus plexus at 2, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360 and 480 min after administration. A sample was also taken at 1 min after i.v. administration. One rat was used for each sampling.

Each compound was simultaneously given to two rats and serum samples from the animals were pooled at each sampling time prior to analysis. The serum, obtained by centrifugation of the blood samples for 10 min at 3000 rpm, was analyzed for β-estradiol and estrone by a radioimmunoassay, the analysis being performed by Statens Seruminstitut, Copenhagen. The sensitivity of the assay was about 10 pg/ml. Separate experiments showed that the β-estradiol esters did not interfere with the estradiol assay. Baseline (predose) serum estradiol concentrations were not subtracted from the postdose sample values because the latter exceeded the endogenous values by a factor of at least 15.

Results and Discussion

The objective of the present work was to examine whether bioreversible esterification of the 3-OH or 17-OH groups in 17β-estradiol may result in increased oral bioavailability of the parent compound using a rat model. In order to estimate the absolute or systemic bioavailability of orally administered 17β-estradiol in the rat, the compound was given both orally and by i.v. administration. The serum concentration-time curves observed after these routes of administration are

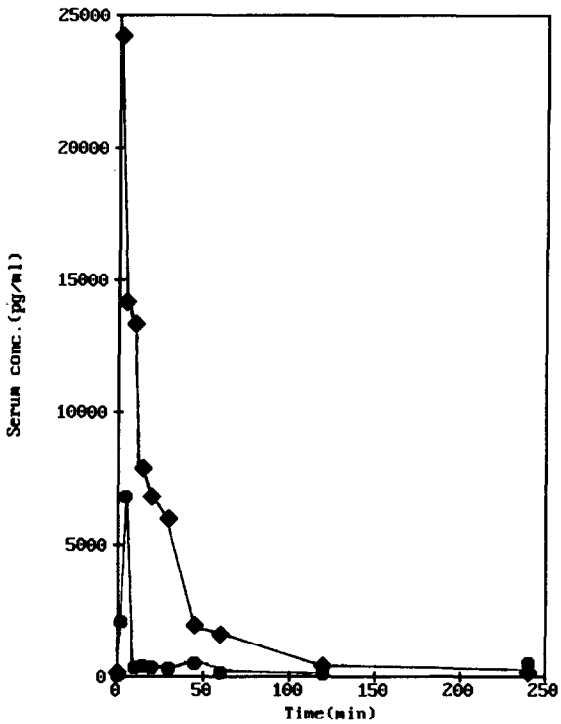


Fig. 1. Serum levels of unchanged 17β-estradiol in rats following i.v. (◆) and oral (●) administration of 17β-estradiol.

shown in Fig. 1. The serum concentrations after i.v. administration declined rapidly with an initial half-life of approx. 7 min. The terminal half-life was 50 min. The absolute bioavailability after oral administration, $F(\%)$, was determined according to:

$$F(\%) = \frac{\text{AUC}_{\text{oral}}}{\text{AUC}_{\text{i.v.}}} \times \frac{\text{Dose}_{\text{i.v.}}}{\text{Dose}_{\text{oral}}} \times 100 \qquad (1)$$

where AUC is the area under the serum concentration-time curves (0–480 min) as calculated using the trapezoidal rule.

At a dose of 40 μg/rat, the absolute oral bioavailability of 17β-estradiol was found to be only 4.3%. This value is similar to that (3.7%) reported previously by Bawarshi-Nassar et al. (1989) after intraduodenal administration of 17β-estradiol to rats at doses of 5–20 μg/rat.

The serum concentration-time curves of 17β-estradiol (E_2) obtained after oral administration of the 17β-estradiol esters **II–VII** are shown in Fig. 2. The AUC values, oral bioavailabilities and E_1/E_2 ratios obtained from the AUC values of both 17β-estradiol and estrone (E_1) are listed in Table 1.

The results obtained show that no ester prodrug affords any significant improvement in oral bioavailability in the rat. The 17-valerate and 17-cypionate esters show 1.6–1.8-fold higher bioavailability than the parent drug but the absolute bioavailability is still quite low. The systemic bioavailability of the 17-valerate ester in humans following the administration of an oral dose of 2

TABLE 1

Area under the serum level curves (0–480 min) for 17β-estradiol (E_2) and estrone (E_1), absolute bioavailabilities (F) of unchanged 17β-estradiol and E_1/E_2 ratio following the oral administration of 17β-estradiol and various ester derivatives in equimolar doses (corresponding to about 40 μg estradiol/rat) to rats [a]

Compound		AUC (ng min ml^{-1})		E_1/E_2	F (%)
		E_2	E_1		
17β-estradiol	**(I)**	132	42	0.3 [b]	4.3
Estradiol-17-acetate	**(II)**	135	49	0.3	4.4
Estradiol-17-valerate	**(III)**	203	40	0.2	6.6
Estradiol-17-cypionate	**(IV)**	233	51	0.2	7.6
Estradiol-3-benzoate	**(V)**	30	38	1.3	1.0
Estradiol-3-acetylsalicylate	**(VI)**	28	142	1.1	4.2
Estradiol-3-anthranilate	**(VII)**	98	43	0.4	3.2

[a] Data were obtained from pooled serum samples from two rats for each compound and each sampling time.
[b] E_1/E_2 ratio after i.v. administration of 17β-estradiol was 0.2.

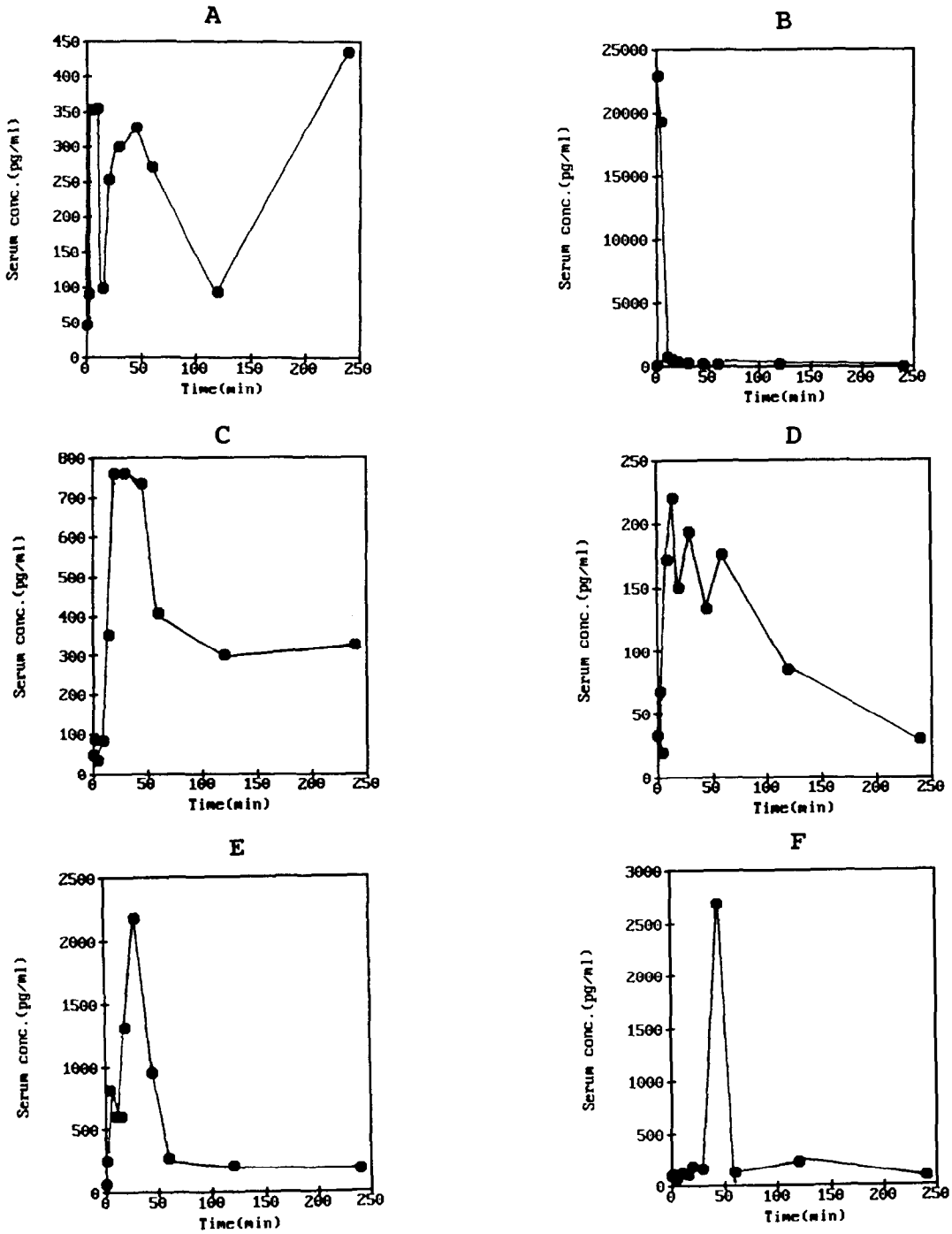


Fig. 2. Serum levels of unchanged 17β-estradiol in rats following oral administration of the 17-acetate (A), 17-valerate (B), 17-cypionate (C), 3-benzoate (D), 3-acetylsalicylate (E) and 3-anthranilate (F) esters of 17β-estradiol.

mg has also been shown to be very low (about 3%) (Düsterberg et al., 1985). The low bioavailabilities found for the 3-acetylsalicylate (**VI**) and 3-anthranilate (**VII**) esters are in great contrast to the findings reported by Hussain et al. (1988). The oral estradiol bioavailability after administra-

tion of esters **VI** and **VII** to dogs was reported to be 17- and 5-fold higher, respectively, than after oral 17β-estradiol. Apparently, there is considerable species variation in the metabolic handling of these esters. It is of interest to note the much higher E_1/E_2 ratio observed with these two esters than with the other compounds (Table 1). The present results indicate that the esters studied are rapidly hydrolyzed before or during the first-pass metabolism in the rat so that no protection of the estradiol molecule is achieved. In separate experiments, it was found that the esters **II–VII** were rapidly hydrolyzed in vitro in a 10% rat liver homogenate, the half-lives being less than 10 min.

In conclusion, esterification of 17β-estradiol at either the 3-phenolic hydroxyl group or at the 17-hydroxyl group with various aliphatic and aromatic acids resulted in no marked improvement of the oral bioavailability of the parent drug as assessed in the rat.

References

Balfour, J.A. and Heel, R.C., Transdermal estradiol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic efficacy in the treatment of menopausal complaints. *Drugs*, 40 (1990) 561–562.

Bawarshi-Nassar, R.N., Hussain, A.A. and Crooks, P.A., Nasal absorption and metabolism of progesterone and 17β-estradiol in the rat. *Drug Metab. Disp.*, 17 (1989) 248–254.

Diczfalusy, E., Franksson, C. and Martinsen, B., Oestrogen conjugation by the human intestinal tract. *Acta Endocrinol.*, 38 (1961) 59–72.

Düsterberg, B., Schmidt-Gollwitzer, M. and Hümpel, M., Pharmacokinetics and biotransformation of estradiol valerate in ovariectomized women. *Hormone Res.*, 21 (1985) 145–154.

Fishman, J., Goldberg, S., Rosenfeld, R.S., Zumoff, B., Hellman, L. and Gallagher, T.F., Intermediates in the transformation of oral estradiol. *J. Clin. Endocrinol. Metab.*, 29 (1969) 41–46.

Hermens, W.A.J.J., Deurloo, M.J.M., Romeyn, S.G., Verhoef, J.C. and Merkus, F.W.H.M., Nasal absorption enhancement of 17β-estradiol by dimethyl-β-cyclodextrin in rabbits and rats. *Pharm. Res.*, 7 (1990) 500–503.

Hussain, M.A., Aungst, B.J. and Shefter, E., Prodrugs for improved oral β-estradiol bioavailability. *Pharm. Res.*, 5 (1988) 44–47.

Lievertz, R.W., Pharmacology and pharmacokinetics of estrogens. *Am. J. Obstet. Gynecol.*, 156 (1987) 1289–1293.

Longcope, C., Gorbach, S., Goldin, B., Woods, M., Dwyer, J. and Warram, J., The metabolism of estradiol; oral compared to intravenous administration. *J. Steroid Biochem.*, 23 (1985) 1065–1070.

Powers, M.S., Schenkel, L. Darley, P.E., Good, W.R., Balestra, J.C. and Place, V.A., Pharmacokinetics and pharmacodynamics of transdermal dosage forms of 17β-estradiol: Comparison with conventional oral estrogens used for hormone replacement. *Am. J. Obstet. Gynecol.*, 152 (1985) 1099–1106.

Schipper, N.G.M., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J. and Merkus, F.W.H.M., Nasal absorption of 17-beta-estradiol and progesterone from a dimethyl-cyclodextrin inclusion formulation in rats. *Int. J. Pharm.*, 64 (1990) 61–66.

Sitruk-Ware, R., Estrogen therapy during menopause. Practical treatment recommendations. *Drugs*, 39 (1990) 203–217.

Yen, S.S.C., Martin, P.L., Burnier, A.M., Czekala, N.M., Greaney, M.O., Jr and Callantine, M.R., Circulating estradiol, estrone and gonadotropin levels following the administration of orally active 17β-estradiol in postmenopausal women. *J. Clin. Endocrinol. Metab.*, 40 (1975) 518–521.