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Oral bioavailability of 17 β-estradiol and prodrugs tested in rats, pigs and dogs

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Abstract

In this work, 15 prodrugs of 17β-estradiol have been synthesized and characterized, and the kinetic profile of the compounds has been studied in rats, pigs and dogs after oral administration. All 15 substances were investigated in the rat model. The five substances, showing the highest bioavailability in this model, were selected for studies in pigs, and the two most promising compounds in pigs were subsequently tested in dogs.

In the rat model, an improvement of the oral bioavailability with a factor three or more was seen from six of the prodrugs. In the pig model, a bioavailability, 2.5–3.7 times higher than that of estradiol was seen from three of five prodrugs.

The dog model appeared extremely sensitive in proving the prodrug effect. Compared with the parent compound, an improved bioavailability of approximately 30 times was observed from the two prodrugs tested. After administration of these substances, the ratio of estrone and estradiol in serum was 0.4–0.6. It can be concluded that in all three animal species, the most promising prodrug properties appeared from the 3-glutarate-17-succinate and 3-benzyl-succinate esters of estradiol.

Keywords: 17 β-estradiol; Prodrugs; Oral bioavailability; Rats; Pigs; Dogs

1. Introduction

1.1. Background

The menopause may be viewed as an endocrinopathy or as a normal female term of life. A fact is that most women suffer from symptoms related to estrogen deficiency in the menopause and benefit from estrogen therapy. During the 1970s, estrogen replacement therapy in postmenopausal women has proven effective in the prevention of osteoporosis (Christiansen et al., 1987). In the 1980s, a lower cardiovascular morbidity and mortality after estradiol treatment were observed. As a result, the use of estrogens by gynaecologists and endocrinologists has greatly increased (Mishell, 1987).

Micronized 17β-estradiol and conjugated and esterified estrogen mixtures, respectively, are the most common used oral route of administration (Sitruk-Ware, 1990). The former is the primary estrogenic hormone produced by the ovary. How-

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ever, the systemic oral bioavailability of 17β-estradiol in humans is only approximately 10% due to an extensive first-pass metabolism of the drug in the gastrointestinal mucosa and the liver (Longcope et al., 1985). The main routes of this metabolism are (1) oxidation of the 17-hydroxyl group to give estrone, and (2) conjugation of the 3-phenolic group in both estradiol and estrone to form sulfate and glucuronide conjugates (Diczfalusy et al., 1961; Fishman et al., 1969; Yen et al., 1975; Longcope et al., 1985). These metabolites have been accused of causing changes in the 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 bypassing the high first-pass metabolism of 17β-estradiol is to administer the drug by a non-oral route such as the transdermal route (Balfour and Heel, 1990) or the nasal route (Bawarshi-Nassar et al., 1989; Hermens et al., 1990; Schipper et al., 1990). Another approach is to administer the drug orally as a prodrug, resisting the metabolism in the gastrointestinal mucosa and the liver. Following the passage of these sites of first-pass metabolism, the prodrug will be cleaved in the circulation and the active drug released. By increasing the oral bioavailability, using this principle, the daily dose can be diminished and still provide the patient with the complete therapeutic effect. Concurrently, the incidence of side effects may be minimized by the lower dose.

1.2. Study objective and design

In this study, the systemic oral bioavailability of a series of estradiol prodrugs has been estimated in rats, pigs and dogs and compared with that of the parent compound. For use in the study, 15 prodrugs of 17β-estradiol were synthesized and adequately identified by NMR-spectroscopy and determination of melting points prior to use. After dissolution in suitable vehicles, each compound was administered to groups of animals, intravenously on day one and orally a couple of weeks later. At appropriate times after administration, venous blood samples were drawn and subsequently prepared for determination of the contents of estradiol, estrone and estrone sulfate by radioimmunoassay. From the individual results, the kinetic parameters, C_{max} (the maximum serum concentration), T_{max} (the time for maximum serum concentration), AUC (area under the time-concentration curve), $t_{1/2\beta}$ (the elimination half life), $F_{absolute}$ (the absolute bioavailability factor), $F_{relative}$ (the relative bioavailability factor), and the ratio of E_1/E_2 levels in serum were calculated. The mean parameters obtained by each prodrug and the parent compound were compared and evaluated.

All 15 compounds were studied in rats. Five of the substances, showing the highest bioavailability rates were subsequently investigated in pigs, and from these studies, the best two prodrugs were selected for kinetic studies in dogs.

2. Materials and methods

2.1. Test compounds

As mentioned above, 15 prodrugs of 17β-estradiol were synthesized for the purpose. The chemical name and structure of the single prodrugs are shown in Fig. 1A and B.

To make it easier to the reader, the prodrugs will be referred to in the following text by their roman numeral as indicated in Fig. 1A and B.

2.2. Synthesis

2.2.1. Apparatus

High-performance liquid chromatography (HPLC) was performed by a Waters apparatus, consisting of a 510 pump, a 717 wisp and a 490 E variable wavelength detector. A deactivated reversed-phase Vydac column (4.6 mm) was used. ^{1}N NMR spectra were run on a Bruker instrument, 400 Mz. BZH 400/52. Melting points were taken in capillary tubes. Chremophor® El was obtained from BASF, Germany, polyethylene glycol (PEG 400) from E. Merck, Darmstadt, Germany, and hydroxypropyl-β-cyclodextrin from Aldrich-Chemie, Steinheim, Germany.

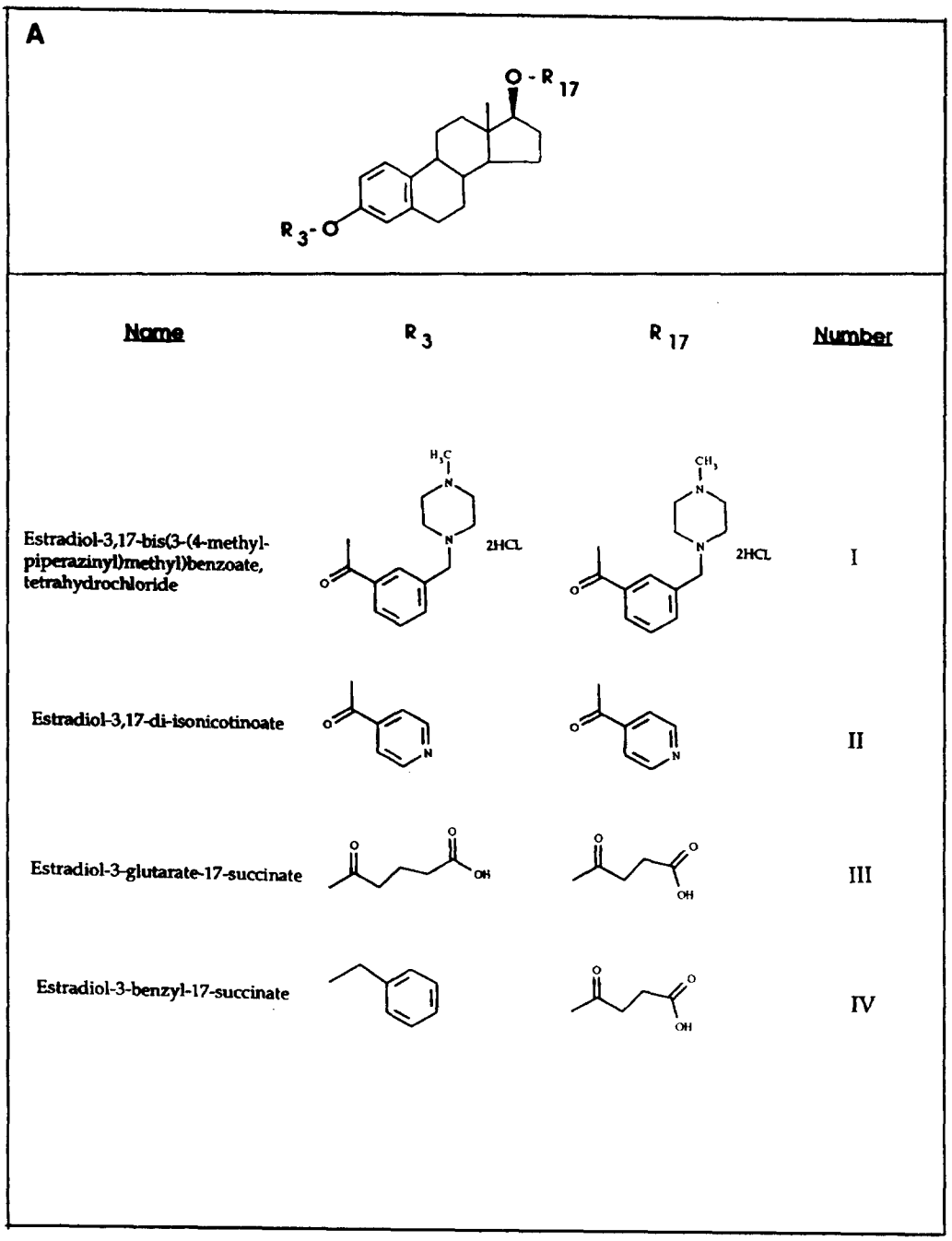
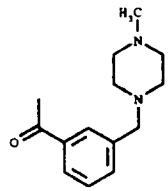
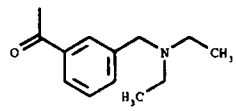
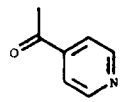
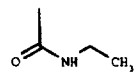
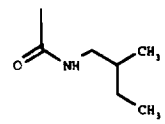
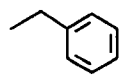
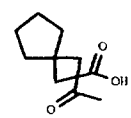
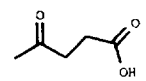
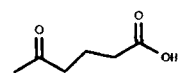


Fig. 1. Chemical name and structure of the single prodrug.

B

Name	R_3	R_{17}	Number
Estradiol-3-(3-(4-methyl-piperazinyl)methyl)benzoate, dihydrochloride			V
Estradiol-3-(3-diethylamino-methylbenzoate), hydrochloride			VI
Estradiol-3-isonicotinoate			VII
Estradiol-3-glutarate			VIII
Estradiol-3-monoethyl-carbamate			IX
Estradiol-3-(2-methylbut-1-yl)-carbamate			X
Estradiol-3-benzylether			XI
Estradiol-3-methylether			XII
Estradiol-17-3,3-(tetramethylenglutarate)			XIII
Estradiol-17-succinate			XIV
Estradiol-17-glutarate			XV

2.2.2. Synthesis of derivatives I, V, VI, VIII, IX, X and XV

I, V and VI were synthesized by H. Bundgaard, The Royal Danish School of Pharmacy, as previously reported (Bundgaard et al., 1989) and VIII, IX, X, and XV were synthesized according to methods from the literature, analogous to previously described by Dittert and Higuchi, 1963; Al-Rawi and Williams, 1977.

2.2.3. The derivatives III, XII, XIII, XIV and 17-β-estradiol

17β-estradiol and compound XII and XIV were purchased from Sigma Chemical Co., St. Louis, USA. Derivatives III and XIII were synthesized by and purchased from Clauson-Kaas, Farum, Denmark.

2.2.4. Synthesis of derivatives II, IV, VII and XI

As described by Dietzel et al. (1990), compound II and VII were prepared, and melting points and ^{1}H-NMR spectra were identical with those reported. IV and XI: 17β-estradiol or equal amount of compound XIV (5.0 g, 18 mmol) was added to a stirred solution of benzylchloride (2.5 ml, 21 mmol) and K_2CO_2) (5.0 g) at 60°C. After 24 h, the product was evaporated in vacuo to leave a yellow oil. The residue was dissolved in chloroform (75 ml). The chloroform layer was washed with water to pH = 7 ($\cong$ 5 × 100 ml). The chloroform layer was dried and evaporated in vacuo. Recrystallization from methanol resulted in colourless crystals: 80%. m.p. 289–293°C. ^{1}H-NMR (400 MHz, Me_2SO-d_6) (XI): δ 0.7 (3H, s, methyl), 1.15-3.65 (16 H, m, alif.), 3.7 (1H, t, 17 α-H), 5.1 (2H, s; CH_2-Benz.), 7.2.-7.6 (5H, m, arom. benz.). (IV): δ 5.1 (2H, s, CH_2-Benz.), 7.2-7.5 (5H, m, arom) 12.1-12.3 (1H, interchangeable with D_2O).

2.3. Preparation of solutions for administration

Solutions suitable for the intravenous use in rats were prepared by dissolving estradiol in a mixture of PEG® 400 and sterile physiological saline (1:9 v/v). The concentration of each compound was chosen to provide a solution containg 10 μg per ml.

For the oral administration of rats, solutions of estradiol and its esters were dissolved in an ethanol, Chremophor® EL, water mixture (1:1:8 v/v). The solutions contained an amount of estradiol corresponding to 80 μg per ml.

Both for intravenous and oral administration in the pigs, solutions were made by dissolving 20 mg of estradiol or an equivalent quantity of prodrug in ethanol, PEG 400 and physiological saline (10:25:65 v/v) to 100 ml, providing 200 μg estradiol or equivalent per ml.

For both routes to the dogs, solutions were prepared by dissolving 20 mg estradiol or an equivalent quantity of prodrug in ethanol, hydroxypropyl-β-cyclodextrin and physiological saline (10:10:80 v/w) to 100 ml. The solution contained 200 μg estradiol or an equivalent prodrug per ml.

2.4. Test systems

As predictive models for the anticipated human absorption rate, three species of laboratory animals were used:

(1) Female Wistar rats, weighing 190–210 g b.w. (body weight) at the start of the study
(2) Premature female pigs of Danish country strain, weighing approximately 40 kg b.w.
(3) Female Beagle dogs, weighing 15–18 kg b.w.

All animals passed a clinical health control, were individually marked with ear tattoo numbers and, during the study period, housed and nursed according to international standards.

The size of the groups included in the study was as follows: 48 rats per dosing group (divided into 12 subgroups of 4 rats per blood sampling time), 2 pigs per dosing group and 2 dogs per group. Prior to analysis, blood samples were pooled. From the day before administration of the estradiol and estradiol esters, all animals were fasted.

2.5. Dosing and sampling

2.5.1. Rats

The groups of rats were dosed intravenously in a tail vein with a bolus of 0.5 ml of estradiol made for parenteral use. This is equal to a dose of 5 μg

estradiol per rat. Other groups of rats received by oral gavage 0.5 ml of the solutions prepared for this use, corresponding to 40 μg per rat or approximately 800 pmol per g rat.

After administration, blood samples were drawn at 0, 2, 5, 10, 15, 20, 30, 45, 60, 120 and 180 min. After intravenous administration, a sample at 1 min was also collected.

Before blood sampling, the animals were anaesthetized by inhalation of carbon dioxide-oxygen (80/20%). From the ophthalmic venous plexus, blood samples of 2 ml were taken from the anaesthetized animals. Only one sample from each animal was taken on the same study day.

For each compound and at each sampling time, blood from 4 animals were collected (see above) and the serum from these 4 samples was pooled before analysis.

2.5.2. Pigs

In the morning of the day of the study, the pigs were placed in individual fixation boxes. A permanent catheter was introduced into one auricular vein and filled with heparin in physiological saline (25 i.e./ml). Through this catheter, the intravenous dose of the estradiol or prodrug solutions was injected as a bolus. Each pig received a dose of 2 mg estradiol or an equivalent amount of prodrug. After appropriate rinsing with physiological saline, blood samples were drawn through the ear catheter at 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 30, 45, 60, 90, 120, 150 and 180 min after administration.

Through a stomach tube, the oral dose was infused and followed by 100 ml lukewarm water. Each pig was given 10 mg of estradiol or an equivalent dose of prodrug. The same time schedule for blood sampling as for the intravenous study was used, except that the samplings at 1, 2, 3 and 6 min were omitted and 25 min were added.

2.5.3. Dogs

As the best results were expected from the double derivatives, and the number of experiments in the dog model was limited, only two of the prodrugs (III and IV) were tested.

In the morning of the day of study, the dogs were placed in individual fixation slings and a Venflon catheter introduced into one forearm vein. As for the pigs, the intravenous dose of estradiol or an equivalent dose of prodrug was infused as a bolus through this catheter. The dose injected was 1 mg estradiol or an equivalent dose.

Through the catheter, blood samples were drawn at 0, 2, 4, 6, 8, 12, 16, 20, 30, 45, 60, 120, 180, 240 and 480 min after administration.

Through a stomach tube, the oral doses of 2 mg estradiol or an equivalent dose of prodrug were infused and each time followed by 100 ml lukewarm water. The same time schedule for blood sampling was used, except that 2 and 6 min were omitted.

2.6. Laboratory assays

The contents of estradiol, estrone and estrone sulfate in serum, obtained by centrifugation of the blood samples for 10 min at 3000 rpm, were analysed using a radioimmunoassay technique at Statens Seruminstitut, Copenhagen. For serum samples $\geq$ 1 ml, the sensitivity of the assay was approximately 10 pg/ml. From previous experiments, it was known that the estradiol esters do not interfere with the estradiol assay. Baseline (predose) serum estradiol concentrations were not subtracted from the postdose serum values because the latter exceeded the endogenous values by a factor of at least 15.

2.7. Handling of data

From the laboratory data, the following parameters were calculated using standard mathematical formulas: T_{max}, C_{max}, $t_{1/2\beta}$ and AUC according to the trapezoidal rule. Based on the AUC figures, the following figures were calculated:

$F_{absolute}$ (%) = $(AUC_{oral}/AUC_{intravenous} \times Dose_{intravenous}/Dose_{oral}) \times 100$

$F_{relative}$ = $AUC_{prodrug}/AUC_{estradiol}$ (at equal doses orally)

Ratio of E_1/E_2 = $AUC_{estrone}/AUC_{estradiol}$ (for the same substance orally)

Table 1
The mean C_{max} and T_{max} values obtained with estradiol and the prodrugs in the three species

Number of compound	Rat		Pig		Dog	
	T_{max} min	C_{max} pmol/l	T_{max} min	C_{max} pmol/l	T_{max} min	C_{max} pmol/l
Estradiol	2	12200	20	2200	12	340
I	10	550				
II	30	3200				
III	30	21400	16	240	45	8300
IV	15	18100	4	2700	90	6300
V	5	145000				
VI	12	69100	25	1900		
VII	30	6200				
VIII	8	105400	180	6400		
IX	12	24200	16	2100		
X	12	22900				
XI	60	14100				
XII	10	8100				
XIII	20	1100				
XIV	5	16200				
XV	15	25400				

3. Results

In the selected animal models, the mean $F_{absolute}$ of 17β-estradiol was low, 4.3% for rat, 1.2% for pig and 0.3% for dog. In the three species, the elimination half lives were nearly the same, 50 min in rat, 49 min in pig and 45 min in dog.

The mean C_{max} and T_{max} values, obtained with estradiol and the prodrugs in the three species, are presented in Table 1.

In the rat model, significant elevated C_{max} values were seen from seven of the 15 prodrugs (III, V, VI, VIII, IX, X and XV), compared with estradiol. The maximum values appear practically at the same T_{max} time as for estradiol. For two of the prodrugs (V and VIII), C_{max} values more than five times the C_{max} values for estradiol were measured. No significant improvements of the mentioned values were seen from the remaining eight prodrugs.

Of the five prodrugs tested in the pig model, significant higher C_{max} values were obtained from two substances (VI and VIII), compared with estradiol. The T_{max} value for one prodrug (VIII) was extremely elevated.

From the two prodrugs selected for investigation in the dog model (III and IV), highly elevated C_{max} and T_{max} values were seen, compared with estradiol. For substance III, the C_{max} value was approximately 24 × higher than that of estradiol, and the T_{max} value was 4 × prolonged. For compound IV, the factors were approximately 19 × higher and 8 × prolonged, respectively.

From the studies of the prodrugs in the three animal models, the mean $F_{relative}$ and ratios of E_1/E_2 in serum are presented in Table 2.

In the rat study, $F_{relative}$ factors higher than 3.0 were seen from six of the prodrugs (III, IV, VIII, IX, XI and XV), compared with estradiol, as reflected in Table 1. For 12 of the substances, the ratio of E_1/E_2 concentrations in serum dropped to 0.4 or below. An increase to 1.0 or more was seen for two of the substances. In the pig model, $F_{relative}$ factors higher than 3.0 were measured for two substances (IV and VIII). In the dog model, bioavailability factors of 28 and 33, respectively were seen from the same two prodrugs tested (III and IV), and E_1/E_2 ratios of 0.4 and 0.6, quite different from the 2.2 value obtained with estradiol.

Table 2
The $F_{Rel.}$ and the ratios of E_1/E_2 in serum obtained in the three animal models. Pooled values are used as follows: $n = 4$ for rats, $n = 2$ for pigs and $n = 2$ for dogs

	Rat		Pig		Dog	
Absolute availabilities (F, % unchanged 17β-estradiol)	4.3%		1.2%		0.3%	
$T_{1/2}$	50 min		49 min		45 min	
Number of compound	Rat (n = 4)		Pig (n = 2)		Dog (n = 2)	
	$F_{Rel\ (\%)}$	E_1/E_2	$F_{Rel\ (\%)}$	E_1/E_2	$F_{Rel\ (\%)}$	E_1/E_2
Estradiol	1	0.6	1	1.7	1	2.2
I	0.4	0.6				
II	1.0	0.4				
III	3.7	0.2	2.5	1.8	28	0.4
IV	3.6	0.4	3.7	1.3	33	0.6
V	2.3	0.3				
VI	2.2	0.3	1.6	2.5		
VII	1.6	0.3				
VIII	4.4	0.4	3.5	0.3		
IX	3.1	0.3	1.6	1.4		
X	1.3	0.4				
XI	3.0	0.4				
XII	1.0	1.3				
XIII	0.2	1.0				
XIV	1.6	0.4				
XV	3.4	0.2				

4. Discussion

In these studies, the potential improvement of the bioavailability of estradiol by esterification of the molecule with prodrug groups have been elucidated in research animals. For this study, the rat, pig and dog have been chosen, as these species are well known as highly suitable for studies of hormones and kinetics, respectively. Compared with the rat and the pig model, the dog kinetic model seemed to be far the most sensitive to prove changes of the bioavailability factor. As described by others, the main part of the absorbed estradiol is rapidly transformed into estrone sulphate in pigs. Subsequently, the serum levels of estradiol remain rather low, even after high doses of both the parent compound and its esters.

Until now, only few data have been published by others on this matter, which are directly comparable. Bawarshi-Nassar et al., 1989 have studied the intravenous and nasal absorption of estradiol in rats and found nearly the same kinetic profile by the intravenous injection as the authors of this study. Hussain et al., 1988 observed an improvement of the oral bioavailability of two prodrugs of estradiol of a factor five and 17, respectively. Longcope et al., 1980 dosed radiolabeled estradiol to dogs and calculated an oral bioavailability of estradiol of a few per cents. The present data are in good accordance with the data stated in these publications. The results from this study indicate that a significant improvement of bioavailability may be obtained by this derivatization procedure. The best absorption data were obtained with estradiol esterified in both the 3- and 17-positions using glutarate-, benzyl- and succinate groups. In all three species, promising improvements of oral bioavailability were seen from the best compounds, which with the selected substances may predict quite good chances for improving the absorption rate in humans.

The authors are reluctant to compare the bioavailability factors obtained from the three species directly, as different solution media were used for administration in the three species. Also the number of pigs and dogs included in the study was too low for exact conclusions. However, there is no doubt that a significant improvement of the bioavailability factor by the esterification of estradiol with prodrug groups was obtained from the animal models used.

5. Conclusion

In rats, pigs and dogs, 15 17 β-estradiol prodrugs were synthesized and studied in vivo for oral bioavailability compared with 17 β-estradiol. As basic data, the oral bioavailability of estradiol was estimated to 4.3% in rats, 1.2% in pigs and 0.3% in dogs. In the three species, the elimination half lives were 50, 49 and 45 min, respectively.

All 15 compounds were studied in the rat kinetic model. The five substances, showing the highest bioavailability in this model were selected for studies in pigs, and the two most promising compounds in pigs were subsequently tested in dogs. In the rat model, a bioavailability factor three times higher than that of estradiol was seen from six of the prodrugs. In the pig study, an improvement of a factor three or more was seen from two prodrugs. In the dog model, a bioavailability factor of 28 and 33, respectively was seen from the same two substances compared with estradiol. Furthermore, a change of the ratio of E_1 and E_2 in serum towards more physiological levels was observed by the oral administration of the selected prodrugs, i.e. in fertile women, E_1 is $< E_2$.

In this study, the most promising prodrug properties in all models were seen from the 3-glutarate-17-succinate and 3-benzyl-17-succinate esters of estradiol, both regarding bioavailability and ratio of estrone/estradiol in serum.

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